



Solvent-based Extraction of Tea Polyphenols and their Health Benefits

Munisha Thakur^{1*}, Bhavika Gupta¹, Kajal Thakur² and Amandeep Kaur²

¹Student, M.Sc. Hons. Biotechnology, School of Bio Engineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India.

²Assistant Professor, School of Bio Engineering and Biosciences Lovely Professional University Phagwara, Punjab, India.

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*Address for Correspondence

Munisha Thakur

Student, M.Sc. Hons. Biotechnology,
School of Bio Engineering and Biosciences,
Lovely Professional University,
Phagwara, Punjab, India.
E.Mail: misha999thakur@gmail.com



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ABSTRACT

Tea is one of the beverages consumed all over the world prepared using buds or leaves of *Camellia sinensis*. It is rich in polyphenolic compounds giving the tea its flavour and aroma as well as various health benefits. Recently, the role of tea in providing antioxidants, anti-inflammatory, antibacterial, and anti-obesity properties, and treating different cardiovascular diseases has been in limelight. To enrich these bioactive compounds for their health benefits, the focus has been shifted to the appropriate extraction of tea polyphenols. One of the traditional methods, relying on solvent extraction, is preferred all over the world due to its high recovery. A number of in vivo and in vitro studies have been conducted to observe the effect of these extracted phenols on the human health. This resent review will summarize the bioactive composition of major type of teas consumed worldwide and different methods used for the extraction of these bioactive compounds highlighting their health benefits.

Keywords: Tea, Polyphenols, Solvent-based Extraction, Bioactive compounds, Therapeutic Benefits

INTRODUCTION

Tea (*Camellia sinensis*), a member of the Theaceae family, is one of the most popular beverages around the globe among coffee and cocoa. Besides its commercial importance, tea is reported to have pharmaceutical potential in treating conditions including cancer and cardiovascular disease and [1,2]. The leaves and buds of the tea plant, are used to prepare the beverage, which was initially developed in China. Tea was first utilised as a therapeutic beverage in China, and it then spread to other nations such as Japan, America, Africa, and Europe. Presently, it is grown in





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about 30 different nations [3]. Catechins which are present in leaves of green tea in abundance is a bioactive compound which have also been employed as food additives and in commercial items. These catechins are utilised in toothpastes, breath fresheners, and mouthwash in the pharmaceutical business in addition to commercial items, the food industry, and both.

There are three main tea types (black, oolong, and green) and the oxidation difference of polyphenols from tea during fermentation distinguishes these different types of tea. Black tea is completely fermented tea, whereas, oolong tea is partially or half fermented, and then green tea does not undergo any fermentation process. The fusion of flavins and theaflavins that results from the fermentation of black tea gives tea its well-known colour and flavour. Although tea has numerous healthy elements, its principal constituents—polyphenols—are what give it its distinctive flavour and health benefits. According to certain reports, green tea contains more polyphenols than black and oolong tea [4]. Yellow tea also includes a lot of polyphenols, in addition to green tea. This tea is very lightly fermented and is similar to green tea as well. But requires a step called sealed yellowing which differentiated it with the green tea [5]. Their polyphenols are powerful antioxidants as well. Which have many health-related benefits.

Catechins in leaves is the majority of polyphenols. Catechins and their derivatives, such as catechin, epicatechin (EC), Gallo catechin (GC), epigallocatechin (EGC), catechin gallate (GC), epicatechin gallate (ECG), Gallo catechin (GCG), and epigallocatechin gallate, are particularly abundant in polyphenols (EGCG) [6]. Flavan-3-ols and their oligomers, phenolic acids, flavanols and their glycosides, hydro stable tannins, theaflavins, and thearubigins are all examples of polyphenols. Each type of tea contains a unique number of polyphenols, which varies depending on how it was made. Because of this, white tea which is least processed and only dried in a controlled environment has a higher concentration of polyphenols than green tea, making it a more powerful antioxidant. White tea is air dried and may be just kept under the sun to dry. Compared to green tea, oolong tea has half the amount of epigallocatechin-3-gallate but double the number of polymerized polyphenols [7].

Tea polyphenols must be extracted in order to get the health benefits they provide. There are different techniques for extraction such as water based, microwave assisted extraction, subcritical water extraction, ultrasound assisted extraction. One other way to extract tea's polyphenols is by solvent-based extraction procedures. In comparison to other approaches, solvent-based extraction gives a better yield. With this technique, soluble chemicals are separated from solid matrixes like plants using a liquid matrix. To prevent the sample from deteriorating, the treatment is conducted at a lower temperature. The volume of the extract and the components we can extract from the sample depend significantly on the different solvents we employ. Following studies on the effects of several solvents and the duration of their infusion, dichloromethane was identified as the most promising solvent for the decaffeination process. The drying and purification technique of the pattern is accomplished on the cease of the entire technique [1].

Tea: The Ancient Beverage

Tea is one of the main non-alcoholic beverages known for its taste all over the world. The main tea-producing countries, accounting for almost 80% of tea production, are China, India, Sri Lanka, Indonesia, and Kenya [8]. China is the largest producer of tea, which is said to be the birthplace of tea. India is the second largest tea-producing country following China. India has three major tea growing regions and its geographical area is ideal for mass cultivation. one in north-eastern India, including West Bengal and the Assam region; the second, the northern region, including Himachal Pradesh; and finally, the small-producing state of Tamil Nadu, southern India. India's famous teas consist of Assam and Darjeeling teas.

There are many types of tea, but he only has main 3 types of camellia tea: one is green tea, black, and finally oolong. The changes between them lie in the fermentation. This refers to the enzymatic and oxidative changes seen in tea leaves during processing. Kaur et al gave the order of consumption of tea around the world is first black tea, second green tea, and lastly oolong tea. As given in fig.1. Among these teas, black tea is the most highly fermented tea. Unlike tea production, green tea is not produced by fermentation black tea fermented to oolong tea (partially



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fermented). The complete fermentation process into BT is the outcome of the action of enzymes on the catechin and number of polyphenols present in the leaves, of the tea tree. It has active ingredients and health-enhancing properties [9]. Green tea is not fermented, black tea is totally fermented and oolong tea is semi or half fermented tea. The major components of the tea leaves are polyphenols, caffeine, theanine, proteins, lignin, carbohydrates, chlorophyll, and organic acids as shown in fig.2 [3].

Three main types of tea

Black tea

Black tea is thought to be created through the fermentation of green tea. Both oxidation and polymerization are involved processes. There is no alcohol or vinegar produced during this fermentation process. Catechins, amino acids, alkaloids, theaflavins, isomers, thearubigins, and other tea polyphenol derivatives are the main ingredients in black tea. The main amino acid present in both black and green tea is L-theanine. And the richest alkaloid in the area is caffeine. Catechins are abundantly found in green tea leaves. Catechins help create a significant amount of theaflavin. Some major theaflavin formation occurs from catechins. Formation of theaflavin (TF1) from EC and EGC, whereas ECG and EGC form theaflavin-3-O-monogallate, further EC and ECG forms theaflavin-3'-O-monogallate (TF2b) and finally formation of theaflavin-3,3'-O, O-di gallate (TF3) by ECG and ECG [10].

Green tea

Green tea, has always been consumed for its health promoting benefits, may it be for cardiovascular, cancer prevention or weight loss related benefits. The green tea benefits for the body are mainly due to presence of polyphenols Proteins, amino acids, carbohydrates, and trace elements including magnesium, calcium, cobalt, nickel, iron, and others can all be found in green tea. Green tea leaves are promptly cooked after harvesting, preventing any fermentation. Because of this, the green leaves retain their green colour and the fermentation's enzymatic activity is quickly stopped. The natural polyphenols are maintained as a result of this phase, which stops the enzyme activity and has health-promoting effects. Oolong and black tea are made from green tea, but they differ in that they contain different theaflavins as a result of the fermentation process [11].

Effects of green tea polyphenols in inhibiting cancer cell proliferation and reducing cancer risk have been studied by numerous scientific studies in the areas of prostate, breast and stomach cancers and pancreatic cancer and been confirmed. Although green tea can support both chemotherapy and preventative effects, it should be emphasized that it cannot replace pharmacological treatment [9].

Oolong tea

Oolong is a classic Chinese tea that is well-liked in southern China. When green tea partially ferments, this tea type is produced. Black tea is created when green tea has fully fermented. The various tea varieties depend on the environmental factors that affect plant growth, harvesting, and tea processing [12].

Bioactives Compounds in Tea

A tea plant contains a variety of substances, but they are distributed in varying amounts. Tea's bioactive ingredients are thought to have positive health effects. The oxidation levels of the various tea kinds affect how the bioactive components differ. The difference in their presence increases with the amount of tea oxidation and fermentation. The tea bioactive compounds consist of polyphenols, caffeine organic acids, theanine, theaflavins, flavonoids, amino acids [13]. In 1g of leaves, the polyphenolic chemicals (catechins, epicatechins) 30-42%, and caffeine 3-6% make up the majority of the bioactive components [4]. The catechins consists of epicatechin Gallo catechin, epigallocatechin, catechin gallate, epicatechin gallate, Gallo catechin, and epigallocatechin gallate and are found in abundance in polyphenols. The presence of the bioactive compounds also depends on when the leaves were collected and what was the environmental conditions of the area too therefore table 1 shows the typical presence of catechins in green tea [3,6]. The dry leaves consist of amino acids (1-4%), Phenolic compounds (18-36%) and where 12- 26% of these phenolic compounds are the catechins [13]. The catechins can be further used in pharmaceutical, food and cosmetic industries too [2,3].





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Polyphenols and catechins

Most of the active ingredients in tea are polyphenols. All teas contain these chemicals and have multiple health benefits. The benefits and taste of tea are also because of polyphenols. Green tea contains significantly more polyphenols than black or oolong tea. Epicatechin (EC), Epigallocatechin-3-gallate (EGCG), Epicatechin-3-gallate (ECG) and Epigallocatechin (EGC) fermented. The various tea varieties depend on the environmental factors that affect plant growth, harvesting, and tea processing [12].

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Antioxidant property of tea

For the antioxidant activity of green tea and black tea the tea catechins and theaflavins are the bioactive phytochemicals which are being responsible. Structural features of green tea catechin that significantly only add to its antioxidant activity are the high-level presence or sometimes absence in the galloyl moieties and, the number and the position of the hydroxyl groups present on the ring of the structure. The latter decides on their ability to be interacting with the biological agents by hydrogen bonding or by electron and hydrogen transfer processes as part of their antioxidant, activity. The, antioxidant mechanisms imply hydrogen atom transfer (HAT) or the single electron transfer reaction (SET), or both. Tea catechins exhibit antioxidant activity by scavenging the lipid alkoxy, and the peroxy radicals by acting as chain- scission, antioxidants [14].



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All the 3 teas give active ingredients such as flavonoids, vitamins etc. Flavonoids are the antioxidants, being a large class present in the body which neutralize free and extra radicals. The free radicals, which are powerful oxidizing compounds that damage cells. Oxidative cell, damage in the body only adds to the cardiovascular disease and cancer. Vitamins also have antioxidant and other anti-carcinogenic properties [15].

Tea Polyphenols and their Extraction

Vuong et al has given many different techniques for extraction from tea as given in table 2 [3]. We in our daily lives have been using and consuming tea by conventional methods therefore in comparison to other solvent approaches using water, organic solvent-based extraction gives a better yield. With this technique, soluble chemicals are separated from solid matrixes like plants using a liquid matrix [1]. This particular technique can make use of a variety of organic solutions. The advantage of utilising polar organic solvents over only water is that they produce a higher yield. Even water can be utilised in this procedure, which uses a wide variety of temperatures to provide superior results. Both hot and cold-water extraction are possible. Acetone, ethanol, methanol, and acetonitrile are a few of the organic solvents employed. Another advantage of employing these solvents is that, following extraction, they require relatively little energy to evaporate [3]. The type of plant employed and the elements we require from the plant are factors in solvent extraction. Depending on the plant, different solvents are employed. Menstruum is the term for the solvent used with medicinal herbs. The difference also depends on whether you extract the polar and non-polar chemicals from the plant using polar or non-polar solvents. Polarity is important in the solvent extraction process; if we are using liquid-liquid extraction, we must remember that the extraction must be soluble in organic solvent and not in water in order to obtain a better yield from the sample [16].

Health benefits of Tea polyphenols

Tea contains many health benefits, including antioxidant, antimutagenic, and anticarcinogenic properties [8]. The medicinal use of tea expands to many benefits including inflammation inhibition, benefits against diabetes, effects against obesity and helps in reduction of many types of cancers. Because of these effects of tea all around the world many products have been produced may it be only as tea or many other supplements having tea present in them [17]. The health improving properties for the body by green tea is totally because of presence of polyphenols, especially flavanols and presence of flavanols. Some clinical, studies done vivo and in vitro experiments have confirmed its antioxidant and, anti-inflammatory properties. Catechins in leaves is the majority of polyphenols in green tea and their anti-oxidant activity stems due to their neutralization of extra nitrogen species and oxygen species and their ability to disturb metal ions in redox-reactions in body. Tea polyphenols have shown some beneficiary results may it be in vivo or invitro studies. These showed some anticarcinogenic effects on lung cancer, breast cancer, prostate cancer, and skin cancer where some in vivo and in vitro studies were done. Tea polyphenols also do help in cardiovascular and neurological diseases where EGCG was the focus of the study. Black tea consists of theaflavins and thearubigins as polyphenolic compounds [4].

Many advantages of polyphenols have additionally been visible together with antioxidant, anti-cardiovascular, anti-inflammation, antimicrobial and anti- obesity. The green tea especially additionally suggests advantages with illness together with associated with Alzheimer disorder, Parkinson's disorder, and diabetes [20]. Parkinson's disease is a neurological condition that should steadily worsen over time. There may be a reduction in the risk of Parkinson's disease with the consumption of tea and coffee, according to studies. There was a positive effect on tea drinkers compared to non- drinkers, according to studies. Depending on the type of tea being consumed, the part of the tea being consumed, and how frequently, the effects of the tea may vary [10]. Tea and coffee have been demonstrated to have benefits for preventing obesity, both the plant and their compounds have showed some protective properties. Not only may the plant be used to cure obesity, but it can also be utilised to prevent and treat heart disease, metabolic issues, and type 2 diabetes [27]. Skin cancer is also a major health problem which comes with costly medical services. One of the major causes may be due to exposure of skin to ultraviolet radiations where it damages the DNA. Studies were done to see the preventive effects of green tea consumption on DNA repair [14,28,29].





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CONCLUSION AND FUTURE PROSPECTS

Tea polyphenols are constantly being researched and are likely to produce more beneficial results. They have many health benefits and have been used for many years. Different extraction techniques offer more ways to improve yields, but they may not be available to everyone. Therefore, traditional solvent extraction methods have been passed on to people and their continued use can make a difference in their health. Therefore, more research should be continued to see what other health benefits polyphenols can provide and how tea can improve your daily lifestyles to a healthier one.

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Table: 1. Catechins present in green tea

| Catechins | % Present |
|-----------|-----------|
| EGCG | 10-15% |
| EGC | 6-10% |
| ECG | 2-3% |
| EC | 2% |

Table: 2. Tea Polyphenols and their Extraction

| S.no. | Extraction techniques | Advantage | Disadvantage |
|-------|--------------------------------|--|--|
| 1 | Cold water | Is more efficient in chelating ferrous ions and scavenging 1,1-diphenyl-2-picrylhydrazyl free radicals as compared to hot water. | Lower yield than hot water extraction. |
| 2 | Hot water | High yield. | Maintain the temperature or the catechins may degrade |
| 3 | Organic solvent extraction | High yield. Require less energy to evaporate. | Can leave unwanted residues. |
| 4 | Microwave assisted extraction. | Shorter extraction time. | Used in less extent to conventional methods for industrial applications. |
| 5 | Subcritical water extraction | Higher extraction rate than conventional methods. | Degradation of catechins at high temperature. |
| 6 | Ultrasound assisted | Has greater penetration ability of | High cost. |





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| | | | |
|---|------------------------------|---|---|
| | extraction | solvent and is effective for bioactive compound extraction. | |
| 7 | Ultrahigh pressure technique | Used to extract caffeine from tea. High yield. Short extraction time. | Further studies needed to see its use in extraction of catechins. |

Table 3: Uses of tea for different therapeutic purposes

| S.no. | Diseases | Studies | Reference |
|-------|-------------------------|--|-----------------|
| 1 | Alzheimer’s disease | Showed daily consumption of tea reduced the Alzheimer’s risk in elderly | [21],[22] |
| 2 | Parkinson’s disease | It was observed that intake of EC (epicatechin) and proanthocyanidin lowered the risk of Parkinson’s. Studies done also showed lower risk of PD with tea intake. | [21], [10] |
| 3 | Lung Cancer | With increase in consumption of tea there was seen decrease in incidence of chronic obstructive lung disease. | [4], [23] |
| 4 | Colorectal cancer | A study in Korea showed that green tea extract supplement helped in prevention of colorectal adenomas to some extent. | [4] |
| 5 | Skin cancer | Consumption of high caffeine reduced basal cell carcinoma to 43% in the consumers than the non-consumers. Green tea polyphenols were seen to have some benefits on the skin and preventing from skin cancer by enhancing DNA repair. | [4], [24], [23] |
| 6 | Breast cancer | A study done in Korea showed that green tea extract supplements reduced percent mammographic density compared with placebo in the younger women than the older women. | [4] |
| 7 | Diabetes | EGCG can be helpful with Type-2 diabetes. | [4], [25]. |
| 8 | Cardiovascular diseases | Treatment with EGCG helped in a study on mice and its protective effects could be seen. Whereas gallic acid is also seen to be helpful with the disease. | [4], [26] |
| 9 | Neurological Diseases | Tea effects on neurological diseases were studied where the polyphenols of tea helped protect the nerve cells and also helped inhibiting stress. | [4], [21] |
| 10 | Obesity | Tea can decrease high fat obesity when studied in a mouse. There were also seen effects of the plants and their constituents how they were health benefiting and reduced obesity. | [26], [27],[25] |

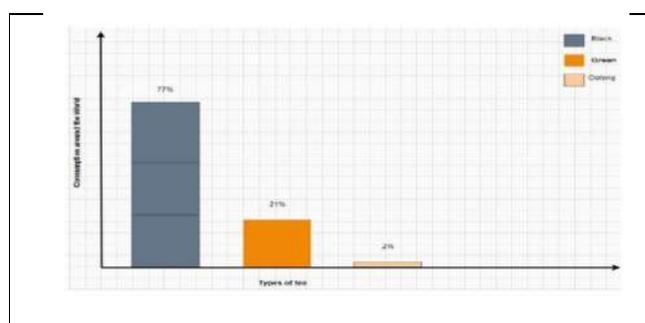


Fig 1. Consumption of Black tea, green tea, and oolong tea around the world

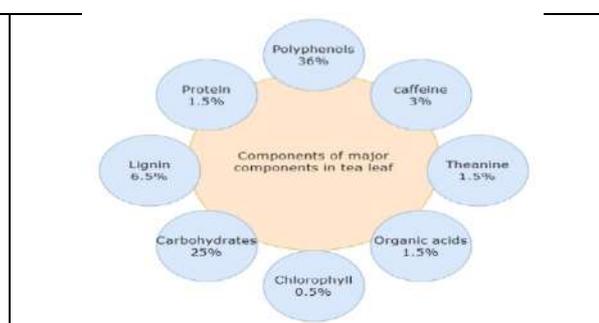


Fig 2: Percentage of different constituents in a tea leaf (dry weight)





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Fig:3 Some solvents used and their properties





Proanthocyanidins : A Analytical Review

Vinay Kumar S¹ and Rashmi N .G^{2*}

¹M.Pharm, Dept. of Pharmaceutical Analysis, Sri Adichunchanagiri College of Pharmacy, B.G.Nagara, Karnataka, India.

²Assistant Professor, Dept. of Pharmaceutical Analysis, Sri Adichunchanagiri College of Pharmacy, B.G.Nagara, Karnataka, India

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*Address for Correspondence

Rashmi N .G

Assistant Professor,
Dept. of Pharmaceutical Analysis,
Sri Adichunchanagiri College of Pharmacy,
B.G.Nagara, Karnataka, India



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ABSTRACT

Proanthocyanidins are a class of polyphenolic chemicals that are extensively distributed in many plants and fruits and are prized for their powerful antioxidant abilities. With regard to proanthocyanidins potential for cardioprotection, antioxidant activity, anti-inflammatory effects, antidiabetic and anticancer capabilities, this review seeks to give a overview of health benefits and There are several spectroscopic methods analysis such as Ultraviolet spectroscopy, and chromatographic methods such as High-performance liquid chromatography, ultra-high performance liquid chromatography.

Keywords: - Proanthocyanidins, Antioxidant, Antidiabetic, HPLC, Analytical methods.

INTRODUCTION

The polyhydroxyflavan-3-ol oligomers and polymers known as proanthocyanidins include monomeric flavan-3-ols as their core units and are connected by extra ether linkages (O-C) or carbon-carbon bonds (B-type proanthocyanidins) or both. Proanthocyanidins have a high structural diversity that is influenced by the two chiral centres in each flavan-3-ol unit, the presence of substitutions (such as galloyl), the degrees of polymerization (DP), the orders and types of linkage between the flavan-3-ol units, and different monomer units, such as epicatechin /catechin (EC), epiafzelechin/afzelechin (EA), epigallocatechin/gal [1]. Since many years ago, proanthocyanidins (PA), monomeric flavonoids, and phenolic acids, which make up the bioflavonoid composition of Pine bark extracts (PBE) from various Pine species, have been used commercially as active ingredients in food and dietary supplement products. The most prevalent flavonoid in PBE, PA, is made up of flavan-3-ols, which typically consist of 2–10 units of catechin and epicatechin linked together by various inter-flavan bonds to produce a variety of oligo- and polymeric



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structures[2]. A subclass of flavonoids known as flavanols is made up of proanthocyanidins that are oligomeric or polymeric and contain two or more catechin components[3]. Here, we discuss the manufacturing process for the P. radiata bark extract Enzogenol along with chemical analyses, safety information from toxicological tests, and results from human research [2]. They are utilised as food additives to improve the microbiological stability, foamability, oxidative stability, and thermal stability of food preparations. Proanthocyanidins provide several advantages for your health. These chemicals' antioxidant, anticancer, and immunostimulating activities have been discovered. According to studies, proanthocyanidins protect the body from sun damage, enhance eyesight, increase flexibility in joints and arteries, as well as in bodily tissues like the heart, and enhance blood flow by fortifying capillaries, arteries, and veins [4].

DRUG PROFILE

Chemical name: (3R)-2-(3,5-dihydroxy-4-methoxyphenyl)-8-[(2R,3R,4R)-3,5,7-trihydroxy-2-(4-hydroxyphenyl)-3,4-dihydro-2H-chromen-4-yl]-3,4-dihydro-2H-chromene-3,5,7-triol

Category: Antioxidant drug

Molecular weight: 592.5 g/mol

Molecular formula: C₃₁H₂₈O₁₂

Melting point: 856.6±65°C at 760mmHg

Solubility: Soluble in water and Methanol

pKa: 5.5-7.4

log P: 0.28

Biological potential of proanthocyanidins.

The therapeutic potentials of several proanthocyanidins are discussed in the sections below. Proanthocyanidins are discussed along with their therapeutic mechanisms.

Antioxidant

Even under settings of normal physiological function, oxidative stress is a major factor in cellular damage. Antioxidants are useful in managing metabolic diseases caused by oxidative stress and cellular harm. By decreasing the burden of free radicals and preventing cellular and molecular damage, antioxidant chemicals act as a barrier against oxidative stress [6]. In human lens epithelial B-3 HLE-B3 cells, grape seed extract reduced H₂O₂-induced phosphorylation of the p38 and c-Jun N-terminal kinase (JNK) proteins of the NF-κB and MAPK signalling, preventing cataract genesis[7]. At 400 mg/kg, grape seed Proanthocyanidin extract has dose-dependent antioxidant effects on produced rat testicular toxicity. Rat models have shown that grape seed Proanthocyanidin extract (GSPE) taken in moderation can reduce oxidative stress and enhance mitochondrial function[8]. As seen in rat models, consumption of grape seed Proanthocyanidin extract (GSPE) at a moderate dosage can reduce oxidative stress and enhance mitochondrial function. Grape seed proanthocyanidin extract reduced liver glutathione alteration in obese rat models[8].

By enhancing the expression of the silent information regulator sirtuin 1/SIRT1, a gene that controls the apoptotic response to DNA damage, Proanthocyanidin from D. kaki peels decreased oxidative damage. The daily intake of 200–300 mg of proanthocyanidins from grape seed extract avoided epigastric discomfort and markedly decreased the intensity, frequency, and demand for narcotic analgesics. Acidosis is caused by oxidative damage, which lowers pH [9].

Lipid lowering and anti-obesity

Postprandial hyperglycemia, which can alter endothelial function, can be caused by eating foods rich in carbohydrates and fat[10]. Proanthocyanidins can help with postprandial hypertriglycerolaemia-related metabolic impairment. By suppressing lipoprotein secretion, grape seed proanthocyanidin extract causes hypotriglycerolaemic effects[11]. A metabolic abnormality is what causes obesity, and obesity can exacerbate the



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disorder. A cohort's level of oxidised LDL was lowered by grape seed extract tablets, reducing the chance of developing arteriosclerosis[12].

Cardioprotective

Atherosclerosis, plaque buildup, calcification, and viscous blood are just a few of the factors that might impair heart function. By relaxing blood vessels and preventing LDL oxidation, proanthocyanidins reduce the symptoms of cardiovascular illnesses[13]. Proanthocyanidins have the ability to prevent oxidised LDL from attaching to the lectin-like oxidised LDL receptor-1 (LOX-1), which plays a role in the development of arteriosclerosis[14]. In endothelium-dependent relaxing action, which involves endothelial NO release and a subsequent rise in c-GMP levels in the vascular smooth muscle cells, a number of proanthocyanidins are implicated. Procyanidins from grape seed extract alter the NO/cyclic GMP pathway, protecting the heart[15]. The capacity to reduce LDL oxidation has been demonstrated by procyanidin fractions from cocoa[16].

Antidiabetic

Diabetes is a metabolic disorder that affects glucose metabolism and is chronic. It is brought on by pancreatic gland damage and an insulin level shortfall[17]. Nephropathy, retinopathy, and neuropathy are all possible complications of severe diabetes. Advanced glycation end product (AGE) production and digestive enzyme inhibition are required for its treatment[18]. In diabetic pancreatic islets, grape seed proanthocyanidins enhanced the amount of normal insulin and lowered the amount of apoptotic cells[19]. Proteins that are misfolded accumulate as a result of endoplasmic reticulum (ER) stress, which also affects calcium homeostasis. ER stress is activated when a person has diabetes[20]. The proanthocyanidin therapy reduced ER stress to some extent. Grape seed proanthocyanidin extract supplementation decreased oxidative stress by preventing lipid peroxidation, improved endothelial function, and decreased the incidence of vascular disease in diabetics[21]. Long-term hyperglycemia results in diabetic encephalopathy. By modifying AGEs/the receptor for AGEs (RAGE)/NF-kB p65 pathway, grape seed proanthocyanidin extract can lessen the damage in the cerebral cortex of diabetic rats[22]. Proanthocyanidins could stop diabetic retinopathy from getting worse.

Extraction of bark Pas.

Flow diagram of the experimental procedure[23]

METHODOLOGY**UV SPECTROPHOTOMETRIC METHOD**

The UV- Spectrophotometer study done by the preparing standard stock solution of Proanthocyanidins was prepared in methanol. From the standard stock further dilution is done. Proanthocyanidins show UV-absorbance maxima at 235 and 280 nm corresponding to the absorbance spectrum of flavan-3-ols.

Reported High Pressure Liquid Chromatographic Methods For Analysis Of Proanthocyanidin Table 1.**CONCLUSION**

Proanthocyanidins, which are prevalent in many plant-based diets, have a number of positive health effects. They are desirable candidates for disease prevention and therapeutic treatments due to their high antioxidant and anti-inflammatory activities as well as possible cardioprotective and anticancer benefits and There are several spectroscopic methods of analysis such as Ultraviolet spectroscopy, chromatographic methods such as High-performance liquid chromatography, ultra-high performance liquid chromatography However, further investigation is required, including carefully planned human clinical studies, to establish the best doses, bioavailability, and potential side effects. A balanced diet that includes Proanthocyanidin-rich foods or supplements may give prospective health advantages and enhance general wellbeing.





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Table 1. Reported high pressure liquid chromatographic methods for analysis of proanthocyanidin.

| DRUG SAMPLE | INSTRUMENT | COLUMN | MOBILE PHASE | FLOW RATE | DETECTION | REFERENCE |
|------------------|------------------|-----------------|--|---|-----------------------------|-----------|
| Proanthocyanidin | Agilent 1100HPLC | RP18e(1003.4mm) | Water containing 1%(v/v) Aqueous acetic acid(A) and Acetonitrile containing 1%(v/v) acetic acid(B) | Binary gradient elution condition : 3.0ml/min , 3% B for 4 min, 3% to 8% B in 10 min, 80% for 2 min. | Diode array detector: 280nm | [24] |





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| | | | | | | |
|----------------------------------|--|---------------------------------|--|---|-------------------------------------|------|
| Enzogenol | Waters 2690 Separation | C18 5 μ (250X4.6mm) | Distilled Water: 20% acetone. | 0.5ml/min | Photodiode Array detector:280nm | [25] |
| Procyanidins | Agilent 1200 HPLC | C18 5 μ (250X4.6mm) | Water(2%acetic acid: methanol | 1.0ml/min, | Diode array detector: 280nm | [26] |
| Procyanidins | Agilent 1200 HPLC | C18 | [A]0.10% v/v aqueous TFA:[B] 0.08% v/v TFA in CH ₃ CN | Gradient flow: 1.0ml/min 8-23%[B] over 25min | Detector: 214,280,320nm | [27] |
| Pine bark and green tea extracts | Agilent 1200 series rapid resolution LC system | Zorbax Eclipse plus C18 | 0.5%Acetic [A]:methanol | multi-step linear gradient was applied: 0.3ml/min. 0 min, 0% B; 5 min, 25% B; 15 min, 35% B; 20 min, 39% B; 38 min, 60% B; 40 min, 70% B; 42 min, 80% B; 44 min, 100% B; 46 min, 0% B; and 48 min, 0% B. | Diode Array detector: 485 and 520nm | [28] |
| Bark PAs | HPLC (Hitachiadduts Merk LaChrom L7000 series, japan | | A(1% v/v of aqueous acetic acid : B(1% v/v of acetonitrile) | 1.0ml/min | Detected: 280nm | [23] |
| Proanthocyanidin | Shimadzu LC-20A HPLC | Hydrosphere C18 (i.d. 4.6 × 250 | solvents A (0.5% formic | 1.0 mL/min. | PDA detector: 273nm | [29] |





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| | system | mm) column | acid) and B (methanol) | | | |
|------------------------------|---|--|---|---|---|------|
| apple extract | Agilent system (HP1260) | Luna Hilic column (150 × 2.0 mm; 3 μm) | 98% acetonitrile and 2% acetic acid (A) and 95% methanol, 3% water and 2% acetic acid (B) | Increasing gradient of (B): 0 min, 7%; 3 min, 7%; 15 min, 30%; 40 min, 49%; 40.1 min, 7% and 45 min, 7% at a flow rate of 0.350 mL/min. | photomultiplier tube gain (PMT) detector : 230 nm for excitation and 321 nm for emission. | [3] |
| Birch Leaf Proanthocyanidins | Merck-Hitachi HPLC-PAD system (Hitachi, Tokyo, Japan) | Superspher 100 RP-18 (75 × 4 mm i.d.; 4 μm). | (A) 1% aqueous formic acid and (B) acetonitrile | 0–2 min, 100% A (isocratic); 2–19 min, 0–9% B in A (linear gradient); 19–35 min, 9–30% B in A (linear gradient); 35–43 min, 30–70% B in A (linear gradient); and 43–51 min, 70% B (isocratic). The flow rate was 1 mL/min. | L-7455 PAD detector: 280 nm | [30] |
| Proanthocyanidin | Acquity UPLC system | BEH Phenyl column | acetonitrile (A) and 0.1% aqueous formic acid | The flow rate of the eluent | diode array detector: 289nm | [31] |





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| | | | | | | |
|---------------------------------|---------------------|--|------------------------------|---|--------------------|------|
| | | | (B) | was 0.5 mL/min. 0–0.5 min, 0.1% A in B; 0.5–5.0 min, 0.1–30% A in B (linear gradient); 5.0–6.0 min, 30–35% A in B (linear gradient); 6.0–9.5 min, | | |
| Alpha Lipoic Acid and Enzogenol | Acquity UPLC system | Waters X-Terra RP18, (150x4.6mm, 2.1µm) column | Water: Acetonitrile (40:60). | 1.0ml/min | PDA detector:280nm | [32] |

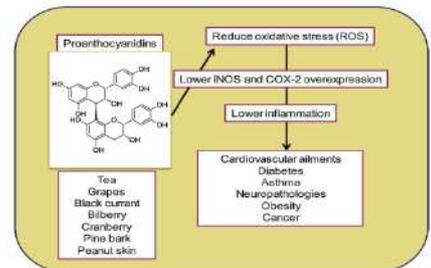
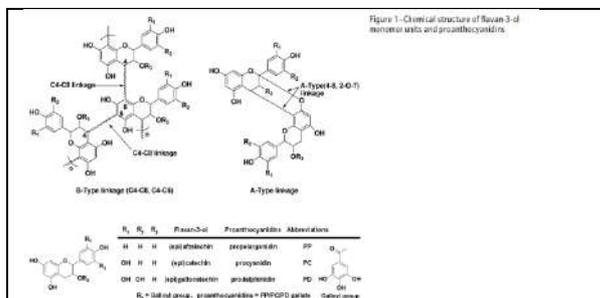


Fig.1. Chemical Structure of flavan-3-ol monomer units and Proanthocyanidins

Fig.2. Proanthocyanidins and their ameliorative mechanisms

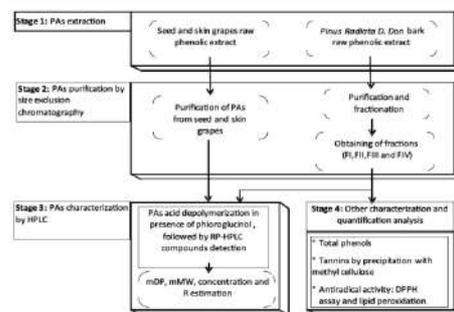


Fig.3. Flow diagram of the experimental procedure[23]





Biodegradation of Plastic Waste using Combinatorial Approaches

K. Bharanipriya¹, P. Keerthana¹, R. Narashimman¹, K. Subash¹, A. Sathish¹ and S. Karthik Sundaram^{2*}

¹II M.Sc Microbiology, Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India

²Assistant Professor, Dr. N.G.P. Arts and Science College, Coimbatore, Tamil Nadu, India.

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*Address for Correspondence

S. Karthik Sundaram

Assistant Professor,

Dr. N.G.P. Arts and Science College,

Coimbatore, Tamil Nadu, India.

E.Mail: karthiksundaram@drngpasc.ac.in



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ABSTRACT

The plastic pollution is one of the anthropogenic effects. Which causes harm to Eco life through toxic compounds. The main objective of this present work is reveals that the effect of Pretreatment of plastic wastes with microbial consortium and analysis of its effect on plant growth. This work includes collection of soil sample from plastic dumped sites, isolation identification, screening compatibility analysis of microbial consortium and effect on plant growth, Pretreatment of polyethylene sheets by physical, chemical and microbial degradation. To make this happen the plastic waste is first treated by chemically through an acid and enzyme treatment. This ensures that the bonds are made available for the microorganisms to break them down. Utilizing microbes that have developed with the plastic wastes for a very long period of time, we would develop compost that would be analyzed for its NPK ratio. The ratio would be enhanced by the addition of other bio fertilizers in the sample. This compost thus developed would be used in presence of toxic end of the composting process and manure without any toxic end products alone would be taken for further process.

Keywords: Plastic pollution, Pretreatment, Microorganisms, Compost formation, Plant growth.

INTRODUCTION

Plastics are a wide a range of synthetic or semi synthetic materials that use polymers as a main ingredient (Praveena Rajan *et al.*, 2020). The word polymer is derived from classical Greek poly meaning “Many” and meres meaning “Parts”. Thus, a polymer is a large molecule (Macro-molecule) built up by the repetition of small chemical units (Robert Oboigbaotor *et al.*, 2000). Approximately 140 million tons of synthetic polymers are produced worldwide each year (Masayuki Shimao *et al.*, 2001). Plastics are made up of linking together by chemical bonds (Swapnil K. Kale *et al.*, 2015). Plastic pollution has been prevalent to the world ever since its use in majority of the areas including



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packaging, electronic industries, building, construction and healthcare etc. This has led to a constantly increasing burden on the environment (Jyoti kaushal *et al.*, 2021). The accumulation of these plastic wastes created a serious threat to the environment that adversely affects wildlife and humans (Haben Fesseha *et al.*, 2019). The polyethylene is the most commonly found non-degradable solid waste that has been recently recognized as a major threat to life. The polyethylene could sometimes cause blockage in intestine of fish, birds, and mammals (Ponniiah saminathan *et al.*, 2014). In a material, any physical and chemical change that is caused by the action of microorganisms is known as bio-degradation. Degradation is an important process in the environmental breakdown of polymer substrate into organic waste. For biodegradable materials composting is being used as an alternative technology to conventional disposal in landfill/incineration. By this process, biodegradable wastes/organic material are transferred into humid substances which are valuable as high quality fertilizer for the agricultural purposes (Thanawadee leejarkpai *et al.*, 2011). PE is highly recalcitrant and inert material hence it is very difficult to degrade in the environment even after buried for several years as landfill. A Polyethylene sheet showed only partial degradation and negligible weight loss when kept in moist soil for 12-13 years. The recalcitrance of PE is due to its water insolubility, the hydrophobicity because of the presence of linear backbone of carbon atoms, degree of crystallinity, and its high molecular weight (Sunil Ghate *et al.*, 2020).

Many microorganisms including fungi and bacteria have been known to possess polyester degrading mechanisms because of various enzymes found in these organisms. These microorganisms use plastics as their sole carbon source (Jyoti kaushal *et al.*, 2021). The extra cellular enzymes are too large to penetrate deeply into the polymer material, and so act only on the polymer surface; consequently, the bio-degradation of plastics is usually a surface erosion process (Ponniiah saminathan *et al.*, 2014). More than 20 bacterial genera have been shown to degrade different types of Polyethylene. These include various Gram negative and Gram positive species belonging to the genera *Pseudomonas*, *Acinetobacter*, *Klebsiella*, *Staphylococcus*, *Streptococcus* and *Bacillus*, etc.. Most of the bacterial strains possess the ability to deteriorate surface and form a biofilm of Polyethylene (Danso D. Chow J *et al.*, 2019). Acids also involved in Bio degradation process. It had a number of favourable properties including Ease of fabrication, Zero toxicity, High Mechanical strength and is compostable. Compost is nutrient rich material that can transform your soil and give plants a dramatic boost. It is created from decomposed Organic matter, with help from bacteria, fungi. Using compost as the only means to maintain/improve the soil fertility in long term. Compost is a good fertilizer because it contains nutrient as well as organic matter. Preparation and use of compost can be a solution to that problem (Madeleine inckel *et al.*, 2005).

Widespread studies on the bio-degradation of plastics have been carried out in order to overcome the environmental problems associated with synthetic plastic waste (Praveena Rajan *et al.*, 2020). The current work is aimed at developed compost using the preprocessed plastic waste and indigenous microbial consortium and analyse of its effect on plant growth and productivity.

MATERIALS AND METHODS

Sample collection

Soil samples were collected from plastic dumped sites at Karumanchirai, Tiruppur, Tamil Nadu. The dump yard was chosen because the microbial communities would have acclimatized to the plastic wastes dumped there and would therefore be a rich source of probable microbes that could degrade the pretreated plastic material (Ziaullah Shah *et al.*, 2013). Polyethylene bags were collected from local market in Kunnathur, Tiruppur, Tamil Nadu.

Isolation of Polyethylene degrading Microorganisms

One gram of soil sample was transferred into a conical flask containing 99ml of sterile distilled water. This content was shaken and serially diluted, (Polyethylene bag) pour plate method was adopted using nutrient agar for bacteria and Potato Dextrose Agar (PDA) for fungi. The plates were then incubated at 37°C for 2-5 days (1). The developed



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colonies were then incubated and sub cultured repeatedly to get pure cultures and it was preserved in conical flask in the form liquid culture (Sharma Prabhat *et al.*, 2013).

Screening of polyethylene degrading microorganisms**Petri plate method**

Isolated bacterial colonies were streaked on minimal salt agar. Polyethylene bag was cut into pieces of 2×2 cm and placed on the minimal salt agar plates. After incubation microbes, Which effectively grew on the top of the Polyethylene strips were chosen for further degradation analysis (Ligi Lambert *et al.*, 2017).

Identification of bacteria and fungi

The identification of bacteria was performed on the basis of microscopic and macroscopic examination and biochemical test according to Bergey's manual of determinative bacteriology. The fungal culture was identified by lacto phenol cotton blue staining technique (R. Usha *et al.*, 2011).

Pretreatment of Polyethylene sheets**Chemical degradation**

The polyethylene sheets were taken and cut into small pieces of 2×2cm. The sheet was treated with Poly L-D lactic acid which was placed in a beaker with 200ml of Poly L-D lactic acid and it was kept for a week. After a week, the pretreated polyethylene sheets were subjected into microbial degradation. Further the chemically treated polyethylene sheets were analyzed for FTIR, which is compared with initial stage of polyethylene sheets. After chemical degradation the process of microbial enzyme degradation is followed.

Degradation of pretreated polyethylene sheets by microbial enzyme

The mass production of polyethylene degrading microorganisms were centrifuged for 2 times to obtain the microbial enzyme which helps in degrading the polyethylene sheets. Now the pretreated polyethylene sheets were aseptically transferred into the beaker containing the 100ml of mass production of isolated and identified polyethylene degrading microorganisms and it was kept for a week. After a week, the sheets were taken away from the beaker and were washed thoroughly using distilled water and diluted ethanol. Sheets were allowed to dry and then weighed to check the weight of plastic sheets.

Weight loss measurement

After the degradation ,the plastic sheets were then disinfected with ethanol for 5 minutes which was dried in hot air oven at 60°C for 20 minutes. These films were weighted by digital balance. The degraded plastic sheets were compared with control. The percentage degradation of polyethylene sheets was calculated by following equation (Sea Cheon Oh *et al.*, 2007): Percentage(%) degradation = $\{(Initial\ weight - Final\ weight) / Initial\ weight\} \times 100$

Bioremediation of Preprocessed plastic wastes through composting

The pretreated Plastic pieces obtained were mixed with a solid biodegradable synthetic material and subjected to aerobic degradation. Then add bio-fertilizer and plastic degrading microbial culture and it was kept for few weeks for plastic degradation process.

Analysis of compost

After finishing the bio-degradation process,chemical analysis was done to find out the influence of the qualitative parameters of the substrate (Dana Adamcova *et al.*, 2013). Also to detect the presence of certain functional groups in polymers using Fourier transform infrared (FTIR),Micro toxicity test is performed by prepared compost material and the quality of compost is determined by N,P,K ratio Further the enigmatically treated polyethylene sheets were compared with chemically treated polyethylene sheets.



**Bharanipriya et al.,****Analysis of Nutrient composition of the compost produced**

Compost typically contains a large amounts of macro-nutrients, such as nitrogen, phosphorus, and potassium, as well as micro-nutrients like calcium, magnesium, and iron. The specific nutrient composition of compost can vary depending on the materials used to create it, such as food waste, yard waste, or animal manure, as well as the composting process itself. In addition to providing essential nutrients to plants and soil, compost can also improve soil structure and water-holding capacity, as well as promote beneficial microbial activity (Thanawadee Leejarkpai *et al.*, 2011).

Analysis of Plastic Decomposition through FTIR

Fourier Transform Infrared Spectroscopy (FTIR) is a common analytical technique used to analyze the decomposition of plastic materials. FTIR can also be used to analyze the effectiveness of various degradation methods for plastic materials, such as chemical and Microbial degradation. By analyzing the infrared spectra of the plastic sample before and after treatment, it is possible to determine the degree of degradation and the effectiveness of the treatment. By comparing the infrared spectra of a degraded plastic sample with that of an undegraded plastic sample, it is possible to identify changes in the functional groups present in the plastic due to degradation (Ebewele *et al.*, 2000).

Analysis for the presence of Toxic end products through Microbial Toxicity Analysis

Microbial toxicity analysis is a process that is used to determine the presence of toxic end products produced by microorganisms. The analysis involves the use of various techniques and methods to detect and quantify the levels of toxins produced by microorganisms. One common technique used in microbial toxicity analysis is the use of bio-assays, which involve exposing living organisms to the sample and observing any effects on their growth or behavior (Pierre Feuilloley *et al.*, 2005).

Plant growth Formation using Compost

The mud layer was prepared by using soil and bio compost in the form of layer by layer. The seed has been added in the prepared bio-compost. Then it was kept in a observation for 2 weeks. The compost has been developed in few days. Further the plant was analyzed for Physical, chemical and enzymatic method. FTIR, NPK and toxicity was analyzed for formulated compost.

RESULTS**Isolation of polyethylene degrading microorganisms**

The serial dilution plates of bacteria and fungi were showed Fig.1&2 respectively. Based on the different colony morphology, several microorganisms were isolated from diluted plates. From this, 14 bacterial colonies and 5 fungal colonies were selected and sub cultured.

Screening of polyethylene degrading microorganisms

The plastic degrading microorganisms were isolated from soil with the help of serial dilution. The bacterial and fungal strains isolated with the ability to degrade and performed basis on the macroscopic and microscopic test. The bacterial culture was screened using the Petri plate method. The piece of plastic was placed on the swabbed culture. After the incubation, it was observed that the bacterial culture was grown on the piece of plastics. So, the result concludes that bacteria have the ability to degrade the plastic material. It was continued for the further process (Praveena Rajan *et al.*, 2020). Figure 5: Shows microbial degradation of polyethylene sheet.

Identification of Polyethylene degrading microorganisms

The screened and isolated organisms were characterized on the basis of macroscopic and microscopic examination and biochemical test according to Bergey's manual of determinative microorganisms (Hilda. A. Emmanuel- Akerele



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et al., 2021). Gram staining of screened bacteria was shown Table 1 and Figure 6, which is Gram positive, Rod shaped and is motile bacteria. The bacterium is further characterized by macroscopic examination of biochemical tests.

Identification of Polyethylene degrading fungi**LPCB Staining**

Among the isolated of fungi, two isolates were reported as potential PE degrading fungi. The selected efficient fungal degraders stained with LPCB shows small conidiophores under microscope (Figure 5) which belongs to the genus *Aspergillus* sp.

Pretreatment of Polyethylene sheets**Chemical Degradation**

The Polyethylene sheets were treated with chemical method using Poly L-D Lactic acid. The results in the formation of aldehydes and acids can negatively influence the fluid viscosity and lubrication and cause corrosion. Polyethylene sheets react within few days with Poly L-D Lactic acid. After few days hard surface of Polyethylene sheets was changed to soft, Smooth and Fluffy.

Degradation of pretreated polyethylene sheets by microbial enzyme

After the process of chemical degradation, the pretreated polyethylene sheets were subjected to the mixed microbial cultures. The mixed culture includes organisms like *Pseudomonas* sp, *Bacillus*, *Staphylococcus aureus*, *Aspergillus* sp. The pretreated polyethylene sheets were again treated with microbial enzymes for one week. In this process the enzymes is attach to the surface of the plastic, hydrolysis to short polymer intermediates, which are ultimately assimilated by microbial cells as carbon source. This bio-degradation of plastic is usually a surface erosion process (Himani Bhardwaj et al., 2012) .

Weight loss measurement

After 14 days of treatment by isolated polyethylene degraders, the weight loss of the pretreated polyethylene sheets were noted and percentage of degradation was calculated (Sea Cheon et al., 2007) The noted weight is mentioned below (Table 3) and Figure 8: Shows the degraded polyethylene sheets.

Formulation of compost

The formulation of compost is prepared by using kitchen waste, mixed microbial culture, paddy straw and soil. This mixer of these materials were kept for few days. After few days the compost is formed. The quality of formulated compost is analyzed by N,P,K ratio, FTIR and Micro-toxicity test.

NPK ratio in Compost

Plants need significant amounts of nitrogen, phosphorus, and potassium to grow and thrive. Nitrogen is needed for leaf growth and is responsible for making plants greener. Phosphorus promotes root development, which helps anchor and strengthen plants. Potassium, also known as potash, helps the plant fight off disease. Table 4: shows that maximum permissible amounts of monitored elements in compost.

FTIR spectrum Analysis for the identification of degraded Polyethylene sheet

Fourier Transform Infrared Spectrophotometer (FTIR) is perhaps the most powerful tool for identifying the types of chemical bonds (Functional group) present in the compounds. The wavelength of light absorbed is characteristic of the chemical bond as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined (Vimala P. P et al., 2015). FTIR spectra were obtained from samples treated showed the presence of several peaks at different period of time. The pretreated Polyethylene sheets were subjected to FTIR and the graph obtained was compared with that of the samples treated at the end of different time period such as Initial stage of Polyethylene sheet, Pretreatment of Polyethylene sheets using Lactic acid and treat with compost.



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The presence of peaks between the range of 1500-1600 cm represents the presence of the C=C stretch which was the lignin of the PE plastic. The reduction in the structure of the PE sheets over a period of chemical action gives rise to the removal of C=C bond, and the formation of new stretches at the range of 2800-3000 cm represents the N-H bond. These functional groups suggest that the PE sheet was reduced and was converted to another ringed structure. The presence of peaks at the range of 3200-3600 cm after the period of incubation suggests that the ringed structure was broken down. The reduction of peaks at the end of treatment showed higher degradation using compost prepared by pretreated PE sheets at the range of 3363cm which proved that the ringed structure was completely broken down.

Micro-toxicity test

The microbial toxicity study on the prepared compost showed the no formation of zone in the compost used and were toxic to test organisms used (*S.aureus*, *Bacillus sp.*, *Pseudomonas*). The micro-toxicity test shows the positive result which indicates in zone of clearance. The below table:5 and (Figure10) shows the Micro-toxicity test result for different microbes.

Plant growth formation using compost

The pretreated plastic pieces obtained were mixed with a solid biodegradable synthetic material and subjected to aerobic degradation. Seedling process were done in the formulated compost. The growth of plant were observed after few weeks. Due to the absence of toxic content in the formulated compost the plant has ability to grow.

DISCUSSION

Plastics are very useful in our day-to-day life to meet our desired needs. Due to its good quality, its use is increasing day by day and its degradation is becoming a great threat. In the natural environment, different kinds of microorganisms play an important role in various steps involved in the bio-degradation of plastics. So the microorganisms is considered as an eco-friendly and cost effective method. In this study, plastic dumped soil samples were serially diluted and microbes were isolated from this for screening of polyethylene degraders. All the isolates may not have the ability to utilize plastic as carbon source and degrade Polyethylene. Therefore they were screened using petriplate method to check their ability of polyethylene degradation. Minimal Salt Medium contains all the other minerals, nutrients as required and polyethylene is used as carbon source by microbes. The isolates, which were able to utilize plastic as their carbon source showed growth on PE strips in petriplate method. Pretreatment of Polyethylene sheets using chemical and enzymatic methods enhance the degradation process and it was subjected to microbial degradation using screened microbes. When PE influence of lactic acid on plastic polymer results in breakdown the complex structure including the outward appearance. The performed plastic were further analyzed for FTIR. The pretreated polyethylene sheets were again subjected to microbial enzymes. Mixed microbial culture includes *Bacillus sp.*, *Aspergillus sp.*, *Staphylococcus aureus* were able to remediate the polyethylene sheets up to 10% respectively. Microbial consortium of fungi and bacteria showed more efficient weight loss. Weight reduction polymer using microbial consortium was generally higher than bacteria and fungi using FTIR analysis.

After the microbial degradation, the plastics were used for preparing the compost. This compost is a controlled, aerobic process that converts organic materials into a nutrient rich soil amendment or mulch through natural decomposition. The prepared compost in further analyzed for N, P, K ratio, FTIR analysis and Micro toxicity test. The compost results in capability for the plant growth promotion. After the plant growth in prepared compost, the compost were analyzed for micro-toxicity against *Staphylococcus aureus*, *E. coli*, *Pseudomonas sp.* Now these microbes involves in degrading the polyethylene sheets. The present study concludes that the isolated microbes are capable of degrading complex polymer sheets to smaller and simple units effectively. The prepared compost is capable of well growth promoting and less cost effective.





CONCLUSION

In recent years, people have shown concern over environmental problems. The problem of plastic pollution is now really a mess of mankind. There is no part of the world untouched from its impact. Before making commercial products globalize, manufacturers must have an idea about the production of our earth. In the present study physical and chemical treatment of PE films enhanced the degradation process. Microbes can be used in both natural and artificial conditions for the purpose of degradation of polymers. Since, this study reveals that the microbial consortium enhances the bioremediation of preprocessed plastic wastes (Praveena Rajan *et al.*, 2020). In the present study, chemical and enzymatic treatment of Polyethylene sheets enhanced the degradation process. The isolated microbes were native to site of polyethylene disposal, and it might show greater capacity of degradation in the natural conditions. We would develop compost that would be analyzed for its NPK ratio, FTIR and Micro-toxicity test. The ratio would be enhanced by the addition of other bio fertilizer in the sample. This compost thus developed would be used in analysis its effect on the growth and productivity of plants. The work also analyzes for the presence of toxic products at the end of composting process and manure without any toxic end products alone would be taken for further process and it can be concluded that good composting conditions were maintained. Here we observed that the quality of compost can be able to promote the plant growth.

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Table 1: Microscopic Examination

| Microscopic observation | Result |
|-------------------------|--------|
| Colour | Purple |
| Shape | Rod |
| Motility | Motile |

Table 2: Biochemical characterization of isolated bacteria

| Biochemical test | Result |
|--|-------------------|
| Citrate test | Positive |
| Methyl Red test | Negative |
| Voges-proskauer test | Positive |
| Indole test | Negative |
| Catalase test | Positive |
| Urease test | Negative |
| Nitrate reduction test | Positive |
| Gelatin hydrolysis test | Positive |
| Gas production | No gas production |
| Glucose | Positive |
| Mannitol | Positive |
| Sucrose | Positive |
| Probable organism is <i>Bacillus sp.</i> , | |

Table 3: shows the weight loss measurement of Polyethylene sheet

| S. No | Treatment | Time Interval | Initial Weight of PE | Final weight of PE | Percentage of Degradation |
|--|-----------|---------------|----------------------|--------------------|---------------------------|
| 1 | Chemical | 7 days | 5 g | 4.83 g | 3.4% |
| 2 | Enzyme | 13 days | 4.83 g | 4.21 g | 12.4% |
| Total Percentage of Degraded Polyethylene sheets = 15.8% | | | | | |





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Table 4: Maximum permissible amounts of monitored elements in compost.

| S.NO | Parameter | Unit | Result |
|------|-------------|-------|--------|
| 1. | Nitrogen | Kg/ha | 188 |
| 2. | Potassium | Kg/ha | 129 |
| 3. | Phosphorous | Kg/ha | 71 |

Table 5: Micro-toxicity studies of the prepared against standard test organisms

| S.No | Test Organisms | Result |
|------|------------------------------|----------|
| 1. | <i>Staphylococcus aureus</i> | Positive |
| 2. | <i>Bacillus sp.</i> | positive |
| 3. | <i>Pseudomonas sp</i> | positive |

| | |
|---|--|
| | |
| <p>Figure 1: Serial diluted plates of Bacteria</p> | <p>Figure 2: Serial diluted plates of Fungi</p> |
| | |
| <p>Figure 3: Microbial degradation of plastic</p> | <p>Fig 4: Gram-positive rod-shaped bacteria</p> |
| <p style="text-align: center;">(a)</p> | <p style="text-align: center;">(b)</p> |
| <p><i>Aspergillus sp.</i>, (A1)</p> | <p><i>Aspergillus sp.</i>, (A2)</p> |
| <p>Fig 5: Microscopic Examination of fungi</p> | |





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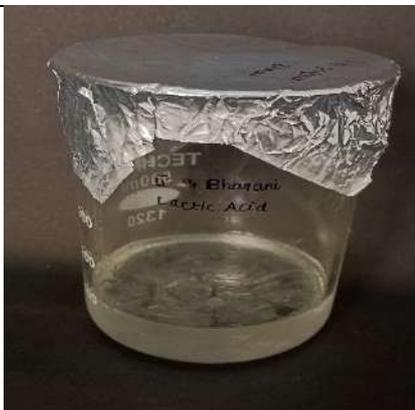


Figure 6: Chemical Degradation



Figure 7: Enzymatic degradation



Fig 8: Degraded plastic sheets

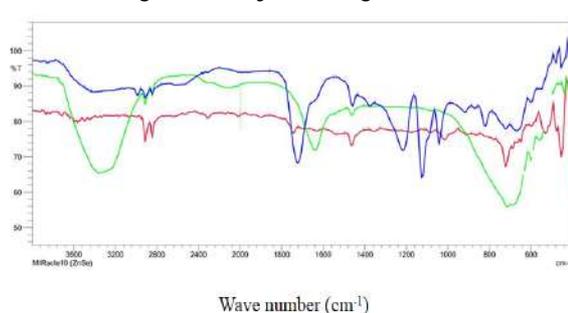


Fig 9: FTIR Spectrograph analysis of PE sheet reduction at various Time period



Figure 10: Micro-toxicity test against standard microbes



Figure 11: Plant growth formation using compost





An Error Analysis of Statistical Packages through Simulation

Manash Pratim Kashyap*

Associate Professor, Department of Statistics, Assam down town University, Guwahati, Assam -781026, India

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*Address for Correspondence

Manash Pratim Kashyap

Associate Professor,

Department of Statistics,

Assam down town University,

Guwahati, Assam -781026, India

E.Mail: mpk.stat@gmail.com



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ABSTRACT

In statistical software, simulation from probability distributions is an important aspect. The paper compares four familiar software viz. Microsoft Excel, SPSS, SAS and R, in their ability to simulate from Bernoulli distribution. The accuracy of the estimate of the parameter of the Bernoulli distribution, from random numbers generated from the different software is measured through the mean sum of squares (MSE). This study empirically establishes that Microsoft Excel is better than SPSS, SAS and R while simulating from the Bernoulli distribution.

Keywords: Microsoft Excel, SPSS, SAS, R, Estimation, Mean Square Error, probability distribution.

INTRODUCTION

Statistical software is increasingly used in scientific research, market surveys, educational research and so on. Perhaps initially we had a very informal, one shot, throwaway software. However, with increasing complexity of computing hardware and increasing sophistication of statistical methodology is no longer desirable or economical to treat software in this way (Kotz and Johnson, 1988). Many commercial statistical software packages have now mushroomed the market, and the quality of such packages has reached its zenith in terms of precision and flexibility (Bhattacharjee and Das, 2005). Thus, it is necessary to compare the ability of the different statistical software. This study focuses a comparison on the ability of simulation from Bernoulli distribution amongst, four software viz. Microsoft Excel, SPSS, SAS and R. The familiarity of the authors with this software and their popularity is the motivating force behind their selection for comparison.



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Simulation is the mathematical model to recreate situation, often repeatedly, so that the likelihood of various outcomes can be more accurately estimated. Simulation forms a central part, because of the relative ease with which samples can often be generated from a probability distribution, even when the density function cannot be explicitly integrated (Sharma, 2003). McCullough (1998) suggest that generation of random numbers should be one of the characteristics of software comparison. Roy *et al.* (2009) considered the strength of simulation in two software viz. MS Excel and R for Poisson distribution. They considered the following steps for comparison.

- (i) Random samples are generated for different sizes for different values of the parameter in MS Excel and R.
- (ii) The size of the sample and the value of the parameter are decided previously.
- (iii) After simulating the data, the maximum likelihood estimates of the parameter are obtained for a fixed sample size and fixed value of the parameter.
- (iv) For a given sample size and a given value of the parameter this is replicated ten times.
- (v) Finally, mean square error (MSE) are obtained for a given sample size and a given value of the parameter.
- (vi) The MSE curves for a given value of the parameter for different sample sizes are then drawn for the competing software packages and conclusions drawn accordingly.

The above mentioned steps are followed in this work as well. But this time it is used for comparing simulation from Bernoulli's distribution from the packages Microsoft Excel, SPSS, SAS and R. The above mentioned procedure is repeated keeping the value of the parameter fixed and varying the sample size. The different MSE values are plotted for different size of the sample for fixed value of the parameter. The calculation is done separately in MS Excel, SPSS, SAS and R. Accordingly, MSE curves are obtained for MS Excel, SPSS, SAS and R are plotted in the same graph. The software providing better simulated values provide smaller MSE values i.e. provide better estimates of the parameter.

LITERATURE REVIEW

A number of reviews of literature concerning statistical software for microcomputers has been provided by the researcher and offered very useful comments to both and users and vendors. Searle (1989) shows that some of the literature review concerning software comparison are comprehensive and general. Francis *et al.* (1975) focused that "*The American Statistical Association*" recommended a comprehensive study of the performance of statistical packages. This study subsequently modified and published monograph form (Francis, 1979, 1981) was the first systematic attempt to evaluate the performance of the software used in academics and industry for critical statistical applications. Dalal (1992) used to compare the different computing packages viz. SAS and SPSS. They analyze unbalance data from fixed model with nested factors. Dallal (1992) found differences between SAS and SPSS results beside some error of calculations of sums of squares in SPSS output. There are certain works that reviewed the literature regarding statistical software comparison include Wampler (1970), Wilkinson and Dallal (1977), Anscombe (1967), Hayes (1982), Wilkinson (1985), Simon and Lesage (1988, 1989), Wilkinson (1994), Buja, Cook and Swayne (1996), Knusel (1998), Rogers, Filliben and others (1998), L'Ecuyer (1999) etc.

Okunade and others (1993) compared the output of summary statistics of regression analysis in common statistical and econometrical packages such as SAS, SPSS, SHAZM, TSP, and BMDP. Oster (1998) reviewed five statistical software packages (EPI INFO, EPICURE, EPILOG PLUS, STATA and TRUE EPISTAT) according to criteria that are of most interest to epidemiologists, biostatisticians, and others involved in clinical research. McCullough (1998) proposed testing the accuracy of statistical software packages using Wilkinson's Statistics Quiz in three areas: linear and nonlinear estimation, random number generation, and statistical distributions. Again, McCullough (1999) used his methodology to the statistical packages SAS, SPSS, and S-Plus. McCullough shows that the reliability of statistical software cannot be taken for granted because he found some weak points in all random number generators, the S-plus correlation procedures, and the one-way ANOVA and nonlinear least squares routines of SAS and SPSS. Zhou and others (1999) reviewed five software packages viz. MLN, MLWIN, SAS Proc Mixed, HLM, and VARC that can



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fit a generalized linear mixed model for data with more than a two-level structure and a multiple number of independent variables. Bergmann and others (2000) compared 11 statistical packages on a real dataset. These packages are SigmaStat 2.03, SYSTAT 9, JMP 3.2.5, S-Plus 2000, STATISTICA 5.5, UNISTAT 4.53b, SPSS 8, Arcus Quickstat 1.2, Stata 6, SAS 6.12, and StatXact 4. They found that different packages could give very different outcomes for the Wilcoxon-Mann-Whitney test.

About the Software Packages

Microsoft Excel is an integral part of Microsoft Office package by Microsoft Corporation. Microsoft Excel is a window-based spreadsheet. It provides the user with several facilities like data analysis and data handling. The Excel worksheet gives a list of function like Financial, Date and Time, Mathematical, Trigonometrical, Statistical, Logical, Database etc. In addition to this Excel's add-in option has a 'Data Analysis ToolPak' that can be used for different types of statistical analysis including simulation from distributions. To activate the Data-Analysis tools one has to click the following- Office button → Excel Option → Add-Ins → Go → Analysis ToolPak → OK. Accordingly under the menu Data, a sub menu Data Analysis would be available where simulation from specified distribution can be performed.

In the late 1960s, Norman H. Nie, C. Hadlai (Tex) Hull and Dale Bent, three Stanford University graduate students, developed a statistical software system. In 1968, Nie and his colleagues founded the Statistical Package for Social Sciences (SPSS). In 1975, SPSS incorporated and established headquarters in Chicago, where the company remains today. In August 1993, SPSS became publicly traded. SPSS is among the most widely used programs for statistical analysis in social science. It is used by market researchers, health researchers, survey companies, government, education researchers, marketing organizations and others. The original SPSS manual (Nie, Bent & Hull, 1970) has been described as 'Sociology's most influential book'. In addition to statistical analysis, data management (case selection, file reshaping, creating derived data) and data documentation (a metadata dictionary is stored with the data) are features of the base software.

SAS was conceived by Anthony Barr in 1966 when he was a North California State University graduate. But with the collaboration of Barr with James Goodnight in 1968, integration of several statistical routines started which were relatively more robust. Now, SAS statistics provides a wide range of statistical software, ranging from traditional analysis of variance to exact methods and dynamic data visualization techniques (www.sas.com). SAS is driven by SAS programs that define a sequence of operations to be performed on data stored as tables. Although non-programmer graphical user interfaces to SAS exist (such as the SAS Enterprise Guide), most of the time these GUIs are just a front-end to automate or facilitate generation of SAS programs. SAS components expose their functionalities via application programming interfaces, in the form of statements and procedures.

R is an integrated software facility for data manipulation, calculation and graphical display. It has a suite of operators for calculations on arrays, a large, coherent and integrated collection of intermediate tools for data analysis, graphical facilities for data analysis and a well developed, simple and effective programming language which includes conditionals, loops, and user defined recursive functions and input and other facilities. Within R many modern statistical techniques have been implemented the benefit of which are enjoyed by many statistical software packages including SPSS. Thus, R has been widely accepted in the scientific world in general and statistical community in particular.

Of the software packages considered for comparison two of them are commercially available software viz. SPSS and SAS. Both the software is highly priced but is popular commercial packages for statistical analysis. However, Microsoft Excel is a spreadsheet and not standard statistical software. Yet Excel offers a wide variety of statistical procedures, in addition there is a 'Data Analysis Toolpak' which extends the statistical capabilities of Excel. The popularity of Microsoft Office (which includes Excel) has made statistical computations with Excel familiar. Numerous texts are available which promotes the use of Microsoft Excel for data analysis, decision making and financial modeling. Since Excel is by and large used for statistical computations, so it becomes necessary to assess the





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statistical capabilities in Excel. R is an open source program set for free distribution. The commercially available statistical software packages are far from the reach of many because of its high price. This has led to the popularity of R of late for statistical analysis. So, to check the efficiency of R relative to the popular commercially available packages is in itself of specific interest. Thus, a comparative study on simulation would help the researcher to understand where each of the software stands relative to each other.

METHODOLOGY OF COMPARISON

James Bernoulli (1654-1705) discovered the Bernoulli distribution. When a trial is such that it has only two outcomes, generally success and failure, then such trials are called as a Bernoullian trial. The random variable X , which represents the outcome of a Bernoullian trial is called a Bernoulli variable and its probability mass function as the Bernoulli distribution. If a random variable X takes two values 0 (for failure) and 1 (for success) with probabilities q and p respectively, where $p + q = 1$, then the probability mass function is given by

$$p(X = x) = p^x q^{1-x} ; x = 0 \text{ and } 1; p + q = 1$$

where, p , the probability of success, is the parameter of the Bernoulli distribution. The distribution is a basic in the study of probability theory, being a model of any random experiment with outcome belonging to two mutually disjoint classes (Kotz and Johnson, 1982). Several other distributions can be reached from the Bernoulli distribution, for example if X_1, X_2, \dots, X_n are independent and identically distributed Bernoulli variates with common parameter p

then the sum of the variates $Y = \sum_{i=1}^n X_i$ follows Binomial distribution with parameters (n, p) . The binomial

distribution under some specified conditions tends to Poisson distribution and for another set of conditions tends to normal distribution. Thus, Bernoulli distribution is the foundation of several other distributions.

If $X = (X_1, X_2, X_3, \dots, X_n)$ is a random sample from the Bernoulli distribution than the maximum likelihood estimator (MLE) of the parameter of the Bernoulli distribution is given by,

$$\hat{p} = \hat{p}(X) = \frac{1}{n} \sum_{i=1}^n x_i$$

The quantity $E_p(\hat{p}(x) - p)^2$ is called the mean square error of $t(x)$ about p .

$$\begin{aligned} MSE(\hat{p}(x)) &= E_p(\hat{p}(x) - p)^2 \\ &= V_p(\hat{p}(x)) + [Bias(\hat{p}(x), p)]^2 \end{aligned}$$

where, $V_p(\hat{p}(x))$ is the variance of t . If t is unbiased for p , $MSE(\hat{p}(x))$ reduces to $V_p(\hat{p}(x))$. For Bernoulli distribution MSE is calculated by the following formula,

$$MSE = \frac{1}{N} \sum_{i=1}^N (\hat{p}_i - p)^2$$

where, N = number of the sample considered, \hat{p}_i gives the estimate of p in the i^{th} repetition and p gives the mean of the Bernoulli distribution. It is obvious from the above formula that a good estimate of p will be closer to the actual value accordingly will have a smaller MSE value.

Simulation and Random Number Generator

In this section we discuss Bernoulli random data generation from softwares used in the study. Following commands can be used for random data generation from Bernoulli distribution



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R Language 2.11.1: *rbinom(n, size, prob)*; where “*n*” is number of random numbers to be drawn, “*k*” is the number of independent trials and “*prob*” is the parameter of the Bernoulli distribution.

SAS 9.1.3: *RANBIN(seed,n,p,x)*; where “*seed*” is the random number seed value, Range of $seed < 2^{31} - 1$, “*n*” is an integer number of independent Bernoulli trials, “*p*” is a numeric probability of success parameter and “*x*” is a numeric SAS variable. A new value for the random variate *x* is returned each time CALL RANBIN is executed.

Minitab 15: *Random n Var; Bernoulli P;* where “*Random*” is Minitab command to generate random numbers, “*n*” is number of random numbers to be drawn, “*Bernoulli*” keyword specifies the Bernoulli distribution and “*p*” is a numeric probability of success parameter.

MS Excel 2007: The simulation can be done by using Analysis Toolpak add-in.

PASW 18: *RV.BERNOULLI(P)*; where “*RV.BERNOULLI*” is PASW keyword and “*P*” is the probability of success Parameter

MSE Curves

The MSE curves are obtained from the MSE values of the estimates obtained (using the procedure discussed in 4.2) and these values are plotted against their sample sizes for a fixed value of *p*. Since the MSE values are obtained from three sources viz. Excel, SPSS, SAS and R accordingly we have four MSE curves in the same graph making the comparison feasible. Since nine values of *p* are considered viz. 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9. So, there are nine graphs in all. It is obvious that if the MSE curves remain closer to the x axis, it provides a better estimate of the parameter.

CALCULATION AND RESULTS

Based on the methodology discussed above, simulations from specific software and relevant calculations are performed and accordingly the graphs are drawn. The MSE values and the graphs of the MSE values are given in Appendix I and II respectively. From the graphs it is obvious that MS Excel is the better simulator of Bernoulli distribution compared to R, SAS and SPSS. It is also observed that SPSS is the better simulator of Bernoulli distribution compared to R and SAS being the worst. Again the graphs focused that with increase in the sample size the values of MSE showed decline i.e. better estimates are obtained as sample size increases.

FUTURE DIRECTIONS

Considering the familiarity and wide acceptability of R, SAS and SPSS, it is strange to see that the two packages cannot provide reliable simulation from Bernoulli distribution. However, SPSS provides better simulation from Bernoulli distribution than R and SAS. But Excel, which is not recommended for statistical analysis by many thinkers of the domain (Knusel; 1998) seems to perform better. With increase in the number of repetitions things may change but very marginally. However, it is essential to take up such comparative study for other commonly used statistical distributions and hence a conclusion should be reached on the simulation capability of R, SAS and SPSS in comparison to Excel.

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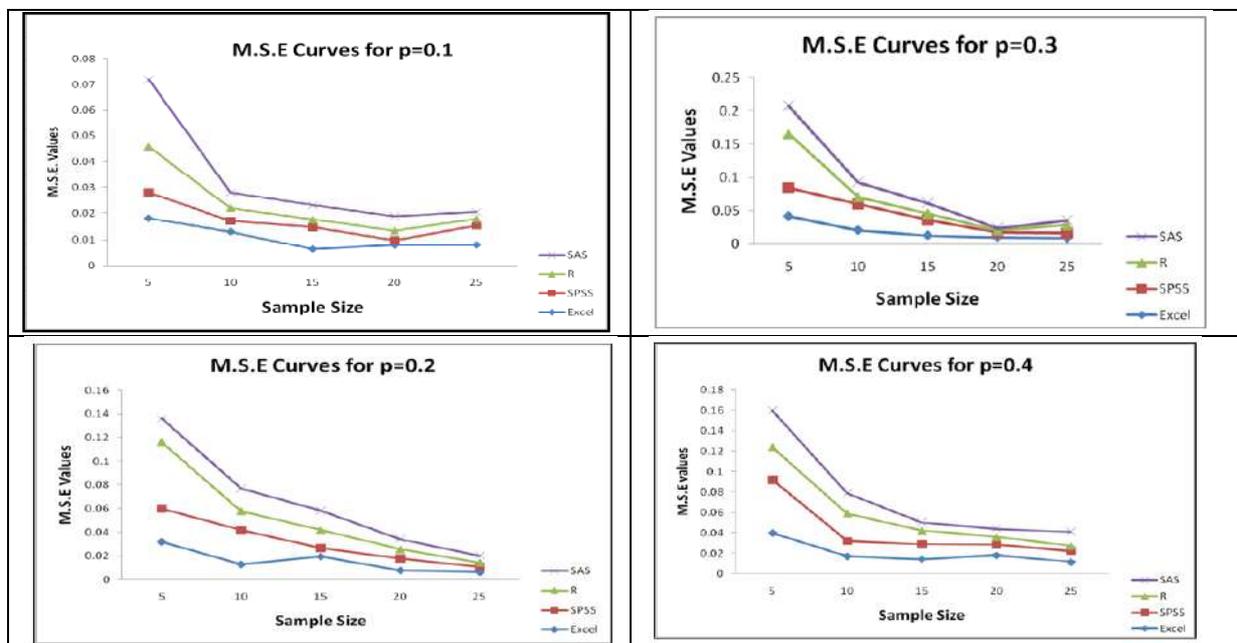
Appendix I: MSE values for different values of p

| p = 0.1 | | | | | p = 0.2 | | | | | p = 0.3 | | | | | p = 0.4 | | | | |
|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|
| n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS |
| 5 | 0.018 | 0.01 | 0.018 | 0.026 | 5 | 0.032 | 0.028 | 0.056 | 0.02 | 5 | 0.042 | 0.042 | 0.082 | 0.042 | 5 | 0.04 | 0.052 | 0.032 | 0.036 |
| 10 | 0.013 | 0.004 | 0.005 | 0.006 | 10 | 0.013 | 0.029 | 0.016 | 0.019 | 10 | 0.021 | 0.039 | 0.011 | 0.022 | 10 | 0.017 | 0.015 | 0.027 | 0.02 |
| 15 | 0.006 | 0.008 | 0.003 | 0.006 | 15 | 0.021 | 0.007 | 0.016 | 0.159 | 15 | 0.013 | 0.023 | 0.01 | 0.016 | 15 | 0.014 | 0.015 | 0.013 | 0.008 |
| 20 | 0.008 | 0.002 | 0.004 | 0.006 | 20 | 0.008 | 0.01 | 0.008 | 0.009 | 20 | 0.011 | 0.007 | 0.004 | 0.004 | 20 | 0.018 | 0.011 | 0.007 | 0.008 |
| 25 | 0.008 | 0.007 | 0.003 | 0.003 | 25 | 0.007 | 0.004 | 0.004 | 0.006 | 25 | 0.009 | 0.007 | 0.013 | 0.006 | 25 | 0.012 | 0.011 | 0.005 | 0.014 |

| p = 0.5 | | | | | p = 0.6 | | | | | p = 0.7 | | | | | p = 0.8 | | | | |
|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|---------|-------|-------|-------|-------|
| n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS |
| 5 | 0.042 | 0.034 | 0.034 | 0.05 | 5 | 0.036 | 0.024 | 0.032 | 0.052 | 5 | 0.042 | 0.018 | 0.019 | 0.042 | 5 | 0.04 | 0.028 | 0.024 | 0.032 |
| 10 | 0.02 | 0.022 | 0.026 | 0.024 | 10 | 0.006 | 0.02 | 0.033 | 0.032 | 10 | 0.01 | 0.01 | 0.011 | 0.014 | 10 | 0.024 | 0.006 | 0.009 | 0.018 |
| 15 | 0.012 | 0.011 | 0.004 | 0.025 | 15 | 0.036 | 0.007 | 0.021 | 0.014 | 15 | 0.009 | 0.014 | 0.016 | 0.013 | 15 | 0.016 | 0.013 | 0.006 | 0.018 |
| 20 | 0.014 | 0.013 | 0.006 | 0.026 | 20 | 0.016 | 0.014 | 0.02 | 0.009 | 20 | 0.006 | 0.007 | 0.017 | 0.019 | 20 | 0.008 | 0.003 | 0.011 | 0.008 |
| 25 | 0.007 | 0.014 | 0.001 | 0.015 | 25 | 0.01 | 0.003 | 0.011 | 0.012 | 25 | 0.007 | 0.006 | 0.003 | 0.014 | 25 | 0.006 | 0.006 | 0.004 | 0.005 |

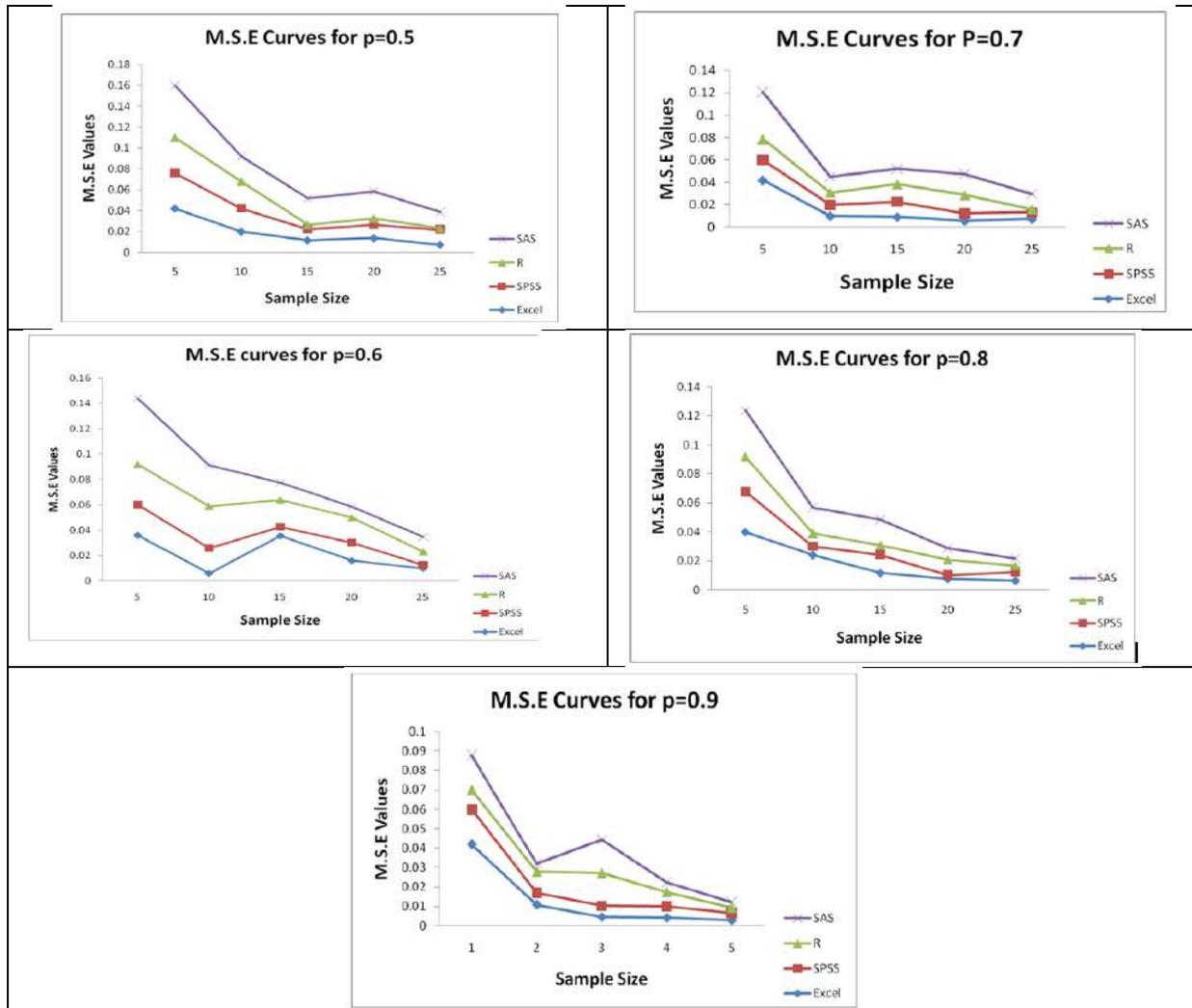
| p = 0.9 | n | Excel | SPSS | R | SAS | n | Excel | SPSS | R | SAS |
|---------|----|-------|-------|-------|-------|----|-------|-------|-------|-------|
| | 5 | 0.042 | 0.018 | 0.01 | 0.018 | 20 | 0.004 | 0.006 | 0.008 | 0.005 |
| | 10 | 0.011 | 0.006 | 0.011 | 0.004 | 25 | 0.003 | 0.004 | 0.003 | 0.003 |
| | 15 | 0.005 | 0.006 | 0.017 | 0.017 | | | | | |

APPENDIX II. MSE curves from Microsoft Excel, SPSS, SAS and R for different values of p





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Bioabsorption of Crystal Violet Stain from water using *Targionia hypophylla* L.

Meenu Mathew¹, Tess Babu², Amrutha S³, Aparna Jamunakumar³, Raisa Jose³ and Abraham Mathew^{4*}

¹CSIR SRF, Bryology Division, PG and Research Department of Botany, St. Peter's College Kolenchery, Kerala, India, 682311

²Research Scholar, Bryology Division, PG and Research Department of Botany, St. Peter's College Kolenchery, Kerala, India, 682311.

³Project Student, Bryology Division, PG and Research Department of Botany, St. Peter's College Kolenchery, Kerala, India, 682311

⁴Assistant Professor, Bryology Division, PG and Research Department of Botany, St. Peter's College Kolenchery, Kerala, India, 682311.

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*Address for Correspondence

Abraham Mathew

Assistant Professor,
Bryology Division,
PG and Research Department of Botany,
St. Peter's College Kolenchery,
Kerala, India, 682311.
E.Mail: abrphyton@gmail.com.



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ABSTRACT

Bryophytes are the amphibians of plant kingdom. Though minute in size, they have paramount influence in ecosystem. They maintain microclimate suitable for small invertebrates and insects and studies indicate high bio-potentiality of the taxa. Dyes discarded from textile and paper industries cause serious environmental problems in aquatic ecosystem. Many are hazardous chemicals that can affect living forms and all of them being coloured can reduce light penetration in water. This can result in trophic cascades leading to reduced energy flow and material cycling. Removal of dyes from industrial effluents is a tedious process and the present paper deals with the use of *Targionia hypophylla* in removing crystal violet dye from water. Dye removal of 92 % was noted at a dye concentration of 0.005% within 1 hr using 3 g plant material.

Keywords :Bryophytes, *Targionia hypophylla*, Crystal violet, Bioabsorption





INTRODUCTION

Bryophytes are poikilohydric forms that can easily gain or lose water from their body depending on the external environmental conditions. They form a diverse assemblage of non-vascular cryptogams with a green gametophytic plant body. Based on species diversity, bryophytes hold third position among plants after angiosperms and algae. Yet studies on bryophytes are minimum owing to small size, low biomass availability and difficulty in identification of taxa. Recent studies indicate bryophyte to have several ecological and economic importance. They play a significant role in water recycling, carbon and nitrogen cycle and biomass production [1]. They are considered as store house of biochemicals. Many of these chemicals are novel and have bioactivities like antimicrobial, anticancerous, antioxidant, piscicidal, larvicidal, antiviral, cytotoxic, plant growth inhibitory, vasopressin antagonistic, muscle relaxing, neurotrophic and antiobesity activities [2]. GC MS analysis of petroleum ether extract of *Pterobryopsis pillifolia* revealed the presence of several volatile compounds, many of which are not seen in higher plants [3]. Several bryophytes are considered as hyper accumulators of metal ions and studies indicate their use in removal of dissolved ions from aquatic system. Their cell wall is thought to have net negative charge and hence absorbs cations from environment [4].

Dyes are widely used in textile and paper industries to impart colour to the product. Many of the dyes are active at high concentrations and thus the leftover solutions have considerable amount of dyes. The spent liquid in most cases are directly discharged into aquatic systems which results in severe pollution and trophic cascades. Dyes alter the light penetrating ability and thus photosynthetic benthic forms get greatly affected. Most of the dyes are also potent carcinogens and teratogens and thus affect aquatic life and pose threat to human health. They are frequently resistant to biodegradation and is difficult to get eliminated from water due to their complex aromatic character and high solubility in water [5]. Wastewater treatment for dye removal include coagulation and precipitation, chemical oxidation-reduction, ozonation, reverse osmosis, membrane filtration and bioabsorption. Most of these techniques have higher operative expenses and many are unproductive in removing dyes. Adsorption is more preferred due to low operating cost and high efficacy. Both natural and synthetic adsorbents have studied in dye removal like corncob and barley husk, cocoa pod husks, palm kernel shell, spent tea leaves, ginger waste, metal-organic frameworks (MOFs), activated carbon, peat, chitin, silica and graphene oxide [6].

Crystal violet is a dye used in paper and textile industry. It is an ingredient in navy blue and black ink. Crystal violet have hazardous effects on living organisms. It is dangerous for humans causing cancer when consumed or inhaled [7]. The present work aims in removing crystal violet from aquatic system using dried thallus of *Targionia hypophylla*.

MATERIALS AND METHODS

Collection of Plant

The bryophyte was collected from different high altitude areas of Kanthalloor area, Idukki District, Kerala during the month of October. The plants were carefully collected in plastic bags and was brought to the lab. The plant was identified based on morphological observation of both gametophyte and sporophyte.

Experimental set up

Crystal violet powder (Merck) was dissolved in water. Six concentrations were made – 0.005 %, 0.01 %, 0.015 %, 0.02 %, 0.025 % and 0.03 %. 100 ml of each solution was taken in 250 ml conical flask. 3g of dried thallus of *Targionia hypophylla* was immersed in the solutions and absorbance of the solutions were read at 580 nm at 10 minutes interval for 1 hr. There after the thallus was taken out and the same initial amount of crystal violet was added to each conical flask, stirred well and the same thalli were immersed in the corresponding conical flasks. This forms the second cycle of bioabsorption. Absorbance of solution was measured at 580 nm at 10 minutes interval for 1 hr.





RESULTS AND DISCUSSION

Gametophytic plant and the sporophyte was viewed under stereo zoom microscope and was identified as *Targionia hypophylla* L (Figure 1). Plant body is a haploid gametophyte. It is flat, prostrate, green and strap shaped. On the dorsal side, areolae with air pores were seen. Anatomically the thallus had an upper photosynthetic region and a lower storage region. Photosynthetic region with uniseriate air chambers with branched assimilatory filaments. Air pores were semibarrel shaped. Storage region composed of compact parenchyma cells. On the ventral surface smooth walled and tuberculate rhizoids were seen. Scales were seen in the ventral surface as two rows on either side of the mid rib. Sporophyte were seen at the apex on the ventral surface. Each thallus had a single sporophyte covered with black bivalve sheath.

The thallus showed bioabsorption of crystal violet dye (Table 1). The dye colour in solution decreased with increase in treatment duration. With dye concentration of 0.005 %, a 10 minute treatment with thallus caused a reduction of 31 % of dye from solution. This got enhanced to 46 % of total dye removal with 20 minutes immersion. With 1 hr immersion, 92 % of dye was removed from solution and the solution became almost colourless. With increase in concentration of dye, there was increase in bioabsorption. A similar pattern of dye removal was seen in all concentration of dye used with 93 % removal of dye by thallus at dye concentration of 0.03 % with a treatment time of 1 hr. Even during the second cycle the thallus continued to show bioabsorption of crystal violet dye (Table 2). Concentration of the dye was double the initial concentration used. The dye colour in solution decreased with increase in treatment duration with 82 % removal of dye by thallus at dye concentration of 0.03 % with a treatment time of 1 hr.

Bryophytes (comprising mosses, liverworts, hornworts and allies) are the second largest group of higher plants after angiosperms, with estimated 15,000 species worldwide [8]. Bryophytes have several ecological and economic importance [9]. On the main feature of bryophyte is its ability to absorb metal ions and dyes. Even dead thallus can hyper accumulate pollutants. Because of certain features, like the lack of roots, unistratose leaves, ion-exchange capacity, and uptake of nutrients from the air, bryophyte can be used for observing regional and local patterns of deposition due to their high accumulation ability [10]. In the current study, thallus showed high degree of bioabsorption with 92 % removal of dye at a concentration of 50mg/l within 1 hr. Fabryanty *et al.* [11] have used bentonite-alginate composite for removal of crystal violet dye. They prepared the composite by using microwave rapid heating method. Crystal violet removal of 80% was observed from water which was much higher when either bentonite or alginate was used alone. In another study, guar gum-g-poly (methyl methacrylate) superabsorbent nanocomposite was used for the removal of crystal violet dye. The adsorption experiments were carried out using batch technique at different pH values and found that the prepared composite showed highest percentage removal of 78% at pH value of 8. The study of adsorption isotherm showed that adsorption process of crystal violet follow both Freundlich and Langmuir isotherm. The kinetics of adsorption was found to follow pseudo second order reaction kinetics [12]. In another study, local soil clay as a low cost and affordable adsorbent for the successful removal of methylene blue (MB) and crystal violet (CV) dyes from an aqueous system was attempted. The study showed maximum adsorption at 2 min and uses the pseudo- second-order kinetics. Maximum removal of MB and CV up to 47.82 and 35.71 mg g⁻¹, respectively was accomplished by dye molecules adsorption onto soil clay [6].

In the present study, same thallus was used twice in removing crystal violet. However compared with first cycle where 93 % removal of dye (dye concentration of 0.03% with a treatment time of 1 hr) was noted, they dye removal got reduced to 82 % in second cycle. Thus thallus can be repeatedly used for dye removal. This indicate the potential of *Targioniahypophylla* in removing crystal violet dye from water.





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CONCLUSION

Dye removal from aquatic system is a real challenge and of the several technologies employed, adsorption is the most feasible methodology. Bryophytes are known to adsorb metal and dyes due to their net negative cell wall charge. Thallus of *Targioniahy pophylla* was successfully used in removing crystal violet dye from water with 93 % removal of dye by thallus at dye concentration of 0.03 % with a treatment time of 1 hr. The crystal violet adsorbed thallus was reused for another cycle of bioadsorption and in this experiment also, there was bioadsorption. The rate of bioadsorption increased with dye concentration. Further studies need to be conducted like the effect of pH and temperature on dye adsorption and also desorption studies and reuse of thallus.

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Table 1 : First cycle of Bioabsorption studies using *Targionia hypophylla* thallus

| Initial Concentration of dye | Concentration of dye absorbed in mg/100 ml | | | | | |
|------------------------------|--|------------|------------|------------|------------|------------|
| | 10 minutes | 20 minutes | 30 minutes | 40 minutes | 50 minutes | 60 minutes |
| 0.005 % | 1.6 | 2.3 | 2.8 | 3.3 | 4.0 | 4.6 |
| 0.01 % | 6.1 | 6.7 | 7.3 | 8.2 | 8.6 | 9.1 |
| 0.015 % | 10.7 | 11.1 | 12.0 | 12.8 | 13.5 | 13.9 |
| 0.02 % | 15.3 | 16.0 | 16.8 | 17.0 | 17.6 | 18.5 |
| 0.025 % | 20.0 | 20.6 | 21.9 | 22.5 | 22.9 | 23.3 |
| 0.03 % | 24.5 | 25.0 | 25.4 | 26.4 | 27.0 | 27.8 |

Table 2 : Second cycle of Bioabsorption studies using *Targionia hypophylla* thallus

| Initial Concentration of dye | Concentration of dye absorbed in mg/100 ml | | | | | |
|------------------------------|--|------------|------------|------------|------------|------------|
| | 10 minutes | 20 minutes | 30 minutes | 40 minutes | 50 minutes | 60 minutes |
| 0.005 % | 1.1 | 1.5 | 2.8 | 3.2 | 3.7 | 4.3 |
| 0.01 % | 5.1 | 5.9 | 6.5 | 7.6 | 8.5 | 9.0 |
| 0.015 % | 9.6 | 10.5 | 11.4 | 12.0 | 12.8 | 13.6 |
| 0.02 % | 14.0 | 14.8 | 15.7 | 16.3 | 16.9 | 17.5 |
| 0.025 % | 16.8 | 18.5 | 19.0 | 19.7 | 20.1 | 21.2 |
| 0.03 % | 19.3 | 22.1 | 22.9 | 23.4 | 24.1 | 24.7 |



Figure 1 : *Targionia hypophylla* Thallus





Design, Development and Evaluation of Microsphere Loaded pH Triggered *In situ* Ophthalmic Gel of Moxifloxacin Hydrochloride as a Method to Control Nasolacrimal Drainage

Shashank Nayak N^{1*}, Sindhu Surendrappa Renuka², Shwetha S Kamath K³, Thimmasetty Juturu⁴ and Srinivas Hebbar⁵

¹Associate Professor, Department of Pharmaceutics, Bapuji Pharmacy College, S.S Layout, Shamanur Road, Davanagere-577004, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India

²Post graduate Student, Department of Industrial Pharmacy, Bapuji Pharmacy College, S.S Layout, Shamanur Road, Davanagere-577004, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India.

³Assistant Professor, Department of Pharmaceutics, Bapuji Pharmacy College, S.S Layout, Shamanur Road, Davanagere-577004, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India

⁴Professor and Head, Department of Pharmaceutics, Bapuji Pharmacy College, S.S Layout, Shamanur road, Davanagere-577004, Karnataka, India. Affiliated to Rajiv Gandhi University of Health Sciences, Bangalore, Karnataka, India

⁵Assistant Professor, Department of Pharmaceutics, Manipal College of Pharmaceutical Sciences, Manipal Academy of Higher Education, Manipal-576104, Karnataka, India.

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*Address for Correspondence

Shashank Nayak N

Associate Professor,
Department of Pharmaceutics,
Bapuji Pharmacy College, S.S Layout,
Shamanur Road, Davanagere-577004,
Karnataka, India.

Affiliated to Rajiv Gandhi University of Health Sciences,
Bangalore, Karnataka, India

E.Mail: shashanknayak87@gmail.com



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ABSTRACT

Eye drops have a significant downside of causing nasolacrimal leakage, which leads to poor patient compliance and acceptance. However, this issue can be resolved by formulating the Moxifloxacin hydrochloride microsphere loaded *in situ* ocular gel using a pH-triggered method. Using 1:10 to 1:16





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ratio of ethyl cellulose and Eudragit RL 100, the moxifloxacin hydrochloride microsphere was developed using the solvent evaporation method. The drug-loaded microsphere was processed for several evaluation parameters, such as drug content, entrapment effectiveness, measurement of particle size, SEM investigations, and XRD studies. The 1:16 drug-loaded microsphere produced encouraging results. Additionally, the improved microsphere was put through a pH-triggered *in-situ* ophthalmic gel production process employing different ratios of carbopol 934 and HPMC K15M. According to the various evaluations that were conducted on the formulation mentioned in the literature. It was discovered that microsphere F4 had a drug content of $90.93 \pm 0.20\%$ and an entrapment efficiency of 89.9 ± 0.22 . Microsphere (F4) had a particle size of $9.65 \pm 1.8 \mu\text{m}$, according to SEM investigations. The ethyl cellulose coating caused the particles to have a negative zeta potential, according to the zeta potential results. The DSC demonstrated how well the API and excipients blended together and how microspheres formed. While the sharp endothermic peak was discovered at 288 degree C, the DSC research showed the sharp endothermic peak at 276.59 degree C. All the initial evaluation trials showed that the prepared *in situ* ophthalmic gel was successful. The best formulae (F4) demonstrated the release for up to 14 hours by using Peppas's model of kinetics and the zero order model. Furthermore, the formulation (F4) also passed the HET CAM test.

Keywords: Moxifloxacin hydrochloride(MFX), Solvent evaporation method, microsphere loaded ophthalmic *in situ* gel, Simulated Tear Fluid (STF).

INTRODUCTION

Ocular drug delivery is sensitive and challenging kind of drug delivery system because of its biological barriers such as aqueous humour drainage, naso-lacrimal drainage, pre corneal tear film in which drug dilution takes place and diffusion barrier of cornea, due of these barriers drug targeting is difficult due to low concentration and lesser bioavailability of drug. Conventional dosage forms (eye drops) which are topically administered are having least ocular bioavailability, especially in case of water-soluble drugs have even less bioavailability, because they are rapidly drained out from action site. Considering all these discomfort and barriers of conventional ocular system various attempts were carried out to increase ocular bioavailability, precorneal retention time and sustain release of drug. Control release dosage forms have vast range of prolonged action which boosts the continuous and sustained release of the ingredients at predetermined time [1]. Novel drug delivery systems like microspheres have an edge over conventional dosage forms for specific site targeting delivery, sustained release, higher residence time, increased bioavailability, and larger drug loading capacity. Microspheres are polymeric carriers of drug which having size range of 1-1000 μm , but for ocular administration its size range should be below 10 μm .

In situ gel are the most promised delivery system which are administered to cul de sac, they are instilled as liquid form and they get converted into gel on contact with cul de sac under different physiological conditions like pH, temperature, and ions activation. *In situ* gels provides dual significant advantages as there liquid phase possess benefits of easy administration whereas gel phase provides benefits of high residence time, sustained release. *In situ* gels are developed to treat anterior segment of eye diseases like dry eye syndrome, cataract, microbial infections like bacterial conjunctivitis and keratitis [2-5]. In recent times a novel approach of drug delivery system like microsphere loaded *in situ* gel which is showing a significant enhancement in ocular bioavailability and improved retention time. Moxifloxacin hydrochloride microsphere loaded pH sensitive *in situ* gel were formulated for ocular administration for improved patient benefits.





The purpose of present investigation is to design, development of moxifloxacin hydrochloride (MFX) microsphere loaded ophthalmic pH triggered *in situ* gel gets transmute from sol to gel at physiological condition there by it increases precorneal residence time [3-4]

MATERIALS AND METHODS

Materials

Moxifloxacin hydrochloride (MFX) was received as gift sample from Micro labs Bangalore, Ethyl cellulose, Eudragit RL100, HPMC K15, Carbopol 934, Methanol, Dichloromethane, Tween 80 were purchased from Rajesh chemicals Co Mumbai, India. Dialysis membrane was procured from Vasa scientific co Bangalore. All the other chemicals belonged to analytical grade as mentioned on the label.

Drug-Excipient compatibility

FTIR was used (Bruker alpha FT-IR instrument) to perform compatibility study. Moxifloxacin hydrochloride, Ethyl cellulose, Eudragit RL100, HPMC K15, Carbopol 934 individually and as well as physical mixtures were scanned according to SOP [6].

Formulation and preparation of microsphere.

MFX-loaded microspheres were prepared using Ethyl cellulose and Eudragit RL100 in combination by using solvent evaporation method. The detailed procedure for the preparation of moxifloxacin hydrochloride loaded microsphere is outlined in the below table 1. Required quantity of different ratio of Ethyl cellulose and Eudragit RL100 were dissolved in 100ml of 1:1 mixture of methanol and Dichloromethane with continuous stirring at room temperature to this solution 100 mg of moxifloxacin hydrochloride was added with continuous stirring. The above solution was transferred into a beaker containing 100ml of water and 0.05% Tween80 was added using micro needle syringe with a continuous stirring 1000-1500 RPM at 40° C for 3 hours. The formulated microspheres were filtered using grade 1 Whatman paper then washed for 3 times with Petroleum ether. Solvents were later subjected to evaporation at room temperature. The procured microspheres were further dried under at room temperature for one day. Further, it was subjected to varied evaluation tests [7,8].

Evaluation of MFX loaded microspheres

Determination of Drug Entrapment efficiency and Drug content:

10mg of MFX loaded microsphere was weighed and transferred into 50 ml of volumetric flask and dissolved in 50 ml of Simulated tear fluid (STF). This volumetric flask was placed in a mechanical shaker bath at 25° C for 3 hours. This solution was filtered and absorbance was measured using UV spectrophotometer at 288nm using STF as blank solution. Drug entrapment efficiency and drug content of microspheres was calculated from the calibration curve generated for MFX over a concentration range of 1-10 µg/ml in STF.⁶

$$\text{Drug Entrapment efficiency} = \frac{\text{Total amount of drug in solution (wt)}}{\text{Total quantity of drug added initially during preparation}} \times 100$$

Zeta Potential analysis

The MFX loaded microspheres were subjected to zeta potential analysis and to determine zeta potential of microsphere using Zetasizer (Malvern Instruments Ltd) JSS drug testing laboratory, Mysore [9,10].

SEM Studies

Topographic studies of MFX loaded microspheres were conducted using JEOL JSM 6100, Japan at SAIF, Chandigarh.¹¹





X-Ray diffraction studies

The studies were done by Rigaku Miniflex600 at SAIF Chandigarh for the MFX loaded microspheres. The XRD was used to investigate the effect of microspheres process on crystallinity of the drug. The results were reported in results and Discussion.

Preparation of moxifloxacin hydrochloride microsphere loaded *in situ* gel by pH triggered method.

The formulae for preparing the moxifloxacin hydrochloride microsphere loaded *in situ* gels given in Table 2. Appropriate amount of HPMC K15M was completely liquified in 10 ml DW, with slow stirring using magnetic stirrer, without formation of lumps of HPMC K15 during stirring. Carbopol 934 was allowed to hydrate overnight by sprinkling the Carbopol 934 over solution. After 24 hrs, stirring was done to disperse Carbopol 934. The Moxifloxacin hydrochloride microsphere was dispersed in Distilled water, Benzalkonium Chloride was later added. Microspheres were then dispersed into the solution containing HPMC -Carbopol solution. The prepared formulae were filled in 25 ml screw capped glass vial and were subjected to autoclaving as specified in literature [12,13].

Evaluation of moxifloxacin hydrochloride microsphere loaded *in situ* gel.

Preliminary evaluations

The formulation display is investigated visually. The clarity check for formulations were done before and after gelling was analysed through visual examination against white and black backgrounds¹². STF was added to the formulation drop by drop with constant mixing, pH was further checked. Drug content was analysed by UV-Spectrometer. 1 ml of *in situ* gel sample was added to 100 ml volumetric flask containing simulated tear fluid further dilution was done to dilute the concentration and absorbance was noted at 288 nm. Later the drug content was evaluated [13].

Gelling strength determination

The formulations with different ratio of HPMC K15M and Carbopol 934 gelling strength were determined by adding 1 drop of formulations in to 1 ml of freshly prepared STF in the screw capped bottles, equilibrated to 37 degree C. To maintain 37 degree C BOD incubator was used, Visual examination was done to assess gel formation [14,15].

Viscosity findings

The formulations rheological character was investigated as pH function¹⁶. The prepared formulations rheological character was determined with Brookfield viscometer (LVDVE) for the gelling solution of pH 4.4 determined using spindle S3 and for the gelling formulation with pH 7.4 was carried out using T bar spindle. The viscosity of the preparations was evaluated at varied shear rate in rpm conditions (5,10,20,30,50,60,100 rpm) [17].

In vitro drug release

It was conducted by using Franz diffusion cell diffusion model in which donor compartment with formulation and receiver compartment with STF with continuous stirring temperature was adjusted to 37°C. 20 RPM was maintained in receptor compartment to mimic blinking phenomenon of eyelids. Samples were later withdrawn at pre-determined intervals. The drug content was analysed at 288 nm using UV spectrophotometer [13].

Sterility studies by direct inoculation method

Sterility of prepared formulation was tested by using FTM and SCDM. These medias are differentiated into three groups in which one as positive (+) control, another as negative control (-) and third one with *in situ* gel formulation. The positive control is seeded with E coli and S aureus and periodic observation were carried up to 14 days to check growth of microorganism. Comparative study is carried to check the sterility of the formulation [18].

Antimicrobial activity

Formulations were subjected to check the antimicrobial activity against E.coli, S.aureus and P. aeruginosa and compared with marketed product. Cup Plate method was used to check the antimicrobial efficacy [19].





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HET-CAM Test

Ocular Irritancy of the formulation was performed on Chorioallantoic Membrane. Approximately 50 to 60 gms weighed hens' egg was selected. Test formulation or diluted solutions, are directly injected on the CAM membrane and visually inspected [19]. Time for reaction on the membrane was recorded in the duration of 5 min. Based on the reaction time, an irritation value was determined and the formulations are classified as different category. i.e. if scoring are categorised as non-irritant (0–0.9), slight irritant (1–4.9), moderate irritant (5–8.9), strong irritant (9–21) [20 – 21].

Positive control: 1% Sodium dodecyl sulphate

Negative control: Normal saline solution, 0.9% NaCl

Test: Moxifloxacin hydrochloride microsphere loaded *in situ* gel.

$$RI = \frac{301 - \text{secH}}{300} \times 5 + \frac{301 - \text{secL}}{300} \times 7 + \frac{301 - \text{secC}}{300} \times 9$$

Where, H indicates haemorrhage, L indicates vascular lysis C indicates coagulation, RI indicates irritation index.

Stability study

Stability study was conducted for the duration of period of 90 days for the formulation F4. The samples were kept at room temperature, 40 degree C at 75% RH and in refrigerator. Sampling was done at 30 days interval and checked for visual appearance, clarity, pH and drug content [22,23].

RESULTS AND DISCUSSION

FTIR(Fourier transform Infrared)

The IR spectra of MFX was compared with IR spectrum of all the excipients to check the interaction. The individual IR spectra of pure drug and polymers as well as drug and polymer mixture, microsphere and polymer mixture which indicate no interaction between drug and polymers which is shown in figure 1 [20].

DSC Differential Scanning Calorimetry

The figure 2 illustrates that thermal characteristic of MFX gave a sharp endothermic peak at 276.59degree C. The MFX loaded microsphere shows sharp endothermic peak at 288.54degree C which is shown in figure 3. The endothermic peak for MFX microspheres with Carbopol 934 and HPMC K15 was observed at 187.56degree C which is represented in figure 4, which is due to low melting point of Carbopol 934 and HPMC K 15 [21].

Drug Content and Drug Entrapment Efficiency

The average calibration equation $y = 0.1005x (R^2 = 0.9901)$ was used to determine the amount of drug entrapped within microspheres. Drug content and entrapment efficiency of microparticles of four different ratio of ethyl cellulose and Eudragit RL100 are shown in the table 3.

Zeta potential determination

The zeta potential was determined by using Malvern particle size analyser. The sample exhibited average zeta potential of -19.8mv with zeta deviation 118mv and conductivity 0.0710 mS/cm was shown in figure 5. The negative zeta potential it is due the negative charge of ethyl cellulose which is coated over the microspheres, indicating that microspheres have ethyl cellulose coating [24].





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Scanning Electron Microscopy (SEM)

SEM reports revealed, the prepared microspheres were having size in micrometers and the particles were nearly spherical in shape. The size of microspheres was measured using image J software at different resolution. The particle size of F4 was found to be $9.65 \pm 1.8 \mu\text{m}$. SEM image of formulation F4 shown in Figure 6.

X-Ray diffraction:

XRD was conducted to investigate and understand crystalline nature of the drug and its status in final pharmaceutical formulations. XRD patterns of moxifloxacin hydrochloride, Eudragit RL 100, ethyl cellulose and microsphere formulation (F-4) are shown in figure 7 [25]. XRD pattern of microsphere formulation F-4 shows extensively reduced peaks of moxifloxacin hydrochloride (figure 7) at 2θ scattered angles 8.65 and 20.32. This suggests that moxifloxacin hydrochloride crystallinity is reduced in the microsphere formulation [26].

Evaluation Parameters of Microsphere Loaded *In Situ* Gel**Preliminary evaluations**

The preparations were transparent in which microspheres was dispersed or distributed like beads and were clear^[9]. The preparation (F1 to F4) were in acidic pH (4.40 to 4.49). Drug-content was in between 97.25% to 99.08%. The data are given in table 4.

Gelling strength determination

The gelling strength of the preparation having varied polymer concentration having different gelling property i.e. The formulation F1 had no gelling property, F2 showed the gelling strength up to 4 hrs, F3 up to 10 hrs and F4 up to 14 hrs [12].

Viscosity findings

The preparations showcased pseudoplastic flow which are shown in figure 8,9 Low viscosity was observed in acidic pH range (pH 4.4) when the preparation is in sol state, as pH is enhanced to 7.4 (pH of tear fluid) by using STF the sol gets converted to gel [27,28].

***In vitro* drug release**

Franz diffusion cell was utilized to demonstrate the *in vitro* drug release kinetics of the prepared formulations. The formulation F2 follows first order kinetics. However, F3, F4 showcased zero order, Peppas. The F4 formulation releases the drug up to 14 hrs, which is greater in comparison to marketed product Moxigram showing the 100% release in one hours which is shown in Figure 10. The drug release kinetics are mentioned in table 5.

Sterility testing by Direct inoculation

The optimized formulae F4 were found to be sterile up to 14 days when seeded into Fluid Thioglycolate medium and Soyabean casein digest media and no precipitation was observed in the sample which is inoculated.

Antimicrobial activity

The Zone of Inhibition was determined with *E. coli*, *S. aureus* and with *P. aeruginosa* for both standard (marketed formulation) and F4 formulation which is listed in the table 6. The comparative zone of inhibition is showed between standard (moxigram-0.5%) and F4 in given in the figure 11. The zone of inhibition of formulation F4 is comparatively less when compared to marketed formulation (Moxigram -0.5%) this is due to ethylcellulose coating of microspheres which retards the burst release of drug from microspheres.

HET CAM test

To the CAM membrane different solution were inserted to check the irritancy, haemorrhage, and coagulation. The results indicated that the preparations did not cause any irritancy, haemorrhage, and coagulation. The results are shown in the figure 12 and table 8.



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The Irritation index of Positive control (1%SDS) is showing sever irritation whereas test solution F4 is having negative RI of -79.57 which causes no irritation indicating solution is isotonic.

Stability study

The optimized formulae F4 passed the stability test which was conducted for a period of 90 days. The results revealed that the formulation F4 did not show any drastic changes in its properties during the stability study.

CONCLUSION

The proposed work revealed that the microspheres of moxifloxacin hydrochloride prepared by solvent evaporation method showcased promising results. The optimized formulae F4 containing microsphere had a drug content of $90.93 \pm 0.20\%$, drug entrapment was $89.95 \pm 0.22\%$, the particle size was found to $9.65 \pm 1.8 \mu\text{m}$ which was determined by image J Software. Negative zeta potential of -0.769 mv which confirmed the Ethylcellulose coating. Further, X-ray diffraction studies suggests that moxifloxacin hydrochloride crystallinity is reduced in the microsphere formulation. After procuring these results the Microspheres were loaded into *In situ* gel preparation using pH triggered method by the use of Carbopol 934 (gelling agent) and HPMC K15M (rate controlling agent). The formulae F4 containing microsphere loaded in situ gel (400 mg of HPMC K15M and Carbopol 934) passed all the preliminary evaluation, *in vitro* release studies followed zero order peppas model non fickian release, the antimicrobial studies indicated the zone of inhibition of microsphere loaded *in situ* ocular gel & marketed eye drops. The HET CAM test reveals that the formulae F4 is non-irritant. The stability studies results indicated the optimized formulae F4 was stable over a period of 90 days. Henceforth, microsphere loaded *in situ* ocular sol to gel preparation is a substitute to the marketed eye drops.

CONFLICT OF INTEREST

The authors do not have conflict of interest. However, we thank Rajiv Gandhi University of Health sciences, Bangalore, and Bapuji Pharmacy College, Davanagere for providing the necessary facilities to do the work.

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Project code 20PHA433.

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Table 1: Formulation of Moxifloxacin hydrochloride microsphere by solvent evaporation method

| Ingredients | F1 (1:10) | F2 (1:12) | F3 (1:14) | F4 (1:16) |
|----------------------------|-----------|-----------|-----------|-----------|
| Moxifloxacin hydrochloride | 0.1 gm | 0.1 gm | 0.1 gm | 0.1 gm |
| Ethyl cellulose | 0.5 gm | 0.6 gm | 0.7 gm | 0.8 gm |
| Eudragit RL100 | 0.5 gm | 0.6 gm | 0.7 gm | 0.8 gm |
| Dichloromethane(DCM) | 50 ml | 50 ml | 50 ml | 50 ml |
| Methanol | 50 ml | 50 ml | 50 ml | 50 ml |
| Distilled Water(DW) | 100 ml | 100 ml | 100 ml | 100 ml |
| Tween 80 | 0.05% | 0.05% | 0.05% | 0.05% |

Table 2: Formulation of microsphere loaded *insitu* gel by pH triggered method

| Ingredients | F1 | F2 | F3 | F4 |
|----------------------------------|-------|-------|-------|-------|
| MFX microsphere (eq wt. to 50mg) | 450mg | 450mg | 450mg | 450mg |
| HPMC K-15 | 100mg | 200mg | 300mg | 400mg |
| Carbopol 934 | 100mg | 200mg | 300mg | 400mg |
| Distilled water | 10ml | 10ml | 10ml | 10ml |
| Benzalkonium chloride (BKC) | 10mg | 10mg | 10mg | 10mg |

Table 3:Percentage drug content and entrapment efficiency of 4 different formulation

| Formulation code | Drug polymer ratio | Drug content (%) (AM±SD) | Drug entrapment efficiency (%) (AM±SD) |
|------------------|--------------------|--------------------------|--|
| F1 | 1:10 | 62.01±0.30 | 68.31±0.20 |
| F2 | 1:12 | 73.03±0.17 | 75.25±0.18 |
| F3 | 1:14 | 80.6±0.17 | 84.64±0.15 |
| F4 | 1:16 | 90.93±0.20 | 89.95±0.22 |

Table 4 :Preliminary evaluation

| Code | Appearance of the preparation | Clarity | pH | % Drugcontent |
|------|---|---------|------|---------------|
| F1 | Transparent with dispersed microspheres | Clear | 4.40 | 97.25 |
| F2 | Transparent with dispersed microspheres | Clear | 4.44 | 98.75 |
| F3 | Transparent with dispersed microspheres | Clear | 4.46 | 99.23 |
| F4 | Transparent with dispersed microspheres | Clear | 4.49 | 99.08 |

Table 5 :Kinetic model data of formulations F2 to F4

| Formulation | Zero order | Peppas | Higuchi | First order | n Value | Best fit model |
|-------------|------------|--------|---------|-------------|---------|-------------------|
| F2 | 0.8989 | 0.9572 | 0.9915 | 0.9339 | 0.4350 | First order |
| F3 | 0.9661 | 0.9525 | 0.9413 | 0.8824 | 0.5699 | Zero order peppas |
| F4 | 0.9911 | 0.9922 | 0.9616 | 0.8096 | 0.7070 | Zero order peppas |

Table 6 : ZOI with varied organisms

| Microbial strain | Zone of inhibition in mm | |
|---------------------|---------------------------------|-----------|
| | Standard (marketed formulation) | F4 |
| <i>E coli</i> | 30.6±0.94 | 7.3±0.47 |
| <i>S aureus</i> | 28.6±0.94 | 8.3±0.47 |
| <i>P aeruginosa</i> | 31±1.63 | 15.6±0.94 |





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Table 8 : Results of HET-CAM Test

| Solutions | Irritation index (RI) | | Inference |
|---|-------------------------------------|--------|-------------------|
| Positive control (1% Sodium dodecyl sulphate) | H=64sec L=98sec C=130sec | 13.81 | Severe irritation |
| Negative control (Normal saline solution, 0.9% NaCl) | H=800sec L=800sec C=800sec | -41.58 | No irritation |
| Test (MoxifloxacinHCL microsphere loaded <i>in situ</i> gel) | H=1440sec L=1440sec C=1440sec | -79.57 | No irritation |

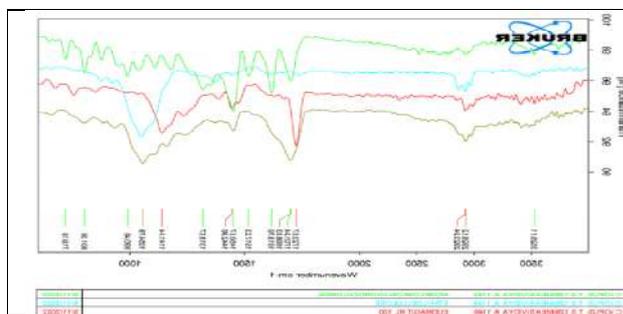


Figure 1:Compatibility study: FT-IR of sample 1(Moxifloxacin Hcl),Sample 2 (Ethylcellulose),Sample 3(Eudragit RL 100), Sample 4 (Moxifloxacin Hcl microsphere + HPMC K15+ Carbopol934)

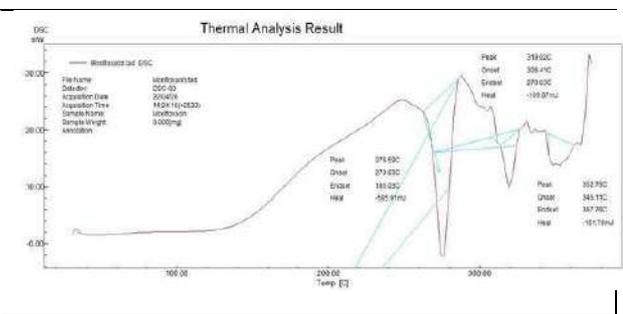


Figure 2: DSC study of Moxifloxacin hydrochloride

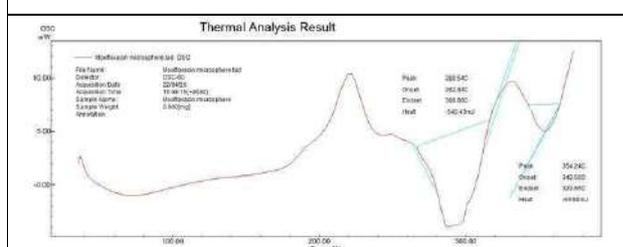


Figure 3: DSC study of Moxifloxacin hydrochloride microsphere

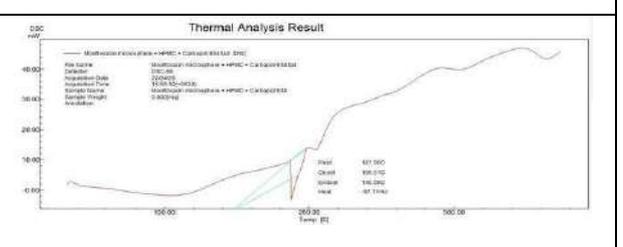


Figure 4 : DSC study of Moxifloxacin hydrochloride microsphere +HPMC+ Carbopol 934

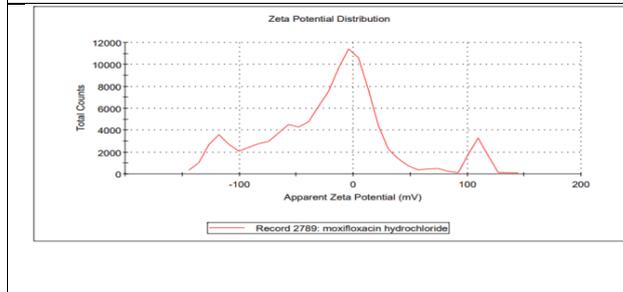


Figure 5: Zeta potential distribution graph of Moxifloxacin hydrochloridemicrospheres

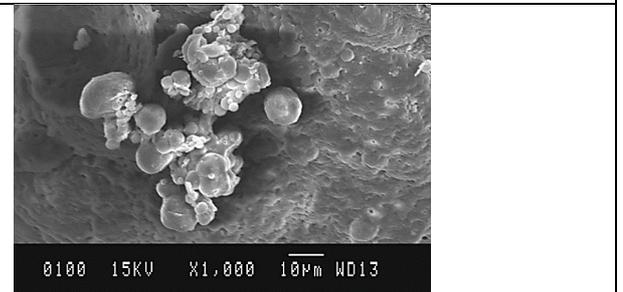


Figure 6: SEM image of F4 MFX microspheres





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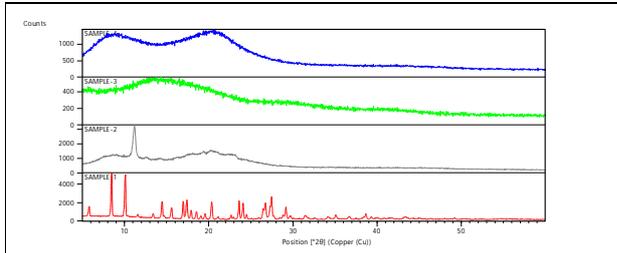


Figure 7 : Combined XRD Spectra of MFX (sample 1), Ethyl cellulose (sample 2), Eudragit RL 100 (sample 3), MFX microspheres (sample 4)

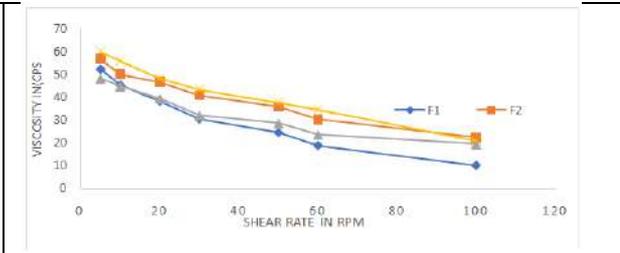


Figure 8: Viscosity of formulations at pH 4.4 (Before gelation at non physiological condition)

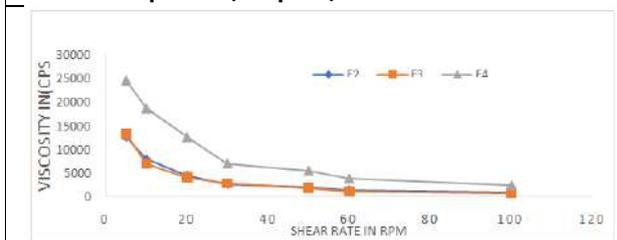


Figure 9 : Viscosity of formulations at pH 7.4 (After gelation at physiological condition)

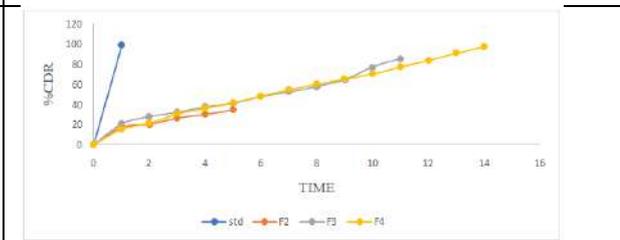


Figure 10: Release study of Moxifloxacin hydrochloride microsphere loaded in situ gel (F2 to F4)



Figure 11 : ZOI of Formulation F4 of Moxifloxacin hydrochloride microsphere loaded in situ gel and marketed product seeded with *E. coli*, *S. aureus* and *P. aeruginosa*

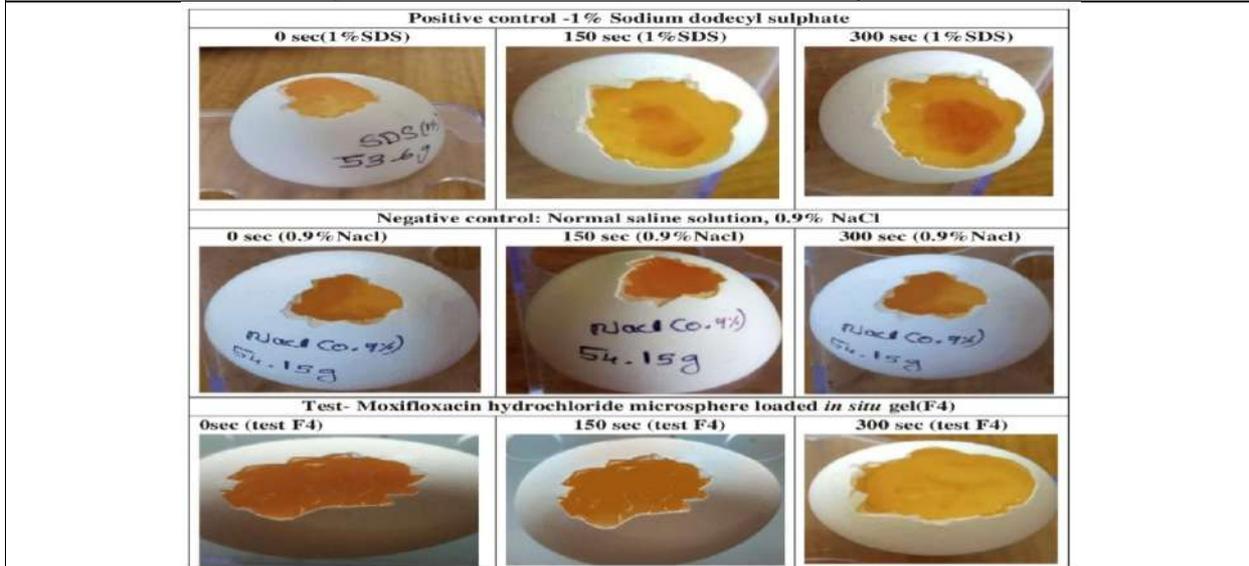


Figure 12: Test- Moxifloxacin hydrochloride microsphere loaded in situ gel (F4)





Development of Moringa Leaf Powder Incorporated Noodles

T.Uma Maheswari^{1*} and A.P.Gokul²

¹Assistant Professor, Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

²Ph.D Scholar, Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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*Address for Correspondence

T.Uma Maheswari

Assistant Professor,

Department of Horticulture, Faculty of Agriculture,

Annamalai University, Annamalai Nagar,

Chidambaram, Tamil Nadu, India

E.Mail: umahorti2003@gmail.com



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ABSTRACT

As noodles are being popular among youngsters, it holds a notion of low protein and micro nutrient contents as the main ingredient for preparation includes refined wheat flour. Hence a study was conducted to develop protein and micronutrient enriched noodles by incorporating moringa leaf in the form of powder. The present experiment was conducted at post harvest laboratory, Dept. of Horticulture, Faculty of Agriculture, Annamalai University to prepare moringa leaf powder incorporated noodles and to evaluate nutritional qualities. Fresh leaves of annual moringa variety PKM-1 was selected for the study. The experiment was conducted in completely randomized design (CRD) with five replications and four formulations (T₁: 5% moringa leaf powder, T₂: 10% moringa leaf powder, T₃: 15% moringa leaf powder, T₄: 20% moringa leaf powder). All the chemical analysis showed that T₃ (15% moringa leaf powder) and T₄ (20% moringa leaf powder) had higher concentration of protein, carbohydrate, calcium and iron. Where as in sensory evaluation T₁ (5% moringa leaf powder) and T₂ (10% moringa leaf powder) had higher score in taste, colour, flavour and overall acceptability.

Keywords: Noodles, Moringa leaf powder.



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INTRODUCTION

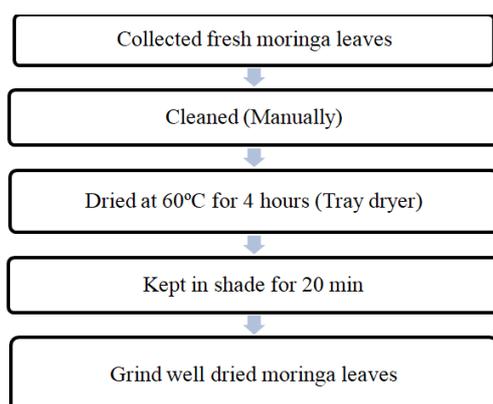
Moringa oleifera is a widely known crop grown throughout India belonging to the family Moringaceae. It is widely called miracle tree as the economic parts include leaves, roots, flowers and pods. Native to South Asia, it is widely seen in South Africa, tropical Asia, Pacific islands, Pakistan, Bangladesh and Afghanistan. *Moringa oleifera* commonly known as *murungai* in Tamil is a well utilized vegetable by major Indians, which is consumed for its medicinal properties. The high nutritious value and its varied use as food and medicine is well known, with leaves being used as forage, trunk used for manufacturing gums, flower nectar as honey and powdered seeds for water purification (Fuglie, 1999).

The main economic part of moringa being leaf as it contains higher amount of micro nutrients which plays a vital role in physiological and metabolic activity of human health. While comparing the nutritive value of moringa leaf with other food products it has been found to contain 7 times higher amount of vitamin C in oranges, 10 times the vitamin A content in carrot, 15 times more potassium than in banana (Gopalan *et al.*, 1998).

The main objective of this study was to find out ways of using dehydrated moringa leaf powder to enrich instant food product. Hence the present study has been carried out to study the incorporation of moringa leaf powder in noodles.

MATERIALS AND METHODS

Fresh leaves of annual moringa variety (PKM-1) was collected from local farmers near Chidambaram. Ingredients used for preparation of noodles had been collected from stores. Completely randomized design was applied for statistical analysis of experimental data. The experiment comprises of four treatment concentrations and five replications. T₁: 5% moringa leaf powder, T₂: 10% moringa leaf powder, T₃: 15% moringa leaf powder, T₄: 20% moringa leaf powder. Collected fresh leaves are manually cleaned to remove stem, dirt and extraneous matter to produce higher quality of dehydrated powder. Cleaned leaves were dried at 60°C for 4 hours in tray dryer. After drying, the leaves are kept in shade for 20 minutes and grinded well to obtain moringa leaf powder.

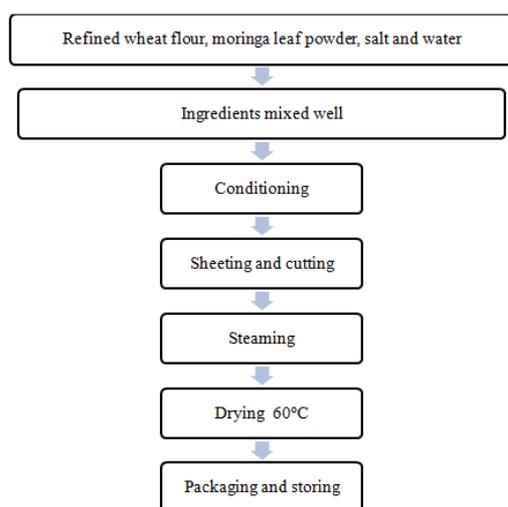


Ingredients for preparation of moringa leaf powder incorporated noodles were moringa leaf powder, wheat flour (100g), salt (3g), water (about 30 ml). Quantity of wheat flour varies for different treatments as concentration of moringa leaf powder varies. Moringa leaf powder (5g, 10g, 15g, 20g) mixed with wheat flour along with salt and mixed well. The dough obtained had a consistency similar to breadcrumbs. Noodles dough is conditioned for half an hour to make it good for sheeting. Using noodle roller the noodles sheet is cut into uniform size noodles at a time.



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Steaming of freshly prepared noodles is done for 20-25 minutes until noodles were partially cooked. The steam cooked noodles were arranged in stainless steel tray and kept under sun for half day and kept in hot air oven for 5 hours at 60°C. Dried noodles was cooled in room temperature and packed in packets and stored in ambient temperature. The flow chart for the preparation of moringa leaf powder incorporated noodles is shown in flow chart 1.



Sensory scoring and nutritional composition of moringa leaf powder incorporated noodles of different concentration had been observed. Sensory analysis was assessed by subjecting to sensory scores for colour, taste, flavour, overall acceptability from ten untrained panelists from Department of Horticulture, Annamalai University. The scores were obtained on 9 point hedonic scale. Chemical analysis of moringa leaf powder incorporated noodles prepared using standardized formulation based on maximum sensory score was carried out for its parameters like carbohydrate, protein, fat, iron, calcium.

RESULTS AND DISCUSSION

Average sensory score of moringa noodles for taste, colour, flavour, overall acceptability was found to be higher in T₁ (5% moringa leaf powder) followed by T₂ (10% moringa leaf powder) as shown in table 2. Similar study conducted by Kolawole *et al.* (2013) observed at the addition of 4 g *Moringa* powder with cake sample found most preferred in terms of colour, taste and general acceptability. As the preference of panel members towards moringa noodles with lesser concentration indicates that bitterness of moringa leaf powder was found to be higher in T₃ (15% moringa leaf powder) and T₄ (20% moringa leaf powder). Similar to that of reported by Mushtaq *et al.* (2018) for characterization of *Moringa oleifera* leaves and its utilization as value added ingredient in unleavened flat bread (chapatti).

Nutritional analysis of moringa noodles was carried out by evaluation of different nutritional properties such as protein, carbohydrate, fat, iron and calcium. The results were presented in table 1. Among the treatments, T₄ recorded higher protein (18.6 g), carbohydrate (65.14 g), fat (4.33 g), iron (3.78 g) followed by T₃ (15% moringa leaf powder). These agree with study on moringa and cake (Olushola, 2006). Concentration of moringa leaf powder had significant influence on nutritional composition of moringa noodles. Similar to that of reported by Srinivasamurthy *et al.*, (2017) characterization of *Moringa oleifera* leaves powder for the production of muffin. Noodles incorporated with higher concentration of moringa leaf powder has higher amount of protein, carbohydrates, iron when compared with treatments with lesser concentration of moringa leaf powder.





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CONCLUSION

In sensory evaluation, T₁ (5% moringa leaf powder) is evaluated as the best treatment when compared with other treatments followed by T₂ (10% moringa leaf powder) as shown in figure 3. While T₃ (15% moringa leaf powder) and T₄ (20% moringa leaf powder) had lesser scoring due to the presence of bitterness in taste as shown in table 2. Whereas T₄ (20% moringa leaf powder) and T₃ (15% moringa leaf powder) were found to be higher in nutritional composition such as protein, carbohydrate, iron, calcium and sodium. In view of increasing the nutritional value of noodles T₃ and T₄ were found to be having huge consumer preference.

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Table 1: Nutritional composition of moringa leaf powder incorporated noodles

| VALUE | PARAMETERS | | | | | |
|--|-----------------|----------------------|------------|------------------|---------------|-------------------|
| | Protein g/100 g | Carbohydrate g/100 g | Fat g/100g | Sodium mg /100 g | Iron mg/100 g | Calcium mg /100 g |
| T ₁ (5% moringa leaf powder) | 9.458 | 58.512 | 2.280 | 1.166 | 2.082 | 27.584 |
| T ₂ (10% moringa leaf powder) | 12.574 | 60.336 | 2.710 | 1.240 | 2.382 | 28.354 |
| T ₃ (15% moringa leaf powder) | 15.712 | 62.596 | 3.334 | 1.312 | 3.204 | 29.342 |
| T ₄ (20% moringa leaf powder) | 18.602 | 65.142 | 4.332 | 1.388 | 3.782 | 29.858 |
| S.Ed | 0.304 | 0.387 | 0.258 | 0.037 | 0.169 | 0.234 |
| C.D. | 0.599 | 0.759 | 0.439 | 0.066 | 0.325 | 0.481 |

Table 2: Sensory scoring for moringa leaf powder incorporated noodles

| Taste panels (TP) | T ₁ (5% moringa leaf powder) | | | | T ₂ (10% moringa leaf powder) | | | | T ₃ (15% moringa leaf powder) | | | | T ₄ (20% moringa leaf powder) | | | |
|-------------------|---|--------|---------|-----------------------|--|--------|---------|-----------------------|--|--------|---------|-----------------------|--|--------|---------|-----------------------|
| | Taste | Colour | Flavour | Overall acceptability | Taste | Colour | Flavour | Overall acceptability | Taste | Colour | Flavour | Overall acceptability | Taste | Colour | Flavour | Overall acceptability |
| TP1 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5 |
| TP2 | 7 | 7 | 7 | 7 | 8 | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| TP3 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 |
| TP4 | 8 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| TP5 | 7 | 7 | 7 | 7 | 8 | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 5 | 5 | 5 | 5 |
| TP6 | 7 | 7 | 7 | 7 | 8 | 8 | 8 | 8 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| TP7 | 7 | 8 | 7 | 7 | 8 | 7 | 7 | 8 | 5 | 6 | 5 | 6 | 5 | 5 | 5 | 6 |
| TP8 | 8 | 8 | 7 | 7 | 7 | 7 | 6 | 7 | 6 | 7 | 6 | 5 | 5 | 5 | 6 | 5 |
| TP9 | 7 | 7 | 8 | 7 | 7 | 8 | 7 | 8 | 5 | 5 | 5 | 6 | 6 | 5 | 6 | 5 |
| TP10 | 8 | 8 | 8 | 7 | 7 | 7 | 7 | 7 | 6 | 6 | 5 | 5 | 6 | 5 | 5 | 5 |
| Grand total | 75 | 76 | 75 | 66 | 74 | 74 | 72 | 75 | 57 | 59 | 56 | 57 | 56 | 54 | 56 | 55 |





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| | | | | | | | | | | | | | | | | |
|---------|--------|-------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|--------|-------|-------|-------|
| Average | 7.5 | 7.6 | 7.5 | 6.6 | 7.4 | 7.4 | 7.2 | 7.5 | 5.7 | 5.9 | 5.6 | 5.7 | 5.6 | 5.4 | 5.6 | 5.5 |
| S.Ed | 0.0164 | 0.017 | 0.164 | 0.017 | 0.0164 | 0.017 | 0.164 | 0.017 | 0.0164 | 0.017 | 0.164 | 0.017 | 0.0164 | 0.017 | 0.164 | 0.017 |
| CD | 0.350 | 0.023 | 0.021 | 0.028 | 0.350 | 0.023 | 0.021 | 0.028 | 0.350 | 0.023 | 0.021 | 0.028 | 0.350 | 0.023 | 0.021 | 0.028 |



Figure 1. Sensory scoring and nutritional composition of moringa leaf powder



Figure 2. Noodles incorporated with moringa leaf powder

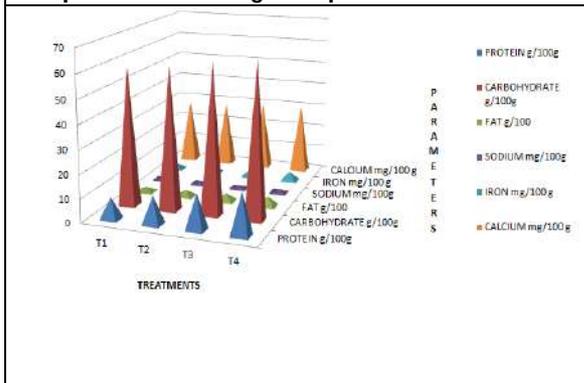


Figure 3. Nutritional composition of moringa leaf powder incorporated noodles

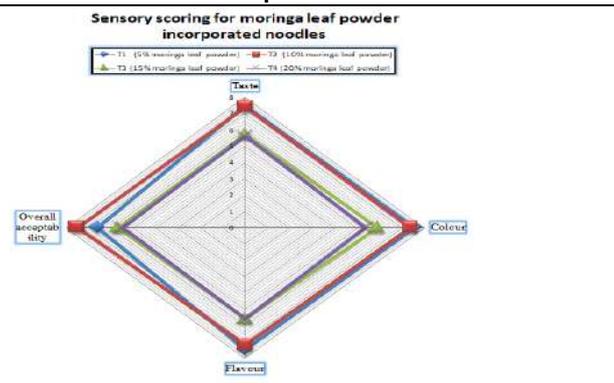


Figure 4. Sensory scoring for moringa leaf powder incorporated noodles





Crystal Growth, NLO, Dielectric and Antibacterial Analysis of Cesium Sulphate-Doped TGS Crystals

S.Jerin Blessy^{1*}, H.Johnson Jeyakumar² and P.Selvarajan³

¹Research Scholar, Reg.No.21212152132003, PG and Research Department of Physics, Pope's College, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

²Head and Associate Professor, Department of Physics, Pope's College, Affiliated to Manonmaniam Sundaranar University, Tirunelveli, Tamil Nadu, India.

³Associate Professor, Department of Physics, Aditanar College of Arts and Science, Tiruchendur, Tamil Nadu, India

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*Address for Correspondence

S.Jerin Blessy

Research Scholar, Reg.No.21212152132003,
PG and Research Department of Physics, Pope's College,
Affiliated to Manonmaniam Sundaranar University,
Tirunelveli, Tamil Nadu, India.
E.Mail: jerinblessy1999@gmail.com



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ABSTRACT

In view of technological importance, crystal growth and characterization has always been an interesting research area among scientists and researchers for many years. In general, crystals can be classified into two categories namely linear and nonlinear crystals. Triglycine sulphate (TGS) crystal is a nonlinear ferroelectric material. Since nobody has studied cesium sulphate-doped TGS (CSTGS) crystal, in this research work, cesium sulphate was added as the dopant (2 mole%) into TGS crystal. Single crystals of CSTGS were grown by aqueous solution technique and the harvested crystals were subjected to studies like XRD, LDT, SHG study, EDS study, dielectric and antibacterial activity studies.. From the results, it is revealed that CSTGS crystal has high LDT, high SHG, and hence the sample is highly suitable for laser, NLO and photonic applications.

Keywords: TGS; doping; solution method; EDS; XRD; LDT; dielectric; antibacterial activity

INTRODUCTION

Triglycinesulfate (TGS) crystal is an interesting ferroelectric and pyroelectric material, which exhibits strong absorbing ability in the most part of infrared region. Due to this property, it is used for the fabrication of infrared

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detectors and pyroelectric vidicon tubes operating at room temperature. It also finds applications in the fabrication of capacitors, transducers and sensors [1-4]. TGS crystal shows a typical second-order ferroelectric phase transition at the Curie point $T_c=49$ °C. Below the T_c , TGS possesses the polar point symmetry of group 2 of the monoclinic system, spontaneous polarization (P_s) arises along the b-axis and above T_c , it possesses the non-polar point group of the monoclinic system. TGS crystal has some disadvantages over doped TGS crystals such as i) the ferroelectric domains possess high mobility at room was obtained by heating the solution of TGS at 50 °C. Temperature as low as 50 °C was maintained in order to avoid decomposition of the salt. TGS single crystals can easily be grown from aqueous solutions and exhibit, among ferroelectric materials, one of the best examples of a second order phase transition with order-disorder character [5-7].

Despite of the complex crystal and chemical structure, the ferroelectric phase transition in TGS follows almost perfectly mean field behaviour and the static dielectric properties can be quantitatively described by a simple Landau Devonshire model. The material is therefore ideal to test a wide range of theoretical predictions on critical phenomena and has been extensively studied in the past [8-10]. It is known that dopants can alter the properties of the host crystals and hence in this work, cesium sulphate was added as the dopant into the lattice of TGS crystals. Ibrahim *et al.* have investigated the effect of doping sulfuric acid on the growth and properties of TGS crystal [11] and they also studied TGS crystals grown in the acidic medium [12]. Manoharan *et al.* synthesized calcium doped triglycine sulphate single crystals [13]. Newman *et al.* have reported that crystals of Triglycine sulphate is a very interesting ferroelectric material and it finds wide application as room temperature IR detectors [14]. Leena Rose *et al.* have grown and studied urea-thiourea doped TGS crystal [15]. Lal *et al.* have investigated on the growth and characteristics of TGS crystal and explored its pyroelectric uses [16]. As unoped TGS crystal is easily depolarizable by application of voltage and stress, many organic and inorganic dopants have been added into the lattice of TGS crystal by many authors to stabilize the internal domains [17-21]. FarhanaKhanum *et al.* have added metal ion dopants like lithium and nickel into TGS crystal to alter its various properties [22]. The molecular formula of cesium sulphate is Cs_2SO_4 and it colourless and water-soluble inorganic substance. It is known that cesium sulphate is isostructural with potassium sulphate [23]. Since no work has been reported on addition of cesium sulphate into TGS crystal in the literature, We have decided to grow a bulk single crystal of the title material using a solution method combined with the slow evaporation technique and to characterize the grown crystals of cesium sulphate-doped triglycine sulphate (CSTGS) using variety of characterization techniques. The results obtained from NLO, LDT, dielectric, antibacterial activity and EDS studies are analyzed in this paper.

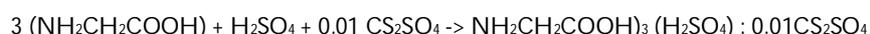
EXPERIMENTAL METHODOLOGY

Chemicals used

- Concentrated sulphuric acid
- Glycine
- Cesium sulphate
- Double distilled water etc.

Crystal Growth

An aqueous solution of TGS was prepared using Analar Reagent (AR) grade of glycine and concentrated sulphuric acid by using molar ratio of 3:1. The solvent used in this work was doubled distilled water. 2 mole% of cesium sulphate was added into the solution. The solution was constantly stirred for about 3 hours using a magnetic stirrer and was filtered using 4 micro Whatmann filter papers. Then the filtered solution was kept in a borosil beaker covered with a porous paper. The growth vessel was kept in an undisturbed place. The allowed growth period was about 25 to 30 days. The chemical reaction for obtaining cesium sulphate-doped TGS (CSTGS) sample is given below:





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The purity of the sample of CSTGS was improved by successive re-crystallization. The schematic diagram for forming the sample of this work is shown in the figure 1 and a good quality harvested crystal of cesium sulphate-doped TGS is shown in figure 2. It is observed that the grown crystal of CSTGS is colourless and transparent and dimensions of a typical crystal (Fig.2) are 18 x 10 x 7 mm³. TGS crystal consists of glycine and sulphate ions and due to presence of glycine, there is a possibility of formation of fungus in the solution during the growth. By adding cesium sulphate as the dopant, it is possible to avoid formation of fungus in the solution and hence quality of the crystal will be enhanced [24].

RESULT AND DISCUSSION

X-ray diffraction analysis

The grown undoped and cesium sulphate-doped TGS (CSTGS) single crystal was subjected to single crystal XRD studies using ENRAF CAD-4 X-Ray diffractometer and the unit cell parameters are provided in the table 1. Single crystal XRD analysis indicates that CSTGS crystal crystallizes in monoclinic structure. The number of molecules per unit cell was found to be 2 and space group is P2₁. When compared to the lattice parameters of undoped TGS crystal, there is no change of crystal structure when TGS is doped with cesium sulphate [25].

Energy Dispersive Spectral (EDS) study

Accurate identification of the elements in a sample is a fundamental prerequisite of a microanalysis system, and in this research, EDAX-INCA, Oxford Instruments, UK, is used to ensure the composite elements of the generated sample. Element Mapping visualizes the elemental distribution by utilizing the X-ray signal from a given energy range. Qualitative mapping provides information about the X-ray intensity distribution within the sample region [26]. The recorded EDS spectrum of CSTGS crystal is shown in figure 3 and it indicates that the grown crystal of CSTGS contains the elements like C, N, O, S and Cs. The experimental percentage of elements in the CSTGS crystal are listed in table 2. Thus, the dopant 'cesium' in form of ion in the interstitial positions of the host TGS crystal. It is noted here that the element hydrogen cannot be identified by EDS method.

NLO studies

The nonlinear optical (NLO) phenomena occur at sufficiently high intense laser fields. As the applied field strength increases, the polarization response of the medium is no longer linear and the induced polarization (P) becomes a nonlinear function of the applied field and it is given by

$$P = \epsilon_0 \chi^{(1)} E + \epsilon_0 \chi^{(2)} E^2 + \epsilon_0 \chi^{(3)} E^3 \dots \dots \quad (1)$$

Where ϵ_0 is permittivity of free space or vacuum, E is the electric field and $\chi^{(1)}$, $\chi^{(2)}$ and $\chi^{(3)}$ are the first-order, second-order and third-order susceptibilities respectively. The second-order susceptibility, $\chi^{(2)}$ is responsible for second-order NLO phenomena like second harmonic generation (SHG), sum frequency generation (SFG), optical rectification etc. SHG efficiency was measured by the Kurtz-Perry technique [27] with 1064nm radiation from a Q switched high-energy Nd:YAG laser with pulse width of 6 ns and repetition rate is 10 Hz. For this experiment, the grown crystal of CSTGS and the reference sample (KDP) were made into fine powder with the particle size of the order of 125-150 μ m. When the sample was exposed to laser radiation of wavelength 1064 nm, green laser radiation of wavelength of 532 nm was emitted. Thus, the wavelength of incident radiation is reduced to half and hence the phenomenon is the SHG. For an input energy of laser radiation of 0.70 mJ/pulse, the SHG signal from CSTGS crystal was obtained to be 1.95 mJ/pulse and that from the reference sample KDP is 8.83 mJ/pulse. Thus, the relative SHG efficiency of CSTGS crystal is 0.22 times that of KDP. Compared to SHG efficiency of undoped TGS crystal (0.09 times that of KDP), CSTGS crystal has more SHG efficiency [28].





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LDT analysis

Laser damage threshold (LDT) value is an important parameter to be studied for NLO and laser crystals. Usually damage occurs on the surface of the crystal when it is exposed to nanosecond or picosecond laser pulses. In this experiment, a Q-switched Nd:YAG laser (pulse width 10 ns and 10 Hz repetition rate) was used and the power of the laser measured by using a power meter (Model No. EPM 200). The surface of the grown crystal of CSTGS was polished using the alumina powder before it was exposed to laser. The energy of the laser beam was increased from 10 mJ and exposure time allowed was 30 s. When 45 mJ of power laser was allowed to fall on the surface of CSTGS crystal, a minor crack and damage appeared. The LDT (power density) value was determined using the following formula

$$P = E / \pi r^2 \tau \quad (2)$$

where E is the energy in mJ, τ is the pulse width (10 ns) in ns and r is radius of the laser spot (0.12 mm). The calculated value of LDT of cesium sulphate-doped TGS crystal is 9.95 GW/cm² and this value is found to be higher than that of tartaric acid-doped TGS, ADP-doped TGS and TGS grown by SR method (Table 3) and hence cesium sulphate-doped TGS crystal is superior material in terms of laser damage [29].

Dielectric studies

Dielectric constant and dielectric loss

Dielectric constant, dielectric loss, AC conductivity and dielectric breakdown are the significant dielectric properties. Dielectric constant can be measured by determining the change in the capacitance of specially designed condenser when the dielectric is inserted between the plates of the condenser. The amount of power loss in a dielectric under the action of AC voltage applied to it is commonly known as dielectric loss. The lower the dielectric loss the more effective is a dielectric material. The dielectric is kept between the plates of the condenser and the dielectric constant can be measured by determining the change in the capacitance of specially designed condenser. There will be a temporal phase shift found to occur between the driving field and the resulting polarization and loss current component appears and it gives the dielectric loss. Atomic polarization of the lattice, orientational polarization of dipoles, electronic polarization are various polarization mechanisms in solids. By studying the dielectric properties as a function of frequency and temperature for crystalline solids, all the types of polarization can be understood [30-32]. In the present study, the capacitance and dielectric loss measurements were carried out using an Agilent 4284A LCR meter at different frequencies ranging from 10² to 10⁶ Hz. For good electrical contact, opposite faces of the sample crystal were coated with good quality silver paint. The dielectric constant (ϵ_r) of CSTGS crystal was estimated using the formula $\epsilon_r = C / C_0$ where the capacitance of the solid is C and the capacitance of the air medium is C₀. The dielectric loss of the sample has been measured directly from the LCR meter. The readings have been taken along the b-axis (spontaneous polarization axis) of the grown CSTGS crystal.

The variations of dielectric constant and dielectric loss of CSTGS crystal at room temperature (30 °C) in the frequency range 10² to 10⁶ Hz are presented in the figures 4 and 5. For comparison purpose, the data of dielectric constant and dielectric loss of undoped TGS crystal are also given in the same figures 4 and 5. It is seen that dielectric properties of CSTGS crystal are more compared to those of undoped TGS crystal and this is due to increase of space charge polarization due to doping and the low dielectric loss of the samples leads to the conclusion that they are good quality dielectric materials [33]. The dielectric constant and dielectric loss factor of CSTGS crystal were measured at different temperatures and they are presented in the figures 6 and 7. From the results it is observed that the ferroelectric transition temperature (T_c) for CSTGS crystal is at 49.8 °C and it is slightly more than that of the undoped TGS crystal (49 °C) [27]. In the figures, both dielectric constant and dielectric loss are observed to be increasing up to the Curie temperature (T_c) and then these values decrease following the Curie-Weiss law. When T < T_c, the grown crystal of CSTGS is a ferroelectric material which shows the non-centrosymmetric behaviour. When T > T_c, CSTGS is paraelectric that shows the centrosymmetric behaviour.





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AC conductivity and activation energy

AC conductivity (σ_{ac}) of the samples was determined using the relation

$$\sigma_{ac} = \omega \epsilon_0 \epsilon_r \tan \delta \quad (3)$$

where ω is the angular frequency of the AC supply, ϵ_0 is the permittivity of free space or vacuum, ϵ_r is the dielectric constant and $\tan \delta$ is the dielectric loss [34]. Frequency dependence of AC conductivity for pure and cesium sulphate-doped TGS crystals is shown in the figure 8. The result indicates that AC conductivity is more for the doped sample and it increases with increase of frequency. AC conductivity of CSTGS crystal at different temperatures was calculated using the values of dielectric constant and dielectric loss and these values are presented in figure 9. The result shows that AC conductivity (σ_{ac}) increases with increase of temperature upto the Curie temperature (T_c) and beyond T_c , it decreases like the behaviour of both dielectric constant and loss factor. Below T_c , AC conductivity of CSTGS crystal obeys the Arrhenius relation as given by

$$\sigma_{ac} = \sigma \exp (-E_{ac}/kT) \quad (4)$$

where k is the Boltzmann's constant, T is the absolute temperature, σ is the constant depending on the material and E_{ac} is the AC activation energy. By drawing a plot of $\ln \sigma_{ac}$ versus $1000/T$ for CSTGS crystal below T_c , the value of AC activation energy was determined. The plots of σ_{ac} versus $1000/T$ for CSTGS crystal at frequencies 10^3 Hz and 10^5 Hz are drawn and they are depicted in the figures 10 and 11. From the results, the activation energy values of CSTGS crystal obtained are 0.952 eV and 1.117 eV respectively at 10^3 Hz and 10^5 Hz. It is observed that the activation energy in the ferroelectric region for the sample is high and it is found to be more when the frequency increases.

3.5.3 Relaxation time and relaxation frequency

For ferroelectric materials, Debye's equation for dielectric constant is given by

$$\epsilon_r (T) = n^2 + (\epsilon_r (\max) - n^2) / (1 + \omega^2 \tau^2) \quad (5)$$

Where $\epsilon_r (T)$ is the temperature dependent dielectric constant, n is the linear refractive index, $\epsilon_r (\max)$ is the maximum dielectric constant at Curie temperature, ω is the angular frequency and τ is Debye's relaxation time [35, 36]. The equation (9) can be written as

$$\epsilon_r (T) - n^2 = (\epsilon_r (\max) - n^2) / (1 + \omega^2 \tau^2) \quad (6)$$

Since n^2 is very small compared to the dielectric constant for ferroelectric crystal, the above equation is written as

$$\epsilon_r (T) = \epsilon_r (\max) / (1 + \omega^2 \tau^2) \quad (7)$$

The relation (13) can be written as

$$\tau = (1/\omega) [(\epsilon_r (\max) / \epsilon_r (T)) - 1]^{1/2} \quad (8)$$

Using the above equation, we can calculate the relaxation time at different temperatures and at different frequencies. The plots of relaxation time versus temperature for CSTGS crystal at frequency of 10^3 Hz and 10^5 Hz are shown in the figures 12 and 13. It can be seen that at $T = T_c$, the relaxation time is equal to zero and this indicates the maximum alignment of ferroelectric domains at Curie temperature. Since relaxation frequency is equal to the reciprocal of relaxation time, the value of relaxation time is less at 10^5 Hz than that at 10^3 Hz. It is observed that the ferroelectric domains will take in the order of microseconds to align themselves in the direction of applied AC field [37].

Antimicrobial activity

The bacteria such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus epidermis* were used for antimicrobial activity studies. The bacterial strains were sub-cultured in nutrient agar medium and





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the bacterial culture was incubated for a day at 37 °C. Antibiotics like Chloramphenicol (standard sample) was used as positive control during antimicrobial activity studies. The grown crystal of CSTGS was evaluated for antimicrobial activity against the four bacterial strains. The Petri dish and metallic borer were sterilized in an autoclave at 120°C for 15 min. The culture media was finally poured into Petri dishes under sterile condition. CSTGS sample was dissolved in DMSO to achieve a final concentration of 20 mg/ml. 20 µl sample was loaded on the dish and activity was analyzed. Formation of zone of inhibition by CSTGS sample and standard sample on the bacterial strains in the Petri dishes is shown in the figure 14. The diameter of the zone of inhibition was measured and the results are given in the table 4. Since zone of inhibition formed by CSTGS sample is more compared to zone of inhibition formed by the standard sample, the grown crystalline sample of CSTGS has high antibacterial activity against the microbes like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus epidermidis* [38].

CONCLUSIONS

2 mole% of cesium sulphate was added into the aqueous solution prepared by glycine and sulphuric acid taking in 3:1 molar ratio and slow evaporation method was adapted to grow the single crystals of cesium sulphate-doped triglycine sulphate (CSTGS). XRD method reveals that CSTGS crystal crystallizes in monoclinic structure. EDS spectral study of the sample reveals that it has the elements like C, N, O, S and Cs and hence the dopant is in the form of Cs^{2+} and SO_4^{2-} in the interstitial positions of CSTGS crystal. LDT value of CSTGS crystal is found to be 9.95 GW/cm² and this value is observed to be more than that of other doped TGS crystals. The relative SHG efficiency of CSTGS crystal is obtained to be 0.22 times that of the KDP sample. The dielectric constant, dielectric loss, AC conductivity, activation energy and relaxation time of CSTGS crystal have been evaluated at different frequencies and temperatures. CSTGS crystal has been tested against the bacterial strains like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Streptococcus epidermidis* and it is observed that the grown crystal has high antimicrobial activity.

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Table 1: Lattice parameters of undoped and cesium sulphate-doped TGS crystal

| Sample | Cell parameters | Volume (Å) ³ |
|---|--|-------------------------|
| Undoped TGS crystal | a= 9.117Å b= 12.983Å , c= 5.741Å $\alpha = 90^\circ, \beta= 105.33^\circ, \gamma= 90^\circ$ | 655.35 |
| TGS crystal doped with 2 mole% of cesium sulphate | a = 9.214(3)Å , b= 12.975 (2) Å , c= 5.752(3) Å $\alpha = 90^\circ, \beta= 106.05 (3), \gamma= 90^\circ$ | 669.85(4) |

Table 2: The weight percentage and atomic percentage of elements in CSTGS crystal

| Element | Line Type | Wt% | Atomic% |
|---------|-----------|-------|---------|
| C | K series | 29.73 | 36.95 |
| N | K series | 24.53 | 26.15 |
| O | K series | 38.39 | 35.82 |
| S | K series | 0.73 | 0.34 |
| Cs | L series | 6.63 | 0.74 |

Table 3: Values of LDT of some undoped and doped TGS crystals

| Sample | LDT value (GW/cm ²) |
|---|---------------------------------|
| Undoped TGS crystal | 8.64 |
| TGS grown in SR method | 8.92 |
| Tartaric acid- doped TGS crystal | 9.74 |
| ADP-doped TGS | 6.06 |
| Cesium sulphate-doped TGS crystal (CSTGS) | 9.95 |

Table 4: Antimicrobial activity of cesium sulphate-doped triglycine sulphate (CSTGS) against some bacterial strains

| Bacteria | Zone of inhibition (mm) | |
|----------------------|-------------------------|-----------------|
| | CSTGS crystal | Standard sample |
| <i>S. aureus</i> | 28 | 20 |
| <i>P. aeruginosa</i> | 23 | 12 |
| <i>K. pneumoniae</i> | 30 | 30 |
| <i>S. epidermis</i> | 31 | 30 |





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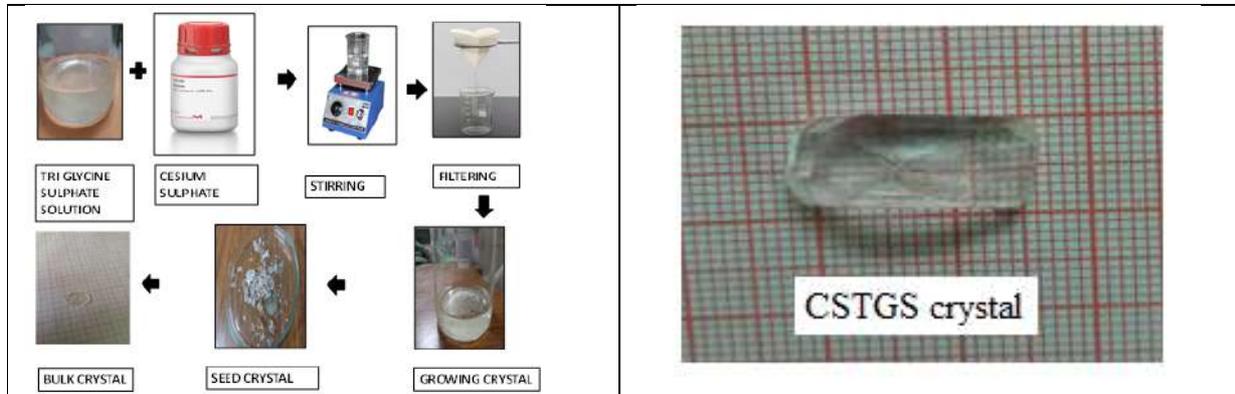


Fig.1: Schematic diagram for growing CSTGS crystal

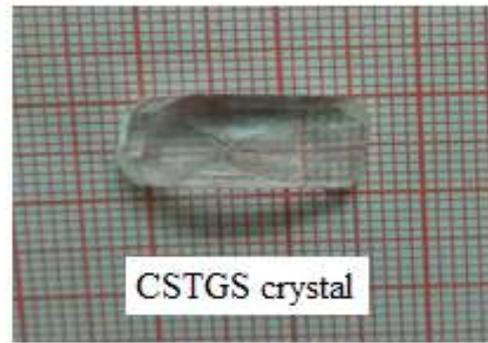


Fig.2: A good quality grown crystal of CSTGS

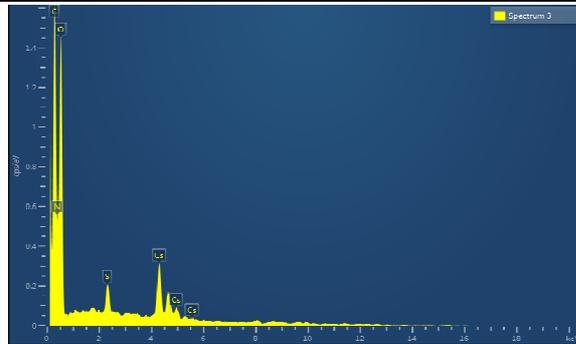


Fig.3: EDS spectrum of CSTGS crystal

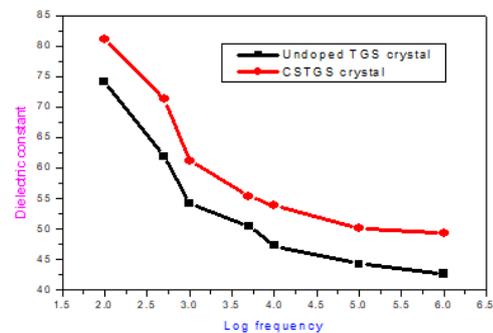


Fig.4: Variation of dielectric constant with frequency for undoped and cesium sulphate doped TGS (CSTGS) crystals

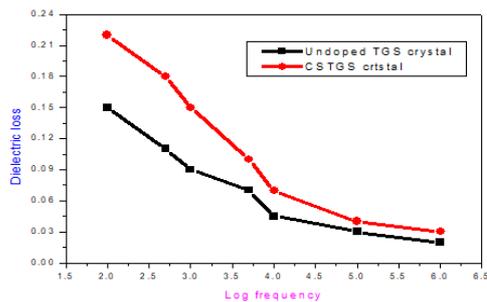


Fig.5: Variation of dielectric loss with frequency for undoped and cesium sulphate doped TGS (CSTGS) crystals

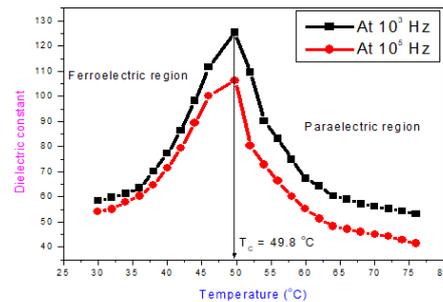


Fig.6: Temperature dependence of dielectric constant for cesium sulphate doped TGS (CSTGS) crystal at frequencies of 10³ Hz and 10⁵ Hz





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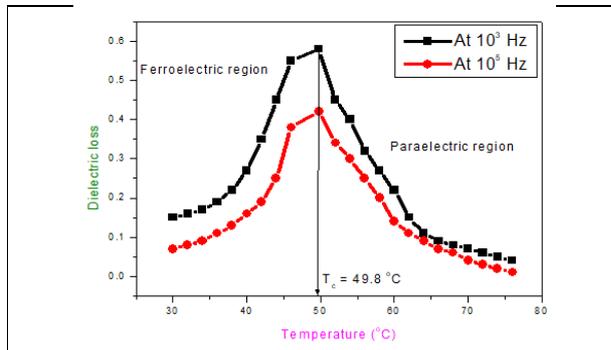


Fig.7: Temperature dependence of dielectric loss for cesium sulphate doped TGS (CSTGS) crystal at frequencies of 10^3 Hz and 10^5 Hz

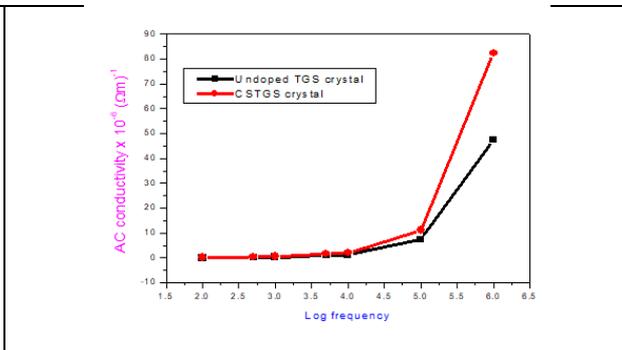


Fig.8: Frequency dependence of AC conductivity for undoped and cesium sulphate doped TGS (CSTGS) crystals

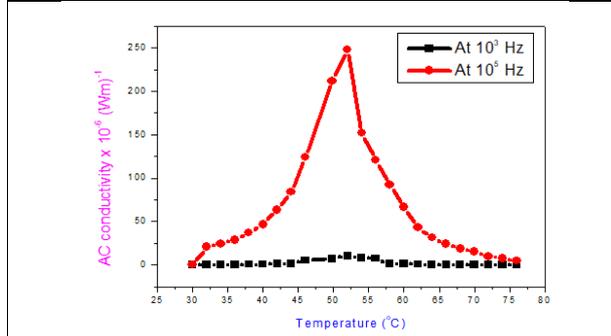


Fig.9: Temperature dependence of AC conductivity for cesium sulphate doped TGS (CSTGS) crystal at frequencies of 10^3 Hz and 10^5 Hz

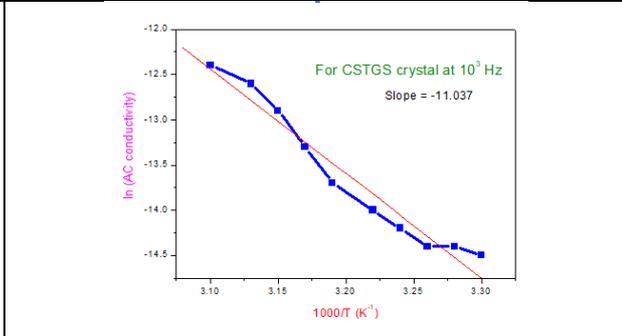


Fig.10: Plot of $\ln \sigma_{ac}$ versus $1000/T$ for cesium sulphate doped TGS (CSTGS) crystal at frequency of 10^3 Hz

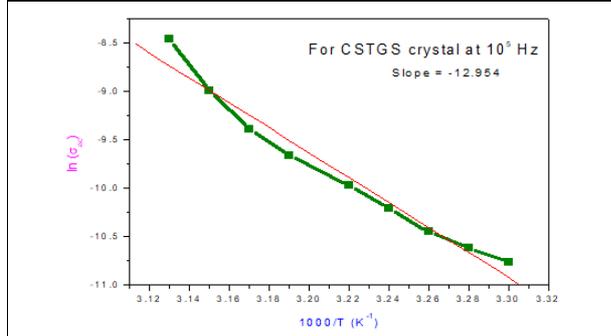


Fig.11: Plot of $\ln \sigma_{ac}$ versus $1000/T$ for cesium sulphate doped TGS (CSTGS) crystal at frequency of 10^5 Hz

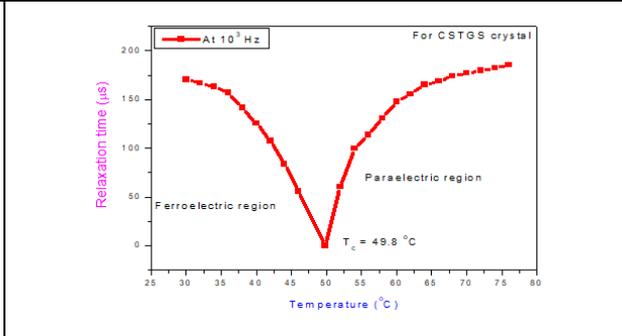
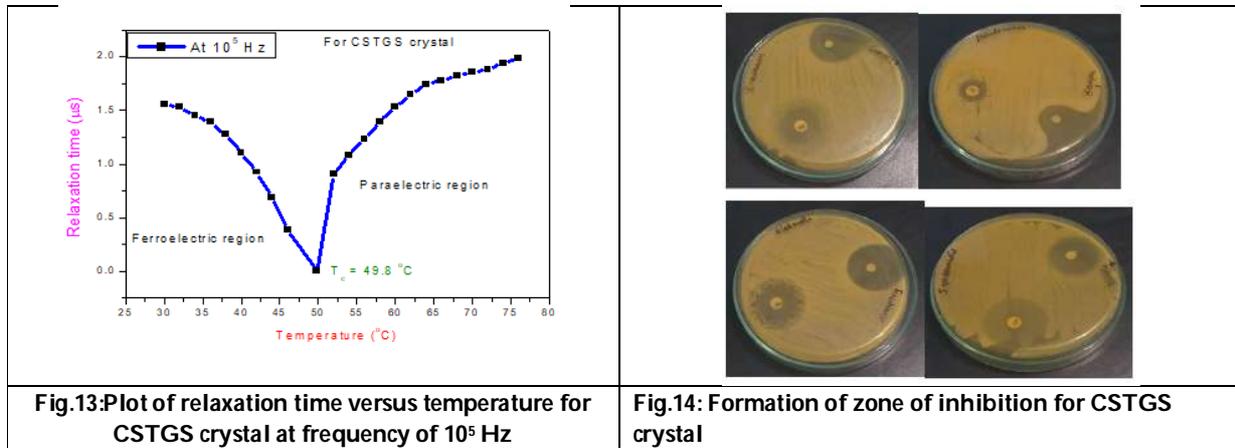


Fig.12: Plot of relaxation time versus temperature for CSTGS crystal at frequency of 10^3 Hz





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Strength and Durability Characteristics of Geopolymer Concrete Incorporated with Clay as Deleterious Material

M. Mahesh^{1*} and B. Madhusudana Reddy²

¹Lecturer, Government Polytechnic, Proddatur, YSR Kadapa, Andhra Pradesh, India.

²Associate Professor, SVU College of Engineering, Tirupati, Andhra Pradesh, India.

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*Address for Correspondence

M. Mahesh

Lecturer,

Government Polytechnic,

Proddatur, YSR Kadapa,

Andhra Pradesh, India.

E.Mail: mahi.malle304219@gmail.com



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ABSTRACT

Sustainability is increasingly a prerequisite for all civil engineering projects due to depletion of natural resources and escalating pollution levels. Several researchers have suggested a wide range of sustainable solutions for various applications in the civil engineering. Among them, geopolymer cement concrete is one of the sustainable approaches which can reduce the high amounts of CO₂ by replacing Portland cement concrete. Further, the presence of deleterious materials in conventional concrete revealed a reduction in performance of concrete structures, But, the studies related to the influence of deleterious materials on properties of geopolymer concrete are limited. So, the present study is aimed to evaluate the influence of black cotton soil as a deleterious material on mechanical behavior of geopolymer concrete. The strength of concrete is evaluated in terms of compressive strength, split tensile strength, flexural strength, shear strength and bearing strength while durability is measured by using chloride intrusion test. The results showed a contrast behavior as compared to the conventional concrete. The presence of clay in geopolymer concrete has showed an increase in strength up to 5% of clay. The same was conformed through the chloride intrusion test conducted on geopolymer concrete

Keywords: Deleterious material, clay, geopolymer concrete, strength, elevated temperature, durability.



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INTRODUCTION

Concrete is typically made of cement, water, coarse particles, and fine aggregates. The strength and durability of concrete constructions can be negatively impacted by the presence of any components, including lignite, coal, pyrites, mica, shale or similar minerals, clay, alkali, soft pieces, marine shells, and organic impurities. Often, these substances are referred to as deleterious materials. The strength and durability of concrete can be dramatically decreased if these components are present in excess of permitted levels. The permitted limits for each type of deleterious material are provided in standard codes for conventional concrete, but the corresponding limits for geopolymer concrete must be obtained through a systematical and focused research in order to prevent the performance of concrete structures from being reduced. Hence, the present article is aimed to evaluate the influence of clay as a deleterious material on strength and durability of geopolymer concrete. French scientist "Joseph Davidovits" first used the phrase "geopolymer concrete" in 1972. Since no Portland cement is utilised in the geopolymer concrete, it is often referred to as zero-cement concrete. Alkali activating solution and silica-alumina rich source materials, including flyash, ground granulated blast furnace slag (GGBS), metakaolin, etc., are used to create geopolymer concrete. In order to prepare geopolymer cement or binder, alkali activating solutions were used to activate the silica-alumina source materials [1]. The composition of geopolymer concrete is shown in figure 1.

Geopolymer mix components including the alkali solution concentration [3, 5, 9, 10, 11, 12], the ratio of silicate solution to hydroxide solution [2, 6, 7, 8], and the ratio of solids to activating solution [4] all have a significant impact on the strength of the mixture. Due to its lower price, sodium hydroxide solution was suggested over potassium hydroxide solution for the preparation of alkali activating solutions. Few research examined the relationship between sodium hydroxide solution concentration and the strength of geopolymer concrete, and they found that increasing sodium hydroxide solution concentration increased the strength of geopolymer concrete.

MATERIALS AND METHODOLOGY

In the present study, the geopolymer binder was prepared by using alkali activating solution (NaOH solution and sodium silicate solution) and flyash. In this study, a powder to liquid ratio of 2.5 and sodium silicate to NaOH ratio of 2 was considered. 15% of geopolymer binder (by weight of mix) is used for preparation of geopolymer concrete. Two series of mixes were prepared using geopolymer binder and ordinary portland cement. Both the concrete mixes were incorporated with black cotton soil (clay) at a dosage of 0, 2.5, 5, 7.5 and 10% by weight of coarse aggregates. A set of cubic specimens, cylindrical specimens and prismatic specimens were prepared with these concrete mixes and subjected to compressive, split tensile and flexural strengths were evaluated. In addition, shear strength, bearing strength of geopolymer concrete and traditional concrete were evaluated. The influence of elevated temperature (200°C) on compressive strength of both geopolymer and traditional concrete was studied. Finally, the effect of clay on durability of concrete (in terms of compressive and flexural strength) was investigated through chloride intrusion of concrete specimen.

RESULTS AND DISCUSSIONS

Compressive strength

A set of 30 cubic specimens (150mm size) were prepared with different dosages of clay for each series of concrete mix and subjected to uniaxial compression as per IS:516-1959. The specimens were cured for a period of 28 and 180 days. The corresponding compressive strengths of geopolymer and traditional concrete specimens are compared as shown in figure 2(a) and 2(b). The results showed a maximum 28-days compressive strength of 49.1MPa and 45.6MPa for geopolymer concrete and traditional concrete respectively. The addition of clay content showed an adverse effect on compressive strength of traditional concrete while the addition of clay content up to 5% showed a positive effect on compressive strength of geopolymer concrete. But, the addition of clay beyond 5% caused a

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considerable reduction in compressive strength. The addition of 2.5% clay reduced the compressive strength (28-days) of traditional concrete by 8% while a reduction of 33% was observed with an addition of 10% clay. The addition of 2.5% and 5% clay increased 28-day compressive strength by 3.4% and 11.1% respectively. The aluminosilicate compounds in clay up to 5% played a key role and reacted with alkali activated solution and beyond this dosage the clay acted as a foreign material and become detrimental to the geopolymer concrete. Due to this reason, the strength was increased up to 5% of clay and then started declining. But, this has to be confirmed by the microstructural studies using scanning electron microscopy, X-ray diffraction. Similarly, the same trend was observed in case of 180-days compressive strength.

Split tensile strength

A set of 30 cylindrical specimens (150mm diameter and 300mm height) were prepared with different dosages of clay for each series of concrete mix and subjected to split tensile load as per IS:5816-1999. The specimens were cured for a period of 28 and 180 days. The corresponding split tensile strengths of geopolymer and traditional concrete specimens are compared as shown in figure 3(a) and 3(b). The results showed a maximum 28-days split tensile strength of 4.72MPa and 4.02MPa for geopolymer concrete and traditional concrete respectively. The addition of clay content showed an adverse effect on tensile strength of traditional concrete as similar to compressive strength of traditional concrete. In case of geopolymer concrete, the addition of clay content up to 5% showed a positive effect on tensile strength of geopolymer concrete.

Flexural strength

A set of 30 prismatic specimens (150mm x 150mm x 700mm) were prepared with different dosages of clay for each series of concrete mix and subjected to 4 point bending as per IS:516-1959. The specimens were cured for a period of 28 and 180 days. The corresponding flexural strengths of geopolymer and traditional concrete specimens are compared as shown in figure 4(a) and 4(b). The results showed a maximum 28-days flexural strength of 5.41MPa and 4.67MPa for geopolymer concrete and traditional concrete respectively. The addition of clay content showed an adverse effect on flexural strength of traditional concrete as similar to compressive strength of traditional concrete. In case of geopolymer concrete, the addition of clay content up to 5% showed a positive effect on tensile strength of geopolymer concrete by increasing 10.6%. The addition of 10% clay caused a reduction of 20.4% and 17.1% for geopolymer and traditional concrete respectively.

Shear strength and bearing strength

A set of 15 concrete specimens were prepared for each series of concrete mix at different dosage of clay as specified. The specimens were cured for a period of 28 days and then subjected to shear loading. Similarly, a set of 15 concrete specimens for each series of mix were subjected to bearing strength test. The corresponding results are presented figure 5 and 6 for shear and bearing strengths respectively. The results showed a maximum 28-days shear strength of 3.67MPa and 3.38MPa for geopolymer concrete and traditional concrete respectively. The addition of clay content showed a considerable decrease in shear strength of traditional. In case of geopolymer concrete, the addition of clay content up to 5% showed a positive effect on tensile strength of geopolymer concrete by increasing 6.4%. The addition of 10% clay caused a reduction of 16.2% and 14.8% for geopolymer and traditional concrete respectively. Similarly, the addition of clay (10%) reduced the bearing strength by 22.1% and 5.8% for traditional concrete and geopolymer concrete respectively.

Effect of elevated temperature

A set of 30 cubic specimens (150mm) were prepared for each series of concrete mix at different dosage of clay as specified. The specimens were cured for a period of 28 days and then subjected to uniaxial compression at an elevated temperature of 200°C. The results showed the same trend for both traditional and geopolymer concrete as described in the compressive strength of these two mixes (figure 7). The elevated temperature reduced the compressive strengths in the range of 17.7-21.4% and 18.2-23.5% for geopolymer concrete and traditional concrete respectively.



**Mahesh and Madhusudana Reddy****Durability characterization**

In this study, the durability of traditional and geopolymer concrete was evaluated in terms of reduction in strength (compressive and flexural) of concrete subjected to chloride intrusion. The results showed same trend of strength with addition of clay for geopolymer concrete and traditional concrete as described in the cases without chloride intrusion. The results are presented in figure 8 and 9 for compressive and flexural strengths respectively. The results showed a reduction of compressive strength in the range of 11.1-15.5% and 12.9-16.0% for geopolymer concrete and traditional concrete respectively. In case of flexural strength, a range of 14.7-18.3% and 14.1-21.2% for geopolymer concrete and traditional concrete respectively.

CONCLUSIONS

- The addition of clay content showed an adverse effect on the strength of traditional concrete while the addition of clay content up to 5% showed a positive effect on the strength of geopolymer concrete. But, the addition of clay beyond 5% caused a considerable reduction in the strength.
- The elevated temperature reduced the compressive strengths in the range of 17.7-21.4% and 18.2-23.5% for geopolymer concrete and traditional concrete respectively.
- The durability studies revealed a reduction of compressive strength in the range of 11.1-15.5% and 12.9-16.0% for geopolymer concrete and traditional concrete respectively. In case of flexural strength, a range of 14.7-18.3% and 14.1-21.2% for geopolymer concrete and traditional concrete respectively.
- Finally, a maximum of 5% of clay can be recommended for geopolymer concrete without compromising the strength and durability of concrete.

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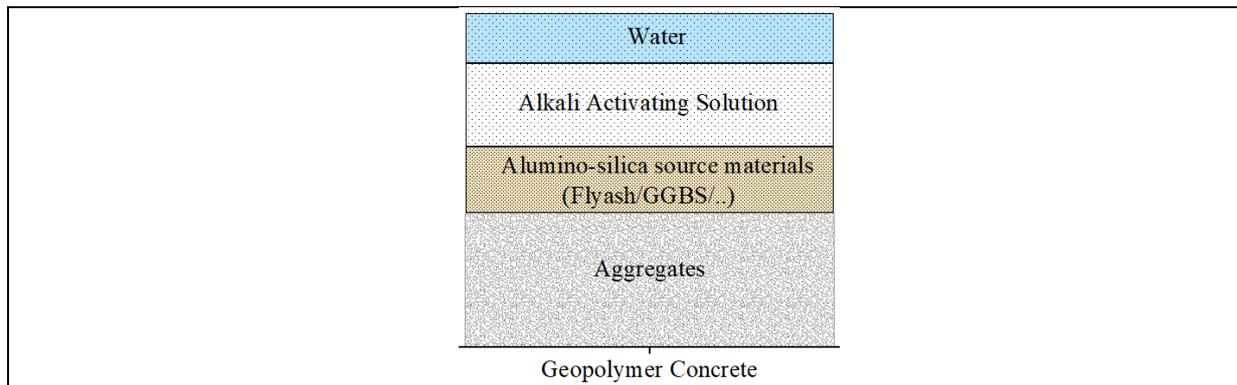


Figure 1. The composition of geopolymer concrete

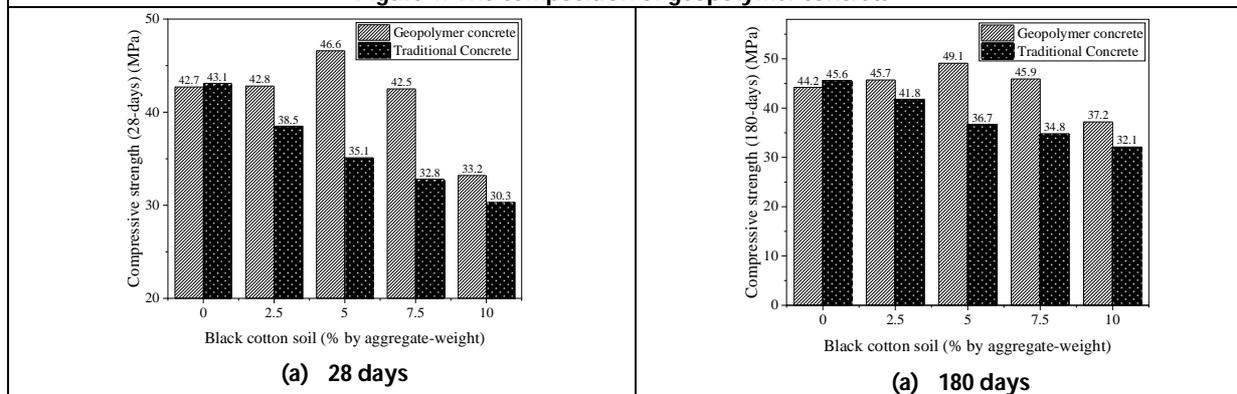


Figure 2. The effect of clay-content on compressive strength

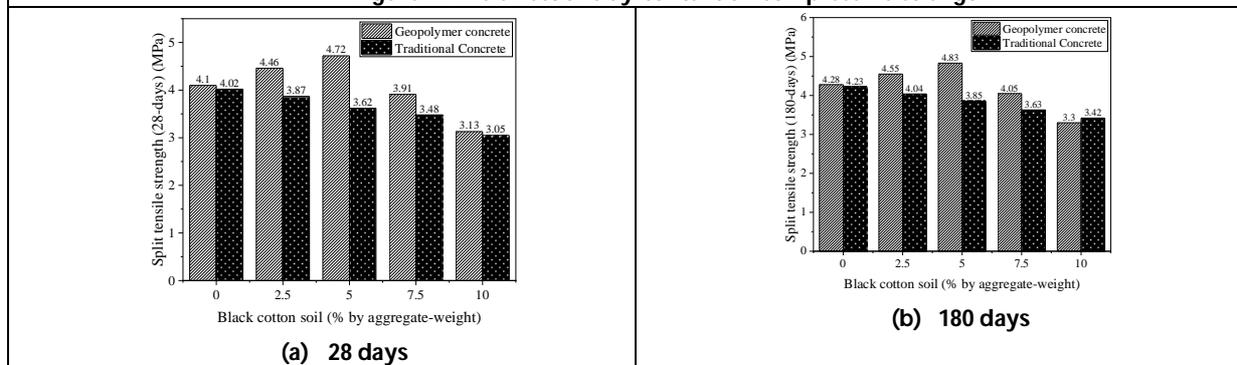


Figure 3. The effect of clay-content on split tensile strength





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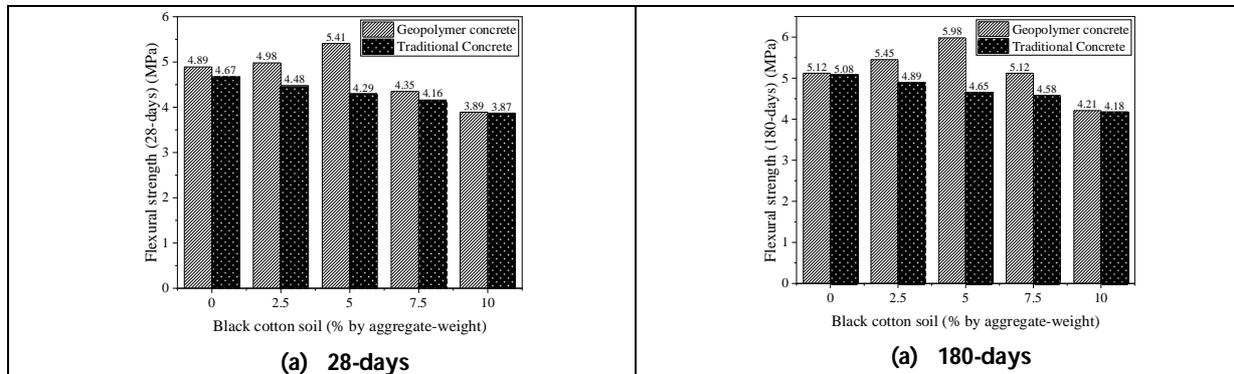


Figure 4. The effect of clay-content on flexural strength

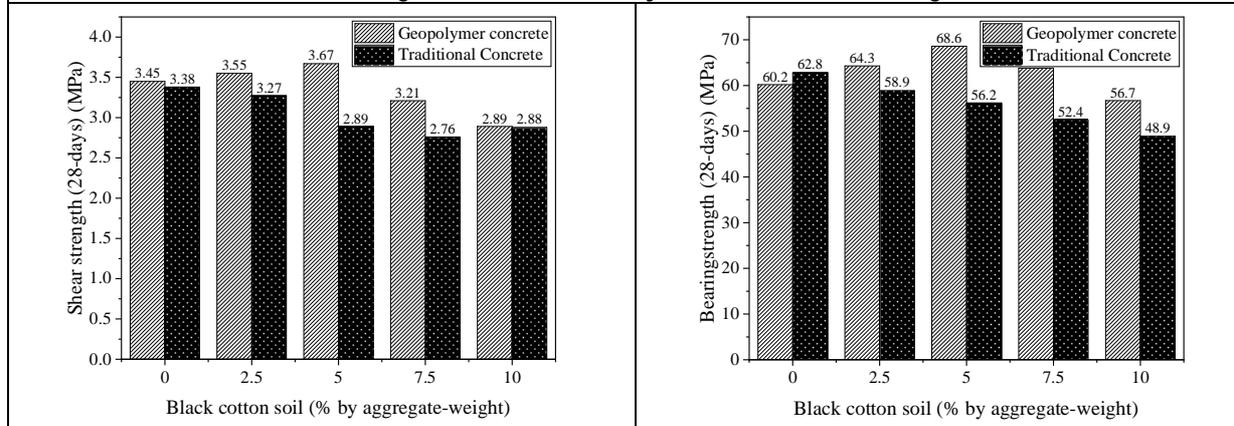


Figure 5. The effect of clay-content on shear strength

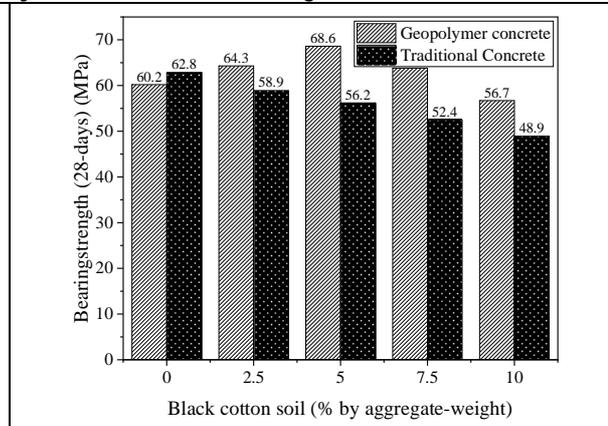


Figure 6. The effect of clay-content on bearing strength

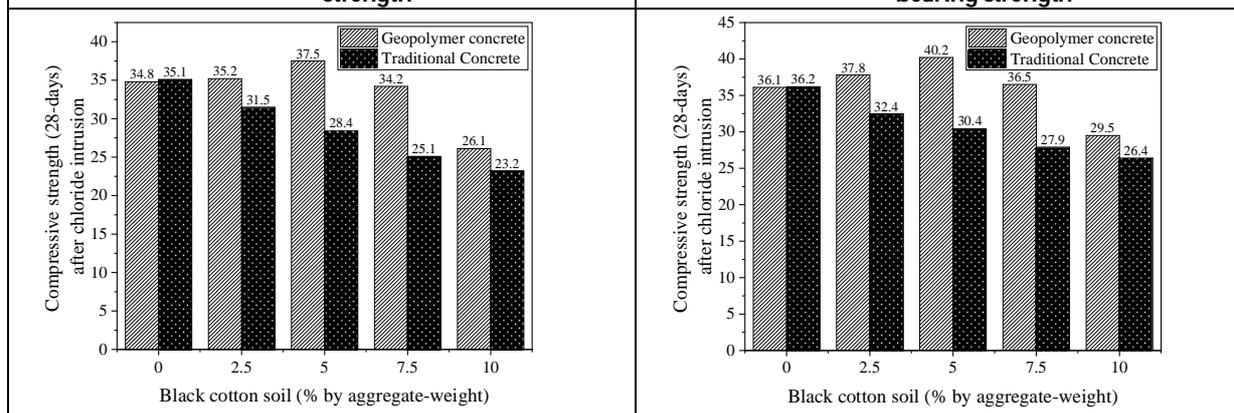


Figure 7. The effect of clay-content on compressive strength at 200°C

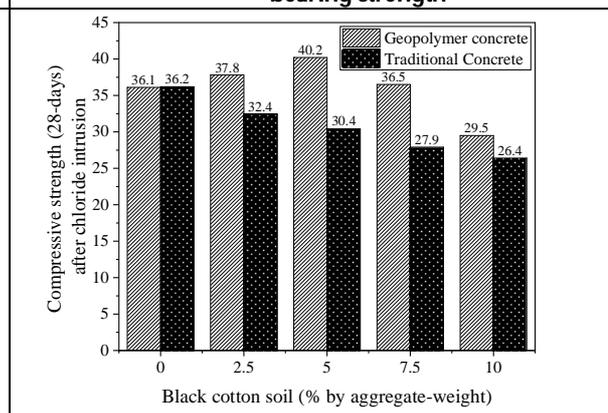


Figure 8. The effect of clay-content on compressive strength after chloride intrusion





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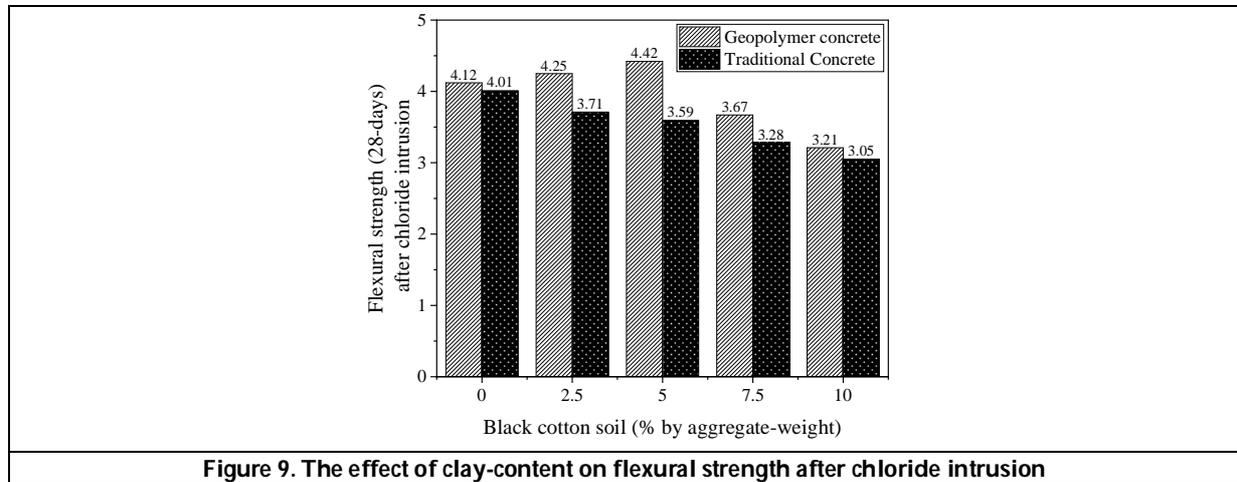


Figure 9. The effect of clay-content on flexural strength after chloride intrusion





Assessing the Relation between Random Blood Glucose Level and Oral Hygiene Status - A Survey

Pratiksha Surana^{1*}, Maya Mhaske², Gargi Deshmukh³, Kalyani Reddy¹ and Nisha Salvi¹

¹PG Student, Department of Periodontology, C.S.M.S.S Dental College and Hospital, Chhatrapati Sambhaji Nagar, Maharashtra, India.

²Professor and HoD, Department of Periodontology, C.S.M.S.S Dental College and Hospital, Chhatrapati Sambhaji Nagar, Maharashtra, India.

³Intern, C.S.M.S.S Dental College and Hospital, Chhatrapati Sambhaji Nagar, Maharashtra, India.

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*Address for Correspondence

Pratiksha Surana

PG Student,
Department of Periodontology,
C.S.M.S.S Dental College and Hospital,
Chhatrapati Sambhaji Nagar,
Maharashtra, India.
E.Mail: pratiksha.k.surana@gmail.com



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ABSTRACT

Periodontal diseases are a group of chronic, progressive bacterial infections resulting in inflammation and destruction of tooth-supporting tissues. The study assesses and evaluates the association between random blood glucose levels and periodontal health. A randomized sample of 100 patients in the age group of 30-80 years was examined for the study by the Department of Periodontology. The periodontal status of the subjects was assessed by missing teeth, tooth mobility, gingival recession, periodontal pockets, suppuration, and furcation. A blood sugar level assessment was done using a glucometer and strips. OHI-S and gingival index were taken. This study shows a significant association between periodontitis and elevated blood glucose level. This study concluded that every patient coming for dental treatment (either surgical or nonsurgical) should undergo systemic investigations like blood sugar level as even in this study we found 2 patients unaware of diabetes, to avoid post-operative complications.

Keywords: Periodontitis, Diabetes, Advanced glycation end products.





INTRODUCTION

Periodontal disease is one of the most common inflammatory diseases in adults [1]. Chronic inflammation of periodontitis aggravates glycaemic control in type 2 diabetic patients through the aggravation of insulin resistance. Increased or decreased release of various inflammatory mediators, such as high sensitivity C reactive protein (hs-CRP), tumor necrosis factor (TNF), interleukin (IL)-6, and adipokines, such as adiponectin, leptin, and resistin, are presumed to be responsible for developing and progressing insulin resistance [2]. Glycaemic control appears to be crucial in the association between diabetes and periodontitis; poorly controlled diabetes has been shown to be associated with greater severity and extent of periodontitis [3]. Diabetes and periodontitis are complex chronic diseases with an established bidirectional relationship. There has long been evidence that hyperglycemia in diabetes is associated with adverse periodontal outcomes. However, given the ubiquity of periodontal diseases and the emerging global diabetes epidemic, the complications of which contribute to significant morbidity and premature mortality, it is timely to review the role of periodontitis in diabetes [4].

Alveolar bone loss is one of the main outcomes of periodontitis, and diabetes is among the primary risk factors for periodontal disease [5]. The association of periodontal disease and diabetes has been termed a “two-way relationship,” with diabetes increasing the risk for periodontal disease and periodontal disease adversely affecting glycaemic control and increasing the severity of complications of diabetes [6]. [Figures 1 and 2] Diabetes increases the RANKL/OPG ratios and enhances the expression level of AGEs, ROS, and inflammatory mediators, which induce osteoblast apoptosis and osteoclastogenesis. This cascade of events contributes to increased bone resorption and reduced reparative bone formation, leading to greater alveolar bone loss in periodontal disease caused by bacterial pathogens. AGE, advanced glycation end product; IL, interleukin; OPG, osteoprotegerin; PDL, periodontal ligament; RANKL, receptor activator of nuclear factor kappa-B ligand; ROS, reactive oxygen species; TNF, tumor necrosis factor [6].

MATERIALS AND METHODS

A randomized sample of 100 patients in the age group of 30-80 years was examined for the study by the Department of Periodontology. Ethical clearance was obtained from the Institutional Ethics Committee.

Exclusion criteria

Patients who had retained roots, third molars, and implants were excluded from the study.

Method

The periodontal status of the subjects was assessed by missing teeth, bleeding on probing, tooth mobility, gingival recession, probing pocket depth (PPD), clinical attachment loss (CAL), suppuration, and furcation.

Periodontal pocket depth was calculated as the distance from the free gingival margin to the bottom of the pocket. Gingival Recession was calculated as the distance from the CEJ to the free gingival margin, and clinical attachment loss (CAL) was calculated as the algebraic sum of recession and PPD measurements. [Figure 3]

A blood sugar level assessment was done using anAccu-Chek Instant S Blood Glucose Monitor and strips. [Figure 4] Indices:

- OHI-S using mouth mirror and No.23 explorer was recorded.
- Gingival index using mouth mirror and the periodontal probe was taken.

Statistics

The data was serialized with all personal information removed and coded so that only the examiner was able to identify the subject. The statistical analysis was performed using IBMSPSS-20 Statistical Package for the Social Sciences (SPSS) software.





RESULTS

From Figure 5 it can be seen that adults ≥ 30 years of age with diabetes suffer from more moderate or severe periodontitis as compared with those that are pre-diabetic and those with normal glucose levels at all age levels. The prevalence percentage at the age group 30-44 for diabetic individuals is about 55%; for prediabetic individuals is 25%; for persons with normal glucose levels is 15%. As age progresses, the prevalence of periodontitis increases in all including diabetic, prediabetic, and persons with normal glucose levels. Error bars represent 95% confidence intervals. As the P value is less than 0.05 there is a significant association between the prevalence of periodontitis and elevated blood glucose level.

DISCUSSION

Periodontitis is very common, with its severe form that threatens tooth retention affecting 10–15% of adults in most populations studied. [7, 8, 9] Moderate periodontitis is even more common, affecting 40–60% of adults. Therefore, periodontitis is a highly prevalent, largely hidden, chronic inflammatory disease. People with diabetes require special attention when it comes to their dental care. This includes post-treatment management and the planning of their treatment. The level of hyperglycemia over time, irrespective of the type of diabetes, is of importance when it comes to the magnitude of its effect on the course of periodontitis.¹⁰ Having the proper coordination with their doctor is very important to ensure optimal glycemic control. For people with diabetes, regular assessments and preventive measures are important to prevent them from developing periodontal disease. If they have this metabolic condition, they should be treated successfully and should have a substantial reduction in periodontal pockets. In addition, they should establish glycemic control and manage other complications in coordination with the patient's physician. Good glycemic control and modification of common risk factors, such as smoking and obesity, as well as complete periodontal therapy and regular periodontal maintenance will help the patient achieve a lifetime of good oral as well as general health [5].

Besides these, regular maintenance and comprehensive periodontal therapy can help prevent the development of periodontal disease. Diabetes and periodontitis are of great interest to physicians and dentists because both these conditions are prevalent with more than 4 in 10 dentate adults suffering from periodontitis and about 1 in 10 adults having diabetes and more importantly. After all, these two diseases are associated. This can have implications for the treatment plan and the expected outcomes thereof. Importantly, the dental team may be the first to suspect undiagnosed diabetes based on the oral health status, first and foremost related to the periodontium.⁴

CONCLUSION

It has long been known that advanced glycated end products produced in diabetic patients are responsible for periodontal destruction and numerous other degenerative changes. Diabetic patients experience delayed wound complications, especially if the blood glucose levels are not controlled. Hence, it is critical to achieving good glycemic control before carrying out surgical procedures or dental implant placement in patients with diabetes. This study concluded that every patient coming for dental treatment (either surgical or nonsurgical) should undergo systemic investigations like blood sugar level as even in this study we found 2 patients unaware of diabetes, to avoid post-operative complications.

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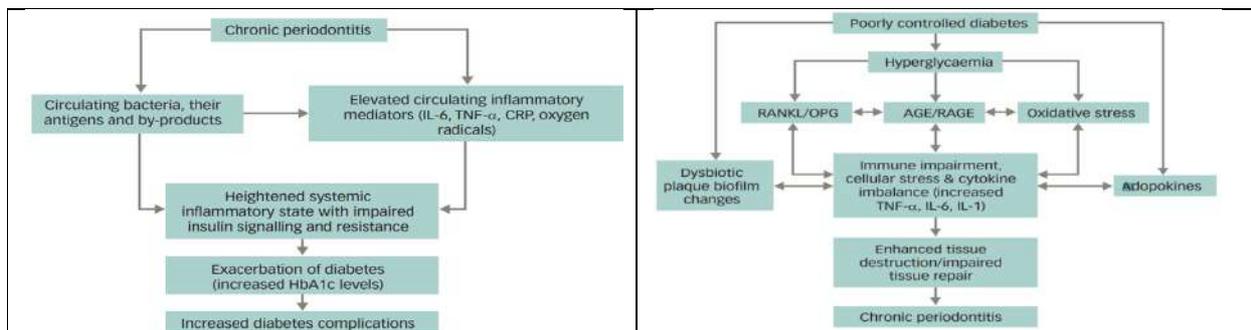
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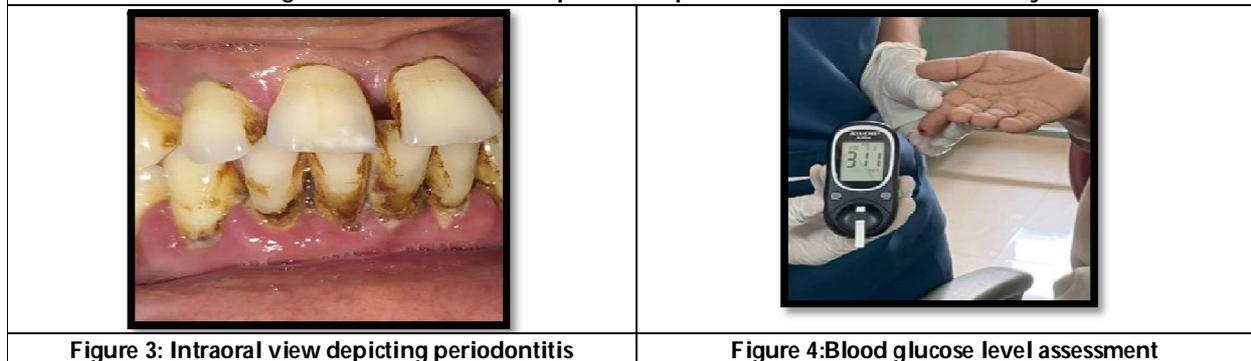


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Figures 1 and 2: Relationship between periodontitis and diabetes – 2-way link





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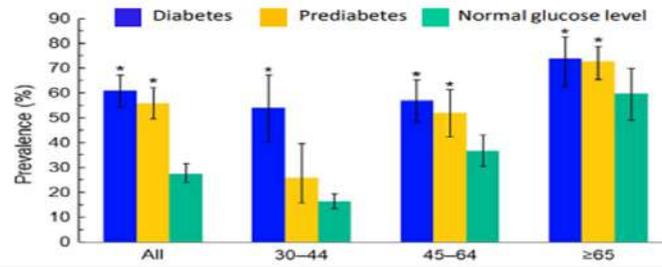


Figure 5: Prevalence of moderate/severe periodontitis among dentate adults aged > 30 years by diabetes status & age group.





Photocatalytic Degradation of TiO₂ Nanoparticles

V. Annapushpam Sangeetha^{1*}, S. Kanagaprabha², A. Mary George Shemona³ and P. N. Selvakumar⁴

¹Research Scholar of Physics, Reg. No. 20212102132010, Pope's College (Autonomous), Sawyerpuram, Thoothukudi - 628251, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli,) Tamil Nadu, India.

²Assistant Professor, Department of Physics, Kamaraj College, Thoothukudi-628003, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli,) Tamil Nadu, India.

³Research Scholar of Physics, Reg. No. 20212152132004, Pope's College (Autonomous), Sawyerpuram, Thoothukudi - 628251, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli,) Tamil Nadu, India.

⁴Assistant Professor, PG and Research Department of Physics, Pope's College (Autonomous), Sawyerpuram, Thoothukudi - 628251, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli,) Tamil Nadu, India.

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*Address for Correspondence

V. Annapushpam Sangeetha

Research Scholar of Physics,
Reg. No. 20212102132010, Pope's College (Autonomous),
Sawyerpuram, Thoothukudi - 628251,
(Affiliated to Manonmaniam Sundaranar University, Tirunelveli,) Tamil Nadu, India.
E.Mail: annapushpam98@gmail.com



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ABSTRACT

The photocatalytic activity of synthesised TiO₂ nanoparticles was examined for Methylene Blue (MB) and Congo Red (CR) dyes under visible light irradiation. The photo-removal efficiency percentages of Methylene Blue and Congo red dyes are calculated. The photocatalytic activity of the synthesised TiO₂ nanoparticles shows enhanced degradation percentages of methylene blue (82%) and congo red (85%) at 20 mg concentrations.

Keywords: Titanium dioxide, nanoparticles, Photocatalyst, Methylene Blue, Congo Red.



**Annapushpam Sangeetha et al.,**

INTRODUCTION

Nanotechnology has enormous possibilities for improving wastewater and water treatment performance while also increasing water supply through the secure use of rare water sources [1]. One of the biggest problems facing the world today is water contamination. In order to use water as drinking water, scientists from all around the world have been working to remove contaminants from it. One of the pollutants that contaminate drinking water is organic dye. The majorities of dyes is hazardous and carcinogenic and are produced by the painting, leather, paper, textile, rubber, garment, fibre, cosmetic, and plastic industries [2-7]. In recent times, the modernization and industrialization of the world have caused so many environmental problems. However, both issues have been responsible for water contamination due to their carcinogenic behavior. Untreated wastewater including organic dyes and pesticides produced by industrial activities is the main cause of water pollution. Apart from that, some dyes and insecticides have toxic properties that affect a variety of human organs [8]. The industries effluents contain molecules of aromatic inert, non-biodegradable, carcinogenic, and mutagenic dyes. By reducing the ability of water to hold oxygen and obstructing sunlight from penetrating through the outer surface of the aqueous phase, the release of these wastes into the environment without the purification may result in water and soil pollution, which will harm the food web in the aquatic system. It also leads to instability in the environment [9, 10]. The removal of dyes from water can often be done using various types of conventional techniques, including reverse osmosis, chlorination, precipitation, filtration, adsorption, ion exchange, biological treatments such as electrochemical approaches, and anaerobic digestion. The highly effective photo degradation process is frequently used for environmental remediation purposes to solve these issues. Because of its easy to use, low cost, environmentally friendly, and generation of non hazardous degraded products [11]. In recent years, the green approach has been used to reduce the use of toxic chemicals because it is nontoxic to the environment, cost-effective and simple, etc [12].

The most significant synthetic dyes are azo dyes, which are commonly used in the production of textiles, paper, cosmetics and photographic industries etc. These dyes can be produced using diazotization and Coupling method. These can be identified by the functional group (-N = N-) interconnecting two symmetrical and/or asymmetrical identical or non-azo alkyl or aryl radicals [13-15]. Methylene blue (MB) and Congo red (CR) are cationic and anionic dyes [16]. One of the most significant pollutants is methylene blue (MB), also known as methyl thioninium chloride. It is a synthetic chemical product having a pungent structure. It is poisonous to all living things and can cause cancer. It affects the respiratory system and irritates the skin and eyes. Drinking water polluted with MB dye is known to be dangerous for both people and animals and can cause subcutaneous tissue-borne sarcoma [17-19]. The secondary diazo artificial organic dye molecule Congo red (CR) is widely used in the cellulose industry, and it will cause low biological demand, high coloration and high chemical oxygen demand in the aqueous system, as well as bladder cancer in humans [20, 21].

Titanium dioxide has been extensively researched for its unique ability to photocatalytic and its uses exhibit high performance for photovoltaic applications. Due to its high activity, TiO₂ has recently generated a lot of interest in the field of environmental purification. Also, it exhibits chemical stability, low toxicity, low contaminants load and cost-effectiveness. Wastewater treatment utilizing TiO₂ photocatalytic degrades many organic contaminants effectively [22]. In this current work, the photocatalytic activity of the synthesized nanoparticles was studied. The degradation efficiency of MB and CR dye under visible light irradiation is calculated at various concentrations.

Mechanism of Photocatalytic Catalytic Degradation of the Dye

The dye is first absorbed on the surface of the catalyst, and then it is illuminated with ultraviolet light to excite valence electrons, allowing electrons to move from the valence band to the conduction band while lifting a positive hole or h⁺, inside the valence band. Adsorbed water molecules will react with the positive holes and free electrons on the surface of the photocatalyst, resulting in the positive holes reacting with water to produce •OH- radicals, and the free electrons reducing the dissolved oxygen to superoxide anion O₂•- radicals. The dye molecules are degraded by

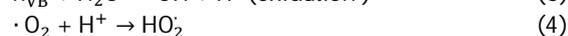
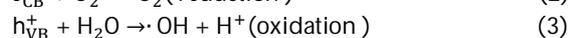
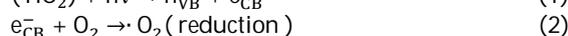




these light-generated radicals into simple molecules like CO₂ and H₂O [23]. The schematic representation of the photocatalytic activity of TiO₂ nanoparticles is shown in figure 1.

Experimental Procedure of degradation of Methylene blue (MB) and Congo red (CR) dyes

The photocatalytic activity of the TiO₂ nanoparticles was evaluated by the degradation efficiency of methylene blue/congo red (MB/CR) under visible light (Philips, 40 W). In each experiment, 10, 20 mg of the TiO₂ nanoparticles was suspended in 10 ml of the aqueous solution of MB (10 mg L⁻¹)/CR (10 mg L⁻¹), and the suspension was magnetically stirred in the dark for 30 min to establish the adsorption/desorption equilibrium of MB molecules on the surface of the catalyst. Subsequently, the mixture was transferred to a test tube and exposed to visible light. At a given interval of time (10 - 60 min), 2 ml of the suspension was taken out and the concentration of MB/CR was analyzed by measuring the absorbance at 650 nm/498 nm using a UV-vis spectrophotometer.



RESULT AND DISCUSSION

Photocatalytic degradation of TiO₂ nanoparticles

In the presence of TiO₂ nanoparticles, the photocatalytic degradation of the dyes Methylene Blue (MB) and Congo Red (CR) was investigated separately. The intensity of absorption reduced after 60 minutes of UV light irradiation due to the discoloration of both dyes. The reduction of initial absorbance at 650 nm and 498 nm for MB and CR in aqueous medium was also decreased upon the addition of NPs at 10 and 20 mg concentrations. The synthesised TiO₂ NPs were estimated with a degrading efficacy of (35% and 82%) and (46% and 85%) respectively. Thus, TiO₂ shows greater efficiency in degrading both MB and CR dyes at higher concentrations. Figure 2 represents the graphical illustration of both MB dye and CR dye degradation while being exposed to visible light under the action of TiO₂ as catalyst. For Methylene blue the absorbance is at 650 nm and for Congo red the absorbance is at 498 nm. Figure 3 illustrates that the TiO₂ nanoparticles exhibit enhanced photocatalytic activity at the 10 mg and 20 mg concentrations of catalyst. Methylene blue and congo red dyes are efficiently degraded by the catalyst at a concentration of 20 mg.

The degradation data of Methylene blue and Congo red is listed in table 1. From the degradation data, it is clear that titanium dioxide nanoparticles showed better degradation performance for both MB and CR dye at a 20 mg concentration.

CONCLUSION

TiO₂ nanoparticles were synthesised by green synthesis using leaf extract. The degradation efficiency of MB and CR dyes was observed at 10 and 20 mg concentrations (35% and 82%) and (46% and 85%) respectively. The degradation activity of the synthesised TiO₂ nanoparticles is high at a concentration of 20 mg for both MB and CR dyes. The synthesised TiO₂ nanoparticles are high-potential catalyst in wastewater removal treatment.



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Table 1. The degradation data of MB and CR dye by the catalyst

| TiO ₂ nanoparticles as catalyst | | | | | |
|--|------------|------------|----------------------------------|------------|------------|
| Degradation of Methylene blue dye | | | Degradation of Congo red dye | | |
| Minutes | 10 mg | 20 mg | Minutes | 10 mg | 20 mg |
| 10 | 0.157 | 0.178 | 10 | 0.172 | 0.192 |
| 20 | 0.141 | 0.138 | 20 | 0.142 | 0.133 |
| 30 | 0.122 | 0.114 | 30 | 0.139 | 0.108 |
| 40 | 0.116 | 0.095 | 40 | 0.13 | 0.083 |
| 50 | 0.107 | 0.04 | 50 | 0.11 | 0.051 |
| 60 | 0.101 | 0.032 | 60 | 0.092 | 0.028 |
| Percentage of degradation | 35% | 82% | Percentage of degradation | 46% | 85% |

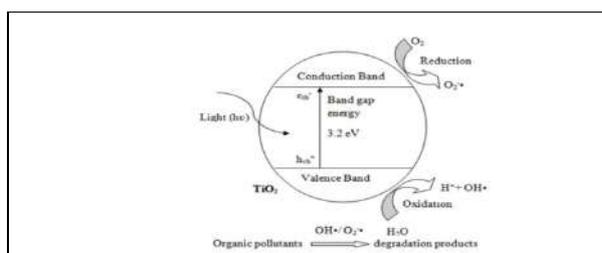


Fig 1: Schematic representation of photocatalytic activity of TiO₂ nanoparticles

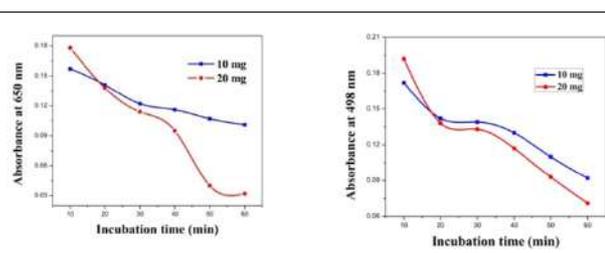


Figure 2. Visible light absorbance spectra for MB and dye

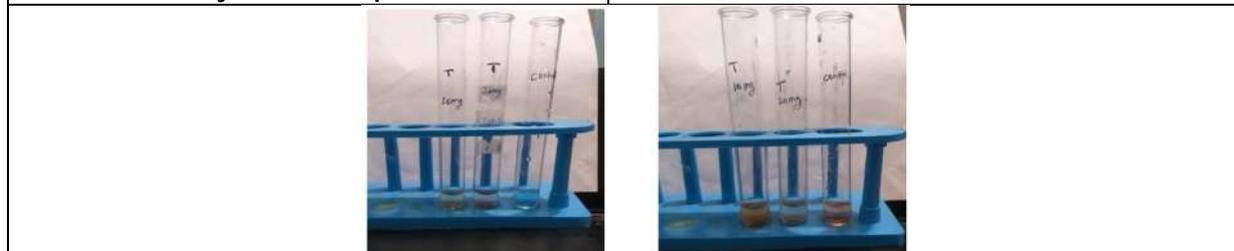


Figure 3. Degradation image of MB and CR dye





A Review on Antidiabetic Potential of Some of the Selected Legumes Consumed in India

Digbijoy Nath¹, Abdul Hadi Umam^{1*}, Manjit Mishra¹, Lakhyajit Borah¹, Himangshu deka² and Josef Yakin²

¹M.Pharm Student, Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam- 781026, India.

²Assistant Professor, Faculty of Pharmaceutical Science, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam- 781026, India

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*Address for Correspondence

Abdul Hadi Umam

¹M.Pharm Student,
Faculty of Pharmaceutical Science,
Assam down town University,
Sankar Madhab Path, Gandhi Nagar,
Panikhaiti, Guwahati, Assam- 781026, India.
E.Mail: hadiumam95@gmail.com



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ABSTRACT

This review examines the antidiabetic potential of selected legumes consumed in India. Type 2 diabetes mellitus (T2DM) is a growing health concern in India, driven by changes in lifestyle and dietary habits. Legumes, a staple in the Indian diet, have garnered attention for their potential role in preventing and managing T2DM due to their nutritional composition and health benefits. This review also focuses on several legumes commonly consumed in India, including chickpeas (*Cicer arietinum*), lentils (*Lens culinaris*), black gram (*Vigna mungo*), and kidney beans (*Phaseolus vulgaris*) etc. These legumes are rich in dietary fiber, protein, and complex carbohydrates, which contribute to their low glycemic index and ability to regulate blood glucose levels, also highlights the role of specific bioactive compounds found in legumes, such as polyphenols, flavonoids, and dietary fibers, in conferring antidiabetic effects. These compounds contribute to the modulation of glucose absorption, insulin signaling, and oxidative stress, thereby influencing glycemic control and insulin resistance. The selected legumes consumed in India demonstrate significant antidiabetic potential. Incorporating these legumes into the Indian diet may offer a natural and cost-effective approach for the prevention and management of T2DM. However, further research is needed to better understand the mechanisms of action, optimal dosages, and long-term effects of legume consumption in individuals with T2DM. Nonetheless, the existing evidence supports the

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inclusion of legumes as part of a comprehensive dietary strategy to combat the growing burden of T2DM in India.

Keywords: Antidiabetic potential, Type 2 diabetes, Lifestyle diseases, Nutritional habits, Dietary interventions, Natural remedies, Anti-diabetic qualities, Traditional dietary patterns, Cardiovascular health.

INTRODUCTION

Diabetes is a category of metabolic disorders characterized by hyperglycemia produced by insulin production, insulin action, or both. Diabetes' persistent hyperglycemia has been associated to long-term organ deterioration, malfunction, and failure, particularly in the eyes, kidneys, nerves, and heart. These vary from autoimmune death of the pancreas' beta -cells, resulting in insulin insufficiency, to insulin resistance-causing anomalies. Diabetes induces changes in glucose, lipid, and protein metabolism as a result of insulin's inefficient action on target tissues [1,4,5]. Polyuria, polydipsia, weight loss, often accompanied by polyphagia, and impaired vision are all indications of severe hyperglycemia. Retinopathy can cause vision loss; nephropathy can cause renal failure; peripheral neuropathy can cause foot ulcers, amputations, and Charcot joints; and autonomic neuropathy can cause gastrointestinal, genitourinary, and cardiovascular symptoms, as well as sexual dysfunction [1,4,5]. Most of diabetes cases fall into one of two major etiopathogenetic types (described more below). One forms of diabetes, type 1, is caused by a complete absence of insulin secretion. Serological evidence of an autoimmune pathologic process in the pancreatic islets, as well as genetic markers, may also be used to identify persons who are more likely to acquire this kind of diabetes. Type 2 diabetes is caused by a combination of insulin resistance and an inadequate compensatory insulin secretory response [1,4,5].

Type 1 diabetes

This type of diabetes, which affects only 5-10% of diabetics and previously was known as insulin-dependent diabetes, type 1 diabetes, or juvenile-onset diabetes, is caused by cellular-mediated autoimmune destruction of pancreas cells [1,4,5]. In this type of diabetes, the pace of cell breakdown varies greatly, being quick in some persons (primarily babies and children) and gradual in others (usually adults). Ketoacidosis, particularly in kids and teens, might be the first sign of the disease. Others have moderate fasting hyperglycemia, which may rapidly increase to serious hyperglycemia and/or ketoacidosis if illness or other stresses are present [1,4,5]. Autoimmune beta-cell destruction has been connected to several genetic predispositions as well as yet-unidentified environmental factors. While individuals with this type of diabetes are rarely fat, the presence of obesity does not exclude the diagnosis. Common autoimmune diseases seen in these people include Graves' disease, Hashimoto's thyroiditis, Addison's disease, vitiligo, celiac sprue, autoimmune hepatitis, myasthenia gravis, and pernicious anemia [1,4,5].

Type 2 diabetes

This form of diabetes, which involves 90-95 percent of diabetics and was formerly referred to as non-insulin dependent diabetes, type 2 diabetes, or adult-onset diabetes, is distinguished by insulin resistance and relative (rather than absolute) insulin shortage. These individuals do not need insulin treatment to survive, at least not at first and perhaps not for the rest of their lives. Although the origins of this type of diabetes are unclear, numerous alternative reasons are plausible [1,4,5]. Obesity causes insulin resistance in most of the people with this type of diabetes. Because hyperglycemia develops gradually and is often not severe enough for the patient to detect any of the conventional indications of diabetes in its early stages, this kind of diabetes frequently stays untreated for many years. Regardless, such individuals are at a higher risk of developing macrovascular and microvascular problems [1,4,5]. Patients with this type of diabetes may have insulin levels that look normal or increased; nevertheless, if their



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cell activity was normal, greater blood glucose levels in these diabetic patients would be predicted to result in even higher insulin levels [1,4,5].

Statistical data

Diabetes, a chronic metabolic noncommunicable disease (NCD), has become a worldwide pandemic. Diabetes affects around 415 million people globally, with a projected increase to 642 million by 2040 [2]. More than 95% of all persons with diabetes have type 2 diabetes mellitus (T2DM). India is one of the epicentres of the worldwide diabetes pandemic, with the second highest number of diabetics (69 million as of 2015) [2,3]. Diabetes is prevalent in Bangladesh, Pakistan, Sri Lanka, and Nepal, as well as other South Asian nations [2].

Common side effects of antidiabetic drugs

Hypoglycemia is linked with almost all anti-diabetic drugs, with the most prevalent reasons being incorrect dose, insufficient condition, pharmacological, and dietary counselling. After insulin delivery, injection site responses such as erythema, edema, and pruritus are usual. Metformin often causes diarrhea, flatulence, indigestion, malabsorption syndrome, nausea, vomiting, headache, and cobalamin insufficiency. Glimepiride and glipizide have been related to dizziness, syncope, headache, and nausea, as well as increased blood levels of liver enzymes. Pioglitazone has also been linked to edema, hypoglycemia, upper respiratory tract infection, heart failure, headache, fractures, myalgia, sinusitis, and pharyngitis. The most frequent gastrointestinal adverse effects of acarbose include diarrhea, flatulence, and stomach discomfort. Saxagliptin and sitagliptin have been related to peripheral edema, headaches, and stomach discomfort [6,7,8].

Legumes

Legumes can be used in agricultural and agroforestry systems as a source of food for people and animals, as well as a supply of wood and soil-improving components. Legumes are one of the three major flowering plant groupings and have a lengthy agricultural history. Many legume seeds are a popular dietary staple across the globe because they are rich in both oil and protein. They have more protein than any other food plant and are almost as nutritious as animal flesh. They are also known as "poor man's meat" since they are a low-cost source of high-quality protein. The presence of root nodules containing nitrogen-fixing bacteria is associated with legumes' high protein content. These Rhizobium bacteria can convert free air nitrogen into a form that plants may utilize to create protein and other nitrogen-containing molecules. Lentil farming improves soil quality by introducing nitrogen-fixing microorganisms. Therefore, farmers often blend legumes with nitrogen-depleting crops. Leguminosae (Fabaceae) is a family of legumes that includes four subfamilies: Papilionoideae, Caesalpinioideae, Mimosoideae, and Swartzioideae [9,10,11].

Cultivation of legumes

Their cultivation and growth procedures Legumes can be divided into two types: dwarf or bush variations (which do not need climbing support and develop early) and climbing kinds (which grow later and require support but have a longer bearing season) (Duffus & Slaughter 13). Because dwarf pea varieties do not bear as heavily as climbing pea types, one or two dwarf kinds should be planted every two weeks to maintain a consistent supply throughout the spring and summer (Rockwell & Peterkin 203) [20,21]. The Shakers Gardener's Manual (1996) demonstrated how to cultivate bush and climbing legume varieties: Drill the bush or dwarf bean 20 inches apart, 2 inches deep, 2 inches apart, and 6 inches apart in the row. Running versus Pole Plant beans three and a half feet apart on each side of a hill. Before planting, we usually build the poles... then dig and loosen the soil, then lay five or six beans in a circle around the pole, approximately 3 inches below, then cover with mellow earth [22]. Legumes are planted largely to improve the texture, fertility, and productivity of the soil. Another important factor is the influence of legumes on the quantitative and qualitative improvement in following crop yields; legumes enhance soil conditions for many species. Farmers believe that the biological potential of leguminous plants to fix nitrogen is also considerable. Plants consume 100% of nitrogen when it is set symbiotically, but only 50-60% of nitrogen when it is set minerally [23].



**Digbijoy Nath et al.,****PLANT PROFILE OF SOME SELECTED LEGUMES FOUND IN INDIA**Cowpea (*Vigna unguiculata*)

Synonym – Chawli

Family: Black eyed pea

Genus: Vigna

Species: *V. unguiculata***Description**

Cowpea (*Vigna unguiculata*) is regarded as "poor man's meat" because it is a rich source of protein, minerals, and vitamins¹ for the rural poor who lack access to protein from animal sources such as meat and fish. Cowpeas are a drought-tolerant food crop that thrives in a variety of climates and soil types. It is extensively produced in Africa, Latin America, Southeast Asia, and the United States. Cowpea is farmed mostly in West and Central Africa, with an annual yield of 3 million tons [24,25,26,27].

Traditional use

Cowpeas are grown largely for its edible beans, but the leaves, green seeds, and pods may also be eaten, enabling the cowpea to be used as a food source before the dried peas are harvested. Koki, also known as moin-moin, is a traditional African dish in which cowpeas are mashed into a paste and baked in banana leaves with spices. Cowpea paste is used as a supplement in baby formula while weaning newborns off milk [28,29,30].

Black gram (*Vigna mungo*)Synonym: *Azukia mungo* (L.) Masam

Family: Fabaceae

Genus: vigna

Species: *V. mungo***Description**

It is a thickly hairy annual shrub that may grow upright, suberect, or trailing. The tap root produces a branching root system with smooth, spherical nodules. The pods are cylindrical, slender, and may reach a length of six centimeters. The plant grows 30-100 cm tall, with large hairy leaves and 4-6 cm long seed pods [31].

Traditional use

Black gram is a common and readily accessible component in all Indian cooking. These beans, also known as urad dal, are high in protein and other vital elements like as calcium, potassium, niacin, and vitamin B. Its medicinal benefits are endorsed by both ayurveda and allopathic practitioners, and frequent consumption avoids a rapid surge in blood sugar levels, reduces hypertension, and protects the heart. It is a miracle drug for those suffering from joint pain and bone disorders.

Chick pea (*Cicer arietinum*)

Synonym: kabuli

Family: chickpea

Genus: Cicers

Species: *Cicer arietinum* L**Description**

It is the most important pulse in India and is often known as chickpea or Bengal gramme. There are two kinds of these: kabuli and desi.



**Digbijoy Nath et al.,****Traditional use**

India produces over 75% of the world's total chickpea crop. Traditional processing methods such as soaking, sprouting, fermenting, boiling, roasting, parching, frying, and steaming are used to prepare the crop for human consumption (Singh, 1985) [32].

Lentils (*Lens culinaris*)

Synonym: Masur

Family: Fabaceae

Genus: *Lens*

Species: *L. culinaris*

Description

The lentil (*Lens culinaris* or *Lens esculenta*) is a species of edible legume. It is an annual lens-shaped plant with lens-shaped seeds. It is around 40 cm (16 in) tall and produces seeds in pods with two seeds each. Canada and India produce most of the world's food crops, accounting for 58% of total output [33].

Traditional use

Lentils are a high-nutrient legume. It is planted for its seed, which is often used to make dhal. Dhal is a split and decorticated seed. The main product is the seed, which contains more protein, glucose, and calories than other legumes and is the most sought-after crop in many lentil-producing areas owing to its high average protein content and rapid cooking ability. It may be served as a main entrée, a side dish, or as a salad ingredient. Seeds may be fried and seasoned for eating; flour can be used with cereals to produce soups, stews, purees, bread, and cakes, and it can also be used as a baby food [34].

Soy bean (*Glycine max*)

Synonym: *G. soja*

Family: Fabaceae

Genus: *Glycine*

Species: *G. max*

Description

The soybean is the most significant bean in the world commercially, giving vegetable protein to millions of people as well as components for hundreds of chemical goods. The soybean is an upright branching plant that may grow to be more than 2 meters (6.5 feet) tall. The self-fertilizing blossoms are white or purple in color. Seeds may be yellow, green, brown, black, or bicolored, however most commercial cultivars have brown or tan seeds [35,36].

Traditional use

Soy milk, from which tofu and tofu skin are formed, is a traditional unfermented dietary usage of soybeans. Soy sauce, fermented bean paste, natto, and tempeh are all examples of fermented soy foods. Fat-free (defatted) soybean meal is an important and low-cost protein source for animal feeds and many packaged meals [36].

Pigeon pea (*Cajanus cajan*)

Synonym: *Cajanus bicolor* DC

Family: Fabaceae

Genus: *cajanuses*

Species: *Cajanus cajan* (L.) Huth



**Digbijoy Nath et al.,****Description**

Pigeon pea (*Cajanus cajan* (L.) Huth) is a popular tropical and subtropical legume grown for its tasty seeds. Pigeon pea is a quick growing, tough, versatile, and drought resistant plant. Because of its drought resilience, it might be considered critical for food security in locations where rainfall is unpredictable and droughts are probable.

Traditional use

It is one of the most popular pulses in India, and it provides a considerable quantity of protein in a vegetarian diet. In areas where it grows, fresh young pods are served as a vegetable in dishes such as sambar. Whole pigeon peas are known as arhar dal in Hindi. In Ethiopia, not only the pods, but also the young shoots and leaves, are cooked and eaten [37].

ANTIDIABETIC ACTIVITY OF THE SELECTED LEGUMES SPECIES**Cow pea (*Vigna unguiculata*)**

Cowpea has recently gained popularity among consumers and researchers worldwide because to its many health benefits, including anti-diabetic, anti-cancer, anti-hyperlipidemic, anti-inflammatory, and antihypertensive qualities. Cowpea phenolic compounds, proteins, peptides, and protease inhibitors have been shown to enhance lipid profile, blood glucose level, and blood pressure [38,39].

Hypercholesterolemic effect

For 4 weeks, hypercholesterolemic hamsters were given diets high in saturated fatty acids and cholesterol, with the sole difference being the protein source (20%): casein (control group, HC), whole cowpea seed (HWS), and cowpea protein isolate (HPI). Plasma total cholesterol and non-HDL cholesterol levels were much lower in hamsters given HWS and HPI. HPI and HC had comparable protein digestibility, which was substantially greater than HWS. HWS-fed animals had considerably greater amounts of bile acids and cholesterol in their faces than casein- or HPI-fed animals. Histological examinations of the liver revealed that the HC diet caused steatosis throughout the hepatic lobule, but the HWS and HPI diets reduced liver steatosis. HWS was more successful than HPI in modulating lipid metabolism, as evaluated by plasma cholesterol decrease and hepatic steatosis. This research found that whole cowpea seed and its protein isolate may help treat diet-induced hypercholesterolemia and steatosis, even in diets rich in saturated fatty acids and cholesterol [40,41].

Antihyperglycemic activity

Because *V. unguiculata* has reported bioactive compounds with antihyperglycemic activity, it was decided to conduct an oral glucose tolerance test (OGTT) and an acetic acid-induced gastric pain model test with methanol extract of *V. unguiculata* ssp. *unguiculata* beans to assess the extract's antihyperglycemic potential. Six groups of five mice each were formed from the animals. Group 1 obtained a vehicle (1% Tween 80 in water, 10 ml/kg body weight) and functioned as the control group, whereas Group 2 received the conventional medicine (glibenclamide, 10 mg/kg body weight). Methanolic bean extract (MEVU) was given to groups 3-6 at dosages of 50, 100, 200, and 400 mg per kg body weight. MEVU, when supplied at dosages of 50, 100, 200, and 400 mg per kg body weight, dramatically lowered blood glucose levels in mice. The % reduction in blood glucose levels at these four dosages was 24.8, 32.2, 42.0, and 51.7, respectively. In example, glibenclamide, a typical antihyperglycemic medication, lowered blood glucose levels in mice by 55.2% when given at a dosage of 10 mg per kg body weight. The findings showed that the extract had significant antihyperglycemic effect at the highest dosage tested and so might be utilized to decrease blood glucose in hyperglycemic patients [42,43].

Black gram (*Vigna mungo*)

The Leguminosae family includes black gram. It is one of the fewer researched legumes, while being extensively utilized in many regions of the globe. It possesses antihyperglycemic, anticholesterolemic, antidiabetic, antioxidant, and other medicinal effects. Whole black gramme has a lot of protein, fiber, vitamins, and minerals including calcium



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and iron. It also contains a lot of phosphorus. Our research concluded that black gramme has substantial therapeutic potential [44,45,46].

Antihyperglycemic activity

Legumes have been shown to reduce glycemic reactions by decreasing carbohydrate absorption or blocking carbohydrate digestion. Dietary fiber and uranic acid in beans are thought to be the culprits underlying these activities. Swiss albino mice weighing between 12 and 16g were utilized. Prior to the actual studies, the animals were acclimatized for three days. Crude methanol extract of non-boiled seeds lowered blood glucose levels by 29.1, 36.5, 42.6, and 48.9% in oral glucose tolerance tests with glucose loaded Swiss albino mice at dosages of 50, 100, 200, and 400 mg per kg body weight, respectively. The percent decreases in blood glucose levels with crude methanol extract of cooked seeds were 24.1, 35.1, 39.4, and 46.5% for the four dosages described above. When provided at a dosage of 10 mg per kg, glibenclamide, a typical antihyperglycemic medication, was shown to lower blood glucose levels by 48.2%. Because *V. mungo* is a leguminous crop, the above may explain the observed antihyperglycemic impact of the seeds, which contain high quantities of fiber and so may have slowed glucose absorption (Khan et al., 2009). [47,48]

Anticholesterolemic Action

Black gram (*Phaseolus mungo*) has previously been shown to have a strong hypolipidemic effect in rats on a high-cholesterol diet. A protein and an insoluble polysaccharide fraction have now been identified that reduce total cholesterol and phospholipid levels in the serum, liver, and aorta of rats given a high-fat, high-cholesterol diet. The observed hypolipidemic impact might be attributed to (a) the kind of carbohydrate present, (b) the type of protein, (c) the type of unsaturated fatty acids, (d) the presence of sitosterol's, or (e) unknown variables. Fatty acids extracted from lipid fractions have a hypolipidemic impact as well (Devi and Kurup, 1972). The globulin fraction from black gramme at 5% dosage and the polysaccharide fraction at 56% dose had a comparable lipid-lowering effect. Thus, the protein fraction seemed to be more efficient than the polysaccharide fraction in lowering total cholesterol and triglyceride levels (Devi and Kurup, 1973). Normal and alloxan-induced diabetic guinea pigs fed a four-week diet containing whole seed of *Phaseolus mungo* showed significant decreases in blood glucose, serum total lipids, triglycerides, and the esterified component of cholesterol. [49,50,51,52]

Chickpea (*Cicer arietinum*)

Chickpea (*Cicer arietinum*) is one of the world's most popular pulses, with over 2.3 million tons entering the global market each year. Several health advantages of chickpea components have been shown in preclinical and clinical investigations, including antioxidant capacity, antifungal, antibacterial, analgesic, anticancer, anti-inflammatory, and hypocholesterolemia capabilities, as well as angiotensin I-converting enzyme inhibition. Chickpeas are high in unsaturated fatty acids (PUFA), resistant starch, dietary fiber, vitamins, minerals, and phytoestrogens. A recent controlled dietary intervention trial found that adding isoenergetic chickpeas to a wheat-based Australian-style diet boosted PUFA intake. This was linked to minor but substantial decreases in blood LDL cholesterol and total cholesterol concentrations in slightly hypercholesterolemic women and men [53,54].

Anticholesterolemic Action

The common connection of hypercholesterolemia with ischemic heart disease has fueled the hunt for medicines that lower blood cholesterol levels. Previously, we found that Bengal gram, also known as "chana," (*Cicer arietinum*), considerably reduces experimentally produced high levels of cholesterol in both blood and tissues of albino rats (Mathur et al., 1963, 1964) and rabbits (Mathur et al., 1965). In cholesterol-cholic acid-fed rats, both protein and fat fractions of Bengal gramme were shown to not only prevent but also reverse experimentally generated elevated levels of cholesterol in both tissues and blood (Mathur et al., 1964). When compared to the hypocholesterolemia effects of tannin, phytate, and pectin in rats, Bengal gramme demonstrated the greatest decrease in blood and tissue cholesterol and triglycerides, as well as increased fecal excretion of bile acids (Sharma, 1984). Fructose-induced hyperlipidemia in rats was efficiently reduced by four active fractions of gramme, including total lipid, fatty acid, globulin, and insoluble carbohydrate fractions. These fractions reduced serum, liver, and aorta levels of cholesterol,



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triglycerides, and phospholipids (Malik et al., 1985). A substantial amount of research has been conducted in human subjects on the hypoglycemic function of chickpeas. A 67-week long-term research in males found that hypocholesterolemia reduced the mean blood total cholesterol level from 206.4 20.0 mg/100 mL to 160.0 24.1 mg/100 ml. The PUFA: SFA (polyunsaturated fatty acids: saturated fatty acids) ratio of 45 free-living adults who consumed 728 g of canned chickpeas per week for 12 weeks, followed by four weeks without chickpeas, increased from 0.39 to 0.47 [55,56,57,58].

Antihyperglycemic Action

Low glycemic index diets have been showed to enhance glucose tolerance in both healthy and diabetic people. Twelve healthy participants ate three 50-g carbohydrate test meals: white bread, wheat spaghetti, and spaghetti in which the wheat was partly replaced by chickpea flour. Blood samples were taken 2 hours after meals to assess the glycemic response and calculate the glycemic index. Pasta's nutritional content and in vitro starch hydrolysis were evaluated. The addition of chickpea flour increased the mineral, lipid, and indigestible component content of the pasta, but not the overall carbohydrate level. Starch hydrolysis was lower in both varieties of pasta than in white bread, although the difference was bigger in chickpea flour pasta. The glycemic indexes (GI) of both forms of pasta were within the usual range for lente carbs, although much lower in the pasta using chickpea flour (GI wheat spaghetti: 735; GI wheat chickpea spaghetti: 586). The addition of chickpea flour as a component in pasta products clearly provides a food with a low glycemic response and may aid in the achievement of a larger choice of low-GI meals for the customer [59,60].

Lentils (*Lens culinaris*)

In Ethiopia, Lens Culinary medik seed has been used in traditional practices to cure a variety of diseases, including diabetes mellitus. Previous phytochemical screening experiments found that germination of *L. culinaris* seeds includes more bioactive components than raw seeds. Although *L. culinaris* germination results in large increases in bio actives, the extract's anti-diabetic impact on germinated seed has yet to be determined. The purpose of this work was to test whether an aqueous methanol extract of germinated *L. culinaris* seed extract has anti-diabetic effect in streptozotocin (Stz)-induced diabetic mice [61,62,63,64].

Antidiabetic Action

Stz-induced diabetic mice were used to test the antidiabetic activity of germinated *L. culinaris* seed extract. The treatment group received an 80% aqueous methanol extract of germinated *L. culinaris* seed at dosages of 100, 200, and 400 mg/kg. Positive and negative controls were libenclamid (5 mg/kg) and dimethyl sulfoxide 2%, respectively. For three weeks, the test extract and controls were administered daily. Weekly measurements of blood glucose and body weight were taken. After 21 days, the extract also improved serum lipid profiles in diabetic mice (p0.05). Weekly measurements of blood glucose and body weight were taken. At the conclusion of each trial, the lipid profiles were measured. To assess the extract's postprandial impact, oral glucose tolerance tests were conducted. In normal mice, the seed extract reduced blood glucose levels significantly following an oral glucose load (p0.05). *L. culinaris* seed extract contains tiny escapable fatty acids, particularly propionate, which inhibit hepatic cholesterol production, lower the activity of the HMG-CoA reductase enzyme, and diminish bile acid and cholesterol reabsorption from the gastrointestinal tract. It is also high in antinutritive substances such phytate, lectins, and tannins, which have been demonstrated to lower blood sugar and plasma cholesterol levels. It also enhanced normal mice's oral glucose tolerance [65,66,67,68,69,70].

Soy bean (*Glycine max*)

Glycine max (L.) Merr. (soybean) seeds, which belong to the Fabaceae family, have been used for centuries in a variety of fresh, fermented, and dried cuisines across Asia (Burton 1997). It is a vital source of high-quality protein and vegetable oil for animal and human nutrition, making it one of the world's most important commercial crops (Meng et al 2007). Soybean is a unique food due to its high nutritional value. Soybean meals are rich in oligosaccharides, fiber, phytochemicals (particularly isoflavones), and minerals, while being low in saturated fat and cholesterol. They also contain oligosaccharides, fiber, phytochemicals (particularly isoflavones), and minerals (Mateos-Aparicio et al.,



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2008). Soy protein and genistein (one of the key isoflavones in soybean) supplements were reported to be effective in treating hyperglycemia and minimizing diabetic sequelae in streptozotocin-induced diabetic mice (Lee 2006). [71]

Antihyperglycemic Action

The present research studied at the antihyperglycemic efficacy of petroleum ether (GM-PEE), alcoholic (GM-ALE), and aqueous extracts (GM-AQE) of Glycine max seeds in alloxan-induced diabetic mice. Glycine max seeds were produced in petroleum ether (GM-PEE), alcoholic extract (GM-ALE), and aqueous extract (GM-AQE) and tested for antihyperglycemic efficacy. In alloxan-induced (80 mg/kg, i.v.) diabetic mice, GM-PEE (100 mg/kg), GM-ALE (100 mg/kg, p.o.), GM-AQE (100, 200, and 400 mg/kg, p.o.), and glyburide (10 mg/kg, p.o.) diabetic animals were treated orally. Serum glucose levels were measured following acute and chronic extract administration. In GM-AQE-treated mice, an oral glucose tolerance test (OGTT) was conducted. Serum glucose levels were not reduced by GM-PEE (100 mg/kg) or GM-EE (100 mg/kg). In acute and chronic tests, GM-AQE (100, 200, and 400 mg/kg) substantially lowered blood glucose levels (antihyperglycemic action). The antihyperglycemic impact of GM-AQE (100, 200, and 400 mg/kg) began at the 2nd hour, peaked at the 4th hour, and was maintained until the 24th hour. In the chronic research, a decrease in blood glucose level was found on the 28th day, as well as after withdrawal for the next 7 days. Increased glucose tolerance was seen in non-diabetic and diabetic mice treated with GM-AQE (100 and 200 mg/kg) in the OGTT. It was determined that GM-AQE, but not GM-PEE or GM-ALE, had antihyperglycemic action. [72,73]

Anticholesterolemic Action

For the first time, the 7S globulin family of soybean storage proteins were discovered to be engaged in vivo in 1992. This research found a direct impact on a 35% decrease in plasma cholesterol levels in rats, with dose and effects comparable to clofibrate. The findings in rats showed a statistically significant decrease in triglyceride levels (Lovati et al., 1992). In rats fed a high-fat diet and then given a single dose of 150, 300, or 450 mg/kg body weight of soybean oligosaccharides, aberrant blood glucose, cholesterol levels, and oxidative stress were considerably reduced (Chen et al., 2010). The hypocholesterolemia effects of soybean protein were demonstrated in hypercholesterolemic men who ate a soybean protein diet for five weeks, followed by a washout period of 10-15 weeks, and then continued the diet for five weeks (Wong et al., 1998). Significant decreases in serum total cholesterol (by 0.22 mmol/l, or 3.77 percent), LDL-C (by 0.21 mmol/l, or 5.25 percent), and TAG (by 0.10 mmol/l, or 7.27 percent) were found to be associated with significant increases in serum HDL cholesterol (by 0.04 mmol/l, or 3.03 percent). Men had lower total cholesterol and LDL-C levels than women. The lipid profile was improved in trials with dosages greater than 80 mg. Only in examinations lasting more than 12 weeks did soy show the greatest reduction advantages. Tablets containing derived soy isoflavones did not help decrease total cholesterol appreciably [74,75,76].

Pigeon Pea (*Cajanus cajan*)

Cajanus cajan, often known as pigeon pea, is a woody perennial legume. This crop is important because of its many purposes as food, medicine, and fuel. It is widely cultivated in India, as well as in Eastern and Southern Africa. Pigeon peas are a popular staple in India, East Africa, and the Caribbean. Almost 90% of the world's supply is produced in the Indian subcontinent. It contributes significantly to African diets owing to its high protein content (20-28%) and palatability [77].

Antihyperglycemic Action

Numerous animal studies on the anti-diabetic potential of pigeon pea have been conducted. Aqueous fractions of *C. cajan* leaves and stems (500 and 1000 mg/kg) demonstrated no hypoglycemic effect in normoglycemic mice. However, in the oral glucose tolerance test (OGTT), it significantly enhanced glucose tolerance after one hour and two hours. Single doses of unroasted *C. cajan* seeds (60 and 80 percent) administered to normal and alloxanized mice resulted in a significant decrease in serum glucose levels after one to two hours and a significant increase after three hours, whereas roasted seeds resulted in a significant increase in serum glucose levels during the three-hour experimental period. After 30 minutes of high-temperature roasting, the hypoglycemic principle was eliminated, whereas the hyperglycemic principle persisted [92]. There have been few human investigations on the hypoglycemic impact of legumes. The prepared diet of *C. cajan* revealed a lower blood glucose response and a lower glycemic index



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when tested on healthy human volunteers. Dietary fiber, amylase content, and antinutrient presence may all lead to a lower glycemic response. Therefore, it may be included to the list of potential foods for diabetics and hyperlipidemic [78,79].

Anticholesterolemic Action

A 10% globulin fraction from red gramme produced a considerable hypolipidemic effect in rats fed a high-fat, high-cholesterol diet. A four-week dose of 200 mg/kg stilbene extract from *C. cajan* L. (sECC) lowered blood and hepatic total cholesterol by 31.5 percent and 22.7 percent, respectively (P 0.05), in hyperlipidemic Kunming mice. Triglyceride levels in the blood and liver were also lowered by 23.0 and 14.4 percent, respectively. Low-density lipoprotein (LDL) cholesterol levels in the blood were also lowered by 53% (P 0.01). HMG-CoA reductase, CYP7A1, and LDL receptor mRNA expression all increased significantly. These studies demonstrated that sECC decreased the atherogenic effects of dietary cholesterol in mice [80,81,82].

CONCLUSION

Changes in lifestyle and dietary habits have had a substantial influence on worldwide health, leading to a rise in the incidence of lifestyle disorders such as type 2 diabetes mellitus (T2DM), which is an abbreviation for diabetes mellitus type 2. This pattern is most obvious in countries that are still in the process of industrialization and economic growth, such as developing nations. The effects of type 2 diabetes not only influence people, but they also place a significant financial burden on healthcare systems owing to the expenses involved with diagnosis and treatment of the condition. As a result, there is an urgent need for solutions that are both effective and economical, which may be used to prevent and treat this illness. The establishment of appropriate food and fitness programs is one strategy that has garnered attention as a potential method. A diet that is high in legumes has been identified as one of the most promising dietary treatments, as well as a natural strategy, for the prevention and treatment of type 2 diabetes. The consumption of legumes, which include beans, lentils, and chickpeas, is commonplace all over the world because of the many health advantages associated with eating them. Studies have shown that legumes contain anti-diabetic characteristics, which contribute to a decrease in blood glucose levels. These benefits may be traced back to the high fiber content of these foods, which slows down the rate at which glucose is absorbed into the bloodstream and avoids sudden increases in blood sugar levels. Additionally, legumes include complex carbs that are slowly digested, which results in a steadier release of glucose into the circulation. This is an additional benefit of eating legumes. Studies has found that eating beans may improve bile salt excretion, which in turn helps keep blood cholesterol levels in a safe range. Bile salts play an important role in both the digestion and absorption of lipids from the diet. Because legumes encourage the outflow of these bile salts, they decrease the amount of cholesterol that is reabsorbed by the body and increase the amount that is expelled from the body. This mechanism contributes to the control of cholesterol levels and aids in the prevention of the development of cardiovascular problems that relate to type 2 diabetes.

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Table. 1: Uncommon side effects of antidiabetics

| Anti-diabetic | Rare and very rare adverse drug reactions |
|--------------------|---|
| Metformin (6*7) | Lactic acidosis (Rare; 0.03 cases per 1000 patient years, with 0.015 fatal cases per 1000 patient years) |
| Glimepiride (6*7) | Epigastric fullness Angioedema, hypotension, shock Agranulocytosis, aplastic anemia, disulfiram-like reaction, hemolytic anemia, leukopenia, pancytopenia, thrombocytopenia |
| Pioglitazone (6*7) | Pulmonary edema and/or congestive heart failure, dyspnea (associated with weight gain and/or edema) |
| Acarbose (6*7) | Ileus and subileus, lymphocytic colitis, Thrombocytopenia Erythema, exanthema, rash, urticarial |
| Saxagliptin (6*7) | Angioedema Anaphylaxis, immune thrombocytopenia |
| Sitagliptin (6*7) | Gastritis (<i>Helicobacter pylori</i>), gastroesophageal reflux disease, Depression, migraine |

Table. 2: Different types of legumes and their scientific name and local names [12,13,14]:

| Legume type | Scientific name | Local name |
|-------------|----------------------------------|---------------|
| Cowpea | <i>Vigna unguiculata</i> | Chawli, lobia |
| Black gram | <i>Vigna mungo</i> | Urad |
| Chick pea | <i>Cicer arietinum</i> | Chana |
| Lentils | <i>Lens culinaris</i> | Masur |
| Soy bean | <i>Glycine max</i> | Soya |
| Piegeon pea | <i>Cajanus cajan</i> | Arhar, Tur |
| Horse gram | <i>Macrotyloma uniflorum</i> | Kulith |
| Green gram | <i>Vigna Radiata</i> | Mung |
| Moth bean | <i>Vigna aconitifolia</i> | Moth, Matki |
| Common bean | <i>Phaseolus vulgaris</i> | Rajma |
| Fenugreek | <i>Trigonella foenum-graecum</i> | Methi |
| Peanut | <i>Arachis hypogaea</i> | Mungphali |



**Digbijoy Nath et al.,****Table. 3: Nutritional value of some legumes [15,16,17,18,19]:**

| Legume type | Energy value(kcal) | Carbohydrate(g) | Protein(g) | Fat(g) | Fiber(g) |
|----------------------|--------------------|-----------------|------------|--------|----------|
| Pinto beans | 245 | 44.84 | 15.41 | 1.11 | 15.40 |
| Great Northern beans | 209 | 37.33 | 17.74 | 0.80 | 12.40 |
| Navy beans | 255 | 47.41 | 14.98 | 1.13 | 19.10 |
| Black beans | 227 | 40.78 | 15.24 | 0.93 | 15.00 |
| Cowpeas | 198 | 35.50 | 13.22 | 0.91 | 11.10 |
| Kidney beans | 225 | 40.36 | 15.35 | 0.88 | 11.30 |
| Chickpeas | 269 | 44.97 | 14.53 | 4.25 | 12.50 |
| Split peas | 231 | 41.36 | 16.35 | 0.76 | 16.30 |
| Lentils | 230 | 39.86 | 17.86 | 0.75 | 15.60 |
| Lupin | 198 | 16.40 | 25.85 | 4.85 | 4.60 |
| Soy bean | 298 | 17.08 | 28.62 | 15.43 | 10.30 |





Secure Data Transmission using ElGamal Key Generation in WBAN

R. Parimala Devi^{1*}, J. Jerlin Adaikala Sundari² and J. Saranya³

¹Assistant Professor, Department of Computer Science, PSG College of Arts and Science, Coimbatore-641014, Tamil Nadu, India

²Assistant Professor, Department of Computer Technology, PSG College of Arts and Science, Coimbatore-641014, Tamil Nadu, India.

³Department of Computer Applications, PSG College of Arts and Science. Coimbatore-641014, Tamil Nadu, India

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*Address for Correspondence

R. Parimala Devi

Assistant Professor,
Department of Computer Science,
PSG College of Arts and Science,
Coimbatore-641014, Tamil Nadu, India
E.Mail: parimaladevi.mohandass@gmail.com



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ABSTRACT

In recent years, Wireless Body Area Networks (WBANs) have been widely used in healthcare applications, such as hospital and home patient monitoring. Wireless medical data are unprotected and subject to eavesdropping, modification, impersonation and replaying attacks than the wired networks. A lot of work has been done to secure the data. The existing solutions can protect the patient data during transmission, but cannot stop the inside attack where the administrator of the patient reveals the sensitive patient data. The main contribution of this paper is securely distributing the patient data in multiple data servers and employing the Paillier and Elgamal cryptosystems to perform statistic analysis on the patient data without compromising the patient's privacy. Wireless Body Area Networks (WBANs) certainly improve patient's quality-of-care without disturbing their comfort. However, there exist many potential security threats to the patient sensitive physiological data transmitted over the public channels and stored in the back-end systems. Typical security threats to healthcare applications with WBANs can be summarized as follows. Eavesdropping is a security threat to the patient data privacy. Impersonation is a security threat to the patient data authenticity. In a home care application, an attacker may impersonate a wireless relay point while patient data is transmitting to the remote location. For this purpose the present study was done using java net beans.

Keywords : ELGAMAL key, WBAN, feasibility





INTRODUCTION

Wireless Body Area Networks (WBANs) are used to monitor patients remotely. Sensors are worn on the body or implanted in the skin to collect some physiological parameters of the human body, such as Electro Cardio Gram (ECG), Electro Encephalo Gram (EEG), body temperature, blood pressure, respiration, body movement. A WBAN is a network that consists of lightweight devices with limited memory, low computation processing, low-battery power and low bandwidth. A physician can use these medical sensor readings to gain a broader assessment of patient's health status. The patient's physiological data may include heartbeat rate, temperature, blood pressure, blood oxygen level. Several research groups and projects are working in health monitoring using wireless networks, for example, CodeBlue , LiveNet , MobiHealth , UbiMon , Alarm-Net , ReMoteCare , SPINE , etc. Thus, healthcare systems are the applications that most benefit from using wireless network technology that can perform patient care within hospitals, clinics and homecare. 2 Wireless medical sensor technology has offered tremendous advantages to healthcare applications, such as continuous patient monitoring, mass-causality disaster

FEASIBILITY STUDY

Preliminary investigation examine project feasibility, the likelihood the system will be useful to the organization. The main objective of the feasibility study is to test the Technical, Operational and Economical feasibility for adding new modules and debugging old running system. All system is feasible if they are unlimited resources and infinite time. There are aspects in the feasibility study portion of the preliminary investigation:

- Technical feasibility
- Operational feasibility
- Economic feasibility
-

TECHNICAL FEASIBILITY

A technical feasibility study assesses the details of how you intend to deliver a product or service to customer. It is the logistical or tactical plan of how your business will produce, store, deliver and track its products or services. A feasibility study is an analysis that takes all of a project's relevant factors into account including economic, technical, legal, and scheduling considerations-to ascertain the likelihood of completing the project successfully. In this project, first sensor produces some medical data and then that is delivered to the many servers and then the main scrambled data is sent to the doctor. Here the technical feasibility study is proved.

OPERATIONAL FEASIBILITY

Operational feasibility is the measure of how well a proposed system solves the problems, and takes advantage of the opportunities identified during scope definition and 7 how it satisfies the requirements identified in the requirements analysis phase of system development. Here the project explains the more security than the existing system. Here this project contains two types of crypto systems and this system shows more security. The scope of this project is to give data security using crypto systems.

ECONOMIC FEASIBILITY

Economic analysis could also be referred to as cost/benefit analysis. It is the most frequently used method for evaluating the effectiveness of a new system. In economic analysis the procedure is to determine the benefits and savings that are expected from a candidate system and compare them with costs. If benefits outweigh costs, then the decision is made to design and implement the system. An entrepreneur must accurately weigh the cost versus benefits before taking an action. This project also includes economic feasibility. It does not need any database so that there is no backend. The sensor produces data and uploads to the server and the server sends to the doctor and then doctor download the data what he wants. There are no complex methods. The system does not contains much more cost. It also contains the simple design methods. The existing system shows image scrambling by encrypting and decrypting which had a complex problems but now data scrambling contains accurate in a easy way.





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METHODOLOGIES

There are two methodologies used here. They are

- Elgamal crypto system
- Paillier crypto system

ELGAMAL CRYPTO SYSTEM

In cryptography, the ElGamal encryption system is an asymmetric key encryption algorithm for public-key cryptography which is based on the Diffie–Hellman key exchange. It was described by Taher ElGamal in 1985.

ElGamal encryption is used in the free GNU Privacy Guard software, recent versions of PGP, and other cryptosystems. The Digital Signature Algorithm (DSA) is a variant of the ElGamal signature scheme, which should not be confused with ElGamal encryption.

PAILLIER CRYPTO SYSTEM

The Paillier cryptosystem, invented by and named after Pascal Paillier in 1999, is a probabilistic asymmetric algorithm for public key cryptography. The problem of computing n-th residue classes is believed to be computationally difficult. The decisional composite residuosity assumption is the intractability hypothesis upon which this cryptosystem is based.

EXISTING SYSTEM

In existing system, the image is scrambled and produces a cipher images. This system uses Compressive sensing, Arnold scrambling, Chaotic scrambling to scramble the images. But in all the times, the images are not used as a data. It leads to a difficult task. It only produces random key generations. It has only less security and low processing. The images given in this paper is scrambled at only one time and so hackers can hack the images easily. The image is transmitted to the doctor to know the patients health condition using 3-D Steganography mechanism.

DRAWBACKS OF THE EXISTING SYSTEM

- It needs special computer power.
- It has less security.
- It does not have a private and public keys to encrypt data.
- It needs to evaluate the data between sender and receiver communication and it takes more time.

PROPOSED SYSTEM

In proposed system, first the sample medical data is taken. The project contains three steps. The first step is to upload the medical data which is given by the sensor. Then the user generates the public key and private key for the data for security. After this process, the user converts the data into blocks and performs scrambling and encryption of data blocks. The second step is that the data is sent to the many servers for security purpose. If the data is sent to one server, it can be easily stolen by hackers. So that the blocked data or scrambled data is sent to many servers. By this process the hackers needs knowledge to hack all the server details. It is a complex process. Here the security is proved. The third step is that the doctor needs to view the patient details in the hospital itself. For that purpose, the doctor needs private and public key and then the granted user needs to download the details and then the user needs to encrypt and decrypt the files using Elgamal and Paillier crypto system.

This is the main aim of this project.

ADVANTAGES OF PROPOSED SYSTEM

- The medical data is encrypted and decrypted twice so that security is high.
- Even if the user can get the encrypted data, he cannot decrypt it without cooperation with all three data servers

SCOPE OF FUTURE ENHANCEMENT





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- Large data sets can be used for scrambling techniques.
- The end-users can view the sensor details in a mobile device.

RESULTS

The result of this project is having more security than the other methods. The wireless body area network contains some sensors which have a confidential data and it should be protected more securely. So, in this project the data is encrypted twice and hackers cannot be able to hack all the details. It contains a manual approach. In later year, it can be done in a automation using machine learning.

CONCLUSION

In order to detect more security for data from sensor network systems, a novel approach is developed using crypto systems. Firstly the sensor data is presented. Secondly, the data are scrambled and encrypted. Next the data sent to the server nodes and then it will reach the granted user respectively.

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Ultrasound Assisted Synthesis, Spectral QSAR Analysis and Antifungal Activities of Some 2-(4- Substituted Phenyl)-Imidazol-5-benzo Ketones

S.Nalini¹, I. Muthuvel² and G. Thirunarayanan^{3*}

¹Assistant Professor, Department of Chemistry, Periyar Government Arts College, Cuddalore-607 001, Tamil Nadu, India

²Assistant Professor, Department of Chemistry, Annamalai University, Annamalainagar - 608 002, (Department of Chemistry, MR. Government Arts College, Mannargudi - 614001), Tamil Nadu, India.

³Associate Professor, Department of Chemistry, Annamalai University, Annamalainagar-608 002, Tamil Nadu, India.

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*Address for Correspondence

G. Thirunarayanan

Associate Professor,
Department of Chemistry,
Annamalai University,
Annamalainagar-608 002,
Tamil Nadu, India.
E.Mail: thirunarayanan.g.10313@annamalaiuniversity.ac.in



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ABSTRACT

More than 90% yields of some 2-(4-substituted phenyl)-imidazol-5-benzo ketones have been synthesized by FeCl₃ / Bentonite catalyzed, Ultrasound promoted condensation of 3,4-diamino benzophenone and various substituted benzaldehydes. These ketones were characterized by their physical constants and spectroscopic data. The spectroscopic functional group frequencies of these ketones were correlated with sigma and Swain-Lupton's constants using single and multi-statistical correlation analysis. From the results of statistical correlation analysis, the quantitative structure activity relationships of these ketones were investigated. Also the antifungal activities of these ketones were measured using Bauer-Kirby disc diffusion method. From the measured antifungal activity, the structure activity relationships of these ketones were studied.

Key words: Imidazole ketones, FeCl₃/Bentonite, Ultrasonication, Infrared spectra, NMR spectra, Regression analysis, Antifungal activity





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INTRODUCTION

Imidazole and its derivatives are an important class of five-membered heterocycles that possess vast applications in synthetic organic chemistry [1] and notorious medicinal activities such as antimicrobial [2], antitubercular [3], anti-HIV [4], anti-oxidant [5], anti-analgesic [5], antihypertensive [6], antiobesity [7], antitumor [8], antiviral [9], anthelmintic [10], antihistaminic [11], anti-inflammatory, antidepressant [5, 12], antidiabetic [13], anticonvulsant [14], antiallergic [15], antirheumatic [16], antiprotozoal [17], antiaging, anticoagulant, antimalarial [18] and antiamebic activity [19]. The chromium salt, imidazolium halo chromates are one of the best oxidizing agents [20]. Numerous synthetic methods such as conventional, solvent free microwave irradiation, neat and Ultrasonication are employed for the synthesis of imidazole, benzimidazole and benzimidazole keto derivatives [1, 21-24]. Chemists and Researchers used these methods for the synthesis of above said derivatives with various catalysts such as montmorillonite K-10 [25], Ball mill agitation [26, BF₃-OEt₂ [27], NH₄Cl [28], activated C-SO₃H [29], ZrOCl₂·8H₂O [22], TiCl₄·SnCl₄·5H₂O [22], HfCl₄ [22], ZrCl₄·TFE/HFIP [22], Cu [30], Amberlite IR-120 [31], SiO₂/ZnCl₂ [32], Dowex-50 W [33], SDS micelles [34], silica sulfuric acid [35], FePO₄ [36], CAN [37], Cu(NO₃)₂·3H₂O [38], K₄Fe(CN)₆ [39] and FeCl₃/Al₂O₃ [40]. Ultrasonicated synthetic methodology is one of the pollution-free synthetic methods due to green solvents such as water and ethanol and low concentrated Lewis acid-bases only applied for this technique [41-44]. This technique was employed for synthesis of various organic substrates including imidazole derivatives [24]. The infrared and NMR spectroscopic data of these derivatives were very useful for prediction of their ground state geometry and stereochemical structures such as *s-cis* and *s-trans* conformers, *E*- and *Z*-configurations [45-47]. The QSAR, QPR, SAR studies of organic substances were carried out with the spectroscopic data and Hammett substituent constants through statistical single and multiple regression analysis [48-54]. Spectral correlation study of various enones and benzimidazole chalcones [53, 54], Schiff's bases [52], pyrazolines [55], isoxazoles [49], oxazine amines [56], pyrimidines [57], triazoles [58], hydrazines [59], phenazines [60], ω-bromo-2-naphthones [61], quinoxaline [62], benzoic acids [63], flavonoids [64], carboxamide [65], carbothioamide [66], trifluoromethylamines [67], acyl bromides [68], sulfonamides [69] and esters [61, 68] were reported. Recently Kamalakkannan et al. [70] investigated the spectral QSAR of some 3-((*E*)-3-substituted phenylacryloyl)benzotrioles. Mala et al., have studied the effect of substituents on (*E*)-3,4-dimethoxyphenyl enones by their spectroscopic data [45]. Within these above view, there is no report available for the correlation of the spectroscopic data of the titled compounds for assessment of QSAR study and antifungal activities. Hence the authors synthesized the titled compounds under Ultrasonication method for the investigation of spectral QSAR and antifungal activities.

MATERIALS AND METHODS

General

Chemicals used in this study were procured from Sigma-Aldrich Chemical company Bengaluru, India. Melting points of the synthesized compounds were measured using Guna make electrical melting point apparatus. IR spectra (ν , 400-4000 cm⁻¹) of all compounds were recorded in Thermo Nicolet: AVATAR-330 FT-IR spectrophotometer using KBr discs. The NMR was recorded on a Bruker 400 MHz spectrometer employed 400 MHz for ¹H NMR and 100 MHz for ¹³C NMR using CDCl₃/DMSO-*d*₆ as solvent with TMS as standard.

General Procedure for the synthesis of (2-(4-substituted phenyl)-1H-benzodimidazol-5-yl)methanones (1-6)

An equi-molar quantity of (3,4-diaminophenyl) (phenyl)methanone (0.01 mol), substituted aryl aldehydes (0.01 mol) and FeCl₃/Bentonite [71] catalyst (0.01 g) in 20 ml ethanol were Ultrasonicated in an ultrasound bath at 40 Hz (Citizen Ultra Sonicator, 40 Hz, 120W, 240V, AC) 15 minutes (Scheme 1) at room temperature. Synthetic reaction was monitored by TLC. After completion of the reaction the catalyst was separated by simple filtration. Evaporation of ethanol gave the crude product. The crude was recrystallized with ethanol, dried and kept in desiccator. These ketones were characterized by their Spectroscopic data and physical constants. All data are well agreed with the data reported earlier [23]. The physical constants and yields of the reaction were given in Table 1.





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Antifungal activity measurement

Antifungal sensitivity assay of the prepared benzimidazole ketones were performed using Kirby-Bauer disc diffusion procedure[72]. In this study, *C. albicans* *Mucor spp.* *Rhizopus spp.* and *A. niger* fungal species were employed for measuring the antifungal activity. Triplicate results were recorded by repeating the same procedure. Ketoconazole was taken as the standard drug.

RESULTS AND DISCUSSION

IR spectral study

The infrared spectral QSAR study was done using the infrared functional vibrations such as NH, CN and CO of the synthesized benzimidazole ketones with substituent constants using single and multi-regression analysis[45-70]. The measured assigned infrared group vibrations are given in Tale 2.

These frequencies were correlated using the Hammett equation (1) as,

$$\nu = \rho\sigma + \nu_0 \quad \dots (1)$$

Where ν is a group frequencies of the substituted system, ν_0 is the group frequencies of unsubstituted system, ρ is the reaction parameters and σ is the substituent constants. Applying an equation 1, the measured infrared spectral frequencies were correlated and the statistical results are given in Table 3. From Table 3, the correlation of NH vibrations of the benzimidazole ketones satisfactorily correlated with sigma and sigma plus constants. The remaining σ_1 , σ_R , F and R parameters were failed in correlation. This is due to the inability of inductive, field and resonance effects to predicts and dominates the effect of substituents on the NH vibrations. All correlations gave positive ρ values. The failure of correlation was attributed to the resonance conjugative structure as shown in Figure 1.

The correlation of CN and CO vibrations of these ketones were failed in correlations. Some of the correlations shows negative ρ values. This implies that the negative correlation was operated in these cases. The failure in the correlation was due to the reasons stated earlier and associated with the resonance-conjugative structure as shown in Figure. 1.

The single regression analysis showed many negative results in infrared vibrations. They are satisfactorily correlated when these vibrations were subjected to multi-regression analysis with Swain-Lupton's parameters[73]. The regression equations (2-7) are as,

$$\nu_{\text{N-H}} = 3378.191(\pm 13.86) + 284.983(\pm 43.50) \sigma_1 + 402.731(\pm 58.62) \sigma_R$$

$$R = 0.974, P > 95\%, n = 6 \quad \dots (2)$$

$$\nu_{\text{N-H}} = 3391.481(\pm 8.53) + 272.073(\pm 26.10) F + 374.04(\pm 31.30) R$$

$$R = 0.990, P > 99\%, n = 6 \quad \dots (3)$$

$$\nu_{\text{CN}} = 1595.98(\pm 15.03) - 6.599(\pm 47.16) \sigma_1 - 18.088(\pm 63.553) \sigma_R$$

$$R = 0.900, P > 90\%, n = 6 \quad \dots (4)$$

$$\nu_{\text{CN}} = 1597.23(\pm 15.13) - 3.586(\pm 46.28) F - 7.115(\pm 55.50) R$$

$$R = 0.901, P > 92\%, n = 6 \quad \dots (5)$$

$$\nu_{\text{CO}} = 1647.98(\pm 13.05) + 32.162(\pm 40.94) \sigma_1 + 54.073(\pm 55.172) \sigma_R$$

$$R = 0.905, P > 91\%, n = 6 \quad \dots (6)$$

$$\nu_{\text{CO}} = 1650.58(\pm 12.93) + 24.972(\pm 39.539) F + 48.184(\pm 47.41) R$$

$$R = 0.905, P > 92\%, n = 6 \quad \dots (7)$$





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¹³C NMR spectral correlations

The assigned ¹³C NMR chemical shifts (δ, ppm) of these prepared ketones were presented in Table 4. The chemical shifts (δ, ppm) of CO, CN and C_{ipso} of the ketones were correlated using Hammett equation with Hammett substituent constants (σ, σ⁺, σ_I, σ_R), *F* and *R* parameters[45-71]. In this correlations, the Hammett equation was employed as shown in equation 6.

$$\delta = \rho\sigma + \delta_0 \quad \dots (6)$$

where δ₀ is the chemical shift in the corresponding parent compound.

These chemical shifts were correlated and the statistical analysis was presented in Table 5. From the table 5, it seems all chemical shifts (δ, ppm) of CO, CN and C_{ipso} carbons of the benzimidazole compounds were failed in correlations. Many correlations shows negative ρ values. This implies that there is a reversible substituent effects operates in these cases. This failure was due to the reasons stated earlier and associated with the resonance conjugative structure as in Figure 1. These carbon chemical shifts gave satisfactory correlation with Swain-Lupton's parameters[73]. The statistical equations (9-14) are as

$$\delta_{CO} = 198.47(\pm 3.41) - 1.080(\pm 10.715) \sigma_I + 15.581(\pm 4.441) \sigma_R \quad \dots (9)$$

R=0.930, P>93%, n=6

$$\delta_{CO} = 198.97(\pm 3.15) - 7.896(\pm 9.643) F + 6.047(\pm 11.562) R \quad \dots (10)$$

R=0.906, P>99%, n=6

$$\delta_{CN} = 153.09(\pm 0.560) + 3.823(\pm 1.752) \sigma_I + 3.396(\pm 2.365) \sigma_R \quad \dots (11)$$

R=0.907, P>90%, n=6

$$\delta_{CN} = 153.34(\pm 0.725) + 2.735(\pm 2.219) F + 2.737(\pm 2.661) R \quad \dots (12)$$

R=0.905, P>92%, n=6

$$\delta_{C_{ipso}} = 144.40(\pm 2.360) - 8.921(\pm 7.404) \sigma_I - 4.110(\pm 9.978) \sigma_R \quad \dots (13)$$

R=0.905, P>95%, n=6

$$\delta_{C_{ipso}} = 143.70(\pm 2.543) - 7.406(\pm 7.781) F - 4.972(\pm 9.330) R \quad \dots (14)$$

R=0.904, P>92%, n=6

Antifungal activity

The measured antifungal activity by means of mm of zone of inhibition of all prepared benzimidazole ketones were given in Table 6. The statistical pictorial of the activity was illustrated in Figure 2. From Table 6, ketones 1-3 and 5 shows good antifungal activity and the compounds 4 and 6 shows lower antifungal activity against *A. niger* fungal species. Here the inductive and +R effects of halogens and methoxy groups enhances the fungal activity. The electronegativity of F atom and mesomeric effects lowers the antifungal activities. Benzimidazole ketones 1-3, 5 and 6 shows good antifungal activity and the ketone 4 shows lower antifungal activity against *Mucor spp.* fungal species. In this case, the inductive effect of Br and Cl atoms, mesomeric and +R effects of methoxy and methyl substituents enhances the fungal activity. The electronegative F atom reduces the antifungal activity against *Mucor spp.* strains. The ketones 1-3 shows good antifungal activity and the compounds 4-6 shows satisfactory antifungal activity against *Rhizopus spp.* fungal species. Here the inductive effect of Br and Cl atoms enhances the fungal activity and the electronegative F atom, +R effect of methoxy and mesomeric effect of methyl groups lowers the antifungal activity. Ketones 1-5 shows good antifungal activity and 6 shows satisfactory antifungal activity against *C. albicans* fungal strains. Here the Inductive effect of halogens, electronegativity of F atom, +R effect of methoxy groups enhances the antifungal activity and the mesomeric effect of methyl group slightly reduces the antifungal activity.





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CONCLUSIONS

More than 90% yields of six 2-(4-substituted phenyl)-imidazol-5-benzo ketones were prepared by ultrasound promoted method. The QSAR of the group frequencies of these ketones were analyzed. From the statistical analysis results infrared NH stretches gave good correlation coefficients with sigma and sigma plus constants. The remaining C=N and C=O stretches were failed in the correlations. The ¹³C NMR chemical shifts of CN, CO and C_{ipso} of these ketones were failed in the regressions. This is due to the inability of the effect of substituents reflects on the functional group frequencies. A satisfactory correlation coefficients obtained in multi-regression analysis. The antifungal activities of these ketones were measured with *C. albicans* *Mucor spp.* *Rhizopus spp.* and *A. niger* fungal species. Many of the ketones shows good antifungal activities against their fungal species.

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Table 1. The physical constants, yields and mass fragments of the synthesized benzimidazole ketones.

| Entry | X | MF | MW | Yield(%) | m.p. (°C) | Mass(m/z) |
|-------|------------------|---|-----|----------|----------------------|---|
| 1 | H | C ₂₀ H ₁₄ N ₂ O | 298 | 91 | 237-238(236-238)[23] | 298[M ⁺] |
| 2 | Br | C ₂₀ H ₁₃ BrN ₂ O | 377 | 93 | 278-279(276-278)[23] | 377[M ⁺], 379[M ²⁺] |
| 3 | Cl | C ₂₀ H ₁₃ ClN ₂ O | 332 | 93 | 283-284(282-284)[23] | 322[M ⁺], 324[M ²⁺] |
| 4 | F | C ₂₀ H ₁₃ FN ₂ O | 316 | 90 | 265-266(264-266)[23] | 316[M ⁺], 318[M ²⁺] |
| 5 | OCH ₃ | C ₂₁ H ₁₆ N ₂ O ₂ | 328 | 96 | 198-199(196-198)[23] | 328[M ⁺] |
| 6 | CH ₃ | C ₂₁ H ₁₆ N ₂ O ₂ | 312 | 95 | 216-217(214-216)[23] | 2312[M ⁺] |





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Table 2. The infrared functional group vibrations(ν , cm^{-1}) of 2-(4-substituted phenyl)-imidazol-5-benzo ketones.

| Entry | X | NH | C=N | CO |
|-------|------------------|------|------|------|
| 1 | H | 3384 | 1615 | 1642 |
| 2 | Br | 3448 | 1577 | 1636 |
| 3 | Cl | 3428 | 1610 | 1677 |
| 4 | F | 3361 | 1601 | 1633 |
| 5 | OCH ₃ | 3264 | 1608 | 1635 |
| 6 | CH ₃ | 3329 | 1578 | 1643 |

Table 3. Results of statistical analysis of infrared frequencies (ν_{NH} , ν_{CN} and $\nu_{\text{C=O}}$ cm^{-1}) of 2-(4-substituted phenyl)-imidazol-5-benzo ketones with Hammett substituent constants (σ , σ^+ , σ_I , σ_R), F and R parameters.

| Freq. (cm^{-1}) | Const. | r | I | ρ | s | n | Correlated derivatives |
|----------------------------|------------|-------|---------|---------|-------|---|--|
| NH | σ | 0.966 | 3365.84 | 123.65 | 1.93 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.981 | 3386.56 | 164.37 | 1.45 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.481 | 3339.32 | 104.75 | 56.77 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.475 | 3407.22 | 171.14 | 66.22 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.246 | 3350.37 | 158.50 | 72.89 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.530 | 3412.95 | 150.70 | 63.78 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| C=N | σ | 0.830 | 1598.23 | -6.710 | 18.52 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.261 | 1596.96 | -10.889 | 17.93 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.232 | 1597.73 | 1.535 | 18.58 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.143 | 1595.31 | 12.758 | 18.40 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.087 | 1598.01 | 0.475 | 18.59 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.059 | 1596.95 | -4.171 | 18.56 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| CO | σ | 0.437 | 1643.98 | 33.113 | 16.75 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.261 | 1645.48 | 10.665 | 17.29 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.121 | 1642.07 | 7.961 | 15.29 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.316 | 1650.57 | 27.941 | 17.48 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.485 | 1645.23 | -2.822 | 18.41 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.404 | 1652.55 | 28.182 | 16.85 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |

r = correlation coefficient; I = intercept; ρ = slope; s = standard deviation; n = number of substituents.Table 4. The NMR chemical shifts (δ , ppm) of 2-(4-substituted phenyl)-imidazol-5-benzo ketones.

| Entry | X | ¹ H NMR | | | ¹³ C NMR | | | | |
|-------|------------------|--------------------|-----------|------|---------------------|--------|--------|---------------|-------|
| | | NH | Ar-H | X | CO | CN | Cipso | Ar-C | X |
| 1 | H | 5.39 | 6.56-8.01 | --- | 197.14 | 153.32 | 142.25 | 110.05-138.34 | --- |
| 2 | Br | 5.23 | 7.26-8.17 | --- | 196.70 | 154.26 | 143.51 | 124.99-138.44 | --- |
| 3 | Cl | 5.22 | 6.79-8.02 | --- | 197.15 | 154.64 | 138.29 | 114.51-132.10 | --- |
| 4 | F | 5.52 | 6.12-8.31 | --- | 184.95 | 152.82 | 142.75 | 112.37-138.42 | --- |
| 5 | OCH ₃ | --- | 6.12-8.31 | 3.39 | 197.11 | 153.55 | 141.82 | 112.61-136.03 | 56.08 |
| 6 | CH ₃ | --- | 7.57-8.43 | 2.09 | 195.11 | 151.92 | 148.17 | 124.36-137.83 | 30.66 |





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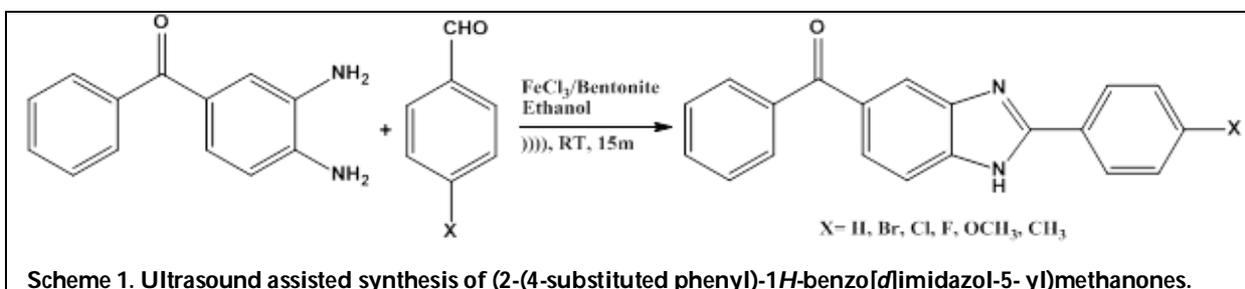
Table 5. Results of statistical analysis of ^{13}C chemical shifts (δ , ppm) of CO, CN and Cippo carbons of 2-(4-substituted phenyl)-imidazol-5-benzo ketones with Hammett substituent constants (σ , σ^+ , σ_I , σ_R), F and R parameters.

| Chem. shift | Const. | r | I | ρ | s | n | Correlated derivatives |
|-------------|------------|-------|--------|---------|------|---|--|
| CO | σ | 0.601 | 194.70 | -1.567 | 5.39 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.205 | 194.66 | -0.305 | 5.40 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.417 | 196.97 | -8.053 | 4.91 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.635 | 198.30 | 16.459 | 4.17 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.665 | 198.30 | -11.341 | 4.03 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.613 | 198.34 | 12.599 | 4.27 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| C=N | σ | 0.637 | 153.87 | 3.044 | 0.84 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.442 | 152.66 | 1.076 | 0.98 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.581 | 153.76 | 2.303 | 0.88 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.550 | 153.48 | 2.891 | 1.09 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.338 | 153.04 | 1.172 | 1.03 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.881 | 153.56 | 0.691 | 1.09 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| Cippo | σ | 0.447 | 142.86 | -6.948 | 3.19 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ^+ | 0.173 | 142.65 | -1.355 | 3.51 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_I | 0.553 | 144.90 | -7.081 | 2.96 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | σ_R | 0.187 | 143.49 | 3.139 | 3.50 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | F | 0.401 | 144.25 | -4.566 | 3.26 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |
| | R | 0.817 | 143.12 | 1.106 | 3.55 | 6 | H, 4-Br, 4-Cl, 4-F, 4-OCH ₃ , 4-CH ₃ |

r = correlation coefficient; I = intercept; ρ = slope; s = standard deviation; n = number of substituents.

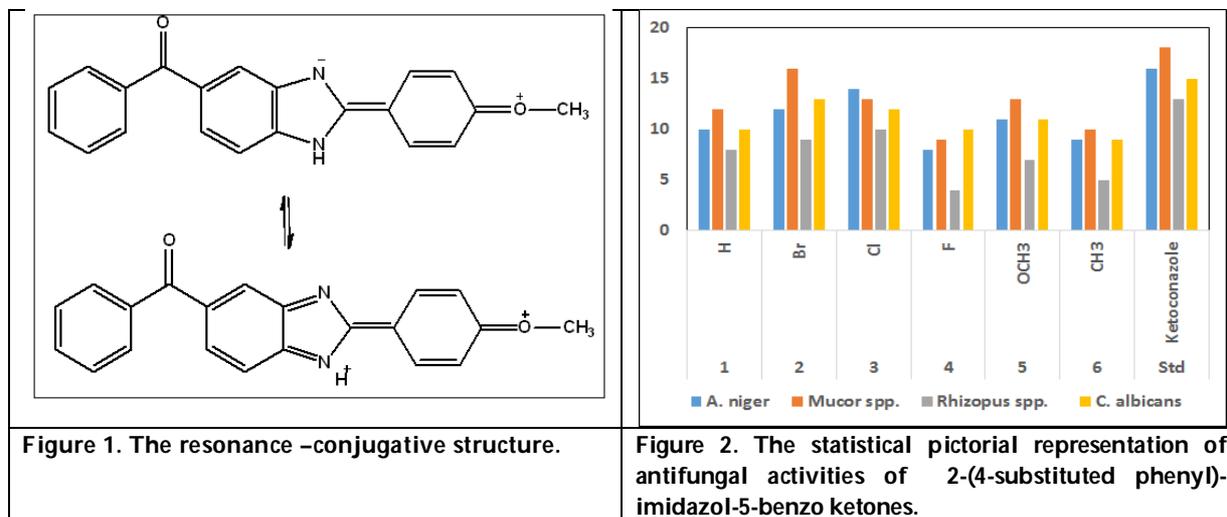
Table 6. Antifungal activities of 2-(4-substituted phenyl)-imidazol-5-benzo ketones.

| Entry | X | <i>A. niger</i> | <i>Mucor spp.</i> | <i>Rhizopus spp.</i> | <i>C. albicans</i> |
|-------|------------------|-----------------|-------------------|----------------------|--------------------|
| 1 | H | 10 | 12 | 8 | 10 |
| 2 | Br | 12 | 16 | 9 | 13 |
| 3 | Cl | 14 | 13 | 10 | 12 |
| 4 | F | 8 | 9 | 4 | 10 |
| 5 | OCH ₃ | 11 | 13 | 7 | 11 |
| 6 | CH ₃ | 9 | 10 | 5 | 9 |
| Std | Ketoconazole | 16 | 18 | 13 | 15 |





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Reliability of Gujarati Version of Foot Function Index Questionnaire

Krupa Mehta^{1*}, Mukul Chauhan² and Himangini Ben Patel³

¹Assistant Professor at Shree K.K Sheth Physiotherapy College, Affiliation with Saurashtra University, Rajkot, Gujarat, India.

²Assistant Professor, Shri Suleshwari College of Physiotherapy, Affiliation with HNGU University, Bhandu, Gujarat, India.

³Physiotherapy Assistant at Addenbrooke's Hospital, Cambridge, United Kingdom.

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*Address for Correspondence

Krupa Mehta

Assistant Professor

Shree K.K Sheth Physiotherapy College,

Affiliation with Saurashtra University,

Rajkot, Gujarat, India.

E.Mail: krupahmehta@gmail.com



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ABSTRACT

This study was designed to establish the reliability of the Gujarati version of Foot Function Index questionnaire for people with foot complaints. 30 subjects having foot complaints, 10 males and 20 females, participated in the study. Test- Retest Reliability was determined by using Intra-class correlation coefficient and Internal Consistency by Cronbach's alpha coefficient. Intra-rater reliability was 0.996 and inter-rater reliability was 0.995. Internal Consistency was 0.927 for the pain subscale, 0.927 for the disability subscale and 0.818 for activity limitation subscale. The Gujarati version of the Foot Function Index was shown to be a reliable instrument for assessing foot complaints.

Keywords: Gujarati FFI, Reliability of FFI.

INTRODUCTION

In the human body feet and ankle are mainly responsible to maintain the balance, both static and dynamic and to transfer internal forces generated by the body that allow locomotion. [1] Any alteration in foot or ankle can have a significant impact on quality of life and independence of patients having foot complaints. [1] Prevalence of foot disorders is more in women and elderly people. [2,3] Foot disorders are often associated with pain which affects the ability to perform activities of daily living. So, it is essential to assess the degree of disability in people with foot complaints due to foot/ankle impairment. Foot Function Index (FFI) questionnaire is frequently used in clinics and for



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research purpose in order to assess various foot pathologies and treatments pertaining to foot and ankle problems. The questionnaire consists of 23 questions divided into 3 subscales: 9 questions for pain, 9 questions for disability and 5 questions for activity limitation. [4] Greater is the score more severe is the pain or limitation of function. FFI has high reliability and validity for patients with rheumatoid arthritis, plantar fasciitis, posterior tibial tendinitis, and ankle instability [5,6]. It is currently available in many languages such as, Chinese [1], Korean [6], German [7], Italian [8], Spanish [9], French [10], Brazilian-Portuguese [11], Turkish [12], and Arabic [13], confirming its validity and credibility. However, reliability of Gujarati version of FFI have not yet been confirmed. So, this study aimed to determine reliability of Gujarati version of FFI (FFI-G).

METHODOLOGY

Ethical clearance was obtained from institutional ethical committee. The present study consisted of 30 subjects having foot complaints and who can understand and knows Gujarati language well. Inclusion criteria for the study were individuals with age groups between 30 to 65 years, who could read and understand Gujarati language. Individuals with metatarsalgia, plantar fasciitis, rheumatoid arthritis and calcaneal heel spur. Subjects were excluded if they had ankle-foot fracture or surgery, foot deformities and subjects who have taken steroids injections before 6 months for ankle-foot painor who had psychological or neurological disorders. Then the subjects were informed about the study, and if they agreed to participate written informed consent was obtained from them before participation.

To evaluate reliability, the test-retest method and internal consistency was used. The Gujarati FFI questionnaire was undertaken by 30 subjects. The subjects completed the relevant questionnaire twice with an interval of 48 hours to minimize any memory of previous answers and any variations in clinical status. Intra-class correlation coefficients (ICC) were used to determine test-retest reliability of the scores on three subscales of FFI and total FFI score. Internal consistency of FFI-G was determined by Cronbach's alpha coefficient.

STATISTICAL ANALYSIS

Statistical analysis was done with use of SPSS version 26. Mean and Standard Deviation (SD) were calculated as a measure of central tendency and measure of dispersion respectively. Intra-class correlation coefficients (ICC) were used to measure test-retest reliability and Cronbach's alpha coefficient was used to measure internal consistency.

RESULTS

Total 30 subjects took part in this study. Out of them there were 10 males and 20 females. Table 1 shows Mean age of them i.e., 40.35 ± 8.97 years. Table 2 and Table 3 shows Intra-Rater Reliability and Inter-Rater Reliability of FFI-G to be 0.996 and 0.995 respectively. Table 4 shows Internal Consistency of FFI-G as 0.927 for the pain subscale, 0.927 for the disability subscale and 0.818 for activity limitation subscale.

DISCUSSION

One in every five middle-aged person presents with foot complaints, and this may compromise walking, may cause balance impairment and a limitation in functional activities of daily living. Pathological conditions related to foot and ankle are under evaluation by healthcare professionals and researchers by using self-reported outcome instruments. Use of these instruments make it possible to get reliable measurements for patients' perceptions, and specific instruments have been standardized in order to take follow up and evaluate the effects of a given intervention. [11,14] In the present study, we reported the reliability of Gujarati version of Foot Function Index Questionnaire. It has been shown to be a reliable (as seen in table 2& 3) and internally consistent (as seen in table 4) self-administered questionnaire for assessment of Gujarati patients with foot complaints. Additionally, this





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questionnaire can be completed in short time so FFI-G was accepted by the people having foot complaints due to foot or ankle impairment.

The results of the present study are consistent with the study of Wu SH et al. He conducted a study to test the reliability and validity of the Taiwan Chinese version of the FFI among patients with plantar fasciitis and ankle/foot fracture. The internal consistency was evaluated by Cronbach's α and it was mostly good for total and subscales, with the exception of the activity limitation subscale. The ICC ranged from 0.74 (pain subscale) to 0.88 (activity limitation subscale), indicating satisfactory test-retest reliability. And it was concluded that the adapted Taiwan Chinese version of the FFI is reliable and valid and can be applied among traumatic and non-traumatic foot disorders.[15] Similarly, In Tae-Sung et al., did research to establish the reliability and validity of the Foot Function Index translated into Korean for use in patients with plantar fasciitis and foot/ankle fracture. 36 subjects with foot complaints participated for that study and test-retest reliability was found to be 0.90 for the pain subscale, and 0.94 and 0.91 for the disability and activity limitation subscales, respectively. The criterion-related validity was established by comparison with the Korean version of the Short Form-36 and Visual Analog Scale. This suggested that Korean version of FFI was a reliable and valid instrument for assessing foot complaints.[6]

The limitation of the present study was associated with the small sample size as the small sample size used is not representative of the entire population of Gujarati people with foot complaints. In future study can be done with larger sample size.

CONCLUSION

The Gujarati version of the Foot Function Index (FFI-G) is a reliable instrument to assess clinical severity in patients having complaints related to foot pathologies and treatments pertaining to foot and ankle problems. Its use is recommended for clinical as well as research purposes.

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Table 1: Demographic data

| Gender distribution | Age (Mean ± SD) |
|----------------------|--------------------|
| Male- 10, Female- 20 | 40.35 ± 8.97 years |

Table 2: Intra-Rater Reliability of FFI-G

| Reliability and test – retest analysis for the Gujarati version of FFI | | | |
|--|------------------|-------|-----------|
| Reliability | Cronbach's Alpha | ICC | p – value |
| Total | 0.996 | 0.996 | 0.00 |

Table 3: Inter-Rater Reliability of FFI-G

| Reliability and test – retest analysis for the Gujarati version of FFI | | | |
|--|------------------|-------|-----------|
| Reliability | Cronbach's Alpha | ICC | p – value |
| Total | 0.995 | 0.995 | 0.00 |

Table 4: Internal Consistency of FFI-G

| Internal Consistency | | | |
|----------------------|-------|------------|---------------------|
| Cronbach's Alpha | Pain | Disability | Activity limitation |
| | 0.927 | 0.927 | 0.818 |





Gas Flooding for Enhanced Oil Recovery with Special Emphasis on Injection of Nitrogen Gas: A Critical Review

Abhishek Kumar Prasad¹, Angshuman Buragohain¹, Anusree Deb¹, Arfaaz Masoom Ahmed¹, Sidhartha Das¹, Jakob Choudhury¹, Sharahnan Puzari¹ and Prasenjit Talukdar^{2*}

¹Student, Department of Petroleum Engineering, DUIET, Dibrugarh University, Assam, India

²Associate Professor, DUIET, Dibrugarh University, Assam, India.

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*Address for Correspondence

Prasenjit Talukdar

Associate Professor,

DUIET, Dibrugarh University,

Assam, India.

E.Mail: prasenjit_duiet@dibru.ac.in



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ABSTRACT

Gas flooding is a widely used technique for enhanced oil recovery (EOR), and nitrogen gas injection has gained significant attention due to its potential advantages. This critical review provides a comprehensive analysis of the key aspects of nitrogen gas injection for EOR applications, including its mechanism of action, advantages, limitations, and challenges. The study identifies several factors that impact the success of nitrogen gas injection, including reservoir properties, injection design, and operating conditions. The review also discusses various techniques and strategies that can be used to mitigate the limitations and challenges associated with nitrogen gas injection. The study concludes that nitrogen gas injection can be an effective EOR method, but its success depends on several factors, and further research is needed to optimize the injection parameters and improve the understanding of the mechanisms of nitrogen gas injection in the reservoir. This critical review provides valuable insights for researchers and industry professionals seeking to explore the potential of nitrogen gas injection for EOR applications.

Keywords: Gas Flooding, Enhanced Oil Recovery, Nitrogen Gas Injection, Viscosity Reduction, Reservoir Properties, Injection Design, Operating Conditions





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INTRODUCTION

In order to improve the recovery of hydrocarbons from reservoirs, the oil and gas sector frequently uses the gas flooding technique. Nitrogen gas has drawn a lot of interest among the several gases used for this because of its distinct physical and chemical characteristics. It has been demonstrated that nitrogen gas injection increases oil recovery by lowering reservoir pressure, displacing oil, and increasing sweep effectiveness. To assess the efficacy of nitrogen gas flooding under various reservoir conditions, a significant amount of experimental and numerical research has been done during the past few decades. However, a thorough critical analysis of the body of research on this subject is still lacking. With a focus on nitrogen gas injection, this review paper seeks to offer a thorough overview of the state of the art in gas flooding research. The basic tenets of nitrogen gas flooding, its benefits and drawbacks, and the many mechanisms that enhance its efficiency in oil recovery will all be covered in this essay. The paper will also emphasise the difficulties and potential future directions in this field while summarising recent improvements and developments in nitrogen gas injection techniques. Overall, the potential of nitrogen gas injection for improving oil recovery in various reservoirs will be critically assessed in this research study. (Alagorni, Yaacob, and Nour 2015a)

Reservoir Screening Criteria for the Injection of Nitrogen Gas

Enhancing oil recovery (EOR) frequently employs nitrogen injection to maximise oil recovery from reservoirs. An essential first step in figuring out whether nitrogen injection is the best EOR method for a certain reservoir is reservoir screening. The following are some of the screening parameters that are frequently used to determine if nitrogen injection in EOR is feasible:

1. Reservoir characteristics: For nitrogen injection to be efficient, the reservoir needs to have adequate permeability and porosity as well as a sufficient pressure and temperature range. Additionally, the reservoir's capacity to store nitrogen should be sufficient to maintain pressure and enhance oil recovery.
2. Oil type: Nitrogen injection often works best with heavy oils because they have a high viscosity and are challenging to make conventionally. It works well for light oils with low API gravities as well.
3. Oil saturation of the reservoir: Since nitrogen injection works best at displacing oil already present in the reservoir, it should have a relatively high oil saturation.
4. Heterogeneity of the reservoir: Nitrogen injection works better in homogenous reservoirs. In heterogeneous reservoirs, oil tends to accumulate in low-permeability zones while nitrogen tends to move through high-permeability zones.
5. Formation damage: The displacement of reservoir fluids caused by nitrogen injection might result in formation damage. Before beginning nitrogen injection, the reservoir's vulnerability to harm should be assessed.
6. Fluid compatibility: The reservoir fluids should be able to withstand the addition of nitrogen without experiencing any negative effects.
7. Cost-effectiveness: To ascertain whether the method is commercially viable, the cost of nitrogen injection should be contrasted with the incremental oil recovery.

Light oils at high pressures and deep reservoirs are required to establish nitrogen miscibility, while steeply sloping reservoirs provide gravity stabilisation. J.V. Taber and others identify the characteristics of rock and reservoir fluid that might be affected by the use of nitrogen, such as API gravity, viscosity, composition, formation type, permeability, transmissibility, temperature, depth, and thickness. (Mogensen and Xu 2020a)

Enhanced Oil Recovery by Inert Gas Injection

The amount of oil that can be collected from a reservoir is increased using enhanced oil recovery (EOR) techniques beyond what is possible with conventional production techniques. One EOR method includes pumping an inert gas into the reservoir, such as nitrogen or carbon dioxide, in order to increase oil recovery. By lowering oil viscosity,



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enhancing gas injection sweep efficiency, and maintaining reservoir pressure, this technology can remove oil from locations that are challenging to access using standard oil production techniques. Maximum recovery efficiency can only be attained by carefully analysing the characteristics of the reservoir and optimising the injection parameters. Inert gas injection is particularly successful in reservoirs containing heavy oil. (Vazquez et al. 2014a).

Laboratory Evaluation of the effect of Pressure and Induced Fractures

The efficiency of nitrogen injection for enhanced oil recovery (EOR) is evaluated in a laboratory setting by simulating actual oil recovery conditions and evaluating core samples from the reservoir. The impact of pressure and caused fractures on nitrogen injection are two aspects that can be assessed in the lab. When determining whether nitrogen injection during EOR is effective, pressure is a crucial factor. To imitate the pressure range in the reservoir during laboratory testing, the pressure can be changed. The outcomes can then be utilised to determine the ideal nitrogen injection pressure to employ in the field. By lowering oil viscosity and raising oil saturation, it has been discovered that increasing pressure can enhance oil recovery.

Another element that can be examined in the lab is induced fractures. Hydraulic fracturing or other techniques can cause fractures in the core samples. The success of the method for enhancing oil recovery can then be evaluated by injecting nitrogen into the samples. To maximise the efficiency of nitrogen injection in the reservoir, the data can be utilised to calculate the ideal fracture design, including the spacing and orientation of the cracks. The impact of nitrogen content, injection rate, and reservoir temperature on oil recovery can all be studied in the lab in addition to pressure and caused fractures. The results of these tests can be used to improve the nitrogen injection process's field-based design.

In general, laboratory testing of nitrogen injection for enhanced oil recovery can give important insights into how well the method works in a particular reservoir. Researchers can optimise the nitrogen injection procedure to maximise oil recovery while minimising costs and any environmental implications by testing different settings and circumstances. Nitrogen has emerged as a viable substitute for carbon dioxide for injection into hydrocarbon reserves around the world to improve subsurface energy recovery. However, the underlying mechanisms of recovery following N₂ injection into fractured reservoirs, which account for a significant fraction of the world's oil and gas reserves, are unknown. The study investigated that efficiency of N₂ injection for increased oil recovery under low and high differential pressures across the core. The findings suggest that N₂ injection in non-fractured cores can reach an eventual oil recovery factor of more than 40% even under immiscible conditions. Molecular diffusion across the fracture-matrix interface is the primary recovery process in fractured media, controlling system dynamics both before and after breakthroughs. The findings not only shed light on how differential pressures and fractures influence the efficacy of N₂ flooding, but they also demonstrate that it can be effective even in immiscible situations. (Fahandezhsaadi et al. 2019b)

Nitrogen injection for enhanced oil recovery

Enhancing oil recovery (EOR) by injecting nitrogen is a method for getting more oil out of reservoirs. In order to decrease the viscosity of the oil, increase its mobility, and shift it towards the production well, nitrogen, an inert gas, is injected into the reservoir. Nitrogen injection works by lowering oil viscosity and increasing mobility, which makes it simpler to dispense oil in the direction of the production well. Additionally, nitrogen provides pressure support, assisting in maintaining reservoir pressure and preventing the rock in the reservoir from collapsing. Nitrogen injection is superior to alternative EOR approaches in a number of ways. It is a reasonably inexpensive technique that requires little modification to be used in already-existing wells. Additionally, nitrogen injection can be used to recover a sizable amount of oil from the reservoir, frequently up to 20–30% of the initial oil present. Nitrogen injection works best in reservoirs with heavy oils or those that have a high gas-to-oil ratio. For nitrogen injection, reservoirs with good permeability and porosity are also appropriate. To determine whether the reservoir is suitable for nitrogen injection, it should be assessed. Nitrogen injection rate should be optimised to ensure that the most oil is recovered at the lowest possible cost. A technique's efficiency can be decreased by channelling, which occurs when nitrogen bypasses the oil due to excessive injection rates. Despite the fact that nitrogen is an inert gas, care must be



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taken when handling and injecting it to avoid mishaps. To stop the accumulation of nitrogen gas in enclosed places, appropriate ventilation and safety measures should be taken. (Vazquez et al. 2014).

Nitrogen immiscible flooding: Gas cap displacement

A gas flooding technique called nitrogen immiscible flooding involves introducing nitrogen gas into an oil reservoir to displace the oil and enhance its recovery. Through injection wells, nitrogen gas is injected into the reservoir during this procedure to form a gas cap on the reservoir's top. The oil is subsequently displaced downhill towards producing wells where it can be recovered by the gas injection. Gas cap displacement, which happens when the injected nitrogen gas forces the oil down into the reservoir and displaces it towards the production wells, is one of the main mechanisms of nitrogen immiscible flooding. In order to properly displace the oil downward, the nitrogen gas cap that is formed at the reservoir's top works as a barrier to stop the oil from rising to the surface. In reservoirs with a high gas-to-oil ratio, where the nitrogen gas may quickly displace the oil due to its low viscosity, gas cap displacement is particularly effective. Furthermore, nitrogen gas displacement efficiency can be increased by injecting it quickly. This aids in maintaining the gas cap and enhances the downward displacement of the oil. However, there are other variables, such as reservoir characteristics, fluid properties, and injection parameters, that can affect how well nitrogen immiscible flooding works. Depending on the unique reservoir conditions, the ideal injection rate, injection pressure, and amount of nitrogen gas needed for displacement will change. In reservoirs with high gas-to-oil ratios, nitrogen immiscible flooding with gas cap displacement is a viable strategy for improving oil recovery. To achieve the highest recovery efficiency, however, detailed analysis of reservoir properties and optimisation of injection settings are essential. (Vazquez et al. 2014a).

Nitrogen miscibility displacement mechanism

There are three types of miscibility including first contact miscibility, multi-contact miscibility, vaporizing mass-transfer miscibility (Li et al. 2013). Multi-contact miscibility: Miscible flood processes involve component transfer from the displaced oil to the injected fluid and from the injected fluid to the oil. Some hydrocarbon gases with a high fraction of intermediate molecular weight components (C_3 , C_4 , and C_5) are miscible with oil, resulting in a transition zone in which both fluids are miscible. This type of miscibility is called multiple-contact miscibility and is subdivided into vaporizing gas drive, condensing gas drive, and gas drive. (Heucke and Ag 2015). Vaporising gas drive: The vaporisation of intermediary components from reservoir oil to injected gas creates a miscible transition zone, which can be removed by infusing natural gas, flue gas, or nitrogen at high pressure. Nitrogen injected at high pressure can generate a miscible slug that facilitates the release of oil from reservoir rock. (Hu et al. 2018). Gravity drainage is a technique used to increase down dip displacement and production of reservoir fluids. It involves extracting oil down-dip and injecting gas up-dip, preventing gas fingering and achieving piston-like displacement. (L. Zhang et al. 2017)

The effect of nitrogen injection duration on the Gas Recovery Factor

In a gas flooding operation, the length of the nitrogen injection is a crucial variable that can have a big impact on the gas recovery factor at the initial gas-water contact. The volume of gas generated to the volume of gas initially present is referred to as the gas recovery factor. The reservoir gas is displaced by the nitrogen gas that is injected during gas flooding, and the amount of this displacement depends on how long the injection lasts. The displacement of reservoir gas increases with injection duration and the gas recovery factor rises. To balance the increased cost of injection with the enhanced gas recovery, the injection period should be optimised. Shorter injection times typically don't yield significant gas recovery because the injected gas can't effectively displace the reservoir gas. On the other side, longer injection times may lead to greater gas recovery, but at a higher cost due to the additional nitrogen gas that must be injected. By carefully analysing the features of the reservoir and optimising the injection parameters, an ideal nitrogen injection period can produce a large amount of gas recovery. The ideal injection duration can be influenced by elements like reservoir permeability, heterogeneity, and initial gas saturation.

Hydrocarbon fields in the final stage of development are characterized by selective water flooding of productive layers and wells under conditions of low reservoir pressures. Water flooding of highly permeable layers causes a



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decrease in well productivity due to the accumulation of a gas-liquid mixture at the bottom-hole. An optimum duration of nitrogen injection into productive reservoirs has been established, with an ultimate gas recovery factor of 58.11%. The technological effectiveness of injecting nitrogen into productive reservoirs is used in order to lower the movement of reservoir water into gas-saturated horizons. (Matkivskyi and Kondrat 2021) .

Effect of CO₂/N₂ Mixture Composition on Interfacial Tension of Crude Oil

Injection gas for enhanced oil recovery (EOR) and enhanced gas recovery (EGR) applications frequently uses nitrogen in the oil and gas sector. The ability of nitrogen injection to lessen the interfacial tension between crude oil and reservoir rock is one of the primary elements influencing its efficacy. The oil is held to the rock surface by the interfacial tension, which also inhibits the oil from flowing freely. By changing the wet ability of the rock surface and facilitating easier oil flow, the injection of nitrogen gas into the reservoir can lessen this tension. The interfacial tension can also be influenced by the nitrogen mixture's composition employed in the injection operation. Different nitrogen mixtures have unique characteristics that may affect how well they reduce interfacial tension. As opposed to pure nitrogen gas, nitrogen that has been combined with a surfactant can more efficiently lower interfacial tension. Laboratory tests and numerical simulations can be used to establish how well the nitrogen mixture's composition reduces interfacial tension. The injection strategy can then be optimised using this information, which will also increase the effectiveness of the EOR and EGR processes. (Gajbhiye 2020)

Natural gas displacement in Consolidated Rocks and Injection Velocity

The oil and gas industry frequently employs nitrogen injection as an enhanced gas recovery (EGR) technique to boost natural gas recovery from reservoirs. Using this method, nitrogen gas is injected into the reservoir to push the natural gas towards the production wells. In consolidated rocks with poor permeability and porosity, nitrogen injection is very successful. The efficiency of recovery can be improved by injecting nitrogen gas into these formations, which can lead to fractures and allow trapped natural gas to escape. Another crucial element in the success of the EGR process is the nitrogen gas injection velocity. High injection velocities can aid in the formation of cracks and improve nitrogen and natural gas interaction, hence enhancing sweep and displacement efficiencies. To avoid gas injection obstructing natural gas, the injection velocity must be properly optimised. An effective method for improving gas recovery in cemented rock formations is nitrogen injection. The EGR process's success depends heavily on the injection velocity and process optimisation. The efficiency and economics of recovering natural gas from reservoirs may be improved with more study and development of this method. (Mohammed et al. 2020)

The Gas–Oil Interfacial Behaviour during Gas Injection into an Asphaltenic Oil Reservoir

The performance of gas injection as an enhanced oil recovery (EOR) approach depends in large part on the behaviour of the gas-oil interfacial during gas injection into an asphaltenic oil reservoir. High molecular weight substances called asphaltenes are present in crude oil and can present considerable difficulties during gas injection. The presence of asphaltenes has an impact on the behaviour of the gas-oil interfacial when gas is injected into an asphaltenic oil reservoir. Asphaltenes have the ability to bind to the gas-oil contact, changing the system's surface tension and interfacial characteristics. As a result, there may be less contact between the injected gas and the oil, which could limit the gas injection's ability to thin the oil's viscosity and improve oil recovery. The gas-oil interfacial behaviour during gas injection into an asphaltenic oil reservoir can be improved using a variety of ways. Utilising surfactants to alter the interfacial tension between the gas and oil is one method. Surfactants can reduce asphaltene deposition and increase the reservoir rock's wettability, improving the interface between the gas and oil that is being injected.

Utilising solvent injection in addition to gas injection is an alternative strategy. The interaction between the injected gas and the oil can be improved by solvent injection, which can aid in dissolving the asphaltenes and reducing their adsorption onto the gas-oil interface. (Escrochi, Mehranbod, and Ayatollahi 2013)



**Abhishek Kumar Prasad et al.,****Increased methane and water production by Nitrogen Injection**

Nitrogen injection is a technology used in the oil and gas sector to increase production rates. As the nitrogen gas injection helps to maintain reservoir pressure, it pushes hydrocarbons away from the reservoir and towards the production well, it can also increase methane and water production. Lowering the viscosity of the oil and enhancing the sweep efficiency of the injected gas, nitrogen injection can aid in the recovery of methane that was lost during primary and secondary recovery processes. Additionally, nitrogen gas injection can aid in the production of water by releasing trapped water from the reservoir. This can boost production rates by lowering the hydrostatic pressure and facilitating easier flow of the hydrocarbons. The efficiency of nitrogen injection in boosting the production of methane and water depends on a number of variables, including the features of the reservoir, the fluid properties, and the injection settings. Depending on the unique reservoir conditions, the ideal injection rate, injection pressure, and amount of nitrogen needed for displacement will change. (Kang et al. 2019)

Effect of Nanoparticle-Stabilized Nitrogen Foam on EOR

A relatively new method called nanoparticle-stabilized nitrogen foam (NSNF) is being researched for its potential to improve oil recovery. In order to displace oil and boost oil recovery, NSNF involves mixing nanoparticles with nitrogen gas to produce a stable foam that can be injected into oil reservoirs. Nanoparticles can considerably increase the stability of nitrogen foam and lessen gas phase coalescence and drainage. As a result, the oil may be displaced from the reservoir more effectively by increasing the contact between the gas and oil.

Additionally, the interactions between the nanoparticles and the reservoir rock may change its wettability and lessen the capillary forces that hold the oil in place. This may increase how much oil is displaced by the injected foam. The type and concentration of nanoparticles, the quality of the foam, and the characteristics of the reservoir are some of the variables that affect how efficient NSNF is at improving oil recovery. Laboratory tests and computer simulations have produced encouraging results, with NSNF greatly increasing oil recovery when compared to traditional nitrogen foam. Nitrogen foam stabilised with nanoparticles has a lot of potential for use in improved oil recovery. Oil displacement and oil recovery from oil reservoirs may be increased by the improved foam stability and interaction with the reservoir rock. To completely assess NSNF's efficacy and optimise its use in EOR procedures, additional research and field tests are required. (Abdelaal, Gajbhiye, and Al-Shehri 2020)

The effect of N₂ gas for the exploitation of natural gas hydrates

A developing technology that has the potential to become a significant supply of natural gas is the use of natural gas hydrates. However, a significant obstacle that restricts their use is the dissociation of petrol hydrates. Nitrogen gas (N₂) can be utilised as an external guest to replace the gas molecules trapped in the hydrate structure as well as an inhibitor to stop the dissociation of gas hydrates. To replace the methane gas molecules trapped in the hydrate structure, N₂ gas can be introduced into the sediments that contain hydrates. The methane gas can be released during this displacement process, making it possible to extract natural gas from hydrate reserves more effectively. N₂ gas can also serve as a dissociation inhibitor for gas hydrates. The hydrate structure becomes more stable and less prone to dissociation when N₂ gas is fed into the hydrate reservoir. This is accomplished by lowering the pressure and temperature of the hydrate structure. This may offer a risk-free and effective method of using gas hydrate resources. The parameters of the reservoir, the pace of injection, and the concentration of N₂ gas are only a few of the variables that affect how well N₂ gas works to extract natural gas from hydrates. To make sure the approach is effective, careful monitoring and optimisation of the injection strategy are required. The use of N₂ gas could be crucial in the development of natural gas hydrates. It acts as an inhibitor to stop the dissociation of gas hydrates and provide a more stable reservoir for the extraction of natural gas. It can operate as an external guest and swap out the trapped gas molecules in the hydrate structure, making it possible to extract natural gas from hydrate reservoirs more effectively. (Mok, Choi, and Seo 2021)



**Abhishek Kumar Prasad et al.,****METHODS OF NITROGEN INJECTION****Miscible gas injection simulations in compositional reservoirs: A new upscaling approach**

A typical enhanced oil recovery (EOR) method used to boost hydrocarbon recovery from reservoirs is miscible gas injection. Due to the presence of numerous components and their interactions, simulating this process in compositional reservoirs is a challenging challenge. As a result, a fresh upscaling strategy has been created to simulate the injection of miscible gas into compositional reservoirs. According to their physical and chemical characteristics, such as molecular weight and phase behaviour, the reservoir's constituent parts are divided up into various clusters in the novel upscaling method. Then, single-component pseudo-components are used to represent the groups, accurately simulating the behaviour of the cluster's actual components. As a result, the compositional model is reduced, making it simpler to simulate and offering precise predictions of the behaviour of the original reservoir. Miscible gas injection simulations in compositional reservoirs can be carried out more quickly and precisely using the novel upscaling method. The streamlined compositional model captures the fundamental physics of the process, such as component interaction and phase behaviour, while lessening the computational cost of the simulation. This strategy can also aid in designing the injection strategy and optimising the injection parameters for optimum recovery effectiveness. (Rios et al. 2019)

Immiscible nitrogen injection: A challenging experience on depleted naturally fractured reservoir

Nitrogen gas is injected into the wellbore using the immiscible nitrogen injection method, which is utilised in the oil and gas sector to increase production rates. Applying this method to depleted, naturally fractured reservoirs, however, can be difficult. Natural fractures and high permeability in these reservoirs can make it so that injected nitrogen gas skips over the oil-bearing zones, leading to ineffective sweep efficiency and low oil recovery rates. The low viscosity of the gas, which might make it challenging to displace the heavier hydrocarbons, exacerbates this issue. Numerous approaches have been developed to address these issues, such as raising the injection pressure to boost sweep effectiveness and using foam or surfactants to lower gas mobility and boost displacement effectiveness. Advanced reservoir characterization methods and numerical simulation models can also be used to enhance recovery effectiveness and optimise the injection strategy. (Vazquez et al. 2014a)

Nitrogen Foam for Viscous Oil Recovery

For enhanced oil recovery, or EOR, the oil and gas sector uses nitrogen foam. In order to improve the displacement of viscous oil from the reservoir, this technique includes pumping a mixture of nitrogen gas and surfactants into the reservoir. This creates a stable foam. Nitrogen foam functions by making the gas being injected less mobile, increasing the stability of the foam and enhancing its capacity to remove heavy oil. The foam also aids in boosting the injected gas's sweep efficiency, ensuring that it contacts a larger portion of the reservoir's surface area and expels more oil. The oil will be able to flow more easily towards the production wells if the foam is able to lessen the capillary forces that keep it imprisoned in the reservoir. Nitrogen foam is very useful for recovering oil that is difficult to remove using traditional production techniques, such as heavy oil or bitumen. The method has been effectively used in both onshore and offshore fields, as well as a range of reservoir types. (Abdelaal, Gajbhiye, and Al-Shehri 2020)

Continuous N₂ injection to improve light oil recovery in multi-wells fractured-cavity unit

An approach that can be used to enhance the recovery of light oil in multi-well fractured-cavity units is continuous nitrogen (N₂) injection. Gravity segregation and viscous fingering, which can cause the injected gas to channel and the oil in the reservoir to be bypassed, are two factors that frequently limit oil recovery in these sorts of reservoirs. By maintaining a steady pressure gradient and encouraging improved sweep efficiency of the injected gas, continuous N₂ injection can aid in mitigating these problems. The overall recovery of the light oil is increased because the N₂ gas pushes the oil out of the pore spaces. N₂ injection can also aid to promote pressure distribution throughout the reservoir and communication between the wells in multi-well fractured-cavity systems. This may lead to improved overall recovery and more effective use of the gas that was injected. The reservoir characteristics, injection rate, and gas composition are only a few of the variables that affect how successfully continuous N₂ injection improves light oil recovery in multi-well fractured-cavity systems. To make sure the approach is effective, careful monitoring and





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optimisation of the injection strategy are required. A possible method for enhancing light oil recovery in multi-well fractured-cavity systems is continuous N₂ injection. The method can improve overall sweep efficiency and improve communication between the wells while reducing gravity segregation and viscous fingering. (W. Su et al. 2017)

New approach of alternating Thickened–Nonthickened Gas Flooding

An enhanced oil recovery (EOR) method called alternate thickened-nonthickened gas flooding includes alternating the injection of thickened and nonthickened gas into an oil reservoir. By lowering gas mobility and raising sweep efficiency, this approach seeks to increase gas displacement efficiency and improve oil recovery. The employment of thickened and nonthickened gas in the same injection cycle is a novel method for this technology. As the injection cycle begins, the mixture is intended to have a high viscosity, which lowers gas mobility and improves sweep efficiency. The mixture's viscosity decreases during the injection process, enabling more effective gas displacement and oil recovery. Increased displacement efficiency, enhanced sweep efficiency, and lower operating costs are just a few advantages that might result from combining thickened and nonthickened gas during the same injection cycle. Additionally, the method may lessen the need for thickening agents during the EOR process, which could be advantageous for the environment. With encouraging results for enhanced oil recovery, this strategy has been researched in lab tests and computer simulations. To completely assess the efficacy of this technology and optimise its use in EOR processes, more research and field tests are required. The novel method of alternately flooding thickened-nonthickened gas holds promise as a powerful method for improving oil recovery. When thickened and nonthickened gas are combined during the same injection cycle, displacement and sweep efficiency can be increased while operating costs and environmental impact are minimised. (AI Hinai et al. 2018)

ADVANTAGES AND DISADVANTAGES OF NITROGEN INJECTION

Advantages of Nitrogen Injection

A common enhanced oil recovery (EOR) technique is nitrogen gas injection, which involves injecting nitrogen gas into an oil reservoir to increase oil recovery. The following are some benefits of employing nitrogen gas for EOR: Nitrogen is the best EOR technique for many reservoirs due to its lower cost than CO₂ and high injectivity. In low permeability reservoirs, nitrogen has strong injectivity and is most attractive both technical and economical solution. Compared to other gases used in EOR, such as carbon dioxide, nitrogen is easily available and very affordable. Nitrogen is a safe choice for use in the manufacture of oil because it is non-toxic and inflammable. Nitrogen may dissolve in oil and lessen its viscosity, which makes it simpler to recover. This property makes nitrogen miscible with oil. Nitrogen gas may be injected into reservoirs at high rates thanks to its strong injectivity, which enhances oil recovery. When compared to other EOR methods that use chemicals or hazardous gases, the use of nitrogen gas has a lower environmental impact. (Cheng et al. 2020) (Jha and Chakma 1991) (Fahandezhsaadi et al. 2019)

Disadvantages of Nitrogen Injection

While nitrogen gas injection is a technique that has many benefits, it also has some drawbacks. Nitrogen is only partially soluble in crude oil, which may limit its ability to thin oil and enhance oil recovery. Compared to other gases used in EOR, such as carbon dioxide, nitrogen gas has a low density. Lower oil recovery rates and decreased sweep efficiency may result from this. Nitrogen gas injection is typically less effective in heterogeneous reservoirs because these environments have a wide range of fluid and geological properties. High nitrogen purity requirements can raise operating expenses because they are necessary for effective nitrogen gas injection. Nitrogen injection necessitates specialized equipment, such as nitrogen generators and compressors, which can raise the cost and complexity of the EOR process. Although nitrogen gas injection as an EOR technique has a number of benefits, it also has some drawbacks that should be carefully considered before use. The precise reservoir conditions, as well as the accessibility of the necessary tools and resources, may have an impact on how well nitrogen gas injection works. (Mogensen and Xu 2020)



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PROBLEMS AND SOLUTIONS OF NITROGEN INJECTION

Problems of Nitrogen Injection

Miscible process suffers from poor mobility due to variations in density and viscosity between injected fluid and reservoir fluid, leading to viscous fingering and gravity override. Transportation of the miscible flooding agent, corrosion of equipment and tubing, and separation and recycling of the miscible flooding agent are all operational issues with miscible processes. Enhanced oil recovery (EOR) using nitrogen gas injection is a technique used to increase the amount of oil that can be extracted from a reservoir. Nitrogen gas is expensive to produce and transport, making it an expensive option for EOR. It has a low solubility in oil, which can lead to poor displacement efficiency and limited oil recovery. Moreover, it tends to sweep through the reservoir quickly, which can lead to poor mobility control and channeling. If nitrogen gas is not injected properly, it can migrate to production wells and cause nitrogen breakthrough, resulting in reduced oil recovery.

Solutions of the problems associated with Nitrogen Injection

Optimizing the injection rate and pressure, it is possible to improve the displacement efficiency of nitrogen gas and reduce the cost of EOR. Combining nitrogen gas with other gases, such as carbon dioxide or methane, can improve the solubility of nitrogen gas and increase oil recovery. Surfactants can be used to improve the solubility and mobility control of nitrogen gas, which can lead to better oil recovery. Proper injection design can help to minimize nitrogen breakthrough and maximize oil recovery. This may include the use of horizontal wells, perforated liners, and other techniques. (Latil 1980) (Memon, Elraies, and Al-Mossawy 2017) (Doe 1928) Nitrogen increase in residue gas poses two problems: It is no longer sealable and too poor to be used as fuel. (Pendleton n.d.). When the displaced and displacing fluids have a significant density difference, gravitational forces acting in the transverse direction may produce separation of the two fluids.

CONCLUSION

Gas flooding has been widely studied and utilized as an effective technique for enhanced oil recovery. Among the various gases used for EOR applications, nitrogen gas injection has gained significant attention due to its potential advantages such as low cost, non-toxicity, and availability. This critical review has provided a comprehensive analysis of the key aspects of nitrogen gas injection, including its mechanism of action, advantages, limitations, and challenges. The primary mechanism of nitrogen gas injection is the reduction of oil viscosity, which results in improved mobility and displacement efficiency. However, nitrogen gas also faces several challenges such as low solubility in oil, limited mobility control, and nitrogen breakthrough, which can hinder its effectiveness. The review also identified several factors that impact the success of nitrogen gas injection, including reservoir properties, injection design, and operating conditions. Various techniques and strategies can be used to mitigate the limitations and challenges associated with nitrogen gas injection. For instance, the optimization of injection rate and pressure, the use of surfactants, and the combination of nitrogen gas with other gases can improve the solubility and mobility control of nitrogen gas and increase oil recovery. Overall, this review provides valuable insights into the potential of nitrogen gas injection as an EOR method. It is evident that while nitrogen gas injection can be an effective technique, its success depends on several factors, and optimization of the injection parameters is critical. Further research is necessary to fully understand the mechanisms of nitrogen gas injection and to develop strategies to overcome the challenges associated with this technique. It also highlights the importance of a systematic approach in the evaluation of gas flooding techniques for EOR applications, and the need for further research to optimize the injection parameters and improve the understanding of the mechanisms of nitrogen gas injection in the reservoir. This study provides a significant contribution to the body of knowledge on gas flooding, and it is hoped that it will encourage further research in this field.





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Mycobiota Associated with Roots of Ashwagandha (*Withania somnifera* (L.) Dunal.) during Storage Deteriorate the Quality of Roots as a Herbal Drug

P.S. Kabnoorkar^{1*}, V. N. Patil² and A. A. Naik³

¹Assistant Professor, Department of Botany, Arts, Science and Commerce College, Indapur-413106 Dist. Pune (MS) India.

²Assistant Professor, Department of Botany, Vidyabharti College, Seloo Dist. Wardha - 442104 (MS) India.

³Assistant Professor, Department of Botany, Savitribai Phule Pune University, Pune- 411007, (MS) India.

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*Address for Correspondence

P.S. Kabnoorkar

Assistant Professor,
Department of Botany,
Arts, Science and Commerce College,
Indapur-413106 Dist. Pune (MS) India.
E.Mail: pkabnoorkar@gmail.com



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ABSTRACT

The quality of *Ashwagandha* roots in the most of cases is not fully assured because of mycobial association. The collection and storage of *Ashwagandha* roots are of traditional type (The roots are piled in heaps, under a shelter or they spread on floor or packed in gunny bags) which leads to the association of fungi with them. In the present study, total 45 fungi were isolated from *Ashwagandha* roots. *A. parasiticus*, *A. flavus*, *A. niger*, *P. expansum*, *Rh. stolonifer*, *Rh. oryzae*, *Ch. spirale*, *T. harzianum*, *Rhizoctonia bataticola*, *Paecilomyces* sp., *F. oxysporum* and *A. terreus* found in higher % incidence. Higher relative humidity favours the maximum association of fungi. In addition some of these moulds also produce toxic metabolites. The most prominent toxin is aflatoxin which is known to cause hazards to the liver, digestive, nervous system, respiratory organs. Total 80 market samples of *Ashwagandha* roots were tested for aflatoxin contamination. Qualitative analysis and confirmation of Aflatoxin B₁, B₂, G₁ & G₂ were analyzed with the help of HPTLC technique.

Keywords – *Ashwagandha*, roots, fungi, percentage incidence, relative humidity





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INTRODUCTION

Botanical name : *Withania somnifera*(Linn.) Dunal

Synonyms : *Withania ashwagandha* Kaul (Khare,2007) *Physalis flexuosa* Linn. (Anonymous,2007, Chopra et al. 1980)

Family : Solanaceae

Vernacular Names (Anonymous, 2007, Chopra et al.,1980, Kirtikar&Basu, 1980, Khare 2007, Uddin et al., 2012).

Arabic :Kaknaj-e-Hindi

Bengali :Ashvaganda, Asvagandha

English : Winter cherry

Gujarati :Asan, Asana, Asoda, Asundha,Ghodaasoda

Hindi : Asgandh, Punir

Malayalam : Amukkiram, Pevetti

Marathi : Askandha, Kanchuki, Tilli

Odiya : Asugandha

Persian : Kaknaj-e-Hindi, AsgandNagaori

Sanskrit : Ashvagandha, Ashvakandika, Gandhapatri, Palashaparni

Tamil :Amukkira, Asubam, Asuvagandi

Telugu :Asvagandhi, Penneru, Pennerugadda,Dommadolu

Urdu :Asgand, AsgandNagori

Ayurvedic- Ashwagandhaa, Hayagandhaa, Ashwakanda, Gandharvagandhaa, Turaga, Turagagandhaa, Turangagandhaa, Vaajigandhaa, Gokarnaa, Vrishaa, Varaahakarni,Varadaa, Balyaa, Vaajikari(A substitute for Kaakoli and Kshirakaakoli)

Cultivated var.: AsgandhNaagori. (Indian botanists consider the cultivated plants distinct from the wild ones.)

Unani-Asgandh.

Siddha -Amukkuramkizhangu.

Geographical Source

Ashwagandha found in the drier parts of India, Sri Lanka, Afghanistan, Baluchistan and Sind and is distributed in the Mediterranean regions, the Canaries and Cape of Good Hope. It is found in high altitude ascending to 5,500 feet in the Himalayas. This shrub is common in Bombay and Western India, occasionally met with in Bengal. It grows wildly throughout India particularly in hotter parts, on waste places and on road sides. It is also cultivated for medicinal purposes in fields and open grounds throughout India. It is widely cultivated in Bikaner and Pilani areas of Rajasthan, Rajputana, Punjab and Manasa (M.P.) (Anonymous, 2007, Chopra et al., 1980, Dey et al., 1973, Dymock et al., 1976, Kirtikar & Basu, 1980, Nadkarni, 1982, Uddin et al., 2012). Bhopal in Madhyapradesh where the best variety of Ashwagandha is obtained, which is called 'Nagori Ashwagandha' (Shalini et al., 2017).

MORPHOLOGY (Fig. - 1) -

A small or middle sized undershrub upto 1.5 m high. Stem and branches erected with minute star-shaped hairs. Leaves upto 10 cm long, ovate, hairy like branches. Flowers pale green, small, about 1 cm long, few flowers borne together in short axillary clusters. Fruit 6mm diameter, globose smooth red enclosed in the inflated and membranous calyx (Jain, 1996)

Part Used – Roots

Young roots are straight, unbranched and conical and in pieces of different lengths. When dry are cylindrical, gradually tapering down, straight, unbranched, Root thickness varies according to age and usually it is 5–12 mm



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below crown. Outer surface is buff to yellow and longitudinally wrinkled. Interior surface is creamy white when broken. They have a short and uneven fracture, a strong odour and mucilaginous bitter and acrid taste (Shah and Seth, 2010, Anonymous, 1982).

Chemical Constituents (Anonymous, 1982, Chopra *et al.*, 1980, Kirtikar & Basu, 1980, Khare 2007, Nadkarni, 1982, Shah and Seth, 2010) –

The root contains several alkaloids, including withanine, withananine, withananinine, pseudo-withanine, somnine, somniferine, somniferinine.

Withanine - Sedative, hypnotic. Withaferin A—major component of biologically active steroids; as effective as hydrocortisone dose for dose. Withaferin A is antitumour, antiarthritic and antibacterial. Anti-inflammatory activity has been attributed to biologically active steroids, of which withaferin A is a major component. The root extract contains an ingredient which has GABA mimetic activity. The free amino acids present in the root include aspartic acid, glycine, tyrosine, alanine, proline, tryptophan, glutamic acid and cystine. The leaves of Indian Chemotype contain 12 withanolides, including withaferin A. Steroidal lactones of withanolide series have been isolated.

Therapeutic Uses (Khare 2007, Nadkarni, 1982, Shah and Seth, 2010) –

Ashwagandha is one of the most widespread tranquillizers used in India, where it holds a position of importance similar to ginseng in China. It acts mainly on the reproductive and nervous systems, having a rejuvenative effect on the body, and is used to improve vitality and aid recovery after chronic illness. It is also used to treat nervous exhaustion, debility, insomnia, wasting diseases, failure to thrive in children, impotence, infertility; multiple sclerosis, etc.

Root - Used as an anti-inflammatory drug for swellings, tumours, scrofula and rheumatism; and as a sedative and hypnotic in anxiety neurosis.

Leaf- Anti-inflammatory, hepatoprotective, antibacterial.

Fruits and seeds - Diuretic.

Important Ayurvedic Formulations (Nadkarni, 1982) -

Ashwagandha Kashayam

Ashwagandha Ghrita

Important Unani formulations containing Asgand are as follows (Anonymous, 2007) :

1. Habbe Asgand
2. Kushta Gaodanti
3. Majoon Salab
4. Majoon Zanjabeel

Marketed Products (Shah and Seth, 2010)–

It is one of the ingredients of the preparations known as Abana, Geriforte, Mentat, Mentat syrup, Reosto, Tentex forte, AntiStress Massage Oil, Nourishing Baby Oil, Nourishing Skin Cream, Anxocare, Galactin Vet, Geriforte Aqua, Geriforte Vet, Immunol, Speman forte Vet, Tentex forte Vet, Ashwagandha tablet (Himalaya Drug Company), Balarishta (Baidyanath), Ashwagandha tablet (BAPS AMRUT). From review of literature it is revealed that Ashwagandha has a immense potential medicinal value & it used from ancient time to treat various disease and to restore the vitality. Roots of Ashwagandha are not stored properly due to faulty storage condition roots may contaminate with fungi. The unscientific methods of harvesting, collection, storage of raw materials, processing and poor storage of herbal drugs, retailed in market openly in unhygienic conditions, are the main causes considered to

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make both, raw materials as well as herbal drugs prone to fungal infections (Essono *et al.*, 2007). The fungal contaminates has been reported to affect the chemical composition of the raw material and thereby, decreases the medicinal potency of the herbal drugs (Roy, 2003, Amal& Al-juraifani, 2011, Aziz *et al.*, 1998, Bungo *et al.*, 2006, Chunyan *et al.*, 2018, Dutta and Roy, 1987, Elshafie *et al.*, 2002, Govender *et al.*, 2006, Hedawoo and Chakranaravan, 2011, Katerere *et al.*, 2008, Mandeel, 2005, Moorthy *et al.*, 2010, Roy and Chourasia 1989,1990a,1990b, Rizzo *et al.*, 2004, Bais *et al.*,2015). There is a possibility of loss of efficacy of raw materials by microorganisms during harvesting, handling and storage (Dubey *et al.*, 2008, Sareen *et al.*, 2010, Truckesses *et al.*, 2008, Takatori *et al.*, 1977,). WHO reports various adverse events may arise from the use of products contaminated with potentially hazardous substances, such as toxic metabolites, pathogenic microorganisms (WHO, 2007& 2004). Therefore, it has been decided to study the fungal association and deterioration of quality of roots of Ashwagandha during storage under the influence of different relative humidity.

MATERIAL AND METHODS

The roots of 'Ashwagandha' were collected from different storehouse/ kashataushdis of Pune. It was brought to the laboratory in separate polyethylene bags to avoid aerial contamination.

Isolation of Mycoflora

Blotter test method, as recommended by International Seed Testing Association (1966) was adopted for isolation of fungi. Agar plate method and surface washing methods were also used. The roots were sterilized with 2% NaOCl solution before plating.

The % incidence of fungi was calculated by using the following formula –

$$\frac{\text{No. of colonies of a particular species}}{\text{Total no. of colonies of all the species}} \times 100$$

Effect of Relative Humidities on Fungal Growth

Test samples were weighed properly and stored in small muslin cloth bags under different relative humidities i.e. 33%, 55%, 75%, 96% RH. Humidities were maintained in sterilized glass desiccators by preparing saturated salt solution of Mg Cl₂ 6H₂O, Mg (NO₃)₂, NaCl and KNO₃ (Wink and Sears, 1950) and by using Environmental Test Chamber (Temperature and % RH Controller). On interval of every 15 days samples were taken out thoroughly washed with 100 ml sterilized glass distilled water to remove the fungal mycelium. The fungal suspension obtained after washing was centrifuged to get residue. The same was diluted by adding 2 ml of sterilized distilled water and inoculated on agar plate. In each case percentage of fungal incidence was recorded.

Analysis of plant samples for aflatoxin contamination

Samples of plant parts used as a drug were analyzed chemically for natural contamination of aflatoxin using the method of Thomas *et al.* (1975) & (Reddy *et al.* 1970).

RESULTS AND DISCUSSION

Data depicted in Table -1 showed total 45 fungi associated with roots of Ashwagandha. Out of these 45 fungi *A. flavus*, *A. niger*, *Rhizoctonia bataticola* observed in higher percentage incidence (15%) followed by *A. oryzae*, *Penicillium expansum*, *Rhizopus stolonifer* (12%). During storage 248 colonies associated with roots which spoil the roots severely and deteriorate the quality of roots as a drug.



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Similar results found by Garg, (2017), Dubey, (2008 &2014), Efuntoye, 2004, Gautam & Bhadauria, (2009 & 2011), Meena *et al.*, 2010. Kneifel *et al.*, 2002, salik *et al.*, 2006. From all these findings revealed that fungal contamination in herbal raw material and products is a serious problem.

The *Withania* roots stored under different relative humidities (33%, 55%, 75%, 96% RH) and calculated the percentage incidence of fungi in each relative humidity (Table -2). Chourasia, (1995), Roy, (2003), Dubey *et al.*, 2004 and Mirsa, (1981), Kabnoorkar & Deokule (2009) also recorded the similar results i.e. role of temperature and relative humidity influence the growth of fungi in crude drugs. Ozay *et al.*, 2008 study revealed Factors influencing fungal and aflatoxin levels in Turkish hazelnuts (*Corylus avellana* L.) during growth, harvest, drying and storage. Maximum association of fungi on roots of *W. somnifera* recorded under the 75 &96% RH while minimum % incidence observed under 55% RH. During the storage period i.e. 90days it was observed the main fungi that produce aflatoxins are *A. parasiticus* and *A. flavus* commonly associated with roots of Ashwagandha. And if such drug material, gets utilized for preparation of medicine/ medicinal products as per the indigenous systems of medicine, then there may be loss of potency and ultimately it may lead to the loss in the authenticity and its efficacy on drugs or drug formulations Roy, (1989).

Ashwagandha roots were tested for aflatoxin contamination and it was observed that out of 80 samples (*Withania*) 15 samples showed positive for BGYF test. Qualitative analysis and confirmation of Aflatoxin B₁, B₂, G₁ & G₂ were analyzed with the help of HPTLC technique and it was found that 4 samples of *Withania* showed the presence of aflatoxin (Table -3) and it was observed that level of aflatoxin was within the safe limit as per guideline of WHO. Aflatoxin contamination of Ashwagandha root is a considerable problem, which poses a risk to human health.

CONCLUSIONS

The fungi isolated from roots of '*W. somnifera*' in various % incidence spoils the roots severely and ultimately affect the quality and efficacy of the therapeutic drug. During the storage (1 to 90 days and 33 to 96% RH) from observations it was observed that 75, 96 % RH showed the significant incidence of fungi on '*W. somnifera*' roots. The roots stored at lower RH showed minimum incidence of fungi. Storage conditions of post-harvest are the main factors causes the fungal association and aflatoxin contamination in crude drugs. The maximum storage period is also responsible for the maximum association of fungi. In addition some of these moulds also produce toxic metabolites (aflatoxin) that usually get associated with roots.

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Table 1– List of fungi with their % incidence of fungi associated with *W. somnifera*

| Sr. No. | Name of fungal species | No. of colonies | % incidence of fungi |
|---------|---|-----------------|----------------------|
| 1 | <i>A. parasiticus</i> Spear | 6 | 2.41±0.48 |
| 2 | <i>A. fumigates</i> Fries | 8 | 3.22±0.48 |
| 3 | <i>A. japonicas</i> Saito | 4 | 1.61±0.48 |
| 4 | <i>A. fonsecaeus</i> Thom & Raper | 5 | 2.01±0.48 |
| 5 | <i>A. thomii</i> Smith | 5 | 2.01±0.48 |
| 6 | <i>A. terreus</i> Thom | 10 | 4.03±0.48 |
| 7 | <i>A. nidulans</i> Winter | 3 | 1.2±0.48 |
| 8 | <i>A. tamariki</i> Kita | 4 | 1.61±0.48 |
| 9 | <i>A. niger</i> Van Tiegh | 15 | 6.04±0.63 |
| 10 | <i>A. oryzae</i> (Ahlburg) Chon | 12 | 4.83±0.48 |
| 11 | <i>A. ochraceus</i> Wilhelm | 2 | 0.8±0.48 |
| 12 | <i>A. flavus</i> Link | 15 | 6.04±0.63 |
| 13 | <i>A. clavato-nanica</i> Batista Maia & Alecrim | 2 | 0.8±0.48 |
| 14 | <i>Alternaria chlamydospora</i> Mouchacca | 4 | 1.61±0.48 |
| 15 | <i>A. infectoria</i> Fucks | 2 | 0.8±0.48 |
| 16 | <i>Curvularia lunata</i> Boedijn | 2 | 0.8±0.48 |
| 17 | <i>Ch. atterimum</i> Ellis & Everhart | 3 | 1.2±0.48 |
| 18 | <i>Ch. amberpetens</i> Reddy | 2 | 0.8±0.48 |
| 19 | <i>Ch. confusum</i> Van Warmelo | 1 | 0.4±0.48 |
| 20 | <i>Ch. olivaceum</i> Cooke & Ellis | 2 | 0.8±0.48 |
| 21 | <i>Ch. elatum</i> Kunze ex Fries | 2 | 0.8±0.48 |
| 22 | <i>Ch. globosum</i> Kunze ex Fries | 8 | 3.22±0.48 |
| 23 | <i>Chaetomium</i> sp. | 3 | 1.2±0.48 |
| 24 | <i>Ch. tortile</i> Bainier | 2 | 0.8±0.48 |
| 25 | <i>Ch. spirale</i> Zopf | 10 | 4.03±0.63 |
| 26 | <i>Ch. aurangabadense</i> Tilak & Reddy | 2 | 0.8±0.48 |
| 27 | <i>Ch. cuneatum</i> Sorgel | 2 | 0.8±0.48 |
| 28 | <i>Cladosporium oxysporium</i> Berk & Curt | 4 | 1.61±0.48 |
| 29 | <i>F. oxysporum</i> Schlechtendahl ex Fries | 4 | 1.61±0.48 |
| 34 | <i>F. solani</i> Appel & Wollenweber | 3 | 1.2±0.48 |
| 31 | <i>Memnoniella</i> sp. | 2 | 0.8±8.9 |
| 32 | <i>Microascus</i> sp. | 3 | 1.2±0.48 |
| 33 | <i>Nigrospora sphaerica</i> Mason | 1 | 0.4±6.3 |
| 34 | <i>P. expansum</i> Link | 12 | 4.83±0.48 |
| 38 | <i>P. digitatum</i> Sacc | 6 | 2.41±0.48 |





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|-----------------------|---|-----------------------------|---|
| 36 | <i>P. purpurogenum</i> Stoll | 4 | 1.61±0.48 |
| 37 | <i>P. chrysogenum</i> Thom | 4 | 1.61±0.48 |
| 38 | <i>Paecilomyces</i> sp. | 10 | 4.03±0.48 |
| 39 | <i>Rh. stolonifer</i> Ehrenberg | 12 | 4.83±0.48 |
| 38 | <i>Thielaviaterricola</i> | 4 | 1.61±0.48 |
| 41 | <i>Rhizoctoniabaticola</i> (Tamb.) Butler | 15 | 6.04±0.68 |
| 42 | <i>T. harzianum</i> Rifai | 10 | 4.03±0.63 |
| 43 | <i>Torulasp.</i> | 4 | 1.61±0.48 |
| 44 | <i>Rh. oryzae</i> Went and Gerlings | 10 | 4.03±0.78 |
| 45 | <i>Non sporulating dematiaceous form</i> | 4 | 1.61±0.48 |
| Total fungal sp. = 45 | | Total no. of colonies = 248 | Total % incidence of fungi = 99.74±2.05 |

Table - 2 : Percentage incidence of fungal organism on *Withaniasomnifera* stored at various relative humidity.

| Mycoflora | Con t. | 33 % RH | | | 55 % RH | | | 75 % RH | | | 96 % RH | | |
|--------------------------|--------|---------|--------|--------|---------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|--------------|
| | | 3 0 | 6 0 | 9 0 | 3 0 | 60 | 90 | 30 | 60 | 90 | 30 | 60 | 90 |
| <i>A. parasiticus</i> | - | - | - | - | - | 0.54± 0.5 | 0.81± 0.6 | 0.54± 0.5 | 1.09± 0.7 | 1.63± 0.9 | 1.36± 0.8 | 1.91±1 | 2.73±1 |
| <i>A. fumigatus</i> | - | - | - | - | - | - | - | 0.54± 0.5 | 0.81± 0.6 | 1.36± 0.8 | 0.81± 0.6 | 1.63± 0.9 | 2.45±1 |
| <i>A. japonicus</i> | - | - | - | - | - | - | - | - | - | - | 0.27± 0.3 | 0.54± 0.5 | 1.09± 0.7 |
| <i>A. fonsecaeus</i> | - | - | - | - | - | - | - | 0.27± 0.3 | 0.54± 0.5 | 0.81± 0.6 | 0.54± 0.5 | 0.81± 0.6 | 1.63± 0.9 |
| <i>A. thomii</i> | - | - | - | - | - | - | - | 0.27± 0.3 | 0.54± 0.5 | 0.81± 0.6 | 0.81± 0.6 | 1.09± 0.7 | 1.91±1 |
| <i>A. terreus</i> | - | - | - | - | - | - | 0.27± 0.3 | 0.81± 0.6 | 1.09± 0.7 | 1.63±0 .9 | 0.81±0 .6 | 1.36±0 8 | 2.73±1 |
| <i>A. nidulans</i> | - | - | - | - | - | - | - | - | - | 0.27±0 .3 | 0.27±0 .3 | 1.09±0 7 | 1.36±0 .8 |
| <i>A. tamari</i> | - | - | - | - | - | - | - | - | - | 0.27±0 .3 | 0.54±0 .5 | 1.09±0 7 | 1.63±0 .9 |
| <i>A. niger</i> | - | - | - | - | - | - | - | 0.81±0. 6 | 1.91±1 | 2.45±1 | 1.36±0 .8 | 2.73±1 | 5.46±1 |
| <i>A. oryzae</i> | - | - | - | - | - | 0.54±0 .5 | 0.81±0 .6 | 0.81±0. 6 | 1.36±0 .8 | 2.45±1 | 0.81±0 .6 | 1.36±0 8 | 3.27±1 |
| <i>A. ochraceous</i> | - | - | - | - | - | 0.27±0 .3 | 0.54±0 .5 | 0.27±0. 3 | 0.54±0 .5 | 0.54±0 .5 | 0.27±0 .3 | 0.54±0. 5 | 0.54±0 .5 |
| <i>A. flavus</i> | - | - | - | - | - | 0.54±0 .5 | 0.54±0 .5 | 0.81±0. 6 | 1.36±0 .8 | 1.63±0 .9 | 1.36±0 .8 | 2.45±1 | 3.82±1 |
| <i>A. clavato-nanica</i> | - | - | - | - | - | - | - | - | - | - | - | 0.27±0. 3 | 0.54±0 .5 |





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|---------------------------------|---|---|---|---|---|---|---|----------|----------|----------|----------|----------|----------|
| <i>Alternariachlamydo spore</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.81±0.6 | 1.63±0.9 | 2.18±1 |
| <i>A. infectoria</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.81±0.6 | 1.36±0.8 |
| <i>Curvularialunata</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.54±0.5 | 0.54±0.5 | 1.09±0.7 |
| <i>Ch. aterrimum</i> | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 1.09±0.7 |
| <i>Ch. amberpetens</i> | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.81±0.6 | 0.81±0.6 |
| <i>Ch. confusum</i> | - | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 |
| <i>Ch. olivaceum</i> | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 1.09±0.7 |
| <i>Ch. elatum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 1.36±0.8 |
| <i>Ch. globosum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.81±0.6 | 1.63±0.9 | 2.73±1 |
| <i>Chaetomiumsp.</i> | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 1.09±0.7 |
| <i>Ch. tortile</i> | - | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 |
| <i>Ch. spirale</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.81±0.6 | 1.63±0.9 | 2.18±1 |
| <i>Ch. aurangabadense</i> | - | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 1.09±0.7 |
| <i>Ch. cuneatum</i> | - | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.81±0.6 | 1.36±0.8 |
| <i>Cladosporium oxysporium</i> | - | - | - | - | - | - | - | 0.54±0.5 | 1.09±0.7 | 1.36±0.8 | 0.81±0.6 | 2.18±1 | 3±1 |
| <i>F. oxysporum</i> | - | - | - | - | - | - | - | 0.54±0.5 | 1.36±0.8 | 2.45±1 | 0.81±0.6 | 1.63±0.9 | 4.09±1 |
| <i>F. solani</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 1.36±0.8 | 0.81±0.6 | 1.36±0.8 | 3.55±1 |
| <i>Memnoniella sp.</i> | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 1.36±0.8 | 2.45±1 |
| <i>Microascus sp.</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 1.09±0.7 | 0.81±0.6 | 1.36±0.8 | 2.18±1 |
| <i>Nigrosporas phaerica</i> | - | - | - | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 1.09±0.7 |
| <i>P. expansum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.81±0.6 | 1.36±0.8 | 0.81±0.6 | 2.18±1 | 3.82±1 |
| <i>P. digitatum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 0.27±0.3 | 0.81±0.6 | 1.91±1 |
| <i>P. purpurogenum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 0.81±0.6 | 1.63±0.8 |
| <i>P. chrysogenum</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 0.81±0.6 | 1.09±0.7 | 2.18±1 |



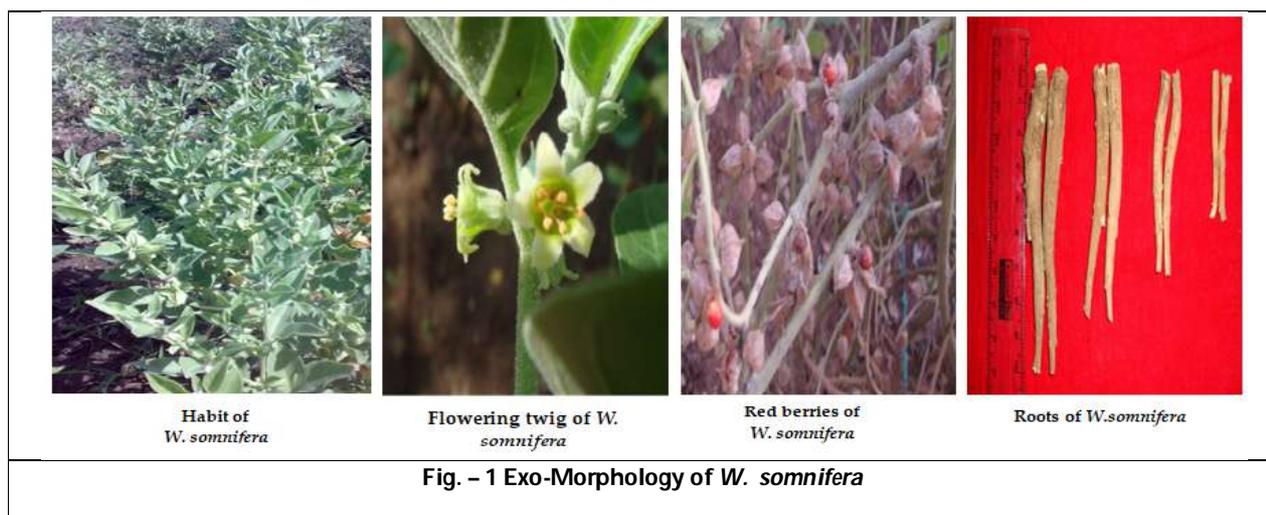


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| | | | | | | | | | | | | | |
|--|---|---|---|---|---|----------|----------|-----------|----------|----------|----------|-----------|---------|
| <i>Paecilomyces sp.</i> | - | - | - | - | - | - | - | 0.81±0.6 | 1.63±0.9 | 2.45±1 | 1.09±0.7 | 2.18±1 | 3.82±1 |
| <i>Rh. stolonifer</i> | - | - | - | - | - | 0.54±0.5 | 0.81±0.6 | 0.81±0.6 | 2.18±1 | 3.55±1 | 1.36±0.8 | 2.73±1 | 4.64±1 |
| <i>Thielaviaterricola</i> | - | - | - | - | - | - | - | 0.54±0.5 | 1.09±0.7 | 1.36±0.8 | 0.81±0.6 | 1.91±1 | 2.45±1 |
| <i>Rhizoctonia bataticola</i> | - | - | - | - | - | - | - | 0.81±0.6 | 1.91±1 | 3.27±1 | 1.09±0.7 | 2.73±0.5 | 4.09±1 |
| <i>T. harzianum</i> | - | - | - | - | - | - | - | 0.54±0.5 | 1.09±0.7 | 1.36±0.8 | 0.81±0.6 | 2.45±0.3 | 3±1 |
| <i>Torulasp.</i> | - | - | - | - | - | - | - | | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 1.09±0.3 | 1.91±1 |
| <i>Rh. oryzae</i> | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.54±0.5 | 1.36±0.8 | 2.45±1 | 1.09±0.7 | 1.91±0.3 | 3.82±1 |
| <i>Non sporulating dematiaceous form</i> | - | - | - | - | - | - | - | 0.27±0.3 | 0.54±0.5 | 0.81±0.6 | 0.54±0.5 | 1.09±0.3 | 1.91±1 |
| % Incidence of fungi | - | - | - | - | - | 2.7±1.2 | 4.32±1.5 | 13.78±2.8 | 27.97±4 | 43.5±5 | 34.04±4 | 58.46±1.2 | 99.75±7 |

Table - 3. Aflatoxin HPTLC Analysis Report

| Sr. No. | Name of Sample | Aflatoxin µg/gm | | | |
|---------|---------------------|-----------------|----------------|----------------|----------------|
| | | B ₁ | B ₂ | G ₁ | G ₂ |
| 1. | <i>W. somnifera</i> | | | | |
| | 1 sample | | | | 0.0009 |
| | 2 sample | | | 0.0023 | |
| | 3 sample | | | | 0.0008 |
| | 4 sample | | 0.0019 | 0.0022 | 0.0014 |





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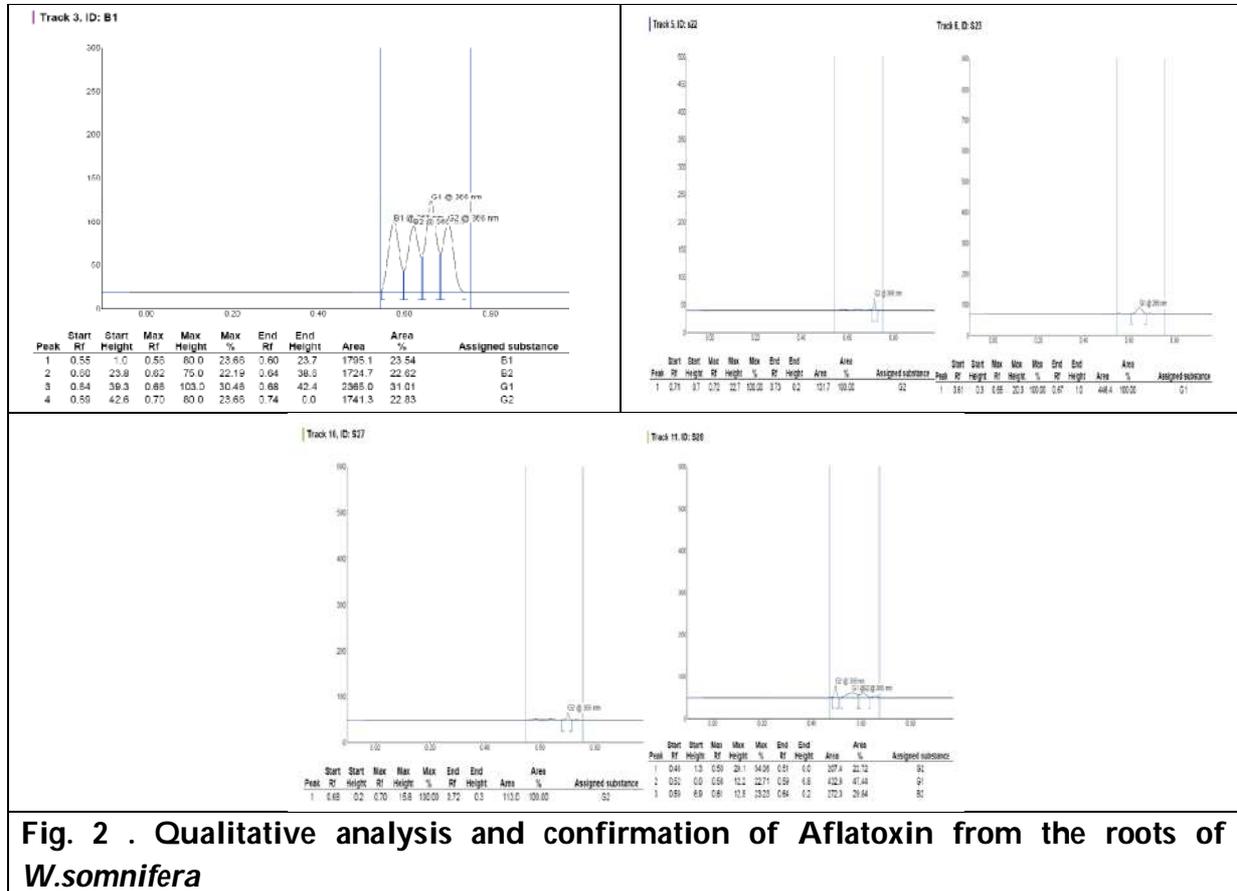


Fig. 2 . Qualitative analysis and confirmation of Aflatoxin from the roots of *W.somnifera*





Pharmacological Evaluation of Anti-Asthmatic Activity of *Azima tetracantha* Lam. Leaves Ethanolic Extracts in Mice

Jeethuri Rajesh¹, Rayadurgam Naveen^{2*}, Vattikutti Uma Maheshwara Rao³, Chilamakuru Naresh Babu⁴, Sunkara Sivananda Swaroop⁵ and Annadurai Gowtham⁵

¹M.Pharmacy, Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research (RIPER) - Autonomous, Anantapur, Andhra Pradesh, India.

²Assistant Professor at Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Autonomous, Anantapur-515721, Andhra Pradesh, India.

³Associate Professor, Raghavendra Institute of Pharmaceutical Education and Research (RIPER)-Autonomous, Anantapur-515721, Andhra Pradesh, India.

⁴Department of Pharmaceutical Chemistry, Raghavendra Institute of Pharmaceutical Education and Research (RIPER) - Autonomous, Anantapur, Andhra Pradesh, India.

⁵Department of Pharmacology, Raghavendra Institute of Pharmaceutical Education and Research (RIPER) - Autonomous, Anantapur, Andhra Pradesh, India.

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*Address for Correspondence

Rayadurgam Naveen

Assistant Professor,

Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Autonomous, Anantapur-515721, Andhra Pradesh, India.

E.Mail: rayadurgamnaveen98@gmail.com



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ABSTRACT

Asthma is a chronic respiratory disease that is increasingly prevalent in India, causing recurrent symptoms like wheezing, breathlessness, chest tightness, and coughing. Severe cases of asthma require high-dose inhaled or systemic corticosteroids or may remain uncontrolled despite treatment. *Azima tetracantha* Lam., a medicinal plant found in Peninsular India, possesses anti-inflammatory, anti-arthritis, anticonvulsant, and other properties that have been traditionally used to treat asthma and other ailments. It includes the leaves are undergone the extraction process, the obtained extract was screened for various phytochemical contents, and Milk induced eosinophilia and leukocytosis were performed in mice. Phytochemical screening shows positive for all chemical constituents except Fats, EEAT in Milk induced leukocytosis includes EEAT 200mg/kg has asignificant effect in eosinophils and leukocytosis, when compared between test vs standard vs induced. This short study concludes that the leaves of *Azima tetracantha* Lam. have an effect of anti-asthmatic activity.

Keywords: eosinophilia, leukocytosis, *Azima tetracantha*, Phytochemical, extract, asthma.





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INTRODUCTION

Asthma is a prevalent chronic respiratory disease that affects millions of individuals worldwide [1], with a growing prevalence in India [2]. The disease is characterized by airway inflammation, bronchoconstriction, and increased airway responsiveness, leading to recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. The severity of asthma is assessed based on the frequency of symptoms, exacerbations, and functional impairment. Severe asthma is defined as asthma that requires high-dose inhaled corticosteroids or systemic corticosteroids or remains uncontrolled despite this therapy [3]. Various stimuli, including allergens and irritants, can trigger asthma, and genetic, environmental, and host factors can influence its development. The pathophysiology of asthma involves inflammatory cell infiltration of the airways, increased T-helper-2-type cytokines, and the release of thymic stromal lymphopoietin by epithelial cells [4]. Poorly controlled asthma can result in significant morbidity and mortality, and it is associated with increased healthcare costs and reduced quality of life.

Researchers are exploring traditional Indian medicinal systems, such as Ayurveda, Siddha, and Unani, for potential treatments for asthma. *Azima tetraacantha* Lam., also known as Needle bush or Sappanwood, is a medicinal plant found in Peninsular India, West Bengal, Orissa, Africa, and tropical Asia that is known for its therapeutic properties. It contains various chemical constituents, such as alkaloids, flavonoids, sterols, terpenoids, volatile oil, and fatty acids, and is used in traditional medicine to treat bronchitis, asthma, cough, fever, diabetes, hypertension, and snakebite [5]. Pharmacological studies have shown that *Azima tetraacantha* Lam has a variety of activities, including, anti-inflammatory [6], anti-arthritic, anticonvulsant [7], hepatoprotective [8], antimicrobial[9], cytotoxic and anticancer[10], antipyretic, and insecticidal activities. The plant also has antidiarrheal and antiulcer properties[11,12] and has been found to exhibit anti-quorum-sensing action. Further research is needed to fully understand the plant's pharmacological properties and potential therapeutic applications for asthma and other conditions

METHODS AND MATERIALS

Plant Collection and Authentication

The fresh leaves of *Azima Tetraacantha lam*were collected from the medicinal garden at Chalapathi Institute of pharmaceutical education, Campus at Guntur, Andhra Pradesh, and Authenticated by Sara Palaparthi, Msc.,(Ph.D.). Head of Department of Botany, Pithapur rajah's government college, Kakinada, Andhra Pradesh.

Preparation of Plant Extracts

The leaves were dried under shade and then placed in a hot air oven at 45°C for five minutes to ensure complete dryness. After that, the dried leaves were crushed into a fine powder using a mortar and pestle. The powdered mass was subjected to maceration using water and soxhlet extraction using ethanol as solvents. The resulting extract was concentrated and used to evaluate the phytochemicals and pharmacological properties [13].

Ethanolic extract (Soxhlet extraction)

Under aseptic conditions, the leaves were gathered, washed, and dried in the shade. The dried leaves were then ground into a powder using a grinder. Hot extraction was performed using 80% ethanol with the aid of the Soxhlet apparatus for 18 hours. The resulting extract was filtered and subjected to vacuum drying (delvac). The semi-solid extract was stored at -20°C for future use [14].



**Jeethuri Rajesh et al.,****Phytochemical Screening [15,16,17]****Test for Steroids****Liebermann - Burchard test**

A sample containing 4mg of extract was treated with 0.5ml each of acetic anhydride and acetic acid. Subsequently, concentrated sulphuric acid was gradually added, resulting in the observation of a blue-green color for terpenoids and a reddish-brown color for steroids.

Salkowski test

To test for the presence of terpenoids, a mixture was prepared by combining 5 ml of an extract with 2 ml of chloroform, and then carefully adding 3 ml of concentrated sulphuric acid to form a distinct layer. The formation of a reddish-blue coloration in the chloroform layer and a green fluorescence in the acid layer indicated a positive result for the presence of terpenoids

Test for Flavonoids**Shinoda Test**

After mixing pieces of magnesium ribbon and concentrated HCl with an aqueous crude plant extract, a pink coloration was observed after a few minutes, indicating the presence of flavonoids

Alkaline Reagent Test

After mixing the extract with 2 ml of a 2.0% NaOH solution, a concentrated yellow coloration was produced, which became colorless upon the addition of two drops of diluted acid. This observation indicates the presence of flavonoids.

Sulphuric Acid test

A portion of the extract was subjected to treatment with concentrated Sulphuric acid, and the formation of an orange color was observed.

Test for Tannins

Ferric chloride test: To examine certain compounds' presence, 0.5g of the dried powdered sample was boiled in 20 ml of water within a test tube and subsequently filtered. A few drops of a 0.1% FeCl₃ solution were added and observed for the formation of a brownish-green, black, or blue-black coloration.

Lead acetate test

To test for the presence of tannins, 2 ml of plant extract was mixed with 2 ml of distilled water, and 0.01 g of lead acetate was added to the solution and thoroughly mixed. The appearance of white turbidity and precipitate indicated the presence of tannins. To test for the presence of tannins, 10 ml of bromine water was added to the 0.5 g aqueous extract, and the bromine water was decolorized.

Test for Saponin

Foam test to test for the presence of saponins, approximately 2 g of the plant extract was mixed with 10 ml of distilled water and vigorously shaken to form a stable and persistent froth. The appearance of froth indicated the presence of saponins. To test for the presence of saponins, approximately 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath, and filtered, and 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously to form a stable and persistent froth. The frothing was then mixed with 3 drops of olive oil, shaken vigorously, and observed for the formation of an emulsion.

Test for Alkaloids**Mayer's Test**

To test for the presence of alkaloids, the filtrates were treated with Mayer's reagent (potassium mercuric iodide), and the formation of a yellow-colored precipitate indicated the presence of alkaloids.



**Jeethuri Rajesh et al.,****Wagner's Test**

To determine the presence of alkaloids, the filtrates were treated with Wagner's reagent (iodine in potassium iodide), and a brown or reddish precipitate formed was observed.

Dragondroff's Test

Filtrates were treated with Wagner's reagent (Potassium bismuth iodide). The formation of an orange-brown precipitate indicates the presence of alkaloids.

Hager's Test

Filtrates were treated with Wagner's reagent (Potassium mercuric iodide). The formation of a yellow precipitate indicates the presence of alkaloids.

Test for Phenols

Approximately 2 ml of the plant extract was mixed with water and heated to 45-50°C. Then, 2 ml of 0.3% FeCl₃ was added to the mixture. The formation of a green or blue color indicates the presence of phenols.

Test for Polysaccharides**Molisch's test**

test involves adding a few drops of 1% alcoholic naphthol to the extract, followed by the addition of concentrated sulfuric acid along the sides of the test tube. A violet ring or coloration at the interface between the two layers indicates the presence of carbohydrates.

Fehling's test

The reddish-orange precipitate obtained after treating the extract with 1 ml of Fehling's solution and heating it in a boiling water bath indicates the presence of reducing sugars such as glucose and fructose.

Benedict's test: To 0.5 ml of the extract, 0.5 ml of Benedict's reagent was added. The mixture was heated in a boiling water bath for 2 min. A characteristic red-colored precipitate indicates the presence of sugar.

Test for Proteins**Millon's reagent**

To the extracts, Million's reagent (mercury in nitric acid) was added. The formation of a white precipitate which turned red on heating indicates the presence of proteins.

Ninhydrin reagent

The extracts are heated with 5% Ninhydrin (in butanol) solution in a boiling water bath for 10 min and the development of purple or bluish color indicated the presence of amino acids.

Biuret test

To the extracts, 4% sodium hydroxide and 1% copper sulfatesolution are added and the formation of violet or pink color indicates the presence of proteins.

Test for Glycosides

Liebermann's Test: 2 ml of acetic acid and 2 ml of chloroform were added to the plant extract. The mixture was then cooled and then H₂SO₄ concentrated was added. The green color shows the entity of aglycone, a steroidal part of glycosides.





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Keller-Kiliani Test

A solution of glacial acetic acid (4.0 ml) with 1 drop of 2.0% FeCl₃ mixture was mixed with the 10 ml aqueous plant extract and 1 ml H₂SO₄ concentrated. A brown ring formed between the layers which showed the entity of cardiac steroidal glycosides.

Salkowski's Test

We added 2 ml H₂SO₄ concentrated to the whole aqueous plant crude extract. A reddish brown color formed which indicated the presence of steroidal aglycone part of the glycoside(15).

Legal's test

To the hydrolysate 1 ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution and observed for pink to red color.

Bomtrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. No color change in the ammonical layer was observed.

Pharmacological Screening

Toxicological studies

The herbal preparations of *Azima tetracantha lam* have been subjected to toxicological studies according to OECD guidelines. Studies were carried out using 25 Albino mice of both sexes weighing 25-30gm. They were obtained from the Chalapathi Institute of Pharmaceutical Sciences, Guntur. All animal experiments strictly complied with the approval of the Institutional Animal Ethics Committee (IAEC). The animals were grouped and housed in polyacrylic cages (38 cm x 23 cm x 10 cm) with not more than 5 animals per cage and maintained under standard laboratory conditions (temp, 25 ± 2°C) with dark and light cycles (12/12 hr). They were allowed free access to a standard dry pellet diet (Hindustan lever limited) and water ad libitum. The protocol was approved (Approval: 09/IAEC/CLPT/2020-21) Institutional by Animal Ethics Committee (Reg no: 1048/PO/Re/S/07/CPCSEA) constituted for animal experimentation as per CPCSEA guidelines. The mice were acclimatized to laboratory conditions for 10 days before the commencement of the experiment. A suspension of ethanolic extract(100,200 mg/kg) of the selected plant was formulated. Mortality, toxic signs, and behavioral changes were observed and recorded for up to 24 hours.

Groups

Each activity includes 5 groups with 5 animals in each group

Group I: Control group

Group II: Standard group

Group III: Inducing group

Group IV: Ethanolic Extract of *Azima tetracantha* (EEAT) (100 mg/kg)

Group V: Ethanolic Extract of *Azima tetracantha* (EEAT) (200 mg/kg)

Anti-asthmatic activity

Milk-Induced Eosinophilia and Leukocytosis

The control group was given distilled water (10 ml/kg, p.o.), whereas the standard group received boiled and cooled cow milk (4 ml/kg s.c.) with dexamethasone 0.5 mg/kg i.p.) as standard, test groups received boiled and cooled cow milk without standard treatment and EEAT(100 mg/kg p.o) and EEAT(200 mg/kg p.o.). After 24 h of milk administration, the blood samples were collected from the retro-orbital plexus under light ether anesthesia. Total eosinophils and leucocytecount were recorded in each group[18].

Determination of Eosinophil Count

Determining the eosinophil count manually from a blood smear slide involves examining the slide under a microscope and counting the number of eosinophils among the total number of white blood cells (WBCs) present in





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10 fields of view. The characteristic morphology of eosinophils, such as their large oval or bilobed nuclei and bright orange-red granules in the cytoplasm, is used to identify them. The eosinophil count is then calculated as a percentage of the total WBC count. However, manual counting methods can be time-consuming and subject to inter-observer variability, which may affect the accuracy and precision of the results. Therefore, automated methods are becoming more popular in clinical practice [19].

Determination of leukocytes

Determining leukocyte count manually involves examining a blood smear slide under a microscope and identifying different types of leukocytes based on their characteristic morphology. The number of each type of leukocyte, including neutrophils, lymphocytes, monocytes, eosinophils, and basophils, is then counted and the total leukocyte count is calculated. However, manual counting methods can be time-consuming and subject to inter-observer variability, which may affect the accuracy and precision of the results. Therefore, automated methods, such as flow cytometry, are becoming more popular in clinical practice[20].

Statistical analysis

The statistical approaches for the data generated were evaluated by the one-way ANOVA using multiple comparisons. The p-value was considered outcomes with p-values below 0.0001S considered to be significant[21].

RESULTS AND DISCUSSION

Phyto chemical screening

Phytochemical screening of *Azima tetraacantha lam* (Family: Salvadoraceae) has revealed the presence of alkaloids, steroids, flavonoids, tannins, saponins, phenols, carbohydrates, and glucosinolates as the major classes of phytoconstituents. The screening for preliminary phytochemicals to identify various chemical components of leaves in the ethanolic extract is depicted in Table No.1

Pharmacological Evaluation:

Milk-Induced Eosinophilia and Leucocytosis

Subcutaneous injection of milk at a dose of 4 mg/kg produced a significant increase in the eosinophilia, and leucocytosis counts after 24 hr of its administration. EEAT at dose 100, 200 mg/kg has inhibited milk-induced eosinophilia, and leucocytosis (***) $p < 0.0001$. The results are depicted in Table No.2

CONCLUSION

The study findings indicate that the ethanolic extract of *Azima tetraacantha* leaves at a dosage of 200mg/kg has shown a significant reduction in eosinophils and leukocyte count when compared to the control and standard. However, further research is required to isolate the active chemical constituent and develop appropriate formulations.

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Table.1: Phytochemical Screening of the Extract of leaves of Azima Tetracanth lam

| Phytoconstituents | Presence or Absence |
|--------------------------|---------------------|
| Alkaloids | + |
| Glucosinolates | + |
| Saponins | + |
| Steroids | + |
| Flavonoids | + |
| Phenols | + |
| Tannins | + |
| Fats | - |
| Proteins and amino acids | + |

Table .2: Effect of EEAT by milk- induced eosinophilia and leucocytosis method in Mice

| Groups | Eosinophils | Leucocytes |
|--------------|---------------|----------------|
| Control | 25.6±1.02 | 75.8±1.15 |
| Standard | 27±0.70**** | 77.6±0.74**** |
| Induced | 159±0.70 | 2203.6±14.60 |
| EEAT100mg/kg | 89.2±0.86**** | 1946±36.95**** |
| EEAT200mg/kg | 60±0.70**** | 1494±35.58**** |

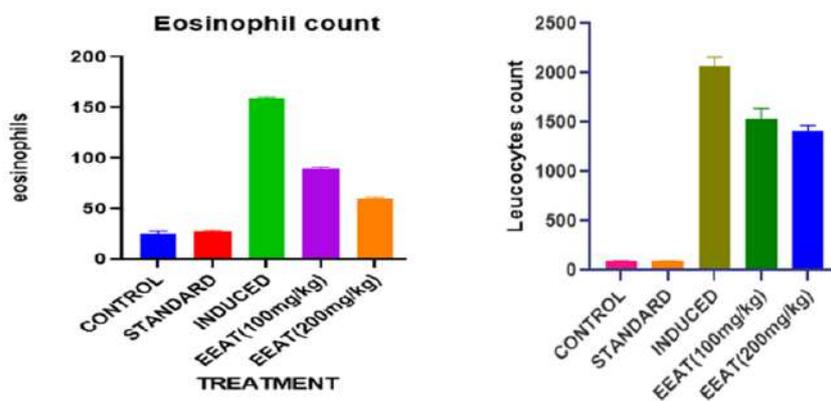


Figure 1: Eosinophil and Leucocytes Count in Milk-Induced Eosinophilia.





Covid-19: A Pandemic Trend

Diptimayee Jena^{1*}, Aman Kumar², Prashishya Bhatta³, Subhashree Sahoo⁴ and Kirtimaya Mishra⁵

¹Assistant Professor, ARKA JAIN University, Jamshedpur, Jharkhand -832108,India

²Project Scholar, ARKA JAIN University, Jamshedpur, Jharkhand -832108,India

³Research Scholar,S 'O' A University, Bhubaneswar, Odisha-751003, India.

⁴Assistant Professor, Usha Martin University, Ranchi, Jharkhand-835103, India.

⁵Professor, ARKA JAIN University, Jamshedpur, Jharkhand -832108,India.

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*Address for Correspondence

Diptimayee Jena

Assistant Professor,

ARKA JAIN University,

Jamshedpur,

Jharkhand -832108,India

E.Mail: diptijena72@gmail.com



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ABSTRACT

The COVID-19 pandemic, which threatens millions of people, is brought on by the novel coronavirus SARS-CoV2. Since humans lack protective immunity and the virus has the ability to evade innate immune responses, it can grow unchecked in tissues that are primarily infected. Following cell death, virus particles and intracellular components are released into the extracellular space, which prompts the activation of immune cells, the formation of immune complexes, and related harm. Infection Later in the course of the illness, the migration of neutrophils and macrophages and/or uninfected immune cells might lead to enormous inflammatory conditions. Pro-inflammatory mediators that are produced out of control are a factor in ARDS and cytokine storm syndrome. Identifying SARS-CoV2 effective defense techniques and the resulting delayed huge immunological response will make it possible to identify biomarkers that predict outcomes as well as phenotypic and disease stage specific therapies, which will likely comprise both Immune-modifying and antiviral therapies.

Keywords: Covid-19, SARS-Co V2, Inflammatory mediators, Macrophages, Cytokine storm syndrome, Immune cells, Biomarkers.



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INTRODUCTION

The coronavirus 2 (COVID-2) that causes serious chronic lung crisis has been connected to a new sickness known as coronavirus disaster 2019 (COVID-19) (SARS-CoV-2). In December 2019, in Wuhan, China, there occurred a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak which has spread to rest regions of the world[1]. Initially the respiratory epithelium may become infected by the virus, but this is likely just a portal of entry. Endothelial injury, which results in the release of cytokines and chemokines, the recruitment of immune cells, and the activation of coagulation and thrombosis, is what causes the important steps in alveolar damage. According to reports, individuals with severe corona virus disease 2019 (COVID-19) frequently have multiorgan damage, including cardiac injury and acute kidney injury. These events start in the lungs but may spread to other organs (AKI)[2-3]. It was just recently reported that 44% of the 701 sequential COVID-19 patients hospitalised in Wuhan had proteinuria, and roughly 27% of them had hematuria. Patients with elevated baseline serum creatinine or elevated baseline proteinuria exhibited a 2- and 5-fold higher risk of in-hospital death than COVID-19 patients with normal renal parameters, respectively, after adjusting for age, sex, illness severity, and leukocyte count[4]. Evidence for the strong relationship between immunological patterns and disease development in virus-infected people is growing. Patients with severe acute respiratory syndrome have a special trait known as a reduction in peripheral T cell subsets (SARS)[5]. Peripheral T cell number can be a reliable diagnostic test for SARS since it can be used to quickly restore peripheral T cell subsets in recovered individuals. Another study that revealed that SARS affected the immune system also described a similar occurrence. In a different investigation, it was discovered that Ebola patients had fewer natural killer (NK) cells than healthy donors. Proinflammatory cytokines increased after the onset of Ebola virus illness symptoms, but patients who had recovered showed low levels of these cytokines [6]. Immune features are now acknowledged as potential biomarkers for disease development as well as potential treatment targets for COVID-19 as the association between immune responses and the disease has been uncovered. The immunological characteristics of COVID-19 are outlined along with possible mechanisms of SARS-CoV-2-induced immune alterations, their impact on disease outcomes, and their implications for potential COVID-19 treatments [7].

Epidemiology

Since the first report from China, the illness has spread widely and cases have exponentially increased. The first case outside of mainland China was discovered on January 11 in Thailand, and within a few months, all continents with the exception of Antarctica had been infected [8]. On January 30, 2020, India announced the first COVID-19 case in the country. By February 3, 2020, there were three cases. In February 2020, there were no new cases reported. However, by the middle of March, the number of infected cases began to rise, and numerous cases from all over India were reported. On March 12, 2020, news of the first COVID-19-related death in India was released. All Indian states with the exception of Sikkim were affected by the disease by the second week of April [9].

Pathophysiology

The pathophysiology of tissue damage caused by COVID-19 is not well understood, but the therapeutic effect of steroids, which are used to stop progression, suggests that it is not primarily caused by viral infection but rather by the inflammatory host immune response, which results in hypercytokinemia and aggressive inflammation [10]. As pathophysiological characteristics with COVID-19 disease are shared a number of pre-existing illnesses or ailments are regarded as COVID-19 comorbidities. The SARS-CoV-2 infection enters the host cells through the S spike protein by attaching to ACE2 for internalisation and by the help of TMPRSS2 protease. The S spike protein's acquisition of a furin cleavage site and changes in the receptor binding domain are responsible for the virus's enhanced infectivity. In people who are susceptible, the virus' interaction with ACE2 may decrease the anti-inflammatory function and increase the effects of angiotensin II [11]. Given the difficulty in treating COVID-19 in individuals with hypertension, some have suggested using (or stopping using) ACE medications and angiotensin II receptor type 1 (AT1 receptor) blockers. Oedema, The pulmonary circulation experiences edoema, deterioration, and cytotoxic alterations as a result of the virus penetrating lung cells, myocytes, and endothelial cells. Interleukin (IL)-6, IL-10, tumour necrosis factor, granulocyte colony stimulating factor, monocyte, chemoattractant protein 1, macrophage inflammatory protein 1,



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and increased expression of programmed cell death 1, T-cell immunoglobulin, and mucin domain 3 (Tim-3) are the main factors contributing to these changes. These alterations contribute to intestinal and cardiopulmonary abnormalities, increased myocardial cell damage, hypoxia-related myocyte injury, body immunological response, and development of lung injury. Hypoxaemia has also been linked to SARS-CoV-2 infection. These modifications cause lactic acid buildup, oxygen free radical accumulation, intracellular pH changes, electrolyte modifications, and further cellular damage[12].

Figure 1: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) initially infects the lungs, where it replicates and causes cytolysis of alveolar cells and an inflammatory burst, diminishing oxygen uptake, but the virus can injure many other organs. Kidney dysfunction is common in patients with severe forms of COVID-19 and is an important risk factor for death. SARS-CoV-2 may bind directly to angiotensin converting enzyme 2 (ACE2) expressed on kidney cells, cause cell injury, and activate the inflammatory response and the complement cascade locally (green box). Kidney damage and dysfunction may also derive from drug nephrotoxicity or systemic events like poor blood oxygenation, lowered blood pressure, or cytokine storms (orange box). AKI, acute Kidney Injury.

Sign and Symptoms

Individuals with COVID-19 have reported experiencing a wide range of symptoms, from minor discomfort to serious sickness. 2 to 14 days after virus contact, symptoms may start to show. Everyone can experience minor to major symptoms. Clinical manifestations comprise cold or a fever, a cough, breathlessness or chest pain, tiredness, and muscular or body aches. Headache, loss of taste and smell, a painful throat, and messy or congested nose, vomiting or nauseating, diarrhoea.

Diagnosis

Chest CT scanning has been utilised as a supplementary method for early diagnosis and evaluation of disease development, and radiography may help corroborate clinical suspicion of COVID-19. Multiple bilateral ground-glass opacities in the peripheral lower lung zones on a CT scan are possible findings, and these are also present in patients with SARS-CoV and MERS-CoV infections [13]. A "positive" chest computed tomography for COVID-19 showed a responsiveness of 97% when using RT-PCR as a standard in 1,014 subjects in Wuhan, China, who underwent both RT-PCR testing and computed Tomographic scanning, whereas selectivity was only 25%[14-15]. Leukopenia, lymphopenia, raised aminotransaminase, elevated lactate dehydrogenase (LDH), and elevated inflammatory markers (such as ferritin, C-reactive protein, and erythrocyte sedimentation rate) are common laboratory results in COVID-19 patients[16].

Treatment

The majority of COVID-19 patients will be capable of recovering at home. Getting enough rest, drinking plenty of fluids, and taking drugs to reduce fever and ease aches and pains are some strategies that work for COVID-19 as well as the flu. Also, the FDA has approved other drugs to stop the progression of COVID-19 in persons who are not hospitalised but are at risk for developing severe illness as well as treatments that may be used for people who have been hospitalised with COVID-19.

Vaccine

Governments should keep working towards immunising at least 70% of their citizens, giving 100% of medical personnel and 100% of the most vulnerable citizens, such as those over 60, immunocompromised individuals, and persons with underlying medical issues, high priority. Some of the vaccines are discussed in the following table.

CONCLUSION

We figure out from this review that the COVID-19 disease profile is vibrant and is still rapidly changing. There are still a lot of COVID-19-related unanswered questions. Our review of the literature reveals that, in contrast to





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subclinical infections that present with positive imaging results on CT, patients with declared COVID-19 infection occasionally have no chest CT abnormalities. It is critical to determine the clinical consequences of chest CT screening in asymptomatic persons. The establishment of any significant advantage on clinical outcomes needs to be more thoroughly analysed in relation to the recognised economic implications and radioactivity relating with CT scanning.

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Table 1: List of Medications used in the treatment of COVID-19

| Name of the Medication | Mechanism of Action | Dose Regimen |
|------------------------|------------------------------|--|
| Remdesivir | RNA Polymerase Inhibitor | 200mg loading dose Followed by 100mg daily infusion |
| Favipiravir | RNA Polymerase Inhibitor | 2,400-3,000mg every 12h loading dose, 1200-1800mg every 12h maintenance dose |
| Lopinavir-Ritonavir | Aspartate Protease Inhibitor | 400/100mg or Standard care |
| Ribavirin & Interferon | Guanosine Analogue | 400mg every 12h |
| Hydroxychloroquine | | 200mg thrice for 10 days |
| Tocilizumab | Monoclonal Antibody | 80-700mg per dose |
| Siltuximab | IL_6 Inhibitors | 11mg/kg |



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Table 2: List of Vaccines used to prevent COVID-19

| Sl. No. | Name of the Vaccine | Brand Name | Works on principle | Producer name |
|---------|-------------------------------------|------------------------------------|---------------------------|---|
| 1 | Oxford–AstraZeneca COVID-19 vaccine | Vaxzevria and Covishield | Viral vector vaccine | British University of Oxford, British-Swedish company AstraZeneca, and the Coalition for Epidemic Preparedness Innovations |
| 2 | Pfizer–BioNTech COVID-19 vaccine | Comirnaty | mRNA vaccine | German company BioNTech and the American company Pfizer |
| 3 | Janssen COVID-19 vaccine | Johnson & Johnson COVID-19 Vaccine | Viral vector vaccine | Janssen Pharmaceutica (a subsidiary of Johnson & Johnson) and Beth Israel Deaconess Medical Center. |
| 4 | Moderna COVID-19 vaccine | Spikevax | mRNA vaccine | American company Moderna, the U.S. National Institute of Allergy and Infectious Diseases, the U.S. Biomedical Advanced Research and Development Authority, and the Coalition for Epidemic Preparedness Innovations. |
| 5 | Sinopharm BIBP COVID-19 vaccine | BBIBP-CorV | Inactivated virus vaccine | China National Pharmaceutical Group (Sinopharm) and its Beijing Institute of Biological Products |
| 6 | Sputnik V COVID-19 vaccine | Gam-COVID-Vac | Viral vector vaccine | Russian Gamaleya Research Institute of Epidemiology and Microbiology |
| 7 | CoronaVac COVID-19 | Sinovac COVID-19 vaccine | Inactivated virus vaccine | Chinese company Sinovac Biotech |
| 8 | Novavax COVID-19 vaccine | Nuvaxovid and Covovax | Viral vector vaccine | Novavax and the Coalition for Epidemic Preparedness Innovations |
| 9 | Covaxin | BBV152 | Inactivated virus vaccine | Bharat Biotech in collaboration with the Indian Council of Medical Research - National Institute of Virology |
| 10 | VLA2001 | Valneva COVID-19 vaccine | Inactivated vaccine | French biotechnology company Valneva SE in collaboration with the |





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| | | | | |
|----|-----------------------------|-----------------|----------------------|--|
| 11 | Sanofi–GSK COVID-19 vaccine | VidPrevtyn Beta | Viral vector vaccine | American biopharmaceutical company Dynavax Technologies Sanofi Pasteur and GSK plc. |
| 12 | Sputnik Light | | Viral vector vaccine | Russian Gamaleya Research Institute of Epidemiology and Microbiology |
| 13 | Convidecia | AD5-nCOV | Viral vector vaccine | Chinese company CanSino Biologics and the Beijing Institute of Biotechnology of the Academy of Military Medical Sciences |

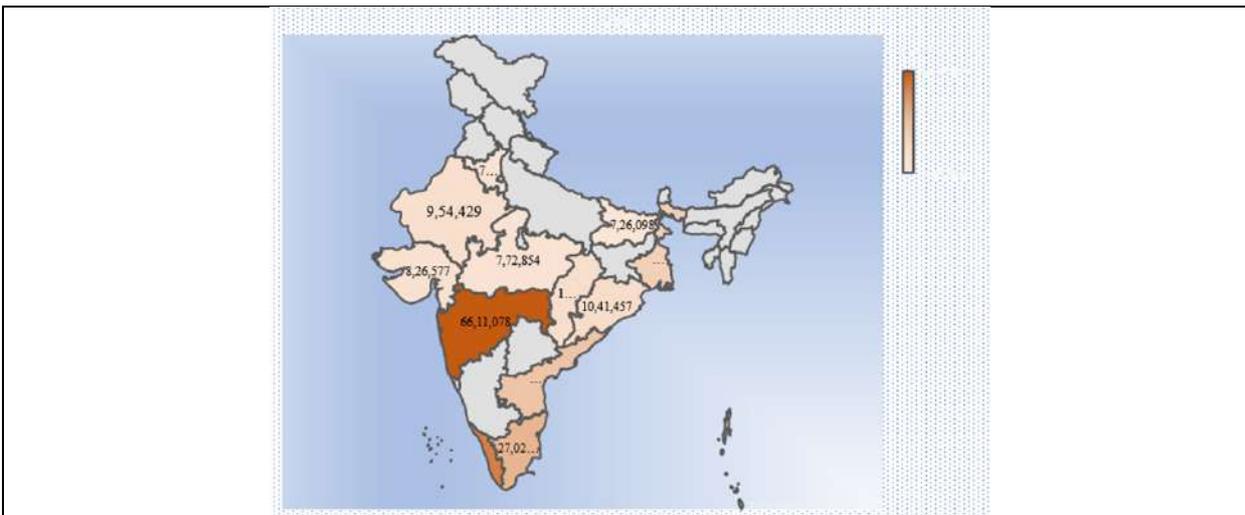


Figure 1: Pictorial representation of Covid-19 in India

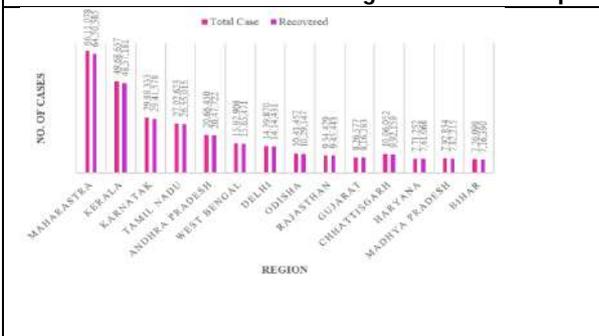


Figure 2: Graphical representation of total no. of cases vs total no. of recovery

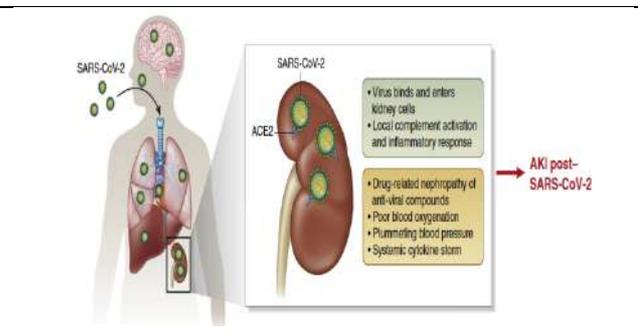


Figure 3.SARS-CoV-2





Potential of *Tinospora cordifolia* in Effective Management of Citrus Greening Disease by Alteration in Life Cycle Stages of the Vector *Diaphorina citri*

Ishu Priya¹, Rashmi Saini^{2*} and Mohammad I. Ali³

¹Research Scholar, Faculty of Life Sciences, Mandsaur University, Mandsaur (M.P.), India.

²Assistant Professor, Department of Zoology, Gargi College, University of Delhi, Delhi, India.

³Associate Professor, Faculty of Life Sciences, Mandsaur University, Mandsaur (M.P.), India.

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*Address for Correspondence

Rashmi Saini

Assistant Professor,
Department of Zoology,
Gargi College, University of Delhi,
Delhi, India.

E.Mail: dr.rashmisaini@gmail.com



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ABSTRACT

Citrus aurantifolia, *Citrus sinensis*, and *Citrus reticulata* are the most significant commercial citrus species which account for 41%, 23%, and 23%, respectively, of all citrus fruits produced in India. Since years, citrus greening disease, commonly referred to as Huanglongbing (HLB), had adverse effect on its production. "*Ca. Liberibacter asiaticus* (CLAs)" a gram-negative, phloem-restricted bacterium, transmitted by the Asian citrus psyllid, *Diaphorina citri* (*D. citri*) is the HLB-causing agent in Asia. The safe and effective management of disease using plant resources has attracted a significant amount of attention due to the rise in insecticide resistance. *Tinospora cordifolia* (Giloy) is a medicinal plant, and its woody stem contains phytoecdysones, which have been proven to interfere with insect moulting. The objective of the current study was to investigate dose dependant effect of *T. cordifolia* on the survival rate and its modulatory effect on life cycle of *D. citri*. There was a significant decrease in the survival rate of *D. citri* with increase in conc. of ethanolic extract of *T. cordifolia* (48%, 44%, 44%, 44%, 36%, 28%, 16% and 8% survival rate with 60%, 65%, 70%, 75%, 80%, 85% and 90% ethanolic extract of *T. cordifolia* respectively) on 4th day of treatment. On the 7th day of treatment, same pattern of significant decrease in the survival rate of *D. citri* with increase in conc. of ethanolic extract of *T. cordifolia* was observed but none of the *D. citri* survived in concentration greater than 50% of ethanolic extract of *T. cordifolia* on 17th day of treatment. In the groups, with ethanolic concentrations greater than 50%, the regulatory impact on the various life cycle stages and changes to the ovulation period were examined. The surviving adults in this cohort were investigated for changes to the ovulation cycle. It was observed that the adult's life cycle stages lasted longer. The dose-dependent investigation revealed a change in the percentage survival rate, growth-

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regulating activity, and a putative insecticidal property of *T. cordifolia* against *D. citri*, which emphasises its role in the control of citrus greening disease.

Keywords: Citrus greening disease, *Diphoriana citri*, Asian citrus psyllid, *Ca. Liberibacter asiaticus* (CLAs), *Tinospora cordifolia*, Giloy, Huanglongbing (HLB)

INTRODUCTION

The citrus fruit crop has a great economic importance and is the most important fruit crop cultivated globally. Citrus is a member of the Rutaceae family, which has about 150 genera and there are about 1600 plant species worldwide, mostly in tropical and warm climates [1]. Citrus is grown in the subtropical and tropical areas of the world between the latitudes of 40° north and 40° south, where the temperature ranges from 15°C to 30°C, and the best citrus fruits are produced there [2,3]. The mandarin (*Citrus reticulata*), sweet orange (*Citrus sinensis*), and acid lime (*Citrus aurantifolia*) are India's three most significant commercial citrus species, accounting for 41, 23, and 23% of the country's total citrus fruit productivity, respectively. Area of citrus plantation is increased from 1091 Ha to 1106 Ha from 2021 to 2022 but production of citrus shows mere increase of 14150 to 14850 (Area in '000 Ha Production in '000 MT) [4]. The principal reason the citrus tree had to be cut down was a bacterial illness that significantly reduced the citrus fruit's yield and reduced its commercial value (FAO, 2012) [5]. Citrus plants are infected by a virus or virus-like pathogen called *Candidatus Liberibacter asiaticus* (CLAs) and *Citrus tristeza virus*, which results in citrus greening disease and affects citrus production all over the world [6,7]. One of the most destructive pests to citrus-growing areas around the world is *D. citri*. As it harbours the Gram-negative bacterium (CLAs) that causes the disease, it is a major vector of citrus greening disease [8,9]. Alternative names for citrus greening disease are yellow shoot (huanglungbin) in China, likubin (decline) in Taiwan, and dieback in India [5].

Yellow shoots, foliar blotchy mottle that may resemble symptoms of zinc deficiency, vein corking, poor blooming, and stunting are all signs that citrus trees have HLB infection. Citrus trees with chronic HLB infection show little foliage, significant twig or limb die-back, and eventual death after three to five years. Citrus with HLB infection shows decreased fruit quality and a production drop of 30% to 100% [10]. Citrus production has lately been reduced globally due to the deadly citrus greening disease. After a long amount of time, infected trees may begin to exhibit defoliation and mineral shortage signs. The most visible signs of rapid decline are typically seen on fruit-laden trees.

The woody stem of a medicinal plant *Tinospora cordifolia*, has been reported to contain sesquiterpene tinocordifolin, sesquiterpene glycosides tinocordifolioside and tinocordiside, arabinogalactan, phytoecdysones ecdysterone and makisterone, and alkaloids berberine, palmatine, and magnoflorine as the main phytoconstituents [11,12]. Phytoecdysones are known for their disruption activity of moulting in insects. Cordifolin, a chalcone reported for the first time from a natural source, has been isolated from the ethanolic extract of the woody stem of *T. cordifolia* [13]. Its chemical structure was established as 1-(2', 3', 4'-trihydroxyphenyl)-3-(4''- methoxyphenyl)-propen-1-one and the compound exhibited good insect growth regulatory activity and has potential larvicidal effect [13]. The goal of the current study was to explore the potential of *T. cordifolia* in the effective management of Citrus Greening Disease by alteration in life cycle stages of the vector *D. citri*.

MATERIAL AND METHODS

Plant Material and Growth Environment

Fifty seven healthy acid lime (*Citrus aurantifolia*) plants were collected from the fields of the Indian Agricultural Research Institute (IARI), Pusa Institute, New Delhi. The average height of each plant was 0.7 metres above the



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substrate, and none of the plants had ever been infected. All citrus plants were given a 12-hour day and 12-hour night photoperiod. 24 to 30°C was maintained as the daily temperature range. All citrus plants were kept for 7 days in isolation to avoid any prior infestation. The adult *D. citri* were then transmitted to the plants after being taken from the fields of IARI, New Delhi. Each treatment group included three plants each (n=3), which were kept separated from each other by a plastic sheet house cover.

Rearing of Psyllids infected with CL as

From the fields of IARI, Pusa Institute, New Delhi, adult *D. citri* were collected from citrus plants that had been infected with the Citrus Greening Bacterium (CGB). Each acid lime plant was transmitted with 25 adult *D. citri* which were kept overnight before transmission.

Preparation of ethanolic extract of *T. cordifolia*

Woody stems of healthy *T. cordifolia* were collected from the fields of IARI, Pusa Institute, New Delhi and were cut into 5-cm pieces. Giloy was sun-dried before being ground into powder using a mortar, pestle, and grinder. The air-dried extract was reconstituted in ethanol at different concentrations (E1-E17, range: 10% - 90%) and were prepared at 100 mg/mL.

Spraying of ethanolic extract of *T. cordifolia*

A hand sprayer was loaded with different concentrations of ethanolic extract of *T. cordifolia*, and was sprayed on the acid lime leaves that had been raised with adult *D. citri* that was CGB positive (n = 3). Acid lime plants (n = 3) with adult *D. citri* infestations with no treatment with ethanolic extract of *T. cordifolia* was also used as control. Spraying of ethanol alone on mature acid lime leaves with *D. citri* infestations (n = 3) and was also used as another control. Acid lime (*C. aurantifolia*) plants were treated with different concentrations i.e. 10% - 90% (n = 3 for each concentration).

Identification of Life cycle stages of *D. citri*:

Adults was observed holding their bodies at a 45° angle to the leaf surface having mottled brown wings which are the distinguishing feature of adult Asian citrus psyllids. Life cycle stages include eggs and five nymphal instars of psyllid which were identified according to Tsai and Liu 2000 [14]. Freshly laid eggs were pale yellow and oval in shape. The first nymphal instar was identified by pair of red compound eyes and a pale pink body while rudimentary wing pads were seen on the second instar's thorax. The third instar was identified as it had well-developed wing pads and signs of antennal segmentation [14]. Metathoracic wing pads extending to the third abdominal segment was considered as the distinguishing feature of the fourth instars while the metathoracic wing pads reaching the fourth abdominal segment was distinguishing feature to identify the fifth-instars [15]. Fifth-instar nymphs were also distinguished from other nymphal stages as they have three setae on each antenna [15]. The life cycle completes when fifth instar metamorphosis into Adult.

Statistical analysis

Results were expressed as mean ± SD. Differences among data were determined using 't' test. Differences between the data were considered significant at p< 0.05.

RESULTS**Determination of Survival Rate of *D.citri* after Treatment**

Citrus plants (n=3 each) were treated with different concentrations of ethanolic extract of *T. cordifolia* and data was compared with the controls. Treatment with 60%, 65%, 70%, 75% , 80%, 85% and 90% ethanolic extract of *T. cordifolia* showed *D.citri* survival rate of 48%, 44%, 36%, 44%, 44% , 28%, 16% and 8% respectively on 4th day of treatment while none of the *D.citri* survived in concentration greater than 50% of ethanolic extract of *T. cordifolia* on 17th day of treatment. The survival rate of *D. citri* at 4, 7, 10, 13 and 17 days after treatment with different concentrations of ethanolic extract of *T. cordifolia* (E1-E17, range 10%-90%) was observed to be dose dependent (Fig. 2).





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Reduction in Egg Laying Capacity of *D.citri*

The dose dependent effect of *T.cordifolia* was observed on the egg laying capacity of *D.citri*. As seen in Fig. 3, the number of eggs laid after 4th day of treatment with different ethanolic concentrations of *T. cordifolia* was found to be significantly decreased as compared to controls. The plants without any treatment was used as one control while the plant treated with ethanol alone was used as another control.

Regulatory Effect on Moulting of *D.citri*

A remarkable dose dependent regulatory effect of treatment with *T. cordifolia* was observed on the moulting of *D. citri*. At concentration above 25% of ethanolic extract of *T. cordifolia* a significant increase was observed in the duration of moulting of fifth instar larva to Adult *D. citri*. This moulting was adversely affected in higher concentrations (30% and 35%) and the duration of conversion of fifth larval stage to adult was found to be increased. The moulting at concentration greater than 40% did not took place and resulted in inability of fifth larval stage to get transformed into adult *D. citri* (Fig. 4).

DISCUSSION

D. citri is the most dangerous pests of citrus producing countries throughout the world due to its function as the major vector of the citrus greening disease. As it feeds, it transmits the causative agent, CLAs, from infected plants to the other plants. *D. citri* mortality can be an effective strategy to manage citrus greening disease. The use of insecticides is the main strategy for controlling psyllid and has increased year by year [16]. *T. cordifolia* hasn't been thoroughly examined in comparison to other plants, including *Mentha piperita*, *Ocimum sanctum*, *Adhatoda vasica*, *Nerium indicum*, *Ipomoea fistulosa*, *Azadirachta indica*, and *Clerodendrum*. The antibacterial capability of *T. cordifolia* has been documented in a number of research studies [17-20], but none of the investigations have shown its insecticidal effect on *D. citri*, the common vector of Citrus Greening Disease.

A phytoecdysone known for interfering with insect moulting has reportedly been found in the woody stem of the medicinal plant *T. cordifolia*. The woody stem of *T. cordifolia* was used as the source for the ethanolic extract that yielded cordifolin, a chalcone that has never before been reported from a natural source. Its chemical formula was determined to be 1-(2', 3', 4-trihydroxyphenyl)-3-(4-methoxyphenyl)-propen-1-one, and the substance showed promising larvicidal action as well as good insect growth regulating activity [13]. The current study demonstrates *T. cordifolia's* potential against *D. citri* and emphasizes its function in the management of citrus greening disease.

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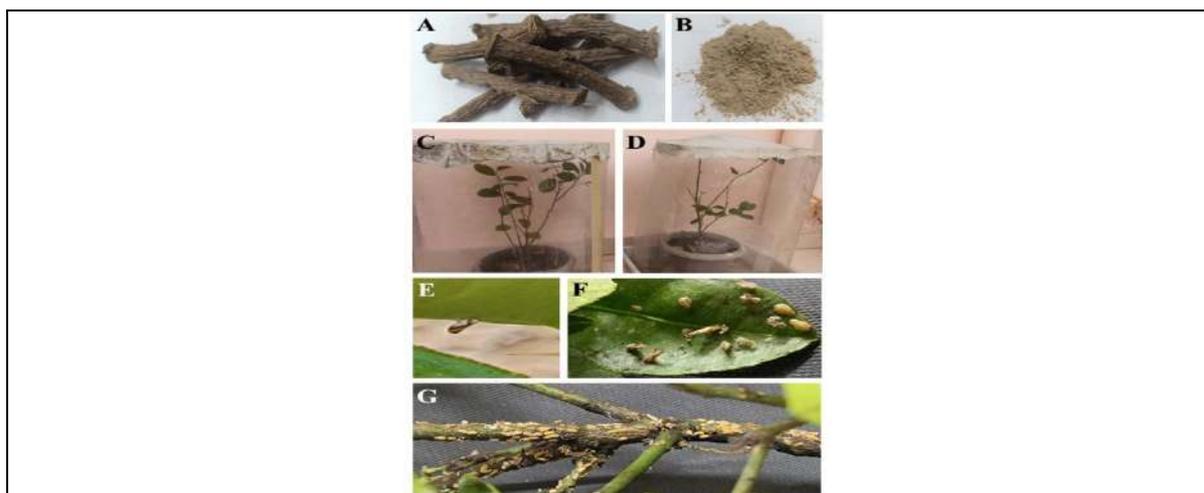
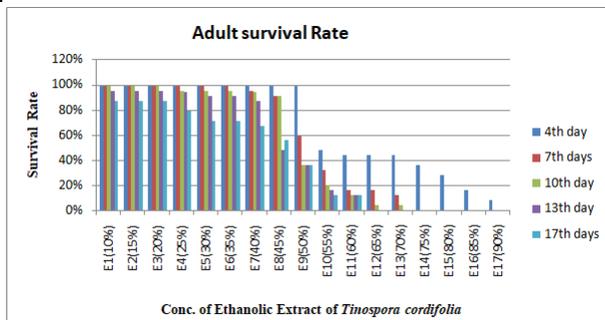


Fig 1. Treatment of ethanolic extract of *T. cordifolia* on *D. citri* infected citrus plants. A-T. *cordifolia* (Giloy) sun dried stems, B-Powdered form of sun dried stem of Giloy, C,D- Citrus plants treated with ethanolic extracts of *T. cordifolia* and were kept separated from each other by a plastic sheet house cover. E- Adult *D. citri* on citrus plant F- Different life cycle stages of *D. citri* on plant leaf, G- Severely infected citrus plant showing adult *D. citri* and its various life cycle stages.



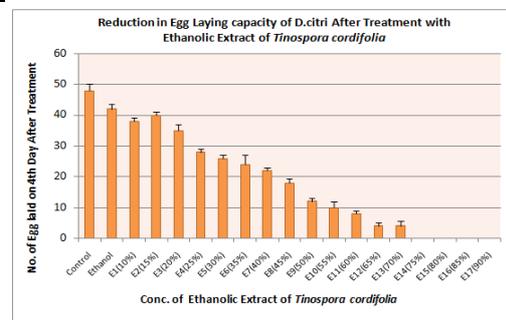


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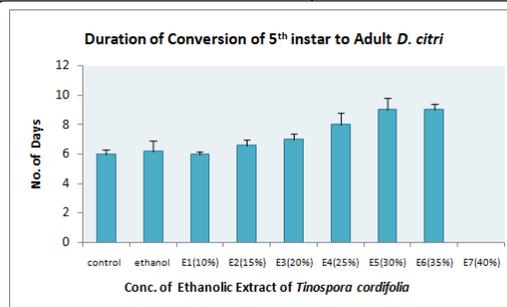
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Fig 2. Dose dependant decline in the survival rate of *D.citri* after 4, 7, 10, 13 and 17 days post-treatment with different concentrations of ethanolic extract of *T. cordifolia* (E1-E17, range 10%-90%).



(p<0.05) at end of figure legend

Fig 3. Reduction in the egg laying capacity of *D. citri* after treatment with *T. cordifolia*. The egg laying capacity of *D. citri* was significantly decreased after treatment with increasing concentrations of ethanolic extract (E1-E17, range 10%-90%) of *T. cordifolia* when compared to controls.



(p<0.05) at end of figure legend

Fig 4. Regulatory effect of *T. cordifolia* on the moulting of *D. citri*. The duration of conversion of the fifth instar larvae to the adult significantly increased with treatment with increasing concentration of ethanolic extract of *T. cordifolia* ((E1-E17, range 10%-90%).





Examining the Impact of Topical Insulin on Wound Healing

Salevendula Sreelekha¹, G Usha Sree², K. Vinod Kumar³ and Shaik Nuzhath^{2*}

¹Student, Department of Pharmaceutical Analysis, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh-India 515721.

²Assistant Professor, Department of Pharmaceutical Analysis, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh-India 515721.

³Professor, Department of Pharmaceutical Analysis, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur, Andhra Pradesh-India 515721.

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*Address for Correspondence

Shaik Nuzhath

Assistant Professor,

Department of Pharmaceutical Analysis,

Raghavendra Institute of Pharmaceutical Education and Research,

Anantapur, Andhra Pradesh-India 515721.

E.Mail: nuzhathpharma@gmail.com



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ABSTRACT

This study investigates the role of insulin in wound healing, a complex biological process. For nearly a century, insulin has been known to speed recovery from various wounds, as demonstrated in numerous human and animal studies. While much research has focused on systemic insulin effects on burn wounds, less attention has been paid to topically applied insulin. This review assesses evidence of topical insulin's impact on wound healing in both diabetic and non-diabetic scenarios. Findings from animal and clinical studies suggest that topical insulin can enhance wound healing through several pathways without causing side effects. Additionally, certain wound dressings, which provide controlled and sustained release of bioactive insulin, can accelerate healing. The increasing recognition of topical insulin in wound healing is evident, but more research is required to fully understand insulin's role in healing diverse wounds.

Keywords: Bioactive insulin, Wound Healing, Sustained release, Clinical Studies.

INTRODUCTION

The frequency of persistent wounds that refuse to heal is strongly linked to diabetes mellitus. In the United States, over 6.5 million people suffer with chronic wounds that cost roughly \$25 billion to treat each year(1). As a result, the



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topic of wound healing is being given more attention as a matter of public health. Growth factors and stem cells have been shown to hasten recovery, but they are quite costly and need further investigation to confirm their safety. Therefore, there would be substantial societal and economic benefits to adopting effective, low-cost, and safe techniques of expediting wound healing. Previous studies have revealed the importance of insulin in the recovery process. As a peptide hormone and growth factor, insulin can repair skin damage at a low enough cost that it could be applied topically to wounds. However, systemic insulin therapy has the downside of inducing hypoglycaemia and hypokalaemia, which can delay wound healing and increase the risk of infection in postoperative patients with diabetes(2). However, research shows that diabetic and non-diabetic patients who use topical insulin experience accelerated wound healing without any changes to their blood glucose levels. Hynick et al. analysed the preliminary data on the effect of insulin on burn wound healing. Despite the abundance of studies examining the results of systemic. Investigations on the wound-healing properties of intravenous diabetes on burn wounds are multiple, while investigations on the efficiency of topical insulin are limited(3). The goal of this investigation is to examine what is known at the moment about how topical insulin affects wound healing in people with and without diabetes.

Phases of Wound Healing:

The Biological Process of Healing A Wound There are several stages in the wound healing process(4). The four phases of wound healing include blood clotting, inflammation, cell division, and structural changes. The first step in mending an injury is called haemostasis, and it entails a number of different processes, including vasoconstriction, platelet aggregation and degranulation, and fibrin deposition. During the inflammatory phase, when capillary permeability and cell migration are at their peak, neutrophils are the first cells to penetrate the injured tissue. They secrete proteases to digest the denatured ECM and disinfect the wound(5). Once there, cytokines such as transforming growth factor (TGF) and others regulate the differentiation of invading monocytes into resident macrophages. The inflammatory response and the proliferation and remodelling stages of wound healing rely on these cells.

The wound healing process includes several stages among them wound healing, immune system responses, cell division, and structural changes to tissues(6). The procedure of he which stops bleeding after an injury, involves vasoconstriction, platelet degranulation and aggregation, and fibrin deposition among them wound healing, immune system responses, cell division, and structural changes to tissues. The procedure of he which stops bleeding after an injury, involves vasoconstriction, platelet degranulation and aggregation, and fibrin deposition(7). During the inflammatory phase, cells migrate to the wound site, including neutrophils, which produce proteins to sweep off decomposed ECM(8). The response to injury, cell spread, and rebuilding phases of the healing process are all affected by cytokines such macrophage a chemo protein-1 (MCP-1), converting development factor- (TGF-), and another. Similarly, monocytes travel to the wound site and differentiate into macrophages. Angiogenesis, fibrin clot breakdown, and re-epithelialization are all processes in the healing process that are supported by the cells involved in the inflammatory response. During the proliferative phase, the wound undergoes re-epithelialization, angiogenesis, and fibroplasia. Keratinocyte migration as well as proliferation are assisted by TGF, angiogenesis is triggered by cytokines like TGF and vascular epidermal growth factor (VEGF), and granulation tissue is generated by fibroblasts releasing extracellular matrix components(9).

The wound undergoes vascular regression, granulation tissue remodelling, and the formation of new extracellular matrix components during the tissue remodelling phase of wound healing. This stage can continue anywhere from 6 months to 2 years, and it entails more tissue remodelling to restore function and appearance. It has been found that applying insulin directly to a wound helps it heal more quickly by reducing the body's inflammatory and oxidative responses. Insulin can reduce the levels of reactive oxygen species, protecting lipids, proteins, and DNA from further damage in rats with burn wounds. Furthermore, topical insulin can promote the early recruitment of neutrophils and have an anti-inflammatory effect by increasing the number of M2 macrophages in the wound. Insulin supports wound healing through, among other mechanisms, stimulating macrophage chemotaxis and phagocytosis, regulating inflammatory mediators, and stimulating keratinocyte migration and differentiation. Insulin induces keratinocyte migration and differentiation via the PI3K-AktRac1 pathway, which requires the insulin receptor and



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EGFR for its proper functioning. Higher levels of hydroxyproline in the control group (13.52.5 days) show that topical insulin therapy on injuries promote the creation of collagen and suppleness in the dermis. Another study found that diabetic rats treated with insulin cream saw faster wound healing than control rats(10). Topical insulin therapy also enhanced the number of newly formed blood vessels in the wound bed, indicating a pro-angiogenic effect(11).

Medical Studies

Several clinical investigations have looked on how well dermal hcg works, in accelerating wound recovery. Topical insulin was compared to standard wound care for the treatment of diabetic foot ulcers in a randomized controlled trial. The results showed that wound healing and complete healing occurred much more quickly in the group given topical insulin compared to the control group(12). Patients with venous ulcers were treated with insulin-containing bandages in a separate experiment, and their wounds were evaluated every week for eight weeks. The results showed that wounds in the group that received dressings containing insulin healed significantly faster than those in the control group(13). According to the evidence you presented, it appears that treatment with topical insulin can hasten the recovery of the epithelium lining the ocular surface following corneal lesions. The therapeutic effects of topical insulin could be improved with further study into its optimal dose and delivery. Found that diabetic rats with corneal abrasions treated with insulin-containing eye drops had faster corneal re-epithelialization and lower wound size than the control group. The tear fluid volume, corneal thickness, and protection of corneal cell shape were all improved in diabetic rats with dry eye syndrome and corneal damage after daily injection of insulin-loaded micro particulate in each eye for 15 days. However, more research is needed to confirm insulin's potential as a treatment for ocular injuries and disorders(13).

Researchers have investigated the efficacy of using insulin cream or solution to hasten wound recovery. Found that administering insulin solution twice daily for 15 days accelerated wound healing in both diabetic and non-diabetic mice by decreasing the amount of time needed for full epithelialization. In a similar vein, shown that wounds caused by a turtle's second purpose can benefit from treatment with insulin solution. Healing of excision wounds can be hastened by using insulin solution, as reported. This is accomplished by hastening the inflammatory response, re-epithelialization, and collagen remodelling. In addition, discovered that diabetic rats treated with insulin cream twice daily for 8 days exhibited accelerated wound healing as a result of restored AKT and ERK signal transduction. More research is needed to determine the efficacy and safety of using insulin cream or solution to speed up wound healing in humans, but these findings are promising. It has also been shown that the expression of vascular endothelial growth factor (VEGF) and stromal cell-derived factor 1 in wounded tissue is elevated after insulin is administered to treat wounds. Studied the outcomes of 26 days of daily application of insulin cream (0.5 U/100 g) on second-degree burns in both control and diabetic rats(14). Researchers found that diabetic rats exposed to insulin cream had greater increases in inflammatory cell infiltration and collagen deposition compared to non-diabetic rats.

Wound dressings made of biomaterials have been studied for their potential to release bioactive insulin slowly and steadily over time. Investigated the bioactive dressing potential of hydrogels containing fibroblasts and insulin in diabetic rats(15). Applying insulin and fibroblast-incorporated hydrogels improved the healing process of diabetes wounds on day 6 after wounding, with higher neovascularization and collagen deposition compared to hydrogels mixed with PBS. Looked into the effects of insulin (50U) complexed with cyclodextrins on excisional skin wounds. Complexed gels significantly boosted keratinocyte proliferation and demonstrated more consistent and persistent neovascularization than insulin gel alone, as found in the study(16).

Nanoparticles and Nano fibrous scaffolds are two examples of insulin delivery technologies that have been the focus of recent research for their potential to speed wound healing. Several formulations of insulin, such as hydrogels, nanoparticles, and topical creams and solutions, have been investigated for use in wound healing(16). Biomaterials, such as dressings, have been shown to be capable of providing both sustained and variable release of insulin. Wound healing after surgical excision was studied in both diabetic and non-diabetic rats by who evaluated the effectiveness of free and Nano-encapsulated insulin(14). The effects of an insulin-chitosan nanoparticle-loaded wound dressing on cutaneous wound healing in rats were investigated by researchers found that by using this treatment on mice,





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wounds healed nearly completely and fibroblast growth was boosted. Nano fibrous scaffolds, insulin conjugated to human hair keratin, and silver nanoparticles loaded with insulin have all shown promise in promoting wound healing in separate studies(17). Collectively, these investigations support the idea that continuously released insulin from dressings can expedite wound healing.

Clinical Studies

In a preliminary investigation, looked at the efficacy on pressure ulcers caused by prolonged lying down. Eight people served as minimise and received only standard nursing care, while six additional served as experimental subjects and received regular insulin (10 U twice daily) for five days. Forty-five patients with non-infected acute and chronic extremities wounds participated in a randomized, double-blind, placebo-controlled trial of topical insulin conducted. By day 15, there was a statistically significant difference between the treatment and control groups in the rate at which wounds healed. The participants were randomly assigned to receive either crystalline insulin sprays (10 U) or saline solution twice daily. The study found that the average rate of wound healing in the treatment group was significantly higher (46.09 mm²/day) than in the control group (32.24 mm²/day), regardless of the initial size of the lesion. In neither the treatment group nor after insulin administration were any symptoms of hypoglycaemia observed. Topical insulin was found to be a safe and effective treatment for nd enhancing the healing of both fresh and chronic wounds on the extremities. Discovered that the effectiveness of topical insulin in healing open, simple cutaneous wounds may depend on the kind and amount of insulin employed(18). In their randomized study with 90 patients, they compared the efficacy of regular crystalline insulin (including zinc), zinc chloride solution in water, and saline. Both the regular insulin group (group I) and the aqueous zinc chloride solution group (group II) increased wound healing when compared to the control group (group III), however the regular insulin group was more effective. There was no discernible change in glucose levels between before and after insulin treatment. To compare the effectiveness of an insulin dressing to that of conventional saline-impregnated gauze in the treatment of pressure ulcers, conducted a randomized, controlled experiment with 50 participants. Insulin dressings or regular saline dressings were applied twice a day for seven days. Mean wound area decreased from 9.61 6.39 cm² (day 1) to 3.68 3.16 cm² (day 7) in the insulin dressing group, while it remained the same at 5.77 4.62 cm² in the saline dressing group (P 0.001). Pressure ulcer healing was aided by insulin dressing more so than by ordinary saline dressing, the study found(19).

Animal studies have looked at various doses and types of insulin to see how they affect wound healing. For instance, wound healing in skin donor sites was improved by injecting a long-acting insulin-zinc solution (0.25U, every other day) locally without any adverse systemic effects (reference omitted). These results from an animal model of diabetic corneal abrasion suggest that topical application of bovine insulin solution (20 I, 4 times daily for 7 days) can promote wound healing and improve epithelialization without causing systemic side effects or hypoglycaemia. Furthermore, the studies demonstrate that topical insulin can influence the inflammatory response, protect corneal cells, keep tear fluid volume normal, and promote collagen remodelling in different types of wounds, such as cutaneous wounds, second-intention wounds, diabetic corneal abrasions, and dry eye syndrome. However, these findings should be interpreted with caution since human studies may not translate to animal studies. Additional clinical study is needed to determine the optimal insulin dosage and form for the various wound forms found in humans. Based on the research we looked at, diabetic rats that were given insulin had significantly faster wound healing times for a wide range of wound types, including burns, full-thickness skin wounds, diabetic corneal abrasions, diabetic dry eye syndrome, cutaneous wounds, second-intention wounds, excision wounds, and corneal injuries. Insulin promotes epithelialization, increases neovascularization and collagen deposition, decreases the inflammatory response, and protects the corneal cell architecture, all of which contribute to faster wound healing. The use of insulin did not increase the risk of hypoglycaemia or cause any systemic side effects. But more study is needed to determine the best insulin application dose, method, and time frame for each wound type(20).



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Experiments using diabetic and non-diabetic rats with surgically removed lesions found that nanoparticles containing recombinant human insulin (at a sustained release rate of 5.2 10³ M) accelerated wound healing on days 12 and 16. In another study, the prolonged release of insulin-loaded chitosan sped up the healing process for full-thickness excision wounds on days 7 and 14(21).

Using topical insulin to treat non-diabetic wounds has been shown in the research reviewed here to have no negative systemic effects or changes in blood glucose levels. Wound infection, bleeding, allergen city, and discomfort were also evaluated as potential detrimental local effects of insulin injection. However, some studies have investigated whether or not applying a topical insulin solution speeds healing of diabetic ulcers. Conducted a randomized, double-blind, placebo-controlled clinical trial of topical insulin in 22 individuals with diabetes and wounds. The patients were split into two groups: those who received insulin cream (n = 11) and those who received a placebo cream (n = 11). The results showed that the insulin cream group had considerably better wound healing than the placebo group. Did a similar study, this time on the effects of local insulin on 8 diabetics with both acute and chronic wounds. Each patient had half of their wound area treated with insulin (10 U) once daily for 14 days(22). Topical insulin administration has been shown to benefit diabetic wound healing in studies. The number of vessels, the percentage of fibrosis, and the average temperature were all significantly different in the insulin-treated and placebo groups, as discovered by Martinez's research. In a study of 10 patients with full-thickness acute wounds, Found that while the amount of fibrosis was not significantly different between the two zones, new vascular formation was significantly better in the insulin-treated region(23).

Examined the impact wound healing in diabetics with foot ulcers and overall blood sugar levels after local insulin injection. Subcutaneous injections of half the calculated insulin dose were given to the experimental group (n = 18) at the base of the ulcer and the other half were given to the placebo (n = 14) group in the abdomen twice daily for 7 days, in a study. Although there was no significant change in fasting blood glucose levels between the two groups, the insulin-treated group dramatically increased the growth of granulation tissue and new blood vessels. Liposomal chitosan gel, a novel insulin delivery method, has been the subject of extensive research into its possible use in wound healing in recent years(24). carried out a scientific trial using double blinding and placebo with 110 people who had diabetic foot ulcers to evaluate the efficacy of topical insulin. Researchers found that after two weeks of treatment, the mean wound width was considerably smaller when insulin gauze dressings were used as opposed to saline gauze dressings. Investigated the effects of insulin mucoadhesive liposomal gel on people with chronic wounds. Wound healing rates in the group given insulin-loaded liposomal chitosan gel were much higher than in the control group, to the absence of hypoglycaemia symptoms were observed(24, 25).

CONCLUSION

In summary, findings from animal and clinical studies collectively suggest that topical insulin aids wound healing through various mechanisms, without producing side effects. Additionally, certain wound dressings, which offer a controlled and ongoing delivery of bioactive insulin, contribute to accelerated wound healing. This has led to growing recognition of topical insulin's value in the wound healing field. Nevertheless, for a more comprehensive understanding of insulin's role in the healing of various types of wounds, further research is clearly required.

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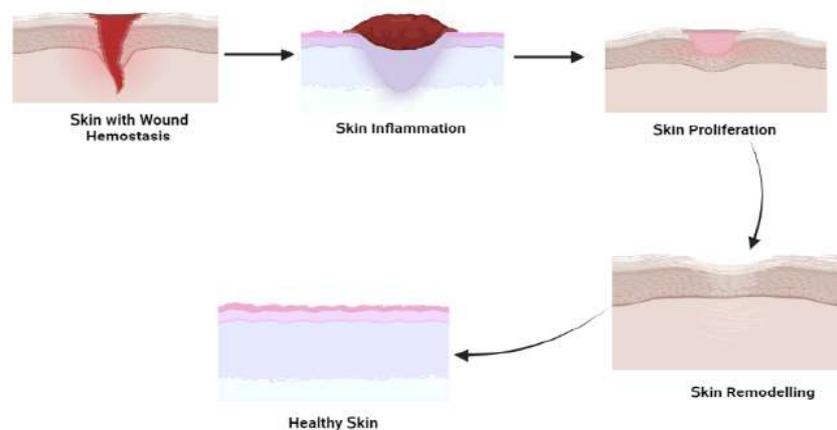
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**Figure 1: Phases of Wound Healing**



Water Retention Characteristics of Soils and Fly Ashes by Pressure Plate Apparatus

D.Gokul^{1*}, G. Manikandan¹, K. Arivazhagan² and P.K. Karthikeyan³

¹Ph.D Scholar, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

²Professor, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

³Assistant Professor, Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

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*Address for Correspondence

D.Gokul

Ph.D Scholar,
Department of Soil Science and Agricultural Chemistry,
Faculty of Agriculture,
Annamalai University,
Annamalai Nagar-608002,
Tamil Nadu, India.
E.Mail: gokuldharmalingam0@gmail.com



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ABSTRACT

The soil moisture is an indication of the amount of water present in the soil. The health of the soil as a plant habitat is determined by its moisture regime, which has storage and percolation properties. The soil water retention characteristics provide information on the ability of soils to store water and the availability of stored water for crop growth and development. The water movement in soils, water uptake by plants and loss to the atmosphere are all energy-related phenomena. The energy relationships between soil moisture and free water are generally expressed in terms of soil-water potentials. In view of this point, a laboratory experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar, to study the water retention characteristics of different textured soils and fly ashes by using pressure plate apparatus. The results of the experiment revealed that the fly ash retained more moisture compared to soils. Among the soils, the clay loam retained more moisture than loam and loamy sand soils. Furthermore, the increased pressure potential from 0.3 to 15 bars decreases water retention capacity in both soil and fly ash samples.

Keywords: Water retention curve, pressure plate apparatus, soil, fly ash, Potentials





INTRODUCTION

Water relationships are among the most important General properties that affect the use of soils for agricultural purposes. The soil water characteristics are a basic soil physical property that is mainly required for the study of infiltration, drainage, irrigation, hydraulic conductivity, solute movement in subsurface soil, plant available water and water stress on the plants. There are many different ways the soil holds water, but not every drop is accessible to plants. Chemical water is an integral part of the molecular structure of soil minerals. It can be securely bound to the surfaces of clay crystals and other minerals by electrostatic forces, but plants cannot use it. The rest of the water is held in soil pores. The amounts of moisture that a soil can store and supply to plants are dependent on the number and size of its pore spaces [8]. The influence of soil moisture on the water, energy and biogeochemical cycles is a major factor in the climate system. Related to this, the field capacity (1/3 bars) and wilting point (15 bars) of the soil play a significant role in crop yield by determining the amount of readily available water.

Coal fly ash is a source of mineral elements, which are also used for plant establishment. Due to its advantageous physicochemical characteristics, coal fly ash application in forestry and agriculture has been rising over time. The use of fly ash in agriculture has been investigated from a wide range of perspectives. These include increasing the essential minerals in the soil, absorbing heavy metals, using fly ash as a pesticide, etc. The application of fly ash can also improve the water-holding capacity of the soil [12]. In the laboratory study of many physical relationships, as well as the extraction of soil solution for chemical analysis, pressure plate extractors have become eminently successful research tools. The pressure plate apparatus has been widely used to measure the relationship between suction and water content in soil samples. It is important to obtain good measurements of soil moisture at the soil moisture constants to use as a comparison to other soil properties such as texture and structure [13]. The pressure plate apparatus provides a convenient, reliable means of removing soil moisture under controlled conditions from soil samples throughout the whole plant growth range without disturbing the soil structure. This apparatus is used to make a soil water retention curve at different pressure potentials. The curve provides a visual interpretation of the relationship between soil water suction and soil water content. This apparatus is an important tool that makes possible accurate measurement of water content over a wide range of matric potentials in a relatively shorter time. It is used, along with the tension plate, to obtain data to construct soil water characteristic curve or water retention curve or water release curve. The wide pressure ranges from very wet to very dry conditions, matric potential is often expressed as the log of the applied air pressure in hPa and it is called pF. The pF curves, also commonly known as soil water retention curves, are often measured using the pressure plate method [5]. This apparatus is also used for the determination of hydraulic conductivity. The results from this apparatus have a variety of practical applications such as soil survey, soil classification and preparing irrigation schedules and drainage for improving crop yields. In view of this context, the present study was undertaken to study the water retention behaviour of different textured soil and fly ash samples at different potentials (0.3, 3, 5, 10 and 15 bars).

MATERIALS AND METHODS

The laboratory experiment was conducted in the Department of Soil Science and Agricultural Chemistry, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Tamil Nadu. The different textured soils, coal fly ash from NTPC DADRI and lignite fly ash from NLC were chosen to study the water retention characteristics by using pressure plate apparatus. Each soil and fly ash sample was replicated twice. The physical properties of soils and fly ashes were given in tables 1 and 2, respectively. Water retained at various suctions were estimated using pressure plate apparatus as per methods described by Bandyopadhyay and Pradhan [1]. The underlying principle is a measurement of water retained in the soil against applied pressure. The matric suction is equivalent to applied pressure and equal in magnitude but opposite in direction. Plotting water retention values against corresponding matric suction gives a water retention curve. For this study, the soil water measurements were made by a porous plate extractor up to 1 bar or a little less and a pressure plate apparatus for higher suction up to 15 bars. The pressure plate extractor model 5 bar ceramic 101 and 15 bar ceramic 1272 were used to analyse the water retention



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characteristics of soil and fly ash samples (Fig. 1). The pressure plate method may be used on disturbed samples or undisturbed soil cores. Through the application of the pressure plate apparatus moisture retention curve may be developed for each soil type. The pressure plate equipment consists of a pressure chamber enclosing a porous plate saturated with water and allowing only water, not air to transfer through its pores. The top surface of the porous plate is at the chamber's applied pressure, while the bottom is at atmospheric pressure. Soil samples have been prepared to a certain bulk density in retaining rings and allowed to saturation by immersion in water. Then the porous ceramic plate was placed in the pressure chamber and a known pressure was applied. When a ceramic plate, with saturated soil on it, is subjected to air pressure above the atmospheric pressure, the higher pressure forces excess water through the microscopic pores in the ceramic plate and out through the outlet system via the passage offered by the screen. The air pressure is provided by a compressor, but it must be always routed through a manifold having pressure regulators and gauges. The water from the outlet till equilibrium against applied pressure is achieved. A known amount of pressure was applied on the ceramic plates in units of bars. At various equilibrium conditions of pressure, the soil water tension or suction was obtained. After that, the soil samples were taken out from the ceramic plate and the moisture percentage was determined by the infrared moisture analyzer balance. By analysing the samples at several different pressures are determined and a moisture retention curve was developed by plotting the known applied pressure versus the water content of the samples. The pressure plate extractor is only able to measure the desaturation curve of the soil moisture characteristics. This is due to the effects of hysteresis, which is the difference in the relationship between the water content of the soil and the corresponding water potential obtained under the wetting (sorption) and drying (desorption) process.

RESULTS AND DISCUSSION

Water retention characteristics of soil

The moisture retention characteristic curves of soils revealed that the water content changed very rapidly at different tension ranges. The data concerning soil water retention at varying potentials are given in table 3. The available soil moisture percentage is maximum in clay loam soil (10.71, 7.86, 7.75, 5.66 and 5.15% were obtained at 0.3, 3, 5, 10 and 15 bars, respectively) followed by loam soil (5.03, 3.71, 2.94, 2.91 and 2.39% were obtained at 0.3, 3, 5, 10 and 15 bars, respectively) and loamy sand soil (3.04, 2.38, 1.99, 1.75 and 1.16% moisture at 0.3, 3, 5, 10 and 15 bars, respectively). The clay loam soil released considerable soil water at 0.3 bars. The soil moisture retention decreased gradually from 0.3 to 15 bars in loamy sand and loam soils (Fig.2). The soil water content raises rapidly when decreases soil potential and drainage mainly occurred in the large pores. With the increase in the soil potential, soil drainage shifted from large pores to small and medium pores [14]. The variation in retention was relatively high under low tensions which could be due to the structure and pore geometry of the soils. Soil texture was highly influenced by the water potential. The effects of soil pore size distribution and hydraulic hysteresis are simulated by embedding the soil water retention curve [7]. The clay fraction commonly increases soil water retention with a higher soil organic carbon reported by Dlapa *et al.* [4]. Clay particles have a larger surface area, which leads to the formation of micropores and a higher affinity for water in comparison to silt and sand, which most often form mesopores and macropores. The organic matter and carbonate levels which create poor soil structure reduce the moisture retention capacity of loamy sand soil [8]. The water-holding capacity of soil highly depends on soil bulk density and porosity [6].

Water retention characteristics of fly ash

The data on water retention of coal fly ash and lignite fly ash at varying potentials are given in table 4. The maximum available moisture percentage was recorded in coal fly ash (33.55, 20.58, 11.98, 11.22 and 9.9% were obtained at 0.3, 3, 5, 10 and 15 bars, respectively) followed by lignite fly ash (31.95, 18.85, 12.07, 11.97 and 8.42% were obtained at 0.3, 3, 5, 10 and 15 bars, respectively). The curve indicated that the increased pressure potentials reduce the moisture percentage or water retention. The water retention of fly ash samples decreased steeply between 0.3 to 3 bars, more gradually between 3 to 5 bars and slowly between 5 to 15 bars (Fig.3). There is less difference in moisture percentage observed between the coal fly ash and lignite fly ash. Furthermore, the application of high suction (15 bars) drains out water from the ashes. This might be attributed to the presence of particulate material and microspheres in fly ash [10]. Normally, fly ash consists of fine, powdery particles which have predominantly



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spherical in shape and mostly amorphous in nature [2]. According to Olipa Nyazambe Lungu *et al.* [9] coal fly ash acts as a cementing agent, which increases the increase of pore spaces. Additionally, it combines with soil particles, which lowers the net charges on the soil exchange complex and results in low water content at wilting point (15 bars). The application of fly ash combined with farm yard manure improved soil permeability as it is micro-sized particles with porous in nature and heavily lower bulk density [3]. The presence of lighter particles gives the lignite fly ash excellent porosity. It contains more than 60% of particles having a size of less than 0.25 mm. It has the highest water-holding capacity of heavy clayey soils and sandy soils. Furthermore, the addition of lignite fly ash up to 15% by weight in clay soil is significantly capable of reducing bulk density and crust formation and improving the soil structure, which in turn improves porosity, root penetration and moisture retention capacity of the soil [11]. Sarkar and Rano [12] reported the greater water retention characteristics of fly ashes are probably due to larger surface area and greater porosity [6].

CONCLUSION

From the results, it may be concluded that clay loam soil showed maximum moisture percentage at different potentials compared to loam and loamy sand soil. With respect to fly ash, it may be concluded that the coal fly ash retained more moisture slightly than lignite fly ash. The water retention capacity of soil and fly ashes highly depended on the size and textural fractions. In general, fly ashes have higher water retention characteristics than soils. Fly ash's high water retention capacity can be applied to a variety of agricultural soils and may improve soil health and crop productivity.

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Table 1: Properties of soil

| Soil samples | pH | EC (dS m ⁻¹) | Organic carbon (%) | Bulk density (g/cc) | Particle density (g/cc) | Pore space (%) | Clay (%) | Silt (%) | Sand (%) | Soil Texture |
|--------------|------|--------------------------|--------------------|---------------------|-------------------------|----------------|----------|----------|----------|--------------|
| Soil A | 6.51 | 0.20 | 0.78 | 1.30 | 2.22 | 41.4 | 35.3 | 37.1 | 26.7 | Clay loam |
| Soil B | 4.53 | 0.17 | 0.81 | 1.32 | 2.40 | 45.0 | 18.4 | 44.9 | 35.8 | Loam |
| Soil C | 7.05 | 0.27 | 0.19 | 1.52 | 2.86 | 46.9 | 7.62 | 10.2 | 81.3 | Loamy sand |

Table 2: Properties of fly ash

| Ash samples | pH | EC (dS m ⁻¹) | Organic carbon (%) | Bulk density (g/cc) | Colour |
|---------------------------|------|--------------------------|--------------------|---------------------|---------------|
| Coal fly ash (NTPC DADRI) | 9.08 | 1.55 | 0.04 | 1.41 | Greyish white |
| Lignite fly ash (NLC) | 8.14 | 1.83 | 0.06 | 1.67 | Grey |

Table 3: Moisture percentage of soils at different potentials

| Soil texture | Potentials | | | | |
|--------------|------------|--------|--------|---------|---------|
| | 0.3 bars | 3 bars | 5 bars | 10 bars | 15 bars |
| Clay loam | 10.71 | 7.86 | 7.75 | 5.66 | 5.15 |
| Loam | 5.03 | 3.71 | 2.94 | 2.91 | 2.39 |
| Loamy sand | 3.04 | 2.38 | 1.99 | 1.75 | 1.16 |

Table 4: Moisture percentage of fly ashes at different potentials

| Fly Ashes | Potentials | | | | |
|---------------------------|------------|--------|--------|---------|---------|
| | 0.3 bars | 3 bars | 5 bars | 10 bars | 15 bars |
| Coal fly ash (NTPC DADRI) | 33.55 | 20.58 | 11.98 | 11.22 | 9.90 |
| Lignite fly ash (NLC) | 31.95 | 18.85 | 12.07 | 11.97 | 8.42 |





Fig. 1. Pressure Plate Apparatus

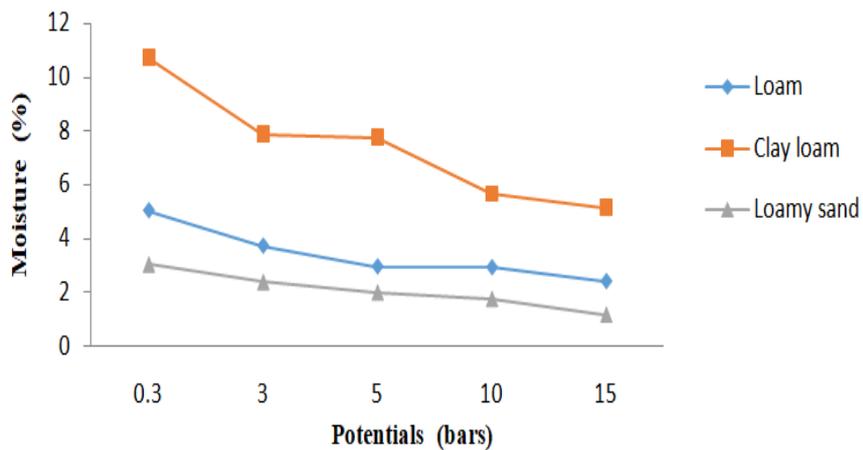


Fig.2. Water retention curve of different textured soils

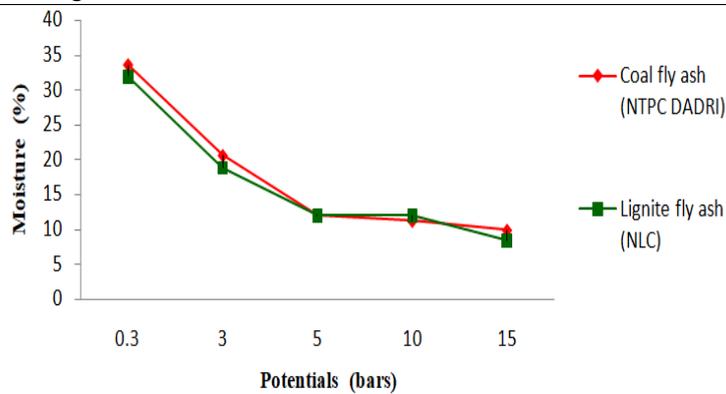


Fig. 3. water retention curve of coal fly ash and lignite fly ash





Regeneration of Cardiomyocytes can be a Cure against Myocardial Infarction- A Review

Yamini BR^{1*}, Mayukh Sarkar², Kimaya Joshi¹, Vinayak Pawar¹

¹M. Pharmacy Student, Department of Pharmacology, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India.

²Assistant Professor, Department of Pharmacology, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, India

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*Address for Correspondence

Yamini BR^{1*},

M. Pharmacy Student,

Department of Pharmacology,

Krupanidhi College of Pharmacy,

Bengaluru, Karnataka, India.



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ABSTRACT

The intricate biological process of heart regeneration is captivating, yet complicated. Heart failure often ensues due to limited capacity for myocardial renewal and the exacerbation of fibrosis. Unfortunately, progenitor cells and heart muscle cells have been removed from the heart are more resistant to damage and not able to restart the cell cycle. However, an optimistic glimmer for developing restorative benefit has been identified by research on the expansion of the heart and renew execution guarded beyond strain. This review explores the biological processes and distinctive functions that contribute to the significant loss of cardiac tissue following a myocardial infarction. With a frantic evaluation of their lead discovery, advancement, and complexity, we specifically review the first interruption one aside from extended or arise nearly scientific utilization.

Keywords: Heart regeneration, Renewing capacity, Heart failure, Cardiomyocytes and Myocardial Infarction.

INTRODUCTION

Myocardial infarction (MI) is a catastrophic disease with a global impact. It is worth noting that in 2019, the number of cases of cardiovascular diseases (CVDs) surpassed half a billion [1]. The rising incidence of CVD-related illness and death should be cause for concern. One of the key factors influencing the development furthermore succession of cardiac infarction is the depletion of cells in the myocardium including their ability for rejuvenation [2].





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Cardiomyocyte replication takes place in embryonic, fetal, and early post-natal stages, but experiences a sudden decline during the neonatal period [3]. Studies have shown that the renewal rate of cardiomyocytes is between 0.5% to 2%, with a decrease in this rate observed as age increases [4,5,6]. The formation of compensatory scar tissue to replace deceased heart tissue can result in impaired cardiac function, ultimately leading to heart failure [7,8]. Research conducted on amphibians and fish suggests that their regenerative capacity persists throughout their lifetime [9,10]. Regeneration fails to take place in these animals, including neonatal mouse and swine, through the rapid growth and maturation of heart stem cells. Instead, it is maintained by existing cardiomyocytes partially dedifferentiating and begins to proliferate again [11,12].

Even while these animal models have shed light on heart regeneration, research has been delayed since there aren't enough genetic resources available to fully understand the fundamental biochemical pathways. The cause of the irreversible termination of cardiomyocyte proliferation after birth antiquated the subject of intensive inquiry in contemporary era. It is important to remember that myocardial infarction damages not only the heart muscle but other cardiac organs as well, including the heart's vasculature. As a result, focusing on these elements might be required for efficient heart repair [13]. Pharmacologic medications, interventional gadgets, and procedures for surgery are among the common treatments for CVDs. None of these remedies, however, can fix the issues with heart regeneration and repair. Numerous researchers are investigating various strategies to boost myocardial proliferative potential and alleviate the cell-cycle interruption of heart muscle cells in adult hearts [14]. The latest developments in cellular and molecular biology have involved significantly to our understanding of the pathogenetic and pathophysiological process underlying cardiovascular diseases. These advancements have also opened up promising opportunities for innovative treatments for cardiac and vascular medical conditions. Gene therapy is another therapeutic approach that shows great potential in improving outcomes for CVDs. In this review, we provide in-depth technical information on each approach, including their respective advantages, potential limitations, and future prospects.

Myocardial Infarction Underlying Pathophysiology

Myocardial infarction (MI) is a condition appertaining to sudden ischemia of the heart muscle tissue, resulting from the obstruction or inadequate blood flow within the coronary vessels, leading to damage of the heart tissue [15]. The most frequent reason for myocardial infarction is coronary artery obstruction, which is often brought on by a build-up of plaque separation within the walls of the coronary arteries. But additional elements, including coronary artery contractions, endogenous myocardial arteries examination, plaque disruption, on-site blood clots and dysfunction of the microvascular system, are all identified as potential causes. [16,17]. Pathophysiological conditions that disrupt the balance between oxygen supply and demand are considerable benefactor to the etiology of myocardial infarction (MI). Several etiological factors, such as hypertension, are major risk factors for stroke, while smoking increases the incidence of MI [18]. Newer research has suggested that in addition to necrosis, apoptosis, another form of cell death, also contributes to tissue damage following myocardial infarction (MI) [19]. Many fatal arrhythmias are thought to be caused by re-entry or a feedback loop, which can be started by inconsistency in potential speed amid damaged and intact tissue. Ventricular fibrillation a highly rapid and unregulated pulse that causes the majority of abrupt cardiovascular deaths, is the most serious of all. A number of life-threatening arrhythmias are thought to be caused by re-entry or a feedback mechanism, which can be initiated by a variation in propagation velocity levels between the affected and unaffected tissue. The most critical of them is ventricular fibrillation, which is characterised by an abnormally quick and rapid heartbeat and is responsible for a great deal of occurrences of cardiac deaths that occur suddenly.

Mechanism for Regeneration of Cardiomyocytes

Regeneration is not limited to morphological repair alone. In order to fully restore function, newly formed myocardium must be able to integrate electrically and mechanically owing neighbouring host tissue. A substantial innumerable study displaying that altered heart muscle cells created in the adult mammalian heart has refuted the popular belief that the heart is a non-regenerative organ due to its ultimate differentiation of heart muscle cells, its predominant parenchymal cell type [20,21]. Cardiomyocytes grow during gestation through hyperplasia, which is the



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division of cells after attaining cellular duplication, and hypertrophy, which is an increase in the size of cells without a surge in cell number, which results in an increase overall mass[22,23]. The primary distinguishing factor between species with regenerative and non-regenerative hearts is their ploidy level. Following a myocardial infarction, cardiomyocyte ploidy increases significantly, particularly in the border region between damaged and healthy myocardium [24,25].The tendency of the proliferation of cardiac cells increases several-fold following cardiac muscle trauma[26].Unlike contrast with adults, the new born mammalian heart shows a remarkable potential for restoration[27]. It is appealing that this exceptional regenerative capacity is only present in new-borns for a brief period duration following birth, declining quickly within the first two to seven days after birth [27,28,29].

Key elements in the regeneration of heart tissue include factors that promote growth, cell cycle regulators, and mirna. Cardiomyocyte development and heart regeneration depend on a number of intrinsic transmission mechanisms. Growth factors such as follistatin-like 1 and insulin-like growth factor 2 have been shown as having part in cardiac renewal through promoting the process of cell rejoining in heart muscle cells [30]. Additionally, it has been discovered that changes in the extracellular matrix can also influence cardiomyocyte replication, with agrin, a fundamental of the new-born ECM, playing a role in stimulating cardiomyocyte division by causing the dismantle of the dystrophin-glycoprotein complex (DGC) associated with the cardiomyocyte sheath[31]. As mammals transition from intrauterine to extrauterine environments, their hearts undergo significant changes that signify the conclusion of cardiac maturation and the start of myocardial maturation.

The extracellular matrix (ECM) of the heart is a constantly changing structure made up of multiple components, comprising protein peptides non-collagenous forming glycoproteins (including fibronectin and laminin), and collagen. During heart growth, homeostasis, and disease, it is essential for conserving the integrity of tissues and coordinating biological processes [32].The earlier heart matrices govern the transition from epithelial to mesenchymal, resulting in the establishment of the atrioventricular and outflow septa, as well as the peristaltic distributing of the developing heart tube. The matrix also contributes to the expansion of the myocardium[33,34,35]. The regeneration of the cardiac muscle is additionally affected by regulators of cell cycle which includes the cyclins and kinases that are cyclin-dependent. In adult heart tissue, it has been demonstrated that the cyclins in B1 and D1, in addition to CDK1 and CDK4, improve cell division[36].Cardiomyocyte proliferation is regulated during the embryonic period by the Wnt/-catenin pathway. Using genetic methods or by employing the tiny molecule BIO, GSK3- β is inhibited in cardiomyocytes, which stabilises β -catenin and causes it to go to the nucleus. Here, the T cell factor group of protein functions as a transcriptional coactivator, causing cardiomyocyte proliferation.[37,38].

The Notch route, which requires cell-to-cell interaction and takes place when the Notch protein is stimulated by one of its ligands, is another signalling mechanism that plays a role for CM proliferation. The result is the transfer from the cleft Notch inner component to the nucleus, where it functions as a transcriptional co-activator [39].The evolutionary conserved kinase cascade called as the Hippo pathway is vital for controlling abnormal conditions, circulation, growth, heart regeneration, and cardiomyocyte rejuvenation[40,41]. When the Hippo pathway is activated, it causes transcriptional co-activators such as YAP, TAZ, and others to become phosphorylated, preventing their determination to the nucleus and limiting the augmentation of cardiomyocytes [40,42].

Soon after birth, cardiomyocytes exit the cell cycle and shift into a quiescent state, resulting in a consequence of the heart's loss of regeneration capacity. The lungs is were blood oxygenation starts, and when extra- and intracardiac shunts close, vascular resistance in the system and oxygen levels suddenly rise [43]. In reality, the cardiomyocytes are exposed to a hyperoxic condition when the arterial oxygen level rises between thirty to one hundred mmHg, which has a substantial impact on their metabolism[44,45].In this stage, cardiomyocytes irreversibly switch from glycolysis under anaerobic conditions and lactate oxidation, which are the primary energy-producing processes during development, to aerobic metabolism. For a short time, they do, however, retain their distinctive resistance to hypoxia and ischemia[46].



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The transition of heart cells to a senescent state is thought to be significantly influenced by changes in the extracellular matrix, also called the ECM, and cytoskeleton architecture. Particularly, it becomes harder for cells to divide as the heart cytoskeleton and ECM grow more rigid and structured[47].

Heart Muscle Cell Renewal in Model Organisms

Vertebrate hearts exhibit distinctive characteristics that set them apart from those of invertebrate species. These variations, such as the emergence of highly developed chambers with internal endothelium lining, are likely the outcome of distinct environmental modification processes[48]. Regeneration often requires the reconfiguration, to varying extents, of embryonic processes that were initially responsible for the generation of the original tissue, leading to the creation of operating tissue [49]. The zebrafish has become the most well-studied model system for heart regeneration to date, due to the extensive regenerative capacity it possesses even in adulthood and the availability of genetic and molecular tools. Following the surgical excision of a small segment of the ventricle (10–20% of the total), heart muscle tissue restores within 60 days after the excess bleeding from the injury ceases by clotting [50].

While full-grown zebrafish and fetal mammalian cardiomyocytes have a glucose-based metabolism and high proliferative capacity, postnatal mammalian cardiomyocytes reside in an oxygen-rich environment and primarily generate energy through oxidative metabolism. Moreover, they have exited the cell cycle [51,52]. Consistent with this observation, it has been proposed that the histological organization of the zebrafish heart is reminiscent of the trabeculated fetal mammalian heart, in which endocardial cells envelop cardiomyocytes and cardiac fibroblasts are relatively sparse [53]. Following surgical apex resection in 1-day-old mice (P1), the amputated region of the heart was progressively restored with minimal fibrosis. However, when the same procedure was performed at P7, the hearts failed to regenerate. Therefore, whereas pre-existing cardiomyocyte proliferation was the principal regenerative mechanism at P1, the response at P7 resembled that of the adult heart, characterized by fibrosis without any indication of cardiomyocyte proliferation [54]. In a related study, P1 and P7 rat neonates were subjected to ventricular resection. While older rats primarily developed scar tissue, younger rats displayed neomyogenesis and maintained cardiac function over the long term, even with reduced perfusion [55]. Following surgical electrocution of rat hearts in the initial, two, three, and four weeks after birth, reports of comparable results were published[56].

In addition, a clinical study revealed that young individuals diagnosed with abnormal left coronary artery from the pulmonary artery (ALCAPA) disease and exhibiting noticeable indications of left ventricle dysfunction and fibrosis were able to recover after corrective surgery with little to no scarring. Based on these results, the authors suggested which the absence of fibrotic scarring might have resulted from compensating heart cells proliferation, which either replaced the injured tissue with fresh functional myocardium or produced next the cardiac muscle to a point where the scar was negligible[57]. Even though cryoinjury replicates various pathological features of myocardial infarction (MI), it is an artificial form of injury, much like apex resection, and is equally susceptible to surgical inconsistencies. LAD ligation seems to most closely reflect adult heart pathophysiology of all neonatal damage models. It is yet unknown, though, whether the heart injury is a result of ischemia damage or a consequence of the procedure's local tissue loss. Neonatal regeneration mechanisms are successful in correcting or preventing the negative effects of pressure overload, as evidenced by the development in the TAC injury model.

The various new-born cardiac injury models offer useful alternatives for researching particular processes implicated with the mammalian reaction to cardiac damage, including myogenesis, angiogenesis, and fibrosis, even though they do not completely reproduce the disease process of the adult heart.

A Comparison of Cardiac Repair Methods based on Cells and Genes

In the area of heart regeneration medicine, a novel strategy has attracted considerable interest and advanced rapidly.





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Cell based Approach

The most promising strategy for repairing damaged myocardium involves remuscularize the tissue by reinstate absent cardiomyocytes between the establishment of cells capable of cardiomyogenesis, with the bone marrow progenitor cell being the most versatile. However, while the therapeutic effectiveness of BMCs in patients with ischemic and non-ischemic heart failure and experimental outcomes appear to support BMC trans-differentiation, there are doubts regarding the plasticity of BMCs. A different report suggests that BMC engraftment in the damaged heart is temporary and hematopoietic in nature, with BMC-derived cardiomyocytes being observed infrequently and only outside the infarcted myocardium [58].

The political atmosphere in the US more than two decades ago may have made a bearing on the quest for adult stem cells with the potential to differentiate into cardiomyocytes. Prior to the growth using pluripotent stem cells that are induced produced from adult cells, restrictions on the usage and investigation of stem cells from embryos were put in place due to ethical concerns. Due to certain studies, mature mice's hearts include Sca-1+ cells that may serve as a source of fresh cardiomyocytes [59,60,61]. Thorough genetic fate-mapping studies have dismissed the significant involvement of c-Kit+ and Sca1+ progenitor cells in the production of newly regenerated cardiomyocytes. Current research attributes the observed benefits of stem cell transplantation mainly to paracrine or immunomodulatory effects. Further research is necessary to determine the specific factors that lead to improved outcomes [62].

As previously discussed, either bone marrow nor any embryonic stem cells have much of the ability to become heart cells. However, being able to create significant numbers of human cardiomyocytes using pluripotent stem cells is a rare opportunity. Unlike adult embryonic stem cells, pluripotent stem cells (PSCs) are indefinitely scalable and capable of developing into almost any form of adult somatic cell, including cardiomyocytes. Only about 10% of the original embryoid bodies (EBs) exhibited unplanned rigid characteristics, as well as just a tiny percentage of cardiomyocytes could be removed from their prevailing over regions via mechanical dissection, making the EBs method laborious and ineffective[63]. Despite the fact that the studies have yielded a range of results, it's likely that real therapeutic or adverse consequences were overlooked or dismissed because of extremely various animal models and people are from each other. The kind and quantity of transplanted cardiomyocytes, the time of cell administration, and the illness model type (I/R vs. MI) may all have contributed to the reported variations in the probability of cardiac arrhythmias in the investigations.

Tissue engineering might be used to solve the issue of poor engraftment rates following cell injection, and it might also offer a reliable way to deliver drugs. For instance, using epicardial patch coated by follistatin-like 1, an amino acid released through epicardial cells, has been demonstrated to stimulate cardiomyocyte proliferation [64]. It has been demonstrated that this strategy improves left ventricular function across both mouse and pig models, and it is currently being investigated for therapeutic usage. The possibility of increasing engraftment rates is one benefit of adopting tissue engineering techniques in this way. In fact, compared to standard cell injection trials, this approach could require fewer cells to accomplish the identical or even greater graft volume and functional effect. Additionally, this strategy may result in mild immunosuppression. However, the problem of electrical coupling has been a significant drawback of this approach so far[65,66,67].

Transplantation of tissue constructs onto the epicardium consistently establishes an obstruction amongst the newly formed heart and the existing tissue, causing an injured tissue cap to emerge. Although non-myocytes such as fibroblasts or macrophages can facilitate electrical coupling, this structural barrier is likely to hinder the integration. Currently, there is a lack of data on electrical coupling from large animal models with more human-like physiology. In the absence of robust hESC or hiPSC lines from guinea pigs, rabbits, or pigs, the current standard for research is xenogeneic studies. However, these studies require a harsh immunosuppressive regimen that cannot be extrapolated to clinical use, as long-term immunosuppression can cause severe problems [68]. Calcineurin inhibitors frequently cause kidney failure, and they also increase the incidence of tumors, such as nonmelanoma skin cancer, compared to the general population.



**Yamini et al.,****Gene based Approach**

Investigation into the uses of gene as a potential therapy for heart disease involves the transfer of therapeutic genetic information to cardiomyocytes to modify the expression and levels of proteins. Current studies in gene therapy are focused on targeting abnormalities in calcium handling, beta-adrenergic signaling, and cardiac regeneration using non coding RNAs that regulate mRNA translation or pluripotent stem cells. The primary objective of these therapies is to enhance cardiac muscle contractility and cytoprotection, promote angiogenesis, or facilitate the homing of stem cells to the myocardium affected by infarction.

Growth factors such as VEGF, FGF, and the growth factor for hepatocytes are all recognised as having the ability to help with heart regeneration. The VEGF family contains several isoforms, including VEGF-A, -B, -C, and -D. VEGF165 as well as HGF genes are concurrently delivered as part of another gene therapy strategy for managing rats experiencing MI symptoms[69]. The porcine myocardium was evaluated for the angiogenic potential of two newly discovered members of the VEGF family, namely VEGF-B186 and VEGF-D Δ N Δ C [70]. VEGF-A is a potent stimulator of endothelial cell growth and angiogenesis, but it alone generates a vascular network that is permeable and non-functional. Adenoviral vectors are a more efficient delivery system for cardiac gene therapy than plasmid DNA, particularly for cDNA delivery.

Consistent findings indicate a substantial decline in lipoprotein (a) concentrations in 50% of individuals with refractory angina, showing the safety and well-tolerance of AdVEGFDNC gene therapy [71]. In contrast to adenovirus, AAV is less immunogenic. However, these vectors should only be used when long-term expression of the transgene is needed. Nevertheless, the immune response triggered by AAV injection may also impede its clinical application in humans.

FGF is part of a group of 22 multifaceted proteins that have been found across humans as well as mice. It plays a role in regulating key processes within heart tissue, especially growth, stability, and illness. It is acknowledged as a cardiomyokine as well. Vascular endothelial cell development has been found to benefit from activation of FGFR1 or FGFR2. In particular, endothelial cells are stimulated by FGF1, FGF2, and FGF4 to express matrix metalloproteinases (MMPs) [72,73].

HGF plays a role in facilitating angiogenesis by stimulating the proliferation, migration, and survival of endothelial cells. In a minipig model of chronic myocardial ischemia, adenovirus-mediated transfer of the HGF gene (Ad-HGF) was used. In order to do this, an Ameroid constrictor had to be wrapped around the coronary artery's left circumflex branch. Skeletal myoblasts improved with HGF were transplanted into a rat model of myocardial infarction, and this led to smaller infarcts and less collagen buildup, higher vascular density, and enhanced heart function[74].

Reprogramming of cardiac cells was achieved in murine MI models through gene transfer using retroviral and lentiviral vectors. However, direct cardiac reprogramming with these conventional vectors has some limitations, including low efficacy of fibroblast to cardiomyocyte conversion and the risk of insertional mutagenesis [75]. Lentiviruses have the potential to cause insertional oncogenesis as they integrate randomly into the target cell, with a preference for gene coding regions. Notably, eight weeks after MI, a lack of TRPV4 was associated with a significant decrease across scar size and the existence of viable cardiac tissue in the infarcted area. Similarly, reduction of LRP6 in a mouse model of MI was linked to considerably reduced scar size, improved cardiac function, and a greater number of new heart cells generated from pre-existing ones.

Enhancing the binding of IL-13 to the type II IL4R α /IL13R α 1 receptor seems to promote DNA synthesis in cardiomyocytes and facilitate recovery from myocardial infarction in situations where heart regeneration is not naturally occurring, rather than affecting cardiac progenitor cells [76]. Elevating the levels of microRNA-199a, which are substantially decreased during hypoxia, enhances contractility of the cardiac muscle, augments muscle volume and diminishes the size of the scar in swine hearts afflicted with infarction [77]. The hydrogel was utilized to hinder rapid metabolism of viral particles by the beating heart, and facilitated slow release of the particles from the gel,



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resulting in an enhancement of therapeutic effects [78]. Injecting Locked nucleic acid (LNA)-based anti-miR-34a through the tail vein resulted in the downregulation of miR-34a expression levels for over a week, leading to improved cardiac function in adult hearts, delayed remodeling, and reduced formation of fibrotic scars seven days after myocardial infarction injury [79].

Due to its ability to improve sarcoplasmic reticulum Ca²⁺ fluxes and increase SERCA2a enzyme activity, S100A1 has been shown to significantly reduce myocardial necrosis and the development of heart failure. The exact estimation of the remarkably comparable S100A1 proteins from pigs and humans, which only differ in their sequences by one amino acid, is essential. Future gene therapy experiments in large animals will be made possible by the accurate assessment of individual S100A1 in pig hearts[80]. Experiments have been carried out where IL-10 was genetically integrated into stem cells using different gene editing technologies such as TALEN and CRISPR/Cas9. These modified cells were then transplanted into a mouse model with ischemic heart disease, and a decrease in pro-inflammatory factors was observed, leading to a reduction in myocardial cell apoptosis and an increase in capillary density. This resulted in the recovery of cardiac function in models of cardiovascular disease [81,82].

Trials that are Currently Active

A research study conducted on male rats found that a single injection of tropoelastin protein resulted in improved cardiac function by making the muscle damaged by a heart attack more flexible. The team observed a smaller scar, an escalation in scar elastin, and improved blood flow through the heart after 28 days. Further studies will involve both male and female animals to explore any potential sex differences in response to the treatment. The goal is to develop a therapeutic approach that could be administered to humans after a heart attack to reduce scarring and improve elasticity of the heart tissue.

The neuroendocrine hormone oxytocin is capable of reprogramming the epicardium, a collection of adult cells in the outer layers of the heart, into stem cells. These stem cells then permeate deeper layers of the injured heart and help cure the wound by secreting healing agents and regenerating deficient cells involving vessels for blood, heart cells, and inflammatory cells. Further research is required to thoroughly investigate this process, including testing on a range of mammalian species before progressing to human trials.

The LAPiS Study is a phase 1/2 open-label study, lasting for 52 weeks, aimed at assessing the assurance and effectiveness of HS-001 in treating advanced cardiac infarction caused by ischemic heart disease. The study will be conducted at multiple locations in Japan, and during open-heart surgery, HS-001 will be transplanted into the damaged tissue of the heart, along with a planned coronary artery bypass graft procedure. Heartseed has prioritized patient safety and benefit in the development of HS-001 to accomplish this objective. The research revealed that ERBB2 doesn't only act as a switch to block differentiation but also has the potential to rejuvenate the heart and increase its resilience. The mice that received the on-off ERBB2 treatment recovered from heart damage, unlike the control group. Notably, a mouse that received the ERBB2 toggle at three months old showed complete recovery from a severe injury two months later, which is equivalent to an 18-year-old being treated for a heart attack at age 50.

CONCLUSION

Innovative approaches to therapy that focus on the pathogenetic framework of these disorders show enormous potential for reducing the significant health and financial burden of cardiovascular diseases. The most recent advances in the investigation of heart regeneration techniques are summarised in this article. Although the heart is a highly complex organ, techniques that influence its regeneration depend on several variables of nontrivial character. While devices, drugs, and heart transplantation will endure to be the mainstays of cardiac infarction treatment, cardiac regeneration is a viable treatment plan for heart muscle cell loss. Before using gene therapy in clinical settings, safety issues such as potential adverse effects, tumorigenesis caused by specific vectors of viruses, and arrhythmogenesis must be addressed. Additionally, preliminary effectiveness testing for potential new treatments





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demands greater accuracy smaller animal simulations that mimic human coronary infarction. With the goal to accelerate up medical translation and make it easier to identify promising strategies, these could include immediate reconfiguration, cell delivery, or activation of native heart muscle cell proliferation potential.

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Aesthetic and Functional Complications of Dental Implant and their Management : An Update and Review

Preeti Prakash Kale^{1*}, Ameet Mani², Gayathri Chinmayee Buyyanapragada³, Kunal Deshmukh⁴, B. Haritej⁵, Rachita Mustilwar¹ and Meenakshi Singh Thakkur¹

¹Lecturer, Department of Periodontology, Rural Dental College and Hospital, Loni -413736, Ahmednagar, Maharashtra, India.

²Professor and HoD, Department of Periodontology, Rural Dental College and Hospital, Loni - 413736, Ahmednagar, Maharashtra, India.

³Lecturer, Department of Periodontics and Implantology, AECS Maaruti College of Dental Sciences and Research Centre, Karnataka, India

⁴Lecturer, Department of Public Health Dentistry, CSMSS Dental College and Hospital, Aurangabad, Maharashtra, India.

⁵Consultant Periodontist and Implantologist, PARTHA Dental, Ananthapur- 515001, Andhra Pradesh, India

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*Address for Correspondence

Preeti Prakash Kale

Lecturer,
Department of Periodontology,
Rural Dental College and Hospital,
Loni -413736, Ahmednagar,
Maharashtra, India.
E.Mail: preetikale20jan@gmail.com



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ABSTRACT

Treatment with dental implants has evolved from earlier much old procedures to a mainstream clinical activity however its potential benefits and high success rates have led to procedures sometimes being incorrectly used with unfortunate outcomes. Implants have evolved from its use as a functional device to a device which is esthetically acceptable. However, treatment is not always successful because implant is a foreign body. The focus of implant research is shifting from descriptions of clinical success to the identification of factors associated with its complications or failure. Present review gives insight about aesthetic and functional complications of dental implant and their management.

Keywords: Dental implants. Aesthetic, complication, implant failure.





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INTRODUCTION

The pioneering work of Professor Branemark and Andre Schroder ushered a new era in Dentistry i.e. "The Era of Implant Dentistry." Branemark and his colleagues created a new field from a serendipitous research thus exemplifying Pasteur's dictum "Chance Favors the Prepared Mind". Branemarks determined research in osseointegration enabled the surgically related and Prosthodontic disciplines an exciting scope in the new world of Dentistry and gave it a global stand. [1] Treatment with dental implants has evolved from earlier much old procedures to a mainstream clinical activity however its potential benefits and high success rates have led to procedures sometimes being incorrectly used with unfortunate outcomes. Implants have evolved from its use as a functional device to a device which is esthetically acceptable. [2] However treatment is not always successful because implant is a foreign body. The focus of implant research is shifting from descriptions of clinical success to the identification of factors associated with its complications or failure. [3]

Esthetics in dental implantology

When considering esthetics and osseointegrated implants one should follow the philosophy of the great Philadelphian inventor-philosopher Benjamin Franklin; "A stitch in time saves nine". Identification of potential aesthetic problem areas prior to fixture installation frequently permits alternative planning and avoids more complex treatment or retreatment at a later date. Pre-existing hard and soft-tissue deficiencies often preclude achieving ideal esthetic outcomes. Carefully assess the hard- and soft-tissue dimensions of the site in relation to the planned implant position. The aim is to maintain 2 mm of thickness of the facial bone. If necessary, augment the site first to optimize the hard- and soft-tissue conditions before placing the implant. [4]

Using guide stents the most frequently encountered esthetic complication of facially angulated screw access holes is often avoided. When ample bone is available guide stents assist the surgeons in angulating the endosseous fixtures so that the prosthetic screw access will be centered within the facial-lingual dimensions of aesthetically prosthetic teeth. The use of diagnostic imaging including OPG, lateral cephalometric films, computerized tomography scans help determine ideal reception sites for the titanium fixtures. With less than ideal available bone, the divergent long axis fixture angulation, tests the creative skills of the prosthodontist. Labially inclined fixtures create the most frequently encountered esthetic problem in the fabrication of fixed tissue integrated prosthesis. Fixed prosthodontic solutions to labial access holes consists of two fundamental methods: One is the use of double casting. The primary casting is fastened either directly to the fixtures or to the labially inclined abutment connectors. This casting contains a set of retentive screw threads positioned in parallel extensions from the framework to permit an over-casting with teeth to be securely fastened, hiding the second set of screw holes on the lingual or occlusal surfaces. The second method of avoiding facial access holes uses intermediary components designed to change the long axis fixture angulation. Example: angled abutment. [5,6]

Lip Line Effects

Maxillary high and mandibular low lip lines may present an esthetic compromise created by either advanced horizontal and vertical bone loss necessitating a space between the tissue integrated prosthesis and the mucosal tissue or abutment fracture, framework and other metallic component visibility. In addition, uneven residual ridges associated with extractions following osseointegration of previously implanted fixtures require esthetic masking. Use of either extended fixed acrylic or porcelain gingival facades provide improved aesthetics but often make oral hygiene more difficult for the fixed tissue integrated prosthesis. An alternative solution to aesthetic complications due to both fixture alignment and high lip line may be the use of an implant supported over-denture. Lip support is best determined by the pre-surgical prosthesis. Traditional guidelines for the tooth position are that they be placed over the crest of the residual alveolar ridge. [7] Because of the resorptive process following tooth loss, the crest of the ridge and the special position of those teeth will usually create a much smaller arch and be positioned more lingually than the anatomic orientation of the natural dentition. In contrast, the bone anchored stability of the tissue integrated prosthesis allows the prosthetic teeth to be positioned off the crest of the severely atrophic residual ridge. With





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restoration of the occlusal vertical dimension and facial cantilevering of the teeth, appropriate lip support may be attained. The result is better facial esthetics. Facial cantilevers can create complications when the cantilevered distance is excessive or when occlusal forms exert loads exceeding the biomechanical limits of the osseointegration interface. The solution to this complication is multifaceted and includes accurate treatment planning, assessment of the quality and quantity of bone and appropriate load distribution through optimal placement of numerous and long fixtures [8].

Functional complications Phonetics

Phonetic complications may occur if the spatial position of the prosthetic teeth are different from the natural dentition or if patients experience long term accommodation to a prosthesis with poor tooth position. Previously existing speech impediments such as lisps or hissing sounds should be noted in the records and pointed out to the patients. It may also be advisable to record the speech difficulty prior to treatment. These records reduce the anxiety levels for both the patient and doctor following the delivery of a final prosthesis and the continuation of a phonetic abnormality. If pre-surgical diagnostics will predict an emergence of the bone anchored unit in a palatal position addition may be made to the pre-surgical prosthesis to simulate the added bulk of prosthetic material a patient may experience with the final prosthesis in the areas of its connection to the osseointegrated fixtures. Phonetic difficulties may also be experienced by patients when the space between the prosthesis and the residual ridge is excessive. Using shorter abutment connections or adding material to the prosthesis to close the space often improves the phonetic difficulty. The mandibular prosthesis may also provide opportunity for phonetic compromise. Patients with long standing edentulism especially in the mandibular posterior often experience an enlargement of the tongue. Early recognition of this condition with pre-treatment discussion with the patient minimizes the potential for frustration when the confining rigidity of the prosthesis restricts the freedom of the tongue. Excessive enlargement of the tongue may require surgical reduction of the tongue following insertion of the tissue integrated prosthesis if patient cannot adapt to the confining feeling of the prosthesis. Reduction of anterior tongue space or impingement on the lingual muscle attachments has been experienced by patients with lingually inclined fixtures. Alteration of the emergence alignment of these fixtures helps to reduce the impingement. [9] Lip, Cheek and Tongue Biting: Functional complications are minimal yet should be noted, as their occurrence is annoying for the patient. Lip, cheek and tongue biting are the most common functional complications with patients who have experienced long term edentulism without the benefit of prosthetic replacement. Increased buccal overjet for correcting cheek or lip biting may be necessary. Widening of the posterior arch form decreases biting the lateral border of the tongue. However, this solution is limited by occlusal considerations, facial appearance and smile line parameters. Cheek bite may also occur due to loss of vertical dimension. Splints to increase the vertical dimension can be used to correct the problem. [10]

Temporomandibular joint Dysfunction

Functional disabilities such as TMJ dysfunction often benefit greatly from restoration of occlusal vertical dimension and posterior dental support provided by fixed tissue integrated prosthesis. However, with long standing severe joint dysfunction dental replacement itself may be inadequate in providing total comfort. The solution to this complication may be additional occlusal appliance therapy or joint surgery. [11]

Para-functional Habits

Para-functional habits such as bruxism and clenching are not contraindication for implant therapy but they do influence the treatment planning. These may produce added muscle exertion or even spasm. Relaxation techniques, physical therapy and time are often the most appropriate and conservative treatment for this condition. Para-functional habit may also create mechanical and biological complications related to the prosthetic components, materials and bone anchored hardware or the state of osseointegration respectively. A pre-treatment diagnosis of severe bruxism or clenching may require the placement of additional fixtures. The mechanical design of tissue integrated prosthesis for patients with noted clenching habits should reduce the cantilevered extension or support those areas with added fixtures. [12]





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CONCLUSION

Failure of implant has a multifactor dimension. Often many factors come together to cause the ultimate failure of the implant. One needs to identify the cause not just to treat the present condition but also as a learning experience for future treatments. Proper data collection, patient feedback, and accurate diagnostic tool will help point out the reason for failure. An early intervention is always possible if regular check-up is undertaken

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Association between Duration of Type 2 Diabetes Mellitus with NCV in Upper Limb and Hand Grip Strength

Shraddha B Jaybhaye^{1*} and Mansi Trivedi²

¹MPT Scholar, Parul Institute of Physiotherapy, Vadodara, Gujarat, India.

²Assistant Professor, Parul Institute of Physiotherapy, Vadodara, Gujarat, India.

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*Address for Correspondence

Shraddha B Jaybhaye^{1*}

MPT Scholar,

Parul Institute of Physiotherapy,

Vadodara, Gujarat, India.



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ABSTRACT

Type 2 diabetes mellitus(DM) is the most common metabolic disorder. Neuropathy is chronic complication of type 2 DM , so diagnosing peripheral neuropathy at subclinical stage and knowing the effect of duration of diabetes in it is necessary. As diabetic patients have increased risk of developing functional disabilities and much less attention has been given to upper limb neuropathy hence the objective of this study was to evaluate nerve conduction study (NCV), hand grip strength(HGS) and associate this 2 variables with duration of type 2 DM. Twenty subjects of type 2 DM with age from 45-60 years in addition to twenty normal individuals with matched age participated in this study. Both groups were evaluated for NCV of median nerve and handgrip strength of dominant hand. After collecting data statistical analysis was done. There was statistically significant difference regarding NCV of study group and control group, and the mean HGS of study group and control group($p < 0.0001$). there was positive correlation between NCV & HGS in study group ($r = 0.84$), there was negative correlation between duration of DM with HGS & NCV($r = 0.82$) , ($r = 0.78$) respectively. There is reduction of NCV & HGS in type 2 DM and this was negatively correlated to duration of diabetes. Patients who are shown to have early onset neuropathy should be educated for hand care which will limit the progression and prevent secondary complication of diabetic neuropathy.

Keywords: Diabetes mellitus, nerve conduction velocity, hand grip strength.





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INTRODUCTION

Introduction

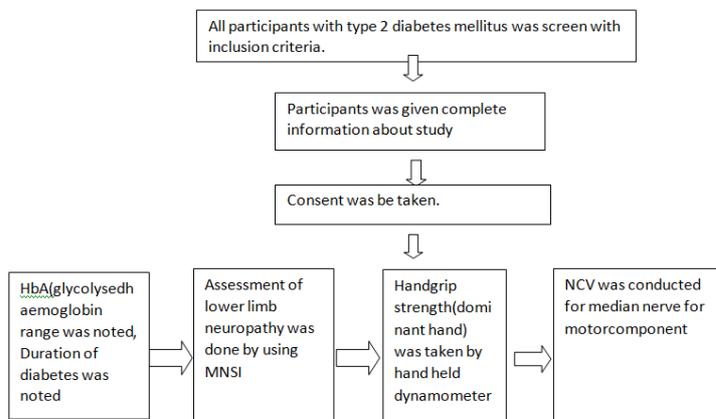
Type 2 diabetes mellitus(DM) is the most common metabolic disorder. According to the World Health Organization (WHO) diabetes mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to damage to the heart, vasculature, eyes, kidneys and nerve [1,2]. The World Health Organization (WHO) describes diabetes mellitus as a chronic metabolic condition marked by high blood glucose levels that, over time, cause damage to the heart, blood vessels, eyes, kidneys, and nerves. T2DM, which is characterised by insufficient insulin secretion by pancreatic islet cells, tissue insulin resistance (IR), and an insufficient compensatory insulin secretory response, accounts for more than 90% of cases of diabetes mellitus. As the illness worsens, insulin secretion becomes unable to keep glucose levels in balance, leading to hyperglycemia [2]. Diabetic Peripheral Neuropathy (DPNN) is the main consequence of T2DM since it also damages nerves. DPN is a well-known micro-vascular consequence of T2DM associated with chronic hyperglycemia and is defined as the presence of peripheral nerve dysfunction in diabetics after exclusion of other causes. Clinically, diabetic neuropathy is a degenerative condition of the peripheral nerve that can cause pain, paraesthesia, problems arising from neurological impairment [4,5]. As duration of diabetes mellitus increases, wallerian degeneration has an impact on every axon. It is true that conduction velocities in diabetic individuals and those with other metabolic neuropathies range from normal to slightly sluggish. The motor and sensory nerve amplitudes are reduced in certain axonal neuropathies.[5]

MATERIALS AND METHOD

Study design

The study was correlational study, it was conducted from December 2021 to September 2022 in Neurological Rehabilitation Department of Parul Sevashram hospital, Parul University. 20 subjects of type 2 DM with were participated in this study. The selection criteria was as follows, type 2 DM with age from 45-55 years in addition to twenty normal individuals with matched age participated in this study. Participants with 5-10 years of duration of type 2 DM and without any limitations in ADL's were included in the study. Participants with CNS dysfunction and musculoskeletal disorder were excluded from the study. Among the subjects who met the selection criteria, the current study included those who received sufficient information and explanation regarding the study and consented to participate in the same. In the act of research consists of human subjects, ethical clearance for this study was acquired from ethical committee of Parul University Institutional Committee for human research. (PUIECHR/PIMSR/00/081734/4510)

PROCEDURE



1.Assessment of handgrip strength by dynamometer^[1]



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The subject was in a sitting position with back support and arm rest. Asked subject to hold a dynamometer, in a dominant hand. Shoulder was adducted, elbow flexed with 90-degree, handle should be rest on middle four fingers. Asked the subject to squeeze dynamometer with maximum isometric effort, maintained it for 5 seconds. 3 maximum contractions will be given with 2 min rest in between. Maximal isometric action was measured by dynamometer before the exercise. The subjects was verbally encouraged.

To evaluate NCV[8]**Motor NCV**

Median nerve

Stimulation site- Elbow:- proximal to elbow crease, medial to biceps tendon

Reference electrode-Distal phalanx of thumb

Ground electrode- on palm

Statistical analysis

Statistical analysis was done by using IBM SPSS version 27.0.1 software. 'p' value for analysed data was ≤ 0.001 . Pearson's correlation coefficient test were used. Microsoft word and excel version 2010 were used to create graph and tables.

RESULTS

There was statistically significant difference regarding NCV of study group and control group, and the mean HGS of study group and control group ($p < 0.0001$). In this study Pearson's coefficient was calculated to study the correlation between HGS and NCV in study group and its correlation to duration of the disease. There was positive correlation between NCV & HGS in study group ($r = 0.84$), there was negative correlation between duration of DM with HGS & NCV ($r = -0.82$), ($r = -0.78$) respectively.

Table no1 shows correlation between handgrip strength with median motor nerve conduction velocity, P value is ≤ 0.001 , pearson's value $r = 0.8417$, which suggest strong positive correlation. Graph No. 1 shows negative linear direction, all of the data points are in pattern trending upwards from left to right, variables are clearly clustered which suggest strong positive correlation

Table no 2 shows correlation between duration of DM with handgrip strength, P value is ≤ 0.001 , pearson's value $r = -0.8219$, which suggest strong negative correlation. Graph no. 2 shows strong negative correlation, as the data points are not following the linear pattern, and variables are scattered which shows strong negative correlation. Table 3 shows correlation between duration of DM with median nerve conduction velocity, P value is ≤ 0.001 , pearson's value $r = -0.7888$, which suggest strong negative correlation. Graph no. 3 shows strong negative correlation, as the data points are not following the linear pattern, and variables are scattered which shows strong negative correlation

Illustrations

DISCUSSION

The present study was done to find out the Association of duration of type 2 DM with NCV in upper limb and hand grip strength in type 2 diabetes mellitus between the age group 45-60 years of both male and female. The hand held dynamometer was use to evaluate the hand grip strength and electromyographic equipment to measure NCV of upper limb of dominated hand of median nerve motor component. The main purpose of this study was to determine correlation of nerve conduction velocity with duration of type 2 DM, handgrip strength in type 2 diabetes mellitus. Ethical clearance was taken from Institutional Ethical Committee, Parul University, Vadodara. The aim of the study was to investigate HGS and median nerve motor NCV in type 2 diabetic patients that whether there is correlation between both variables and its relation to duration of diabetes.





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Hyperglycemia causes a non enzymatic covalent bonding of glucose with proteins that alters their structure and inhibits their functions so these glycosylated proteins can lead to diabetic neuropathy [1]. Reduction in grip strength is associated with poor glycaemic control and increased systemic inflammatory cytokines such as tumor necrosis factor & interleukin-6 which have detrimental effects on muscle function [5]. On the other hand, a cross-sectional study done in 2017 in India has concluded that increasing duration of diabetes was a significant risk factor for abnormal nerve conduction velocity, but it agrees that age has no statistical significance in terms of velocity. In another Indian study conducted in 2018, the result was that there was a significant decrease in the NCV among the diabetic population aged five years or more compared with those with duration of less than five years, concluding that the risk of complications of neuropathy increases with increasing duration and severity of hyperglycaemia[9]

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Table No. 1 Correlation Of Handgrip Strength With Median MNCV.

| | Mean | Std Deviation | r-value | p-value |
|-------------|-------|---------------|---------|---------|
| HGS | 29.17 | 1.67 | 0.8417 | ≤0.001 |
| Median MNCV | 43.01 | 2.39 | | |

Table No. 2 Correlation Of Duration Of Dm With Handgrip Strength

| | Mean | Std Deviation | r-value | p-value |
|----------------|-------|---------------|---------|---------|
| DURATION OF DM | 6.94 | 0.39 | -0.8219 | ≤0.001 |
| HGS | 29.17 | 1.67 | | |

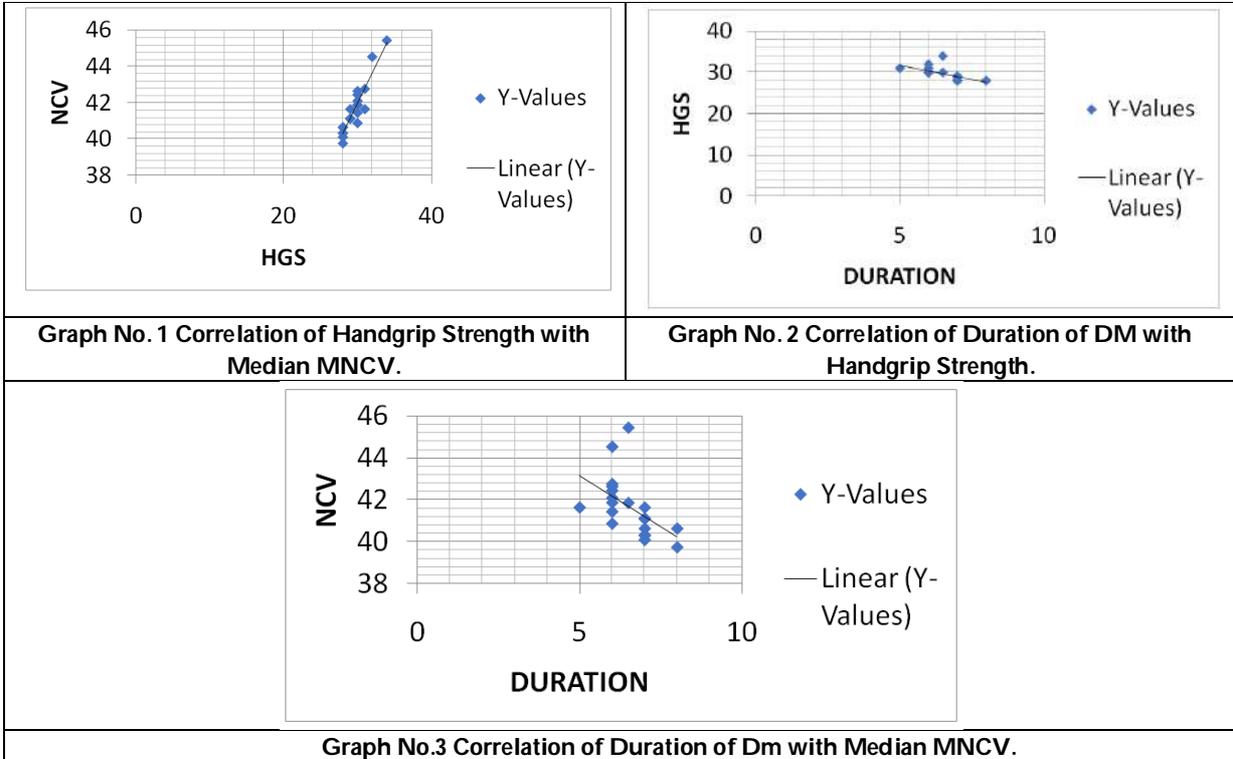
Table No.3 Correlation Of Duration Of Dm With Median MNCV.

| | Mean | Std Deviation | r-value | p-value |
|----------------|-------|---------------|----------|---------|
| Duration of DM | 6.94 | 0.39 | - 0.7888 | ≤0.001 |
| Median MNCV | 43.01 | 2.39 | | |





Shraddha B Jaybhaye and Mansi Trivedi





Phytochemical Characterization of Ethyl Acetate Extract of *Bixa orellana* Leaves using Gas Chromatography-Mass Spectrometry Analysis

Sabarinath.C^{1*}, Kalaichelvan.VK² and Senthil Kumar R³

¹Research Scholar, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India

²Associate Professor, Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India.

³Professor, Department of Pharmaceutical Chemistry, SwamyVivekanandha College of Pharmacy, Elayampalayam-637205, Tamil Nadu, India

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*Address for Correspondence

Sabarinath.C

Research Scholar,
Department of Pharmacy,
Faculty of Engineering and Technology,
Annamalai University, Annamalai Nagar-608 002,
Tamil Nadu, India.
E.Mail: revathi.sabari@gmail.com



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ABSTRACT

Identification of phytochemicals are play an important role in herbal drug research. Gas chromatography-mass spectrometry analysis is one of the important tools for the identification of phytochemicals present in herbal drugs. *Bixa orellana* is an important medicinal herbal under the family of Bixaceae. Current study aimed to evaluate the phytochemical characterization of ethyl acetate extract of *Bixa orellana* leaves using Gas chromatography-mass spectrometry analysis. There are 16 compounds were identified in GC-MS analysis using NIST Library. The four compounds [17.412 %: Capric Acid Isopropyl Ester, 15.476%: Octadecane, 1-(Ethenyloxy)-, 26.598%: Cyclopentaneundecanoic Acid, 8.865%: 2-Dodecenoic Acid] having the maximum peak area compared with all other compounds. It confirms that these phytochemicals are majorly present in the ethyl acetate extract of *Bixa orellana* leaves. Further, isolation and evaluation of the biological activities of identified compounds are needed.

Keywords: GC-MS, Phytochemical analysis, Herbal medicine, Bixaceae, *Bixa orellana*.





INTRODUCTION

Any plant that has components in one or more of its organs that may be utilized therapeutically or that serve as building blocks for the creation of effective medications is considered a medicinal plant [1]. By using this description, it is possible to distinguish between plants that are considered medicinal but have not yet undergone a full scientific investigation and plants whose therapeutic capabilities and ingredients have been scientifically verified [2, 3]. *Bixa orellana* is an herbal plant in the family of Bixaceae. The Brazilian native plant known as *Bixa orellana* also thrives in other parts of South and Central America [4]. *Bixa orellana*, sometimes known as Annatto, is a condiment that is frequently used in cooking to enhance the color of cuisine. However, it is now used in a wide range of industrial production sectors. Due to studies demonstrating the health advantages of its usage, it is now applied to the skin in the form of sunscreen and cosmetics, and its producers are grateful that they were able to cultivate it [5, 6]. Consequently, as we continue to study bioactive compounds from diverse plant families and species. More than 20 different compounds have so far been identified from *Bixa orellana* seeds [7]. In addition to bixin and norbixin, the seeds of annatto have also been shown to contain isobixin, beta-carotene, cryptoxanthin, lutein, zeaxanthin, bixein, bixol, crocetin, ishwarane, ellagic acid, salicylic acid, threonine, tomentosic acid, tryptophan, and phenylalanine [8-10]. However, there is no phytochemical characterization and the identification of secondary metabolites was not explored yet in the ethyl acetate extract of leaves of *Bixa orellana*. The Gas chromatography-mass spectrometry (GC-MS) analysis is one of the sophisticated techniques for the identification of phytochemicals present in herbal drugs [11, 12]. So, the current study was designed to characterize the phytochemicals present in the ethyl acetate extract of *Bixa orellana* leaves using Gas chromatography-mass spectrometry analysis.

MATERIALS AND METHODS

Materials

The ethyl acetate was analytical grade and purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

Plant material collection and authentication

The fresh leaves of *Bixa orellana* were collected from Mettur, Salem District, Tamil Nadu, India. The collected plant material was prepared as a herbarium and the same was identified by Dr. P. Radha, Research Officer (Botany), Central Council of Research in Siddha, Mettur, Tamil Nadu, India.

Extraction

The plant material was dried under shade and powdered using a mechanical mixer. And about 200 g of powdered leaves of *Bixa orellana* was defatted with n-hexane and extracted with ethyl acetate using the Soxhlet extraction technique [13, 14]. The obtained extract was concentrated using distillation and ethyl acetate extract was selected for GC-MS analysis.

GC-MS Analysis

Agilent Model 8890 GC System with Single Quadrupole Mass Spectrometer (5977B MSD) analyzer was used for the separation and identification of phytochemicals from ethyl acetate extract of leaves of *Bixa orellana*. The analysis parameters for GC-MS as follow: Agilent 30 m x 250 μ m x 0.25 μ m column, Syringe Size: 10 μ L Injection, Volume: 1 μ L, Initial temperature: 75 $^{\circ}$ C, Pressure: 11.367 psi, Flow: 1.2 mL/min, Average Velocity: 40.402 cm/sec, Ion Source: EI Source, Temperature: 230 $^{\circ}$ C, Quad Temperature: 150 $^{\circ}$ C and Fixed Electron Energy: 70 eV. NIST Library was used for matching mass spectra and identification of phytochemicals [15-18].





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RESULTS AND DISCUSSION

The results of GC-MS analysis of ethyl acetate extract of leaves of *Bixa orellana* were shown in Table 1 and the spectrum of the same was shown in Figure 1. The reported activities of identified compounds in ethyl acetate extract of leaves of *Bixa orellana* were obtained from <https://pubchem.ncbi.nlm.nih.gov/>. There are 16 compounds were identified in GC-MS analysis of ethyl acetate extract of leaves of *Bixa orellana* using NIST Library. The 5 compounds had the above 5 % of peak area in GC-MS analysis out of 16 compounds. Only the four compounds [17.412%: Capric Acid Isopropyl Ester, 15.476%: Octadecane, 1-(Ethenyloxy)-, 26.598%: Cyclopentaneundecanoic Acid, 8.865%: 2-Dodecenoic Acid] having the maximum peak area compared with all other compounds.

CONCLUSION

In conclusion, the Capric Acid Isopropyl Ester, Octadecane, 1-(Ethenyloxy)-, Cyclopentaneundecanoic Acid and 2-Dodecenoic Acid are majorly identified compounds in ethyl acetate extract of leaves of *Bixa orellana*. Further, the isolation of these compounds and evaluation of biological activity is needed.

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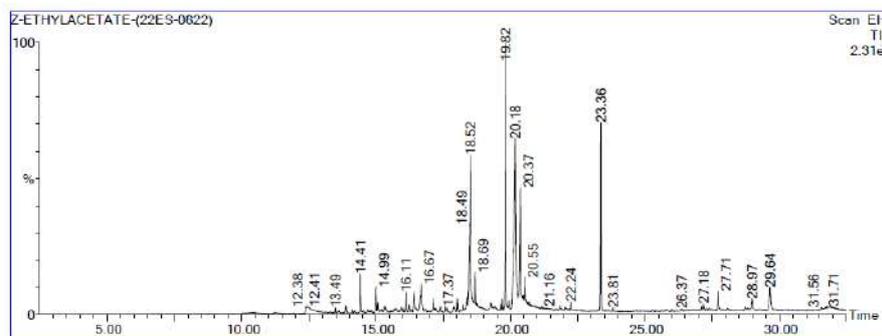


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Table 1: Identified compounds from ethyl acetate extract of *Bixa orellana* leaves using GC-MS

| S.No. | RT | AREA | AREA % | COMPOUND NAME | MOLECULAR FORMULA |
|-------|--------|-------------|--------|---|---|
| 1. | 14.413 | 928,911.7 | 2.474 | Z,Z,Z-4,6,9-NONADECATRIENE | C ₁₉ H ₃₄ |
| 2. | 14.993 | 538,120.7 | 1.433 | METHYL CIS-2-(3-CYCLOPROPYL-7-NORCARANYL)ACETATE | C ₁₃ H ₂₀ O ₂ |
| 3. | 16.109 | 549,018.0 | 1.462 | 9-DECEN-2-ONE | C ₁₀ H ₁₈ O |
| 4. | 16.404 | 730,054.9 | 1.945 | 1,2-DIOXOLAN-3-ONE, 5-ETHYL-5-METHYL-4-METHYLENE- | C ₇ H ₁₀ O ₃ |
| 5. | 16.669 | 1,353,089.2 | 3.604 | 2-TRIDECENAL, (E)- | C ₁₃ H ₂₄ O |
| 6. | 18.030 | 317,145.9 | 0.845 | CARBOISOPROPOXY ISOPROPOXY SULFIDE | C ₇ H ₁₄ O ₃ S |
| 7. | 18.520 | 6,536,438.0 | 17.412 | N-CAPRIC ACID ISOPROPYL ESTER | C ₁₃ H ₂₆ O ₂ |
| 8. | 18.695 | 654,290.4 | 1.743 | 2-UNDECENE, (Z)- | C ₁₁ H ₂₂ |
| 9. | 19.815 | 5,809,613.0 | 15.476 | OCTADECANE, 1-(ETHENYLOXY)- | C ₂₀ H ₄₀ O |
| 10. | 20.175 | 9,985,136.0 | 26.598 | CYCLOPENTANEUNDECANOIC ACID | C ₁₆ H ₃₀ O ₂ |
| 11. | 20.365 | 3,328,049.0 | 8.865 | 2-DODECENOIC ACID | C ₁₂ H ₂₂ O ₂ |
| 12. | 20.546 | 496,903.2 | 1.324 | PROPANENITRILE, 3-(HEXYLOXY)- | C ₉ H ₁₇ ON |
| 13. | 23.357 | 4,110,513.5 | 10.950 | BENZENEPROPIONIC ACID, 4-TETRADECYL ESTER | C ₂₃ H ₃₈ O ₂ |
| 14. | 27.713 | 618,566.9 | 1.648 | BENZYL ALCOHOL, 3,5-DIACETOXY-, ACETATE(ESTER) | C ₁₃ H ₁₄ O ₆ |
| 15. | 28.974 | 466,698.6 | 1.243 | 7,11-HEXADECADIENAL | C ₁₆ H ₂₈ O |
| 16. | 29.639 | 1,117,959.4 | 2.978 | ACETOXYACETIC ACID, NONYL ESTER | C ₁₃ H ₂₄ O ₄ |

Figure 1: GC-MS spectrum of ethyl acetate extract of leaves of *Bixa orellana*



Co-Curricular Activities Programmes of Government and Private High Schools Students in Thiruvannamalai District

N.Suthakar¹ and R.Jeyanthi^{2*}

¹Research Scholar, School of Education, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India

²Associate Professor, School of Education, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600117, Tamil Nadu, India

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*Address for Correspondence

R.Jeyanthi

Associate Professor,

School of Education,

Vels Institute of Science, Technology and Advanced Studies (VISTAS),

Pallavaram, Chennai-600117,

Tamil Nadu, India



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ABSTRACT

This study attempts to explore comparative research on extracurricular activities programs in public and private high schools. This article focuses on students' understanding and organization of extracurricular activities related to physical development, literary and academic development, aesthetic and cultural development, social welfare and civic development. According to research results, public high schools have enough staff and equipment to organize extracurricular activities in the school. But in terms of implementation time, it lags behind private high schools. Research shows that public and private high schools do not meet the standards needed to achieve the appropriate values of extracurricular activities. The results can help improve the implementation and organization of successful extracurricular programs and develop students' hidden talents.

Keywords: Education, extra curricular, co-curricular activities, curriculum

INTRODUCTION

Education is a process of developing a child's personality and inherent abilities. Therefore, the aim of education should be to develop each individual to his or her full potential and to give them the opportunity to achieve as much in life as their natural abilities allow. Modern education recognizes that when the child goes to school, the child enters mentally, physically, spiritually, socially and professionally and as such they must be educated in all of them.



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These activities have been found to be valuable vehicles for developing in students appropriate attitudes, habits, interests and ideals. This specific goal can be maximally achieved through the proper organization of extracurricular activities in the school. Extracurricular activities are now considered an integral part of educational endeavors in schools. Until recently, they were called extracurricular activities. But now these have been recognized as part of the regular curriculum for the holistic education of the child. Instead of being called extracurricular activities, they are now called extracurricular activities. Due to their importance in education, they have been renamed "extracurricular activities" because they are an integral part of the school curriculum. As such, school and extracurricular activities are carried out in parallel. Board of Education of India has emphasized the importance of extracurricular activities as the totality of the learning experience that the school provides to the students through a variety of activities whether inside or outside the school, which are performed under his supervision. The new education system pays special attention to the needs of extracurricular activities. Children can learn more to succeed academically than school assignments fail to convey. Without these activities, the education of children will face many shortcomings and disabilities. Productive and creative activities help develop harmonious 4H - Head, Heart, Hands and Health.

These activities allow children to showcase their many talents. A large number of potential risks to children remain unexplored without facilities for activities. They forge and develop their talents after the process of discovery. Extracurricular activities such as athletics, games and sports promote children's physical development. They are very helpful for children's motor and sensory development. With the current concept of training transformation, if boys and girls want to adapt to society, they must successfully participate in social activities. They must have experience in giving and receiving invitations, giving presentations, conducting interesting conversations, playing games at parties and dancing properly, which can be earned by participating in a variety of activities during the semester. not by getting high scores. in English and social sciences.

More and more people suffer from nervous and mental breakdowns, and as Jacobson rightly points out, relaxation is one of the most essential requirements of physical and mental hygiene. Sports, games, hiking, excursions and other school activities help meet this need. Extracurricular activities are useful to test students' likes and dislikes, to find out their interests and abilities in these areas, and to develop their interests and skills. Qualities that cannot be developed through conventional curriculum such as quick and clear thinking, adaptability, good judgment, initiative, confidence, broad vision, tact, patience, tolerance, integrity, willingness to work, enthusiasm, faith, courage, connection, selflessness, etc. can be developed through extracurricular activities. Extracurricular activities such as drawing and painting, painting, modeling clay, making toys, etc. develop students' aesthetic sensibility.

In fact, extracurricular activities must provide many wonderful opportunities to unleash the creative impulses that are buried under the debris of school routine. Psychologically, these activities are considered necessary because they provide outlets for the student's excess energy flow. They are recognized as a source of replenishment and revitalization of the school curriculum. On the strength of its immense value in education, the Secondary Education Commission commented: "Schools are more than just places. formal learning whose primary concern is the communication of a specified amount of knowledge rather than an organic and living community, primarily concerned with training its students in what we call the "art of living gracefully." Knowledge and learning are certainly valuable, but they must be acquired as a by-product of self-interested activity, only then can they become an important part of the student's spiritual and personal life and influence his behavior. But the "art of living" is a much more global concept than the acquisition of knowledge, however intelligently planned it may be. It involves learning the habits and graces of social life and the ability to work cooperatively in a team: it requires patience, good humour, sincerity, camaraderie and discipline. . These can only be cultivated within the framework of social life and many school activities must find a recognized place in every school"

According to the 2005 National Framework, we must establish Plan and pay attention in the following areas:

1. Connect knowledge to life outside of school.
2. Accordingly, learning will move away from rote methods.
3. Enrich the curriculum to ensure the all-round development of children instead of just focusing on textbooks.



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4. Make exams more flexible and integrated into classroom life and,
5. Nurturing a primal identity informed by benevolent concerns in the country's democratic politics. In the current context, the inclusion of extracurricular activities (ACC) in the curriculum has become one of the leading guiding

Principles for achieving educational goals.

Extracurricular activities as an integral part of general education form a child's personality. As for T.P. Nunn, school is not a place to acquire knowledge but also a place where children have the habit of doing certain activities. These activities are closely related to the actual existence of human life. The primary goal of extracurricular planning is the maximum participation of all students in the school. By providing a coherent program of varied, stimulating activities, the school will not waste students' time and energy but will enhance their intellectual capacity while at the same time training them. Teach them better qualities. The after-school program promotes equal opportunity and learning regardless of caste, creed, gender and economic status.

Considering the above facts, this study was chosen to compare the extracurricular activities practices of public and private high schools in Thoubal district of Manipur. Among the different categories of extracurricular activities, the study focused on five important categories of CCA, which are (i) physical development activities (ii) literary and academic development activities (iii) aesthetic and cultural development (iv) Social welfare activities and (v) civic development activities due to their high degree of conformity with the standards for junior high school students. This research could be helpful in determining the extent to which high school extracurricular activities programs benefit students in shaping their careers from early childhood and in reducing problems. adolescence and rationalize to well-form hobbies and leisure activities.

Objectives of the Study

1. Know the level of awareness about the extracurricular program in public and private high schools.
2. Research on the organization of extracurricular activities programs aimed at physical development, literary development, academics, aesthetic development, culture, social welfare activities and civic development of middle schools. public and private high schools.

Hypotheses of the study

1. There was no significant difference in knowledge of extracurricular activities between public and private high schools.
2. There are no significant differences in the organization of CCA programs for physical development between public and private high schools.
3. There are no significant differences in the organization of CCL programs for literary and academic development between public and private high schools.
4. There are no significant differences in the organization of CCA programs aimed at cultural and aesthetic development between public and private high schools.
5. There are no significant differences in the organization of CCA programs for social welfare activities between public and private high schools.
6. There are no significant differences in the organization of CCL programs for civic development between public and private high schools.

RESEARCH METHOD

Research Methodology refers to the overall strategy you choose to integrate the different components of your research in a logical way, thus ensuring that you solve the research problem effectively. For this study, the researcher chose survey method. Surveys allow the researcher to collect a large amount of data in a relatively short period of time.



**Suthakar and Jeyanthi****Sample**

In this study, only 160 students were selected by simple random sampling from eight (8) high schools in Thiruvanamalai district, Tamil Nadu. The sample included 80 students from four (4) public high schools and 80 students from four (4) private high schools, a truly representative proportion of the population.

Research Tools

In this study, the researcher used a questionnaire to survey the opinions and attitudes of public and private high schools towards after-school activity programs.

Statistical Analysis

To analyze and interpret the test results, the investigator used the mean and standard deviation and the t-test was used to determine the difference between the two samples

INTERPRETATION, RESULT AND DISCUSSION**Difference in the Awareness of Co-curricular Activities Programmes Between Government and Private High Schools Students.**

It was observed mean scores were obtained from 56.05 and 58.91 with SDs of 4.16 and 7.05, respectively. The ratio "t" between the mean scores of the two groups is 3.14, significant at the 0.01 significance level. This means that there is a significant difference between public and private high school students in their perceptions of after-school programs. Arguably, private high school students have a better understanding of after-school programs than public high school students.

Difference between Government and Private High School Students Towards Co-curricular Activities Programmes for Physical Development.

Mean scores of 31.61 and 36.28 were observed with SD levels of 5.09 and 8.28, respectively. The "t" ratio between the mean scores of the two groups is 4.28, significant at the 0.01 significance level. From the above discussion, we can understand that private high school students benefit from better facilities and organized CCA programs for physical development than public high school students.

Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Literary and Academic Development.

Mean scores of 28.61 and 40.04 were observed with SDs of 9.25 and 7.33, respectively. The 't' ratio between the mean scores of the two groups was 6.57, significant at the 0.01 significance level. It can be argued that students in private high schools benefit from better facilities and the organization of CCA programs for literary and academic development than students in public high schools. .

Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Aesthetic and Cultural Development.

Mean scores of 28.31 and 39.68 were observed with SDs of 8.36 and 5.54, respectively. The "t" ratio between the mean scores of the two groups is 10.15, significant at the 0.01 level of significance. It can be argued that private high school students benefit from better facilities and organization of CCA programs for aesthetic and cultural development than public high school students.

Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Social Welfare Activities.

Mean scores of 26 and 33.91 were observed with SDs of 6.66 and 9.37, respectively. The ratio "t" between the mean scores of the two groups is 6.18, significant at the 0.01 significance level. It can be said that students of private high



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schools benefit from better facilities and organization of CCA programs for social welfare activities than students of public high schools.

Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Civic Development Activities.

Mean scores of 35.09 and 38.93 were observed with SDs of 7.99 and 5.91, respectively. The ratio "t" between the mean scores of the two groups is 3.46, significant at the 0.01 significance level. It can be argued that students in private high schools benefit from better facilities and the organization of CCA programs for civic development activities than students in public high schools. .

CONCLUSION AND SUGGESTIONS

1. Public secondary schools have sufficient personnel and equipment to organize extracurricular activities in the school. But in terms of implementation time, it lags behind private high schools.
2. Public and private high schools do not meet the standards necessary to achieve the appropriate values of extracurricular activities.
3. It can be said that students in private secondary schools benefit from better facilities and better organization of extracurricular activities than students in public secondary schools.
4. The analysis also shows that students in private high schools have a better understanding of extracurricular activities than students in public high schools.
5. This study found that programs and activities implemented in extracurricular activities act as a process of finding students' innate talents. This increases each student's competitive spirit, an opportunity to tap into their own potential.
6. Research has found that through after-school programs, students' minds expand with knowledge of cultural awareness. Their social milieu increased and they were no longer misaligned. They can develop the wisdom within themselves to become successful citizens.
7. Analysis provides a good reason why extracurricular programs help students maintain self-confidence and self-discipline. They develop desirable human values of cooperation, solidarity and self-reliance. Their work culture reinforces their innate stamina both physically and mentally.
8. The analysis of this study shows that extracurricular activities give students the opportunity to choose a subject specialization for their future career.

SUGGESTIONS

1. The system includes a standardized test in certain specific extracurricular activities that should be made mandatory in all schools. So there needs to be a minimum standard for a student to achieve at different stages/standards.
2. Due to a certain shortage in the organization of extracurricular activities for public high schools, the competent authority (A.I./D.I.) should periodically inspect their relevant schools and collect data necessary, needs.
3. The government or educational policy makers should create a common platform on which all schools, regardless of government high schools and private schools, can be assessed for student performance. in extracurricular activities and different points should be assigned based on the above. result. Schools that fail to meet the minimum score will be penalized with a fine or unsubscribed.
4. Workshops or seminars related to extracurricular activities should be held regularly to raise organizational standards and not deviate from generally accepted principles for the organization of extracurricular activities. lock up.
5. To strengthen the role of parents in motivating their children to participate in extracurricular activities, a joint parent-teacher conference should be held on this issue and parents should participate in it. extracurricular activities in schools as much as possible.





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6. Teachers and educational planners should organize value-oriented extracurricular activities. They should apply techniques such as goal observation, self-assessment, cumulative recording, etc. aims to assess students' academic performance in various extracurricular activities.

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Table-1. Difference in the Awareness of Co-curricular Activities Programmes Between Government and Private High Schools Students.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 56.05 | 4.16 | 0.92 | 3.14 | 0.01 |
| Private | 80 | 58.91 | 7.05 | | | ** |

** stands for significant at 0.01 level.

Table-2. Difference between Government and Private High School Students Towards Co-curricular Activities Programmes for Physical Development.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 31.61 | 5.09 | 1.09 | 4.28 | 0.01 |
| Private | 80 | 36.28 | 8.28 | | | ** |

** stands for significant at 0.01 level.

Table-3. Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Literary and Academic Development.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 28.61 | 9.25 | 1.74 | 6.57 | 0.01 |
| Private | 80 | 20.04 | 7.33 | | | ** |

** stands for significant at 0.01 level.

Table-4. Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Aesthetic and Cultural Development.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 28.31 | 8.36 | 1.12 | 10.15 | 0.01 |
| Private | 80 | 39.68 | 5.54 | | | ** |





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** stands for significant at 0.01 level.

Table-5. Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Social Welfare Activities.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 26 | 6.66 | 1.28 | 6.18 | 0.01 |
| Private | 80 | 33.91 | 9.37 | | | ** |

** stands for significant at 0.01 level.

Table-6 Difference Between Government and Private High School Students Towards Co-curricular Activities Programmes for Civic Development Activities.

N=160

| Type of school | N | M | SD | SED | t-ratio | Significance |
|----------------|----|-------|------|------|---------|--------------|
| Government | 80 | 35.09 | 7.99 | 1.11 | 3.46 | 0.01 |
| Private | 80 | 38.93 | 5.91 | | | ** |

** stands for significant at 0.01 level.





Enzyme Activity of Arbuscular Mycorrhizal Fungi (*Glomus fasciculatum*) on Zinc Tolerance in Groundnut (*Arachis hypogaea* L.)

P. Munnaji* and T. Ravi Mycin²

¹Ph.D., Research Scholar, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

²Professor, Department of Botany, Annamalai University, Annamalai Nagar, Tamil Nadu, India

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*Address for Correspondence

P. Munnaji

Ph.D., Research Scholar,
Department of Botany,
Annamalai University,
Annamalai Nagar, Tamil Nadu, India.
E.Mail: munnaji21021998@gmail.com



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ABSTRACT

The aim of the current study was to determine the impact of various zinc concentrations affected the enzyme activity of groundnut (*Arachis hypogaea* L.). The groundnut seeds were exposed to 2.5, 5, 7.5, 10, and 12.5 mg of Zn as a untreated seeds were used as control. A randomised block design was used, with three replications of each treatment. Complete observations on the groundnut peroxidase and catalase enzyme activities were made. All results when compared to control demonstrate that zinc metal negatively impacts groundnut growth by decreasing enzyme activity combined with AMF on the enzymatic activity of groundnuts cultivated in pot cultures.

Key words: AM Fungi, Zinc chloride, *A. hypogaea* L.

INTRODUCTION

Environmental contamination is expanding year after year, causing major and irreversible harm to the globe. There are several sorts of environmental pollution, including noise, air, water, soil, and light pollution. The biological system is harmed by them. There are a number of techniques now in use to rid the environment of these types of toxins, but the majority of them are expensive and fall short of their potential. The word "mycophytoremedial" refers to a range of methods that employ plants to decrease, eliminate, degrade, or immobilise environmental toxins, mostly those of anthropogenic origin, with the goal of rehabilitating the area's sites so they may be used for either private or public purposes [1].





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Heavy metals (HMs) are unique products, and as a result of their uniqueness, they cannot be converted into non-toxic forms. Both natural and man-made sources, such as mining, industry, and automobile emissions, release heavy metals into the environment.[2]Zinc (Zn) is one of the important micronutrients required for plant growth and development. Zn deficiency is a major problem and hence adequate Zn supply during crop developmental stages are recommended to improve the nutrient content in the edible parts and also to improve productivity [3]. The present study examines the interactions between Zn and other nutrients in soil, behaviour in plant growth. It stresses the need for identification of the factor responsible for any Zn response to the addition of another nutrient compound [4].Zn is an imperative constituent of particular proteins called as zinc fingers that attach to DNA and RNA and plays major role in their stabilization and regulation [5].

The peanut (*Arachis hypogea*) is one of the most important oil crops worldwide ,with high nutritional values. Yield of groundnut is constrained owing to diverse biotic and abiotic factors[6].Globally, peanut by-products (skins and hulls) are cultivated as trash to be disposed of whereas peanut seeds are grown for the manufacture of oil, peanut butter, confections, snacks, and protein extenders. The value-added properties of peanut skins and skin extracts have been reported in several research as an antioxidant, functional food component, animal production feed ingredient and antibacterial agent [7].

Arbuscular mycorrhizal fungi (*Glomus fasciculatum*) have obligatory symbiotic relationship with more than 80% of terrestrial plant species. AMF symbiosis acclimatizes plants for their better survival, enhanced growth and development in biotic as well abiotic environment, thereby promoting sustainable growth and development of plants[8]. Arbuscular Mycorrhizal (AM) fungi are obligatory biotrophic symbionts living in the roots of most terrestrial plants. AM fungi (AMF) have a positive effect on plant growth and plant nutrition, especially under stress conditions[9].

MATERIALS AND METHODS

The seeds Black gram (CoGn-4) was obtained from Tamil Nadu Agricultural University (TNAU), Coimbatore and Tamil Nadu. The uniform seeds are selected for the experimental purpose. Source of $ZnCl_2$ stock solution prepared by dissolving the molecular weight of $ZnCl_2$ in different concentrations viz.,(garden soil Control,T₁-2.5mg,T₂-2.5mg+AMF,T₃-5mg,T₄-5mg+AMF,T₅-7.5mg,T₆-7.5mg+AMF,T₇-10mg,T₈-10mg+AMF,T₉-12.5mg,T₁₀-12.5mg+AMF) of $ZnCl_2$ the solution were prepared freshly at the time of experiments. The pots were filled with 5 Kg of garden soil, selected groundnut seeds were sown in the pots and irrigated with normal tap water. Untreated pot was maintained as the control.

AM Fungi

The AM Fungi (*Glomus fasciculatum*) were collected from Department of Microbiology Tamil Nadu Agricultural University (TNAU), Coimbatore, Tamil Nadu, India.

Enzymatic Activities

The activities of catalase and peroxidase were estimated and recorded at different days intervals.

Catalase

Catalase Activity

Catalase activity was assayed as described by Chandlee and Scandalios (1984) [10]. Extraction Five hundred milligrams of frozen material was homogenized in 5 ml of ice-cold 50 mM sodium phosphate buffer (pH 7.5) containing in 1mM PMSF. The extract was centrifuged at 4 for 20 minutes at 12,500 rpm. The supernatant was used for enzyme assay



**Munnaji and Ravi Mycin****Assay**

The activity of enzyme catalase was measured using the method of Chandlee and Scandalios (1984) with modification that the assay mixture contained 2.6 ml of 50 mM potassium phosphate buffer (pH – 7.0), 0.4 ml of 15 mM H₂O₂ and 0.04 ml of enzyme extract. The decomposition of H₂O₂ was followed by the decline in absorbance at 240 nm. The enzyme activity is expressed in units 1 mM of H₂O₂ reduction per minute per mg protein.

Peroxidase Activity

Peroxidase activity was assayed by the method of Kumar and Khan (1982) [11]. Assay mixture of peroxidase contained 2 ml of 0.1M phosphate buffer (pH 6.8), 1ml of 0.01M pyrogallol, 1ml of 0.005M H₂O₂ and 0.5 ml of enzyme extract. The solution was incubated for 5 min at 25°C after which the reaction was terminated by adding 1ml of 2.5N H₂SO₄. The amount of purpurogallin formed was determined by measuring the absorbance at 420 nm against a blank prepared by adding the extract after the addition of 2.5N H₂SO₄ at zero time. The activity is expressed in unit mg⁻¹ protein. (One unit is defined as the change in the absorbance by 0.1 min⁻¹ mg⁻¹ protein).

RESULT AND DISCUSSION**Catalase Activity**

The effect of different concentrations of Zinc with AMF on Catalase contents (Units min⁻¹ mg⁻¹ protein). In leaf of groundnut at 20, 40, 60, 80 and 100 DAS is given in Table 1. The highest Catalase content of leaf was observed as (4.58, 8.36, 10.47, 21.53 and 15.36 Units min⁻¹ mg⁻¹ protein.) was recorded in 2.5mg(Zn)+AMF(T₂) treated plants at 20,40,60, 80 and 100 DAS respectively. The lowest catalase content of leaf (0.42, 0.66, 2.35, 2.96 and 2.27 min⁻¹ mg⁻¹ protein.) was observed in 12.5mg(Zn)T₉ concentration of Zinc treated plants at 20,40,60, 80 and 100 DAS respectively.

Peroxidase Activity

The effect of different concentrations of Zinc with AMF on Peroxidase contents (Units min⁻¹ mg⁻¹ protein.) in leaf of groundnut at 20,40,60, 80 and 100 DAS is given in Table 2. The highest peroxidase content of leaf (6.93, 9.38, 21.38, 24.17 and 10.36 min⁻¹ mg⁻¹ protein.) was recorded in 2.5mg (Zn)+AMF Zinc treated plants at 20,40,60, 80 and 100 DAS respectively. The lowest Peroxidase content of leaf (1.65, 2.15, 3.25, 7.24 and 3.14 min⁻¹ mg⁻¹ protein.) was observed in 12.5mg(Zn) T₉ treated plants at 20,40,60, 80 and 100 DAS respectively. It's reported that the enzymes like catalase and peroxidase were low in AMF than the control [12]. They also found that the peroxidase and catalase increased with increasing heavy metal concentrations. Antioxidant enzymes such as CAT, APX and SOD play a vital role in increasing defensive mechanisms towards more ROS production. On the contrary, in this work, it is found that the catalase and peroxidase increased with treatment of AMF and they decreased with increase in concentration of Zn. It's reported that catalase and peroxidase activity significantly increased in all stress conditions when seeds are treated with (Brassinolide)BS or without BS [13]. As PEG stress increased from -5 bar to -15 bar catalase and peroxidase activity also increased. This is in accordance with the current study. It's suggested that the AMF is able to maintain mycorrhizal symbiosis in Zn toxic soils and significantly increase the plant growth, productivity and nutrient contents [14]. The same result is observed in this research. The study reported that the effects of autochthonous microorganisms (AMF and/or plant growth promoting bacteria) on the antioxidant activities of plants growing in a multi heavy metal contaminated soil [15] showed that enzyme activities including SOD, CAT, POD, GR, and APX increased in tomato plants under Cd stress, and that inoculation with AMF further raised their activity. This continued rise in antioxidant activity points to AMF's potential function in mediating the rapid scavenging of ROS. It's revealed that Pb and Zn increased the superoxide dismutase (SOD), peroxidase, glutathione synthetase (GSH), malondialdehyde (MDA), and catalase levels with increased concentrations from 100 mg/kg to 200 mg/kg significantly (p < 0.05) compared to the control Maize plants [16;17]. On the contrary, in the current study, the amount of peroxidase and catalase decreased with the increased concentration of Zn in groundnut, but increased when treated with AMF.



**Munnaji and Ravi Mycin****CONCLUSION**

Zinc (Zn) is one of several heavy metals that cause severe environmental contamination in soil, sediments and groundwater. There are a number of techniques now in use to rid the environment of these types of toxins, but the majority of them are expensive and fall short of their potential. The word "mycophytoremedial" refers to a range of methods that employ plants to decrease, eliminate, degrade, or immobilise environmental toxins, particularly those of anthropogenic origin, with the goal of rehabilitating the area's sites so they may be used for either private or public purposes. AMF (*Glomus fasciculatum*) are among the most prevalent types of soil fungi, and the majority of plant species have relationships with AM fungal species.

The effect of Zinc and AM fungi on seed enzyme activities of groundnut plants the coGn-4 varieties of groundnut seeds were obtained from the Tamil Nadu agricultural university Coimbatore. When $ZnCl_2$ salts were employed as a therapy, enzyme activity measures including catalase and peroxidase were higher at AMF than in the control group. But because zinc levels were higher in the AMF group compared to the control group, fewer enzymes like catalase and peroxidase were inhibited. It was then discovered to rise with rising heavy metal concentrations. It was then discovered to rise with rising heavy metal concentrations. The current study has significantly increased productivity by utilising AM fungi, a symbiotic fungal association with higher plants, in an effort to lessen the toxicity of industrial waste, particularly with regard to zinc, and to develop life-saving mechanisms to shield agricultural fields from zinc antagonistic effects.

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Table-1: Effect of zinc and AMF on the catalase activity of groundnut (*Arachis hypogea*.L)

| Treatments with | Zinc + AMF | Catalase(units min ⁻¹ mg ⁻¹ protein) | | | | |
|---------------------------------|------------|---|-------------------|--------------------|--------------------|--------------------|
| | | Day After Sowing (DAS) | | | | |
| | | 20 | 40 | 60 | 80 | 100 |
| Control | | 3.97±0.119 | 5.48±0.164 | 7.37±0.221 | 10.73±0.322 | 9.27±0.278 |
| 2.5 mg ZnCl ₂ | | 3.31±0.099 | 4.37±0.131 | 5.97±0.179 | 8.37±0.251 | 7.46±0.224 |
| 2.5 mg ZnCl ₂ + AMF | | 4.58±0.137 | 8.36±0.250 | 10.47±0.314 | 21.53±0.646 | 15.36±0.460 |
| 5 mg ZnCl ₂ | | 3.14±0.094 | 3.46±0.104 | 4.83±0.145 | 6.87±0.206 | 5.47±0.164 |
| 5 mg ZnCl ₂ + AMF | | 3.28±0.098 | 5.38±0.161 | 6.12±0.183 | 7.78±0.233 | 6.27±0.188 |
| 7.5 mg ZnCl ₂ | | 2.22±0.066 | 3.34±0.100 | 4.38±0.131 | 4.96±0.148 | 2.37±0.071 |
| 7.5 mg ZnCl ₂ + AMF | | 2.91±0.087 | 3.87±0.116 | 4.63±0.139 | 5.26±0.157 | 3.88±0.116 |
| 10 mg ZnCl ₂ | | 0.87±0.026 | 2.98±0.089 | 3.36±0.101 | 3.98±0.119 | 3.26±0.098 |
| 10 mg ZnCl ₂ + AMF | | 0.98±0.029 | 3.25±0.097 | 3.97±0.119 | 4.47±0.134 | 3.37±0.101 |
| 12.5 mg ZnCl ₂ | | 0.42±0.012 | 0.66±0.019 | 2.35±0.070 | 2.96±0.088 | 2.27±0.068 |
| 12.5 mg ZnCl ₂ + AMF | | 0.83±0.024 | 2.34±0.070 | 2.97±0.089 | 3.87±0.116 | 2.56±0.076 |

± Standard deviation





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Table-2: Effect of zinc and AMF on the Peroxidase activity of groundnut (*Arachis hypogea*.L)

| Treatments with | Zinc + AMF | Peroxidase (units min ⁻¹ mg ⁻¹ protein) | | | | |
|---------------------------------|------------|--|-------------------|--------------------|--------------------|--------------------|
| | | Day After Sowing (DAS) | | | | |
| | | 20 | 40 | 60 | 80 | 100 |
| Control | | 0.96±0.029 | 2.26±0.067 | 2.29±0.068 | 2.96±0.088 | 2.15±0.064 |
| 2.5 mg ZnCl ₂ | | 4.97±0.149 | 6.88±0.206 | 9.38±0.281 | 23.26±0.698 | 8.47±0.254 |
| 2.5 mg ZnCl ₂ + AMF | | 6.93±0.208 | 9.38±0.281 | 21.38±0.641 | 24.17±0.725 | 10.36±0.311 |
| 5 mg ZnCl ₂ | | 3.16±0.095 | 4.36±0.131 | 5.47±0.164 | 7.35±0.220 | 5.15±0.154 |
| 5 mg ZnCl ₂ + AMF | | 3.87±0.116 | 5.16±0.154 | 7.84±0.235 | 21.24±0.637 | 7.18±0.215 |
| 7.5 mg ZnCl ₂ | | 3.66±0.109 | 3.96±0.118 | 4.97±0.149 | 7.16±0.214 | 4.99±0.149 |
| 7.5 mg ZnCl ₂ + AMF | | 3.78±0.113 | 4.14±0.124 | 5.16±0.155 | 18.16±0.544 | 5.10±0.153 |
| 10 mg ZnCl ₂ | | 2.95±0.088 | 3.16±0.095 | 3.86±0.116 | 8.14±0.244 | 4.25±0.127 |
| 10 mg ZnCl ₂ + AMF | | 3.15±0.094 | 3.83±0.115 | 4.16±0.124 | 11.17±0.335 | 4.97±0.149 |
| 12.5 mg ZnCl ₂ | | 1.65±0.049 | 2.15±0.064 | 3.25±0.097 | 7.24±0.217 | 3.14±0.094 |
| 12.5 mg ZnCl ₂ + AMF | | 2.86±0.086 | 2.95±0.088 | 3.45±0.103 | 7.97±0.239 | 4.15±0.124 |

± Standard deviation





On Continuity of γ^* GRWC Sets

M.K.Eswarlal^{1*} and R.Bhavani²

¹Assistant Professor, Department of Mathematics, Sourashtra College, (MKU22PFOS10531), Affiliated to Madurai Kamaraj University, Madurai, Tamil Nadu, India.

²Assistant Professor and Research Supervisor, PG and Research Department of Mathematics, MTN College, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

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*Address for Correspondence

M.K.Eswarlal

Assistant Professor,
Department of Mathematics,
Sourashtra College, (MKU22PFOS10531),
Affiliated to Madurai Kamaraj University,
Madurai, Tamil Nadu, India.
E.Mail: eswarlalmaths@gmail.com



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ABSTRACT

Here continuity of γ^* grwc (γ^* Generalized Regular Weakly Closed) sets are discussed. For this purpose the γ^* grwc sets are introduced and used. Further investigated and analyzed its relationship with various sets in a topological space (TS).

Key words: γ^* grwc set.

INTRODUCTION

Topology plays a vital role in Mathematics and it is considered as one of the useful branch. Norman Levine explained the Generalized closed sets [9] in 1970 and derived many properties in a TS. Here continuity of γ^* grwc sets were analyzed. This paper is framed as follows, in section 2 - literature Review and preliminary concepts were presented. section 3 - deals with continuity of γ^* grw closed set and finally the conclusion is given in section 4.

LITERATURE REVIEW AND PRELIMINARIES

In this section, some of the literatures were reviewed related to study area and preliminaries were mentioned. Generalized closed sets was introduced by Levine [9] (1970) and studied some of its properties. Balachandran et al [2] (1996) has discussed g – continuous maps, that contains continuous as well as gc – Irresolute maps. Mashour et al [12] (1981) investigated α - continuous and α - open mapping in a TS. Sundaram et al [16] (1991) has introduced a class of semi generalized continuous maps. In this paper, we introduce γ^* grwc sets and studied its continuity in a TS.





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Basic concepts of Topology

Definition 2.1 Closure (C_l) and Interior (I) of a set.[8]

Let X be a TS and $A \subseteq X$, $I(A)$ is defined as the union of all open sets contained in A and $C_l(A)$ is defined as the intersection of all closed sets containing A .

Definition 2.2

Let (X, τ) be TS and a subset A of X is said to be

- (i) α open [10] if $C_l(A) \subseteq I(C_l(I(A)))$ and α closed set[13] if $C_l(I(C_l(A))) \subseteq A$.
- (ii) regular open [14] if $A = I(C_l(A))$ and regular closed [4] if $A = C_l(I(A))$.
- (iii) δ closed [8] if $\delta-C_l(A) = A$, Where $\delta-C_l(A) = \{x \in X : I(C_l(H)) \cap A \neq \emptyset, H \in \tau \text{ and } x \in H\}$
- (iv) Weakly closed (w -closed) [12], if $C_l(A) \subseteq H$ whenever $A \subseteq H$ and H is semi open in (X, τ) .
- (v) γ open [2], if $A \subseteq I(C_l(A)) \cup C_l(I(A))$ and γ closed [4] if $C_l(I(A)) \cap I(C_l(A)) \subseteq A$.
- (vi) Semi open set [7] if $A \subseteq C_l(I(A))$ and semi closed set [8] if $I(C_l(A)) \subseteq A$.

Definition 2.3

Let (X, τ) be TS & $A \subseteq X$ is called

- (i) Generalized closed set (g closed) [6] if $C_l(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ) .
- (ii) δg closed [3], if $\delta C_l(A) \subseteq H$ whenever $A \subseteq H$ is open in (X, τ) .
- (iii) θg closed set [4] if $C_{l_\theta}(A) \subseteq U$ whenever $A \subseteq U$ and U is open.
- (iv) Generalized α closed ($g \alpha$ closed)[8] if $\alpha cl(A) \subseteq H$ and H is α open in (X, τ)
- (v) αg closed [8], if $\alpha C_l(A) \subseteq H$ and H is open in (X, τ)
- (vi) Regular generalized weakly set (rgw closed) [15], if $C_l(I(A)) \subseteq U$ whenever $A \subseteq U$ and U is regular semi open in X .
- (vii) sg closed [1], if $sC_l(A) \subseteq U$ whenever $A \subseteq U$ and U is semi open in X .

$G \gamma$ 'grw closed set

In this section, we introduce, investigate a new class of continuous functions using the new set called γ 'grwc (γ ' Generalized Regular Weakly closed) Sets.

Definition 3.1

A function $f : (X, \tau) \longrightarrow (Y, \sigma)$ is called γ 'grw continuous if the inverse image of every closed set in (Y, σ) is γ 'grwc set in (X, τ) .

Theorem 3.1

Every continuous function is γ 'grwc continuous.

Proof:

Let $f : (X, \tau) \longrightarrow (Y, \sigma)$ be a continuous map. Let V be a closed set in (Y, σ)

Then $f^{-1}(V)$ is closed in (X, τ) . Since every closed set is γ 'grwc. Therefore $f^{-1}(V)$ is closed in (X, τ) . (i.e) f is γ 'grwc continuous.

Remark 3.1

The converse of Theorem 3.1 need not be true.

Example 3.1

Let $X = \{e, m, s, h\}$, $\tau = \{ \emptyset, X, \{e\}, \{m\}, \{e, m\}, \{e, m, s\} \}$ and

$\tau^1 = \{ \emptyset, X, \{h\}, \{s, h\}, \{e, s, h\}, \{m, s, h\} \}$





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γ *grw closed sets of (X, τ) are $\{\emptyset, X, \{e\}, \{m\}, \{s\}, \{h\}, \{e, m\}, \{e, s\}, \{e, h\}, \{m, s\}, \{m, h\}, \{s, h\}, \{e, m, s\}, \{e, m, h\}, \{m, s, h\}, \{e, s, h\}\}$

Let $Y = \{e, m, s, h\}$, $\sigma = \{\emptyset, Y, \{e\}\}$ and $\sigma' = \{\emptyset, Y, \{m, s, h\}\}$

Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = h, f(m) = s, f(s) = e$ and $f(h) = m$.

Here $\{m, s, h\}$ is closed in (Y, σ) but $f^{-1}(\{m, s, h\}) = \{e, m, h\}$ is not closed in (X, τ) .

Hence f is not continuous. But $\{e, m, h\}$ is γ *grw closed set. Therefore f is γ *grw continuous.

The proof of the following theorems is based on the above definitions and known results,

Theorem 3.2

- (i) Every rgw, θg and α continuous is γ *grw continuous.
- (ii) Every $\gamma, g\alpha$ and αg continuous is γ *grw continuous.
- (iii) Every $g, \delta g$ and semi closed continuous is γ *grw continuous.
- (iv) Every w continuous is γ *grw continuous.
- (v) Every wg and sg continuous is γ *grw continuous.

Remarks 3.2

The following examples 3.2 to 3.6 shows that the converse of the above theorem need not be true (except rgw continuous).

Example 3.2

Let $X = \{e, m, s, h\}$, $\tau = \{\emptyset, X, \{e\}, \{m\}, \{e, m\}, \{e, m, s\}\}$ and

$\tau' = \{\emptyset, X, \{h\}, \{s, h\}, \{e, s, h\}, \{m, s, h\}\}$

Let $Y = \{e, m, s, h\}$, $\sigma = \{\emptyset, Y, \{h\}\}$ and $\sigma' = \{\emptyset, Y, \{e, m, s\}\}$

(i) Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = e, f(m) = h, f(s) = m$ and $f(h) = s$.

Here $\{e, m, s\}$ is closed in (Y, σ) and $f^{-1}(\{e, m, s\}) = \{e, s, h\}$ is closed in (X, τ) .

Hence f is rgw continuous also $\{e, s, h\}$ is γ *grw closed. Therefore "every rgw continuous is γ *grw continuous"

(ii) Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = e, f(m) = m, f(s) = s$ and $f(h) = h$.

In this case $\{e, m, s\}$ is closed in (Y, σ) but $f^{-1}(\{e, m, s\}) = \{e, m, s\}$ is not closed in (X, τ) . Thus θg is not continuous. But $\{e, m, s\}$ is γ *grw closed. (i.e.) f is γ *grw continuous.

(iii) Let $Y = \{e, m, s, h\}$, $\sigma = \{\emptyset, Y, \{h\}, \{e, h\}\}$ and $\sigma' = \{\emptyset, Y, \{m, s\}, \{e, m, s\}\}$

Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = s, f(m) = m, f(s) = e$ and $f(h) = h$.

Here $\{m, s\}$ and $\{e, m, s\}$ is closed in (Y, σ) how ever $f^{-1}(\{m, s\}) = \{e, m\}$ and $f^{-1}(\{e, m, s\}) = \{e, m, s\}$ is not closed in (X, τ) . Which is γ *grw continuous but not α continuous.. (i.e.) f is γ *grw continuous.

Example 3.3

Consider $X = \{e, m, s, h\}$, $\tau = \{\emptyset, X, \{e\}, \{m\}, \{e, m\}, \{e, m, s\}\}$ and

$\tau' = \{\emptyset, X, \{h\}, \{s, h\}, \{e, s, h\}, \{m, s, h\}\}$

Let $Y = \{e, m, s, h\}$, $\sigma = \{\emptyset, Y, \{e\}, \{m\}, \{e, m\}\}$ and $\sigma' = \{\emptyset, Y, \{s, h\}, \{m, s, h\}, \{e, s, h\}\}$

(i) Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = s, f(m) = m, f(s) = e$ and $f(h) = h$.

Here $\{s, h\}, \{m, s, h\}$ and $\{e, s, h\}$ are closed in (Y, σ) but $f^{-1}(\{s, h\}) = \{e, h\}$ is not closed in (X, τ) , consequently f is γ *grw continuous not γ continuous.

(ii) Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = s, f(m) = m, f(s) = e$ and $f(h) = h$.

We see that $\{s, h\}$ and $\{m, s, h\}$ is closed in (Y, σ) but $f^{-1}(\{s, h\}) = f^{-1}(\{e, s, h\}) = \{e, h\}$ and $f^{-1}(\{m, s, h\}) = \{e, m, h\}$ is not closed in (X, τ) . Which is γ *grw continuous but not $g\alpha$ continuous. (i.e.) f is γ *grw continuous





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(iii) Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = s, f(m) = m, f(s) = e$ and $f(h) = h$.

Now $\{s,h\}$ and $\{m,s,h\}$ is closed in (Y, σ) but $f^{-1}(\{s,h\}) = f^{-1}(\{e,s,h\}) = \{e,h\}$ and $f^{-1}(\{m,s,h\}) = \{e,m,h\}$ is not closed in (X, τ) . Which is γ^* grw continuous but not αg continuous. (i.e) f is γ^* grw continuous

Example 3.4

Take $X = \{e,m,s,h\}, \tau = \{\emptyset, X, \{e\}, \{m\}, \{e,m\}, \{e,m,s\}\}$ and

$\tau' = \{\emptyset, X, \{h\}, \{s,h\}, \{e,s,h\}, \{m,s,h\}\}$, Let $Y = \{e,m,s,h\}, \sigma = \{\emptyset, Y, \{s\}\}$ and $\sigma' = \{\emptyset, Y, \{e,m,h\}\}$. Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = e, f(m) = h, f(s) = m$ and $f(h) = s$.

Here $\{e,m,h\}$ is closed in (Y, σ) but $f^{-1}(\{e,m,h\}) = \{e,m,s\}$ is not closed in (X, τ) . Hence f is not $g, \delta g$ and semi closed continuous. But $\{e,m,s\}$ is γ^* grw closed. Obviously f is γ^* grw continuous.

Example 3.5

let $X = \{e,m,s,h\}, \tau = \{\emptyset, X, \{m\}, \{e,m\}, \{e,m,s\}\}$ and

$\tau' = \{\emptyset, X, \{h\}, \{s,h\}, \{e,s,h\}\}$

Let $Y = \{e,m,s,h\}, \sigma = \{\emptyset, Y, \{e\}, \{e,h\}\}$ and $\sigma' = \{\emptyset, Y, \{m,s,h\}, \{m,s\}\}$

Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = e, f(m) = m, f(s) = s$ and $f(h) = h$.

Certainly $\{m,s,h\}$ and $\{m,s\}$ are closed in (Y, σ) . Now $f^{-1}(\{m,s\}) = \{m,s\}$ and $f^{-1}(\{m,s,h\}) = \{m,s,h\}$ are not closed in (X, τ) .

Hence f is not w continuous. (i.e.) f is γ^* grw continuous.

Example 3.6

Let $X = \{e,m,s,h\}, \tau = \{\emptyset, X, \{e\}, \{m\}, \{e,m\}, \{e,m,s\}\}$ and

$\tau' = \{\emptyset, X, \{h\}, \{s,h\}, \{e,s,h\}, \{m,s,h\}\}$

Let $Y = \{e,m,s,h\}, \sigma = \{\emptyset, Y, \{s\}, \{m,s\}\}$ and $\sigma' = \{\emptyset, Y, \{e,h\}, \{e,m,h\}\}$

Let $f : (X, \tau) \rightarrow (Y, \sigma)$ be defined as $f(e) = e, f(m) = h, f(s) = m$ and $f(h) = s$.

Here $\{e,h\}$ is closed in (Y, σ) but $f^{-1}(\{e,h\}) = \{e,m\}$ is not closed in (X, τ) .

Which is γ^* grw continuous but not wg and sg continuous. (i.e) f is γ^* grw continuous.

Remark 3.3

Thus fig (i) represents the relationship between γ^* grw continuous and $g, \delta g, \theta g, \gamma$, and α continuous.

Remark 3.4

Relationship between γ^* grw continuous and $rgw, sg, w, wg, g\alpha$ and αg continuous are represented in Thus fig (ii).

CONCLUSION

We found the relation between γ^* grw continuous set and various sets. These results can be extended to other TS in general, to Grill Topological spaces in particular.

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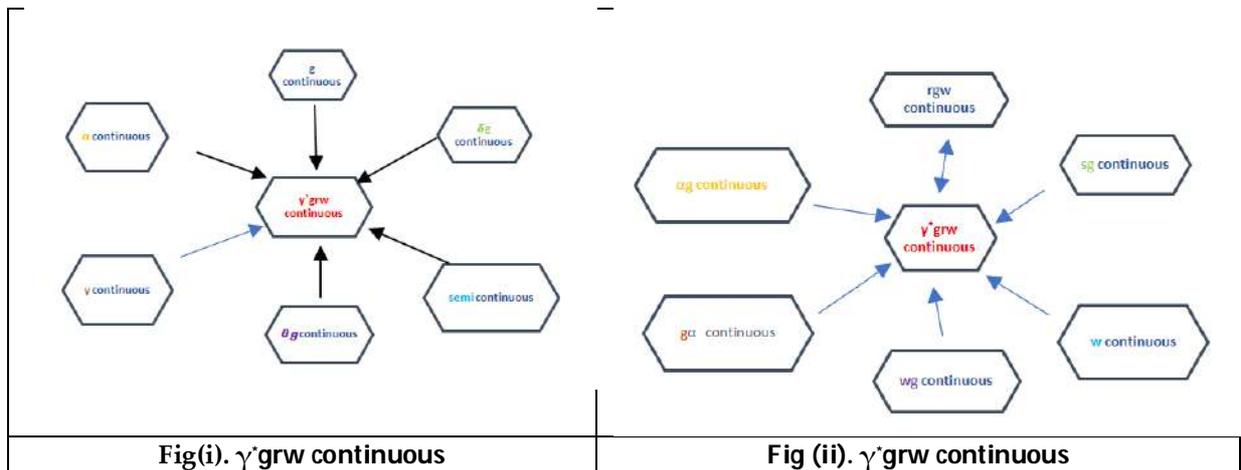
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Improved Accumulation Efficiency of Phosphorus Solubilizing Bacteria on *Solanum lycopersicum* L.

N.Uma Maheswari^{1*} and V.Subiksha²

¹Head and Vice Principal, PG and Research Department of Microbiology, STET Women's College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli) Sundarakkottai, Mannargudi, Thiruvarur, Tamil Nadu, India.

²Research Scholar, PG and Research Department of Microbiology, STET Women's College (Autonomous), (Affiliated to Bharathidasan University, Tiruchirappalli) Sundarakkottai, Mannargudi, Thiruvarur, Tamil Nadu, India.

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*Address for Correspondence

N.Uma Maheswari

Head and Vice Principal,
PG and Research Department of Microbiology,
STET Women's College (Autonomous),
(Affiliated to Bharathidasan University, Tiruchirappalli)
Sundarakkottai, Mannargudi, Thiruvarur, Tamil Nadu, India.
E.Mail: umasamyamf@gmail.com



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ABSTRACT

Phosphorus (P) is one of the important component for the plant growth metabolism and development of crop. Phosphorous Solubilizing Bacteria (PSB) enhance plant growth and solubilisation and reduce the negative effects of overused agricultural fertilizer in farmland and protect the soil and environment. Hence, the present study was planned to isolate and identify of phosphorus solubilizing bacteria (PSB) from rhizosphic soil Thiruvarur Dt, Tamilnadu, India. Agricultural field Soil sample was selected, serial dilution was performed for isolation. Bacterial species were identified by Biochemical tests, pure PSB screened and identified by Pikovskaya medium. Hence the bacteria were identified as *Pseudomonas* sp. The pot experimental was designed as control T1, carrier based phosphorus solubilizing inoculant T2 (*Pseudomonas* sp) with *Solanum lycopersicum* L and liquid biofertilizer (*Pseudomonas* sp) with *Solanum lycopersicum* L. T3 In this study liquid biofertilizer treated with *Solanum lycopersicum* L T3 treatment showed the best result in the parameters such as Carotenoids 0.653 ± 0.58 , Protein 7.63 ± 0.53 , Carbohydrate 6.564 ± 0.46 , Total Chlorophyll 1.0655 ± 0.626 and height 25.7 ± 0.40 . Further study is to be planned to isolate indigenous efficient of PSB and field trial. Hence the study was performed the PSB microbes to maintain the sustainable agriculture and to make green environment.

Keywords: Phosphorus, PSB, *Pseudomonas* sp, Liquid biofertilizer, *Solanum lycopersicum* L.





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INTRODUCTION

Bio - fertilizers are one of the best modern tools for agriculture. It is a gift of our modern agricultural science. Biofertilizers are applied in the agricultural field as a replacement to conventional fertilizers. Conventional fertilizers contain compost, household wastes and greets more. Scientist have developed biofertilizers to prevent pollution and make this world healthy for everybody in a natural way. Phosphorus is one of the major essential macronutrients for plants and its applied to soil in the form of phosphate fertilizers. However, a large portion of soluble inorganic phosphate applied to the soil as chemical fertilizer is immobilized rapidly and becomes unavailable to plants (Goldstein, 1986). Microorganisms are involved in a range of process that affect the an formation of soil phosphate and thus an integral part of the soil cycle . In particular , soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P and through solubilization and mineralization (Hilda and Fraga , 1999). Currently , the main purpose in managing soil phosphorus is to optimize crop production and minimize P loss from soils.

Phosphate solubilizing microorganism (PSM) solubilizing phosphate by producing various organic acids , plant take up this available Phosphate. Some of the soil bacteria are characterized to solubilize the phosphate compound , mainly by producing inorganic acid and so called Phosphobacteria which attracted considerable attention for their potential use as biofertilizer. Hence the present investigation is to be performed for isolation and identification PSB from rhizospheric soils and prepare liquid biofertilizer treated with *Solanum lycopersicum* L.

MATERIALS AND METHODS

Collection of soil sample (Atlas,2012)

Soil samples were collected from the agricultural field of Thiruvarur District, Tamilnadu, India given below. Five spots were fixed in a plot for taking one composite mixture of the soil. The surface of the field was scrapped away to obtain uniformly thick slice of soil from the plough depth from each place. A " V " - shaped cut was made with a spade to remove 1 to 2 cm slice of soil. The sample was collected in a clean bag and marked properly. The mouth of the bag was tied carefully. The same soil was also collected for pot culturing of the plants.

Analysis of physicochemical parameters of the soil Before Treatment (Jackson,1973)

After removing the debris, the soil samples was suspended in distilled water (1:2 w/v) and allowed to settle down the sand particles. Physico-chemical parameters of soil such as pH, Temperature, Moisture, Electrical conductivity, Nitrogen, Phosphorus content were assessed by electrometric and turbidity method respectively.

Isolation of PSB from the soil sample

Serial dilution Method (Aneja, 2002)

1 gram of soil sample was suspended in 10 ml distilled water. To make 10^{-1} dilution from 1 ml sample was mixed with 9 ml distilled water to make 10^{-2} dilution and this sample were serially diluted up to 10^{-8} dilution. 0.1 ml sample was from 10^{-5} , 10^{-6} and 10^{-7} tubes and spreaded over the nutrient agar medium. The plates were incubated in a incubated at 37° for 24 hours. The isolated bacterial strains were identified based on their cultural, morphological and biochemical characteristics to identify the bacteria (Cappuccino and Sherman, 1999). The bacteria was isolated by serial dilution and total cell count technique. The Phosphate Solubilizing bacteria was identified by Gram's staining, Motility test and biochemical tests.

Screening of phosphate solubilizers

Appropriate dilutions is to be plated on Phosphate -containing (Pikovskaya medium -modified by Rao and Sinha 1963) for obtaining microorganisms capable of dissolving Phosphates. The plates is to be incubated for 4-5 days. Transparent zones of clearing around microbial colonies indicated the extent of Phosphate solubilization. Extent of solubilization is to be determined quantitatively. Number of colonies on the respective dilution is to be calculated

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and expressed as colony forming units (CFU g⁻¹) of soil. The colony of the largest halo zone from each of the soil samples is to be isolated and further tests is to be performed to confirm the presence of PSB. Purified PSB isolates is to be subjected to various staining techniques such as Gram Staining (Gram, 1884) and Endospore staining technique (Schaeffer and Fulton, 1933) as an initial screening.

Indole acetic acid production by PSB (Tien et al., 1979)

The potential for the production of IAA is to be determined in culture filtrates of the isolates grown in King's broth supplemented with 100 ppm tryptophan as per the standard procedure. Based on the IAA production potential, the isolates is to be selected for the further study.

Mass culture of PSB

Based on screening efficiency of PSB (*Pseudomonas* sp) was used for pot culture treatment. These cultures were separately inoculated and incubated in 2 days for 500 ml conical flasks at 32°C as submerged culture used to inoculate fresh media with inoculum loads of 50% (v/v). Treatment 1 is Control. Treatment 2 is Carrier based phosphorus solubilizing inoculant (*Pseudomonas* sp) with *Solanum lycopersicum* L. Treatment 3 is Liquid bio fertilizer (*Pseudomonas* sp) with *Solanum lycopersicum* L.

Morphological parameters

After treated with microorganisms, the morphological parameters analysed with standard procedure such as, Height of the plant (in cm), Number of leaves (per plant), Number of roots (per plant), Root length (in cm), Shoot length (in cm). The biochemical components such as Protein, Carbohydrate, Chlorophyll, Carotenoid were analysed by proper standard procedure.

RESULTS

In the present study, the soil was collected and the nutrient properties of soil and microbial colonization were studied. The phosphate solubilizing bacteria were isolated using culture medium and identified based on morphological and biochemical characteristics of phosphate solubilizing bacteria were determined by Gram staining and biochemical tests. The serial dilution and plating method used for the isolation of bacterial colonies. These colonies were identified by Gram's staining and biochemical tests. The isolated bacteria PSB 1 was identified as gram negative, motile rod shaped organism using Gram's staining and hanging drop method (Table 1). The bacterial isolates showed positive for Voges Proskauer, Citrate utilization, catalase, and negative results for Indole, Methyl red and Urease test. Hence, the organism was identified as *Pseudomonas* sp (PSB 1). The liquid biofertilizer (treatment 3) showed the best results compared with treatment 2 and treatment 1. In the overall treatments 45th day, T3 (liquid biofertilizer) was showed in higher activity in Carbohydrate 6.564±0.46, Protein 7.643±0.53 µg and Carotenoids 0.653±0.58 mg. Liquid *Pseudomonas* inoculants was significantly increase the parameters such as shoot, root, length, number of roots, height, chlorophyll content. Our study was clearly highlighted that environment and economy friendly liquid *Pseudomonas* inoculants that potentially promotes sustainable agricultural development in the long term.

DISCUSSION

In this study correlated to inoculation of Phosphate solubilizer (*Bacillus* sp) was evaluated for the growth promotion of cotton P and flux of available P in soil at different P level viz 30, 50 and 90 kg ha. Results demonstrated that phosphate solubilizing and enhanced the yield components of cotton compared to inoculated control (Rodríguez *et al*, 1996). PGPR having the potential of phosphate solubilisation enhanced the growth and production availability of phosphorus and rate of nitrogen fixation (Ponmurugan and Gopi, 2006). *Bacillus* sp enhanced the seed cotton yield, plant height, NP content in cotton leaves and available P in the soil.





PSB are common in the rhizosphere and secrete of phosphatases are common method of facilitating the conversion of insoluble forms of P to available forms (Rodriguez and Fraga , 1999) . The P solubilizing activity is determined by the microbial to produce biochemical ability to produce and release organic acids , which through their carboxylic groups : chelate to phosphate converting them the cations (mainly Ca) bound (Kpoblekou and Tatabai , 1994) .

CONCLUSION

The present study concluded that, use of PSB as the liquid biofertilizer as a easy and low cost technology. The use of PSB as the form of liquid biofertilizer to improve the plant growth and productivity. Day by day, the application of chemical fertilizers to the soil will make it sterile in the future i.e., making it inorganic and unfavourable for cultivation of crops. Thus to prevent environmental pollution and to reduce the extensive use of chemical fertilizers, the effective microorganisms can be recommended to the farmers to ensure public health and a sustainable agriculture. Future study have to be taken to introduce organic farming to the agrarians to achieve the goal of protecting the fertility of their cultivable lands for field trial.

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Table 1: Morphological characteristics of Phosphate solubilizing Bacteria (PSB)

| Morphological characterization | <i>Pseudomonas</i> sp |
|--------------------------------|-----------------------|
| Gram Staining | Gram negative |
| Motility | Motile |
| Shape | Straight curved,rod |

Table 2: Analysis of Physico-chemical parameters of soil sample Before and After treatment with PSB

| Physico-chemical parameters | Before treatment | After treatment |
|-----------------------------|------------------|-----------------|
| pH | 6.2 | 6.6 |
| Temperature (°c) | 7.4 | 8 |
| Moisture (%) | 62 | 70 |
| Electrical conductivity | 1.20-1.80 | 1.26-1.36 |
| Nitrogen (N) mg/L | 80.4 | 90.6 |
| Phosphorus (P) mg/L | 75.2 | 82.4 |
| Potassium (K) mg/L | 35 | 41 |
| Carbon (C) mg/L | 0.78 | 0.99 |

Table 3: Screening of PSB by Organic acid production

| Organic acid production | <i>Pseudomonas</i> sp |
|-------------------------|-----------------------|
| Lactic acid | +Ve |
| Malic acid | -Ve |
| Isovaleric acid | +Ve |
| Isobutyric acid | +Ve |
| Acetic acid | -Ve |

-Ve indicates -Negative

+Ve indicates -Positive

Table 4: Effect of PSB on different morphological parameters in *Solanum lycopersicum* L.

| Treatments | Height of the plant (cm) | No.of. leaves | Shoot length (cm) | Root length (cm) | No.of. Roots |
|------------|--------------------------|---------------|-------------------|------------------|--------------|
| T1 | 15.2±0.25 | 18.0±0.10 | 15.7±0.30 | 5.3±0.10 | 10.2±0.50 |
| T2 | 19.3±0.29 | 20.1±0.12 | 17.2±0.50 | 7.5±0.15 | 15.3±0.60 |
| T3 | 25.7±0.40 | 27.7±0.30 | 23.3±0.90 | 10.2±0.20 | 21.6±0.73 |

Values are presented as Mean ± standard Deviation

Table 5: Biochemical constituents analysis in *Solanum lycopersicum* L.

| Treatment | Carbohydrate (mg/g) | Protein (ug/g) | Carotenoids (mg/g) |
|-----------|---------------------|----------------|--------------------|
| T1 | 4.250±0.24 | 5.436±0.45 | 0.425±0.20 |
| T2 | 5.432±0.35 | 6.436±0.49 | 0.530±0.40 |
| T3 | 6.564±0.46 | 7.643±0.53 | 0.653±0.58 |

Values are presented as Mean ± standard Deviation





Therapeutic Alternatives for Treating the Metabolic Effects of Polycystic Ovary Syndrome

Janvika Varma^{1*} and Aparna Pandey² and Arun Kumar Kushrestha³

¹Assistant Professor, Department of Humanities and Science, Sankalchand Patel University, Visnagar – 384315, Gujarat, India.

²Associate Professor, Department of Humanities and Science, Sankalchand Patel University, Visnagar – 384315, Gujarat, India.

³Assistant Professor, ITM Vocational University Vadodara, Gujarat, India.

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*Address for Correspondence

Janvika Varma

Assistant Professor,
Department of Humanities and Science,
Sankalchand Patel University,
Visnagar – 384315, Gujarat, India.
E.Mail: janvikaverma1612@gmail.com



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ABSTRACT

Poly-cystic is a typical endocrine condition that affects women of reproductive age is ovarian syndrome (PCOS). This is metabolic manifestations, such as insulin resistance, dyslipidemia and obesity, significantly increase the risk of long-term metabolic diseases including type 2 diabetes, cardiovascular disease and non-alcoholic fatty liver disease. There is no single therapeutic approach to managing the metabolic aspects of PCOS, thus the aim of this review is to discuss the therapeutic options currently available. We focus on lifestyle modifications, pharmacological interventions and bariatric surgery as potential therapeutic strategies. Clinical professionals and patients now have more options thanks to recent advancements in pharmaceuticals, which have increased the number of possible therapeutic options. Metformin, an insulin sensitizing drug, has historically been used to manage the metabolic elements of PCOS; however, compared to newer drugs like in cretin mimetic, phenytoin and the SGLT2 antagonists they are less effective in terms of reducing weight and cardiovascular risk. In addition, modern therapies like twin-cretins, Potential therapies for T2DM include triple GLP-1 agonists, glucagon receptor antagonists, and Imeglemin. In comparison to the standard infertility medication clomiphene citrate, the aromatase inhibitor Letrozole looks to be more effective in managing poly-cystic ovarian syndrome-related infertility. Despite the fact that, the androgen antagonist most widely used for treating irregular periods in PCOS individuals who do not want to conceived is oral contraception. We conclude that lifestyle modification should be the primary therapy for managing the metabolic aspects of PCOS, while pharmacological interventions and bariatric surgery should be considered in cases where lifestyle

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modifications fail. Understanding the therapeutic options available can help clinicians to provide individualized, evidence-based management for women with PCOS.

Keywords: Bariatric surgery, GLP-1, incretins hormone, poly-cystic ovary syndrome, SGLT 2 inhibitors, sodium-glucose cotransporter 2 inhibitors, combined oral contraceptive pills, menstrual irregularity, hyperandrogenism, lifestyle interventions.

INTRODUCTION

Elevated androgen levels, irregular menstruation, and/or tiny cysts on one or both ovaries are all symptoms of the complex disease known as poly-cystic ovary syndrome [1]. Poly cystic ovaries are one example of a morphological disease, but it can also primarily be biochemical. An-ovulation, micro cysts in the ovaries, inhibition of follicular growth, and changes in menstrual patterns are all symptoms of hyperandrogenism, a clinical feature of PCOS [2]. According to research, PCOS affects 5% to 10% of females between the ages of 18 and 44, making it the most prevalent endocrine disorder in females of reproductive age [3]. PCOS is commonly found in female patients who need treatment for conditions like obesity, acne, amenorrhea, excessive hair growth, and infertility. Endometrial malignancy, cardiovascular disease, dyslipidemia, and type 2 diabetes mellitus are more prevalent in women with PCOS [4]. The pharmacotherapeutic management of PCOS is addressed in this review.

Etiology

As an oligogenic disease, PCOS is characterized by a heterogeneous, clinical, and biochemical phenotype that is determined by the interaction of numerous genetic and environmental variables [5]. A family history of PCOS is fairly prevalent, but it is unclear whether this has any relationship to the genetic cause of the condition, which is still unknown. A formal discrimination study is impossible without sufficient phenotypic data. But according to recent research, the clustering of PCOS in families replicates an autosomal dominant pattern. In addition to toxins and infectious diseases, poor dietary habits and physical inactivity can aggravate aspects of the environment related to PCOS, such as obesity [6]. A change in lifestyle, such as losing weight and exercising more, can sometimes improve the reproductive and biochemical symptoms of PCOS [7].

Metabolic consequences of PCOS

Menstrual abnormalities, poly-cystic morphology of the ovaries, and extra androgen levels (hirsutism and acne) are biochemical and clinical characteristics of PCOS [8]. Impairment of glucose tolerance and insulin resistance, two factors that contribute to type 2 diabetes mellitus, are more common in women with PCOS. (T2DM) [9]. Nearly 50% of PCOS-affected females eventually develop full-blown metabolic syndrome. (MS) [10]. Dyslipidemia, central adiposity, hypertension, and impaired glucose tolerance are all symptoms that are associated with MS and increase the risk of developing diabetes and coronary heart disease [11]. Additionally, an array of complications like infertility, an increase in body weight, endometrial cancer, and an elevated chance of cardiovascular disease are linked to PCOS. (CVD) [12, 13]. As sustained prevention of disease is positively correlated with effective weight loss for cardio metabolic disorders in PCOS, a therapeutic strategy focusing on weight control is the cornerstone of PCOS management [14]. Weight loss in women with PCOS is still difficult to accomplish, though. The first-line of treatment is lifestyle changes, such as nutrition and exercise. Even so, it rarely results in weight loss of more than 10 kg [15]. The evidence that is currently available on lifestyle intervention is mainly underwhelming [16]. There are psychotherapies to avoid and manage metabolic comorbidities in PCOS, but they are underutilized because their efficacy is still largely unknown these therapies usually play a role in medical management of additional illnesses including T2DM. Although surgical weight loss has been an option for a long time, it hasn't been applied commonly as a means of treatment in the care of PCOS. Researchers aimed to offer a description of the therapeutic and preventative advantages of the therapy options for the metabolic abnormalities during PCOS in this review with an



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emphasis on changes in lifestyle, T2DM pharmaceuticals, and surgical weight loss. The review followed the guidelines from the worldwide evidence-based guideline for the diagnosis and treatment of poly-cystic ovary syndrome [17].

Managing comorbidities with a metabolic component: Additional therapeutic alternative

Non-pharmacological approaches

Treatment is focused on the symptoms because the main cause of PCOS is unknown. Few therapeutic methods completely alleviate the syndrome, and even when symptoms are present, the patient may choose not to receive therapy because she wants to become pregnant [18]. Correcting an-ovulation, preventing androgens from acting on specific tissues, and lowering insulin intolerance are all desirable outcomes of treatment.

Lifestyle Modifications

More than half of PCOS patients are overweight or obese [19], so weight loss is the main recommendation for PCOS patients since it can increase metabolism, Reduced insulin, androgen, and luteinizing hormone (LH) levels and facilitate weight loss effectively [20]. Additionally, it aids in regulating menstruation, increasing the likelihood of getting pregnant [21]. PCOS patients are obese, have elevated blood cholesterol levels, and have problems with hormonal balance. It is crucial to know that exercise by itself will never be sufficient to aid in weight loss. A healthy food is more crucial than anything else. Diet is hardly a top concern for Indian ladies. Protein and fiber should make up one gram per kilogram of body weight of a nutritious diet. It should be mentioned that a 30% calories deficits constantly, must be maintained. Obese individuals may lose weight, and women with PCOS who are infertile have irregular ovulation and are more responsive to ovulation induction medications, which increases the likelihood of pregnancy and live birth. Research has shown that losing up to 5% of one's initial weight can aid in reestablishing normal menstruation and improve the response to ovulation and reproductive drugs [22].

In several instances, this is possible with only a modest loss of weight, and lifestyle changes like diet control and exercise are highly advised, especially those who are prediabetics since they may delay the onset of T2DM [23, 24]. It would seem that a low-glycemic-index diet would be a desirable option for weight loss given the strong correlation between obesity, insulin resistance, and metabolic issues; however, no one diet has emerged as superior to others, despite the fact that the studies that have been conducted so far are minuscule, brief, and contain a variety of different configurations. Following a minimal-crab, ketogenic food plan for a period of six months, a brief study of women with PCOS discovered improvements in their body mass index, hormone profiles, and ovulation [25]. In women with PCOS, a moderate reduction in carbohydrate consumption decreased fasting and challenged insulin levels, according to a study examining the impact of isocaloric diets on insulin sensitivity [26]. A recent systematic review looked at the effects of different food combinations on the metabolic and reproductive outcomes in women who suffer from PCOS. The results showed that monounsaturated fat led to greater reduction in weight, healthier menstrual with using a meal plan reduced in carbohydrates along with significant decrease regard to resistance to insulin [27]. According to an evaluation of fitness programs in Poly-cystic ovarian syndrome from an extensive overview, Short-term frequent physical activity at a moderate level enhanced fertility and menstruation periodicity markedly and helped a overweight adolescent patient having Poly-cystic ovarian syndrome to lose weight and improve their insulin sensitivity [28].

Medicine formulation

Anovulation

Clomiphene

The drug preferred in inducing ovulation in Poly-cystic ovarian syndrome is clomiphene citrate, while the precise mechanism of effectiveness remains unknown. Within the anterior pituitary, CC acts a kind of anti-estrogen by increasing the heartbeat width of gonadotropin-releasing hormone and boosts the production of follicle-stimulating hormone (FSH) by inhibiting oestrogen receptors in the hypothalamus. Follicle growth is aided by luteinizing hormone (LH), a hormone. The initial dosage is 50 mg/day for 5 days. For the following cycles, 50 mg/day for 5 days is maintained if ovulation occurs but no pregnancy develops. For women with PCOS who are refractory to CC, CC

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can be used in combination with metformin. (conditional evidence-based recommendations, moderate-quality evidence). About 30% of successful conceptions are caused by clomid, but 20% pregnancies end with an abortion in many cases. Enlarged ovaries, hyper-stimulation symptom, aggregate gestation, hives, abdominal discomfort, gas and exhaustion are a few of the side effects [29]. Usually, after three rounds of therapy, further treatment is not advised; however, up to six cycles may be tried before considering further therapy.

Letrozole and aromatase inhibitors (AI)

Androgens are converted to estrogen by aromatase. Letrozole is the most popular non-steroidal selective AI for ovulation induction in the third generation. Ovarian estradiol production is inhibited by Letrozole. When the pituitary secretes more FSH, the follicles become more sensitive to it, raising the ovulation rate. This results from a brief increase in androgens in the ovary and the discharge of negative feedback from the hypothalamus [30]. Studies comparing the effectiveness of anastrozole (Arimidex, AstraZeneca) and Letrozole for inducing ovulation and comparing their effects on fertility not be regarded as statistically important [31]. Anastrozole was compared with clomiphene 50 mg/day over the course of a 5-day regimen in a phase 2 dose-finding trial. Comparing all three dosages of anastrozole to clomiphene, ovulation rates were higher [32]. Due to the potential for congenital abnormalities, individuals who cannot be candidates for clomiphene or gonadotropins, or who are clomiphene-resistant may want to consider taking an aromatase inhibitor [33].

Gonadotropins

HMG and FSH can also be applied to start ovulation if clomiphene and/or metformin treatment are ineffective. Treatment with gonadotropins for PCOS sufferers who are an-ovulating, Thought of this having an alternate option to SERM with AI for patients who have not responded to first-line oral ovulation stimulation medications such as clomiphene citrate [34]. Continuing clomiphene citrate for an additional six cycles is a possibility for women who ovulate while taking the drug. In a study involving 302 females, 132 females received weekly increments of 25 units of low-dose FSH (50 units subcutaneously) on cycle day 4 and 123 patients were given clomiphene (50 mg) for 5 days beginning on day 4 with the dose being tit-rated up to 150 mg/day [35]. There were more live deliveries with FSH (52% vs. 39%, respectively; $P = 0.04$) and higher pregnancy rates with FSH compared to clomiphene (58% vs. 44%, respectively). Although gonadotropins may be more successful than clomiphene for causing ovulation, clomiphene's comparative affordability and simplicity of administration made it the preferred first-line treatment for PCOS fertility. It should be noted that this research used low-dose FSH because high doses are linked to a higher risk of multiple pregnancies and OHSS [36].

Antiandrogens

Antigens like spiro lactone, flutamide, and finasteride help PCOS sufferers with their hirsutism and acne issues. These antigens may be advantageous for people with elevated lipid levels, which are common in PCOS patients. The outcomes of flutamide 250 mg, spironolactone 100 mg, and finasteride 5 mg were studied for six months in 40 hirsute women. All three medicines were successful, even though there were no notable differences between the groups. The most popular anti androgen is spiro lactone (25–100 mg twice daily), which is safe, readily available, and inexpensive. Contraception is advised for PCOS patients who use antiandrogens due to the increased risk of teratogenicity to the male foetus (opposing genital development).

Medication for diabetes

Impaired insulin secretion and function are part of the pathogenesis of PCOS [37]. The elevated levels of androgens in PCOS are known to be influenced by high blood sugar and insulin resistance [38]. The ovaries are susceptible to high insulin levels and insulin regulates ovarian function. High levels of androgen are produced by theca cells in reaction to excessive insulin, which in turn causes follicular maturation to be arrested, which increases the risk of poly-cystic ovarian morphology, a sign of PCOS [39]. In addition to having a crucial part in the pathology of PCOS, insulin resistance negatively affects the females suffering from poly-cystic ovarian syndrome by predisposing them medical conditions over the long term, such as T2DM and coronary heart disease. Acanthosis nigricans is an ancient indicator of insulin resistance. Long-term insulin resistance may result in adverse systemic consequences. As a





consequence, managing insulin resistance through medication and dietary modifications is essential for PCOS treatment [40].

Metformin

It has been established that the biguanide drug metformin is both secure and efficient. Metformin is one of the most frequently prescribed insulin sensitizers in controlling poly-cystic ovarian syndrome and was also utilized for decades to treat T2DM; Metformin has been used for many years to treat type 2 diabetes and is one of the most frequently used insulin sensitizers in managing PCOS, even though it is still an authorized use. Through increased glucose uptake, increased glucose utilization, and decreased hepatic glucose synthesis, metformin increases insulin sensitivity in peripheral tissues. Diarrhea, nausea, vomiting, and abdominal distension are some of the adverse effects of metformin. Diabetes type 2 and prediabetics are more common in PCOS individuals. Despite their obvious connection, PCOS and type 2 diabetes mellitus (T2DM) are frequently misunderstood when it comes to obesity. Since among individuals who have PCOS, metformin treatment had been found to lower the occurrence of T2DM prevention is crucial in this group. Patients with PCOS have a poorer lipid profile than the general population, with lower levels of high-density lipoprotein (HDL) and higher levels of triglycerides, both of which are important indicators of cardiovascular problems. Treatment for cholesterol is therefore essential in PCOS. By immediately lowering hyperinsulinemia or by changing the liver's metabolism of free fatty acids, metformin lowers dyslipidemia. Several studies have shown that metformin has a substantial impact on dyslipidemia, even though it had no effect on total cholesterol levels. Women with PCOS are given metformin at a starting dosage of 500–850 mg per day; if tolerated, the dose may be increased to 2000 mg per day. Higher dosages of metformin can aid in weight loss and lipid profile improvement, particularly in obese and PCOS patients. Long-term metformin use has also been linked to vitamin B12 insufficiency. Initially administering a modest dose of 500 mg, and then progressively increasing it to a maximum dose of 1500 mg once per week, can help avoid the mild gastrointestinal side effects of metformin [41]. The most typical signs and symptoms include stomach discomfort, diarrhea, nausea, vomiting, and light weight loss. Due to metformin intolerance and its related side effects, several therapy options should be considered for PCOS patients who are unable to take metformin. A significant decrease in BMI was seen in both groups of women with PCOS who participated in a research comparing metformin and lifestyle interventions; however, the metformin group was the only one to experience a decrease in androgen levels [42].

A significant drop in BMI without a change in lifestyle was observed during RCT examining the effect of metformin on body weight in PCOS patients who were obese and/or morbidly obese [43]. In a trial of 3234 non-diabetic patients with increased fasting plasma glucose who were randomly assigned to either metformin or lifestyle intervention with a mean follow-up of nearly 3 years, a lifestyle intervention reduced the new incidence of T2DM by over 60%. On the other hand, metformin lowered it by just over 30%, but this benefit was completely lost following the washout period. This was further supported in a related research where metformin's effects vanished after 12 months of withdrawal [44]. Because of their hyperinsulinemia, elevated androgen levels, obesity, and dyslipidemia, women with PCOS are also more likely to develop cardiovascular disease [45]. Evidence suggests that PCOS and obesity have separate effects on arterial endothelial function⁴³; however, obesity is not a factor in the link between elevated insulin levels and cardiovascular disease (CVD) [46, 47]. Women with PCOS usually have low levels of high-density lipoprotein (HDL), which is a strong predictor of CVD, and high levels of triglycerides, which are worse lipid profiles compared to the healthy population [48] [49]. Therefore, dyslipidemia management is essential in PCOS. The liver metabolism of free fatty acids is improved by metformin either directly or indirectly by lowering hyperinsulinemia [50]. According to numerous studies, metformin significantly affects dyslipidemia; however, there was no positive influence of metformin on total cholesterol levels [51-53].

Thiazolidinediones

A peroxisome proliferate-activated receptor-gamma (PPAR-) ligand, pioglitazone is a thiazolidinedione. Increasing peripheral glucose absorption and controlling adipogenesis and insulin action are the main mechanisms by which pioglitazone works. Women with PCOS have reported that it helps with ovulatory dysfunction, hyperandrogenism, and insulin intolerance [54]. Pioglitazone's impact was examined in a randomised control experiment (RCT).





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Pioglitazone significantly decreased fasting serum insulin levels and the free androgen index while increasing SHBG levels in a randomized control study (RCT) comparing the effectiveness of the drug versus placebo in PCOS [55]. Metformin and pioglitazone were compared for their ability to treat PCOS in a meta-analysis, the pioglitazone group showed a substantial improvement in ovulation and menstrual cycle. In contrast to the metformin group, the pioglitazone group's body mass index (BMI) score significantly increased [56]. A randomized open-label experiment assessing the effects of pioglitazone, metformin, and orlistat on mean insulin resistance (IR) and its biological variability in women with PCOS discovered a significant overall decline in IR and IR variability [57]. Despite pioglitazone's beneficial impact on PCOS's metabolic metrics, there is a lot of worry about the medication's potential to cause pulmonary oedema brought on by fluid retention, cardiac damage, and congestive heart failure [58]. The use of thiazolidinediones in obese PCOS patients raises concerns about weight gain, even though the absolute risk is minimal in young women with the condition.

Glucagon-like peptide-1 receptor analogue

Potentiating proteinogens are those that boost glucose-dependent insulin secretion, particularly after a meal, such as glucagon-like peptide-1 (GLP-1) and glucose-dependent unguided polypeptides (GIP). Responsiveness to incretins Changes in incretin activity are associated with insulin resistance, particularly type 2 diabetes. In a recent investigation, researchers discovered that people with PCOS have lower incretin hormone levels. With better glycemic control and weight reduction in type 2 diabetes patients, targeting this system as a treatment for the disease has therefore emerged as a viable option. A promising medication called Mimetics can be used to treat PCOS in a variety of patients because it specifically addresses a metabolic target [59]. The incretin effect, or the stimulation of glucose-dependent insulin release by incretin hormones such as glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic poly peptide (GIP), is well recognized [60]. Although the primary function of GLP-1 is not to increase insulin secretion, their ability to reduce weight may indirectly enhance insulin sensitivity. Yaribeygi et al.'s latest study suggests that these compounds may also increase insulin sensitivity by affecting eight different molecular pathways, including those involved in inflammation, oxidative stress, lipid metabolism, GLUT-4 expression and translation, -cell function, endoplasmic reticulum (ER), and insulin signal-ling [61].

GLP-1 agonists include, for example, the commercially available medications liraglutide, semaglutide, dulaglutide, and exenatide. It has been demonstrated that liraglutide (1.2 mg or 1.8 mg) administered intravenously once day improves glycemic control in T2DM patients (HbA1c lowered by 1.5% with concurrent weight loss) [62]. Similar results (HbA1c lowered by 1.4%) and a considerable weight loss were seen with semaglutide therapy [63]. Liraglutide dramatically decreased the risk of cardiovascular disease in the LEADER study [64]. Only one observational study has looked into the possible effects of liraglutide on weight loss in obese and overweight people with PCOS, and the results are encouraging. In this research, 84 Obese women with PCOS were given subcutaneous liraglutide injections every day for four weeks before being observed for more than 27 weeks (beginning dose: 0.6 mg, daily increase: 1.2 mg, then 1.8 mg if tolerated). Weight and BMI significantly decreased, according to the findings [65]. A recent in-depth examination and meta analysis found that GLP-1 agonists were found to be significantly more effective than metformin in improving insulin sensitivity, lowering BMI, and decreasing abdominal circumference in PCOS-affected women [66]. Similar studies on PCOS women treated with GLP-1 receptor analogues found positive outcomes testosterone levels and weight loss, but no discernible impact on insulin levels or sensitivity.(RAs) [67, 68]. A substantial decrease in weight and atherothrombotic markers, such as endothelial function and clotting, was observed in a study contrasting the impact of liraglutide (1.8 mg od) medication on atherothrombotic risk in PCOS-afflicted obese women [69]. Another study that looked at how liraglutide (1.8 mg) affected obese PCOS patients' quality of life (QOL) and depression revealed a substantial improvement in QOL due to a significant weight loss [70].

Sodium-glucose co-transporter-2 inhibitors

A family of oral drugs called sodium-glucose co-transporter-2 inhibitors (SGLT-2) is used to treat T2DM. Examples of these drugs include dapagliflozin and empagliflozin. Their mechanism of action involves inhibiting SGLT-2, which decreases glucose re-absorption and increases urine glucose excretion, in the proximal convoluted tubule





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(PCT) of the kidney [71]. The glycemic parameters significantly improve as glucose is removed and its plasma levels fall 81. The risk of hypoglycemia is minimal because this mechanism of action is exclusively glucose-dependent and insulin-independent, unlike those of other drugs [72]. SGLT2 inhibitors are increasingly being used to treat obesity. Besides the fact that they protect against cardiovascular and renal problems, their body weight impact is encouraging [73]. In addition to lowering blood sugar levels, they can also increase insulin sensitivity by reducing glucotoxicity and lipotoxicity, enhancing β -cell function, reducing oxidative damage and inflammatory processes, and promoting caloric deposition and weight loss, among other molecular pathways [74]. In recent studies involving PCOS patients, SGLT2 inhibitor therapy has produced encouraging outcomes. Treatment with empagliflozin showed a significant improvement in a 12-week randomized open-label trial comparing empagliflozin to metformin in obese women with PCOS found no differences in the metabolic parameters, however there were changes in the anthropometric measures and body composition. This indicates that SGLT2 inhibitors may be advantageous in the treatment of PCOS. The SGLT2 antagonists frequently cause vulvovaginal candidiasis, vulvovaginal infections, vulvovaginitis, and genitourinary tract infections [75]. The main outcome (MACE) was reduced by 14% of the 10,142 T2DM patients at high CVD risk who participated in the CANVAS (for canagliflozin) trial [76].

However, results from the DECLARE-TIMI trials (for dapagliflozin) did demonstrate a reduced incidence of cardiovascular deaths and hospitalization for heart failure, despite the fact that they did not demonstrate a significant impact on MACE outcome [77]. In recent studies involving PCOS patients, SGLT2 inhibitor therapy has produced encouraging outcomes. Treatment with empagliflozin showed a significant improvement in during a 12-week randomized open-label experiment comparing empagliflozin to metformin in obese women with PCOS, changes were observed in anthropometric measurements and body composition but not in the metabolic parameters [78]. This implies that SGLT2 antagonists might be helpful in the treatment of PCOS. Genital infections, genitourinary tract infections, vulvovaginal candidiasis, and vulvovaginitis are common adverse reactions to SGLT2 antagonists [79].

Dipeptidyl peptidase-4 inhibitors

Gliptins, commonly known as dipeptidyl peptidase-4 (DPP-4) inhibitors, are oral hypoglycemic drugs used to treat type 2 diabetes. They are typically used as a second or third-line therapy after metformin [80]. They function by impeding the activity of DPP-4, a universal enzyme that breaks down GLP-1 generated internally [81]. The drug type that has received the most research to date is sitagliptin. Sitagliptin's impact on blood glucose levels and visceral fat (VAT) in PCOS-affected women was examined in a double-blind crossover research, which found that it decreased the maximal glucose response to oral glucose tolerance and VAT [82]. Sitagliptin improved β -cell function (HOMA-B) and insulin sensitivity, it was investigated as a potential therapy for PCOS patients with metformin intolerance in a pilot randomized research [83]. In a research on rats with PCOS, sitagliptin improved ovarian fibrosis, decreased androgen levels, and decreased fasting blood glucose [84]. The most frequently documented side effects of gliptin therapy are pharyngitis, breathing issues, and headaches [85]. Saxagliptin therapy has been associated with hypersensitivity reactions, including angioedema, anaphylactic response, and dermatological reactions, though these are uncommon [86].

Myo-inositol

A nutritional supplement called inositol helps insulin signaling. Its function in controlling the metabolic and molecular elements of PCOS is not well understood. Menstrual cycles and ovulation can be improved, claims a recent research. Inositol has few benefits, but it also has a low chance of side effects and is inexpensive, so this recommendation cautions against using it [87]. It exists in nine distinct isomeric forms, the most prevalent of which are in several intracellular signal transduction pathways, such as insulin signal transduction, myo-inositol (MYO) and d-chiro-inositol serve as the structural foundation for a variety of second messengers. serves a crucial role. While d-chiro-inositol-based second messenger activation promotes glycogen synthesis, Activation of second messengers based on MYO improves the function of glucose transport proteins and regulates glucose uptake. Type 1 and type 2 diabetes have been linked to abnormal inositol production and metabolism [88]. Growingly more people are turning to inositol as a dietary supplement for the therapy of a number of diseases, such as T2DM, GD, and PCOS [89]. In



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comparison to controls, women with PCOS who received MYO treatment had a substantially lower risk of GD, according to a comprehensive study [90]. MYO consumption has been shown to help PCOS-affected women who might have been infertile ovulate and respond to assisted reproduction [91]. MYO supplements helped PCOS-related hormonal and reproductive issues, according to a recent systematic study that evaluated their effectiveness in treating PCOS in women. Additionally, it improved follicular growth and egg maturation [92]. Since MYO supplements are typically used in the management of PCOS is generally well tolerated at the recommended doses of 2-4g/day with few safety concerns worthwhile to take into account.

Medication for dyslipidaemia

Statins

One of the most important indicators of cardiovascular risk in PCOS women, dyslipidemia is characterized by elevated triglycerides, low HDL cholesterol, and high LDL cholesterol. dyslipidemia, which is characterized by high LDL-C, triglycerides, and reduced HDL-C in PCOS women. Therefore, an effective PCOS therapy would involve lowering the risk of cardiovascular disease and raising the lipid profile. It has been demonstrated that statins can aid in the therapy of PCOS. A statin is a medication that stops the production of cholesterol. It also goes by the names atorvastatin, fluvastatin, pravastatin, rosuvastatin, and simvastatin. The production of cholesterol in rodents depends on the enzyme 3-hydroxy-3- methylglutaryl coenzyme A (HMGCoA) reductase. When this enzyme is blocked, HMG-CoA is converted to mevalonate, which reduces the production of cholesterol. A treatment with atorvastatin reduced the oxidative stress marker malondialdehyde (MDA) in the blood of fat women with PCOS. Additionally, androstenedione and dehydroepiandrosterone sulphate are decreased by atorvastatin [93]. When compared to placebo, atorvastatin significantly reduced hyperandrogenism, inflammatory markers, and insulin resistance in PCOS-affected women in a randomized, placebo-controlled experiment [94]. In terms of HOMA-IR (homeostasis model assessment insulin resistance), free androgen index (FAI), total testosterone, and sex hormone-binding globulin (SHBG), the atorvastatin-pretreated group significantly outperformed the placebo-pretreated group after receiving metformin for an additional 12 weeks, demonstrating that atorvastatin enhances the effects of metformin [95]. In this research, atorvastatin treatment for 12 weeks substantially decreased acylation stimulating protein (ASP), interleukin-6 (IL-6), and monocyte-chemo-attractant protein-1, which are markers of inflammation and adipose tissue dysfunction. (MCP- 1). The amounts of testosterone and HOMA-IR then significantly increased [96]. The same research also looked at atorvastatin's impact on pancreatic beta-cell function (HOMA-), and found that it significantly decreased HOMA-. The fact that this finding persisted after receiving metformin therapy for an additional 12 weeks suggested that rather than a decline in -cell function, there may have been an improvement in insulin resistance, which would have reduced the need for -cells [97]. Additionally, atorvastatin therapy significantly lower levels of serum malondialdehyde (MDA), an indication of oxidative stress, were found in PCOS-affected obese women [98]. Additionally, in this group of PCOS-affected women, atorvastatin markedly decreased the amounts of androstenedione and dehydroepiandrosterone sulphate (DHEAS) [99]. When compared to placebo, a 12-week course of atorvastatin substantially increased the level of serum vitamin D (25OHD) in PCOS-affiliated women. However, it should not be used in young women of reproductive age due to its potential teratogenicity and until more trustworthy evidence are available to explain its efficacy.

Oral contraceptives

Oral contraceptives may be an option for PCOS sufferers who don't want to get pregnant (OCs). The main mechanism of action of OCs in the treatment of PCOS is menstrual cycle management. Additionally, these drugs diminish testosterone, which lessens hirsutism, acne, and hirsutism. The most popular OCs used to treat hirsutism and acne brought on by PCOS is oestrogen and progesterone combos. Theoretically, these drugs are better at treating androgenic symptoms than earlier versions. After six months of OC therapy, the majority of women with hirsutism reported better clinical symptoms. The findings also suggest that synergy between antigens and OCs may exist. PCOS should be treated to alleviate symptoms as well as to avoid long-term effects. Antigens and oral contraceptives are routinely prescribed by doctors to reduce testosterone levels, treat symptoms, and protect the endometrium. Due to the increased risk of thromboembolic events, dependence on ethinylestradiol and cyproterone acetate should be minimized and they shouldn't be used as first-line COCP medicines [100, 101].





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Medications to lose weight**Orlistat**

Orlistat treatment dramatically decreased body weight and blood levels, according to a study that examined the effects of the medication on biochemical and hormonal variables in women with PCOS compared to those of metformin. A lipase inhibitor called orlistat reduces the absorption of dietary fat by preventing the breakdown of triglycerides in the stomach and pancreas. Levels of androgen are higher than those of metformin [102]. Additionally, Orlistat reduced IR indicators, testosterone, and total cholesterol. Orlistat also decreases blood pressure, and because it helps people lose weight, it may help people in this high-risk category prevent type 2 diabetes. The recommended dosage of 120 mg of Orlistat three times a day with meals has been associated with increased lipodystrophy, diarrhea, stomach pain, and gas. Additionally, it might result in a lack of fat-soluble vitamins. Orlistat may be useful in the management of obesity, although there is debate over how well it manages the metabolic component of PCOS. Visceral adiposity Index (VAI) levels were higher in overweight and/or obese PCOS patients as compared to peer controls and non-obesity PCOS patients, and they were associated with a number of metabolic and inflammatory parameters [103]. According to a study, Orlistat treatment for PCOS patients improved lipid profiles, weight, BMI, and waist circumference when compared to metformin plus lifestyle changes [104]. Orlistat also decreased total cholesterol, IR markers, and androgen levels [105, 106]. In this high-risk population, Orlistat also slightly lowers blood pressure and contributes to the prevention of T2DM, probably due to its effect on weight loss [107].

Sibutramine

Sibutramine, an appetite suppressant, is used to treat obesity together with dietary and lifestyle modifications. It prevents monoamines from reuptaking. It blocks the absorption of neurotransmitters such as serotonin, norepinephrine, and dopamine [108]. As a result, it enhances their availability in synaptic clefts, helping to decrease appetite, improve fullness, and lead to decreased food intake [109]. Women with PCOS saw a significant weight loss of 7.8–5.1 kg after six months of treatment with sibutramine at a daily dose of 15 mg, compared to placebo (2.8–6.2 kg) [110]. Additionally, another RCT with a lower daily dose of 10mg revealed even larger weight loss (15.41.1 versus -11.11.9) [111]. However, sibutramine significantly increases the risk of myocardial infarction, stroke, and cardiovascular death [112]. Sibutramine use for weight loss in women with PCOS who have a high cardio-metabolic risk is thus debatable.

Rimonabant

A cannabinoid 1 (CB1) receptor blocker called rimonabant is used to treat obesity and anorexia. Rimonabant decreased body weight and alanine aminotransferase (ALT) in women with obese PCOS who did not have nonalcoholic fatty liver disease (NAFLD) [113]. Rimonabant significantly increased GIP after 3 months of treatment, whereas metformin had no effect, in a study comparing the effects of rimonabant and metformin treatment on incretin hormones in obese women with PCOS [114]. In addition, rimonabant therapy increased the weight loss effect and metabolic benefit of metformin therapy in obese women with PCOS [115]. Rimonabant, when compared to metformin, had a better effect on weight loss, improved insulin resistance, and decreased testosterone levels in PCOS women [116]. However, research from clinical trials revealed that rimonabant led to serious psychiatric issues such as depressive illness, mood swings, and suicide thoughts [117]. Due to their side effect profiles, rimonabant and sibutramine have both been taken off the market.

Naltrexone/bupropion

With regard to eating habits, the opioid receptor antagonist naltrexone has a high affinity for the μ -opioid receptor. Animal studies have demonstrated that naltrexone decreases dopamine release, which in turn reduces food intake, consumption, and binge eating behaviour. Recently, the US Food and Drug Administration (FDA) approved it for use in treating drug and alcohol addiction. Bupropion, an antidepressant, can be used to treat depression and help people quit smoking. Dopamine re-uptake is prevented as a result of its action. In clinical trials, weight loss was the most typical adverse event. Scientific studies have demonstrated that combining these medications causes a significant weight decrease even though none of these medications have been FDA-approved for the treatment of



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obesity. For instance, the drug combination naltrexone/bupropion (N/B), marketed as Cont-rave and available orally, has just been approved for the management of obesity in the US and EU. As a result, the metabolic component of PCOS may benefit clinically from weight loss while using naltrexone and bupropion. Its surprise that this recommendation now covers reproductive therapy, infertility surgery, and antimicrobial medication. The effectiveness of both treatments should be investigated despite the lack of information on PCOS and fertility. Pregnant women should carefully consider delaying pregnancy until they have established nutritional stability after losing weight because of the possible risks pregnancy poses to the unborn child. There are two challenges that must be addressed: neonatal mortality and growth restriction. Along with co-morbid conditions like type 2 diabetes, hypertension, and dyslipidemia, it can also benefit patients who have pre-eclampsia, gestational diabetes, large-for-gestational-age children, and other related conditions.

Bariatric surgery

The treatment of metabolic disorders, such as insulin resistance and excess body weight, by bariatric surgery has been practiced for many years. The Roux-en-Y gastric bypass (RYGB), the adjustable gastric band, and the sleeve gastrectomy are the three procedures that are most frequently used for bariatric surgery [118]. To treat obesity and T2DM remission, RYGB, as opposed to sleeve gastrectomy, has been found to be more beneficial [119, 120]. The most recent recommendations for treating obesity include bariatric surgery for individuals with a BMI of 40 kg/m² or higher, or a BMI between 35 kg/m² and 40 kg/m² with associated comorbidities such T2DM or high blood pressure [121]. Despite the fact that PCOS is more frequent in very obese women of reproductive age, very few research have looked at the effects of bariatric surgery in this group. A prospective study followed 17 PCOS women with an average age of 30 years prospectively for 26 months after biliopancreatic bypass surgery or a laparoscopic RYGB. For the majority of patients, menstruation returned to normal, and a 41kg weight decrease on average was recorded. Additionally, the indices of insulin resistance (HOMA-IR was reduced by 50%), hirsutism, and androgen profile all showed a significant improvement [122]. In addition, a retrospective analysis of 24 PCOS-positive, morbidly obese women with an average BMI of 50 kg/m², a mean age of 34 years, and a mean body weight of 306 44 lbs who underwent RYGB and were monitored for more than two years straight was published. Within 3.5 months, all of the ladies had their periods again, there had been a weight loss of about 56.7% at 12 months, and the HbA1c level had dropped from 8.1% to 5.1% within 3 months [123]. Since many women with PCOS are obese, PCOS poses a significant risk for MS, and bariatric surgery may offer a longer-term therapeutic alternative in some patients [124].

Other therapies

Medroxyprogesterone acetate

Patients with PCOS who do not desire to get pregnant and are not at risk for conception can use medroxyprogesterone acetate (MPA) to treat dysfunctional uterine bleeding or amenorrhea. The recommended dosage range for MPA is 5 to 10 mg/day for 10 to 14 days each month. Monthly progesterone therapy prevents aberrant endometrial proliferation but does not stop the generation of ovarian androgen. 13 Patients with PCOS may benefit from MPA since it may increase lipid profiles and insulin sensitivity [125-127].

Glucagon receptor antagonist

The pancreatic beta cells that produce the hormone glucagon actively control glucose homeostasis during fasting periods by promoting hepatic gluconeogenesis and glycogenolysis [128]. It has been noted that diabetes patients have elevated glucagon levels and a higher glucagon to insulin ratio [129]. As a result, it would be expected that inhibiting glucagon receptors would cause the liver to produce less glucose, which would enhance glycemic control. medications that aim to limit the activity of glucagon are being developed as potential treatments for type 2 diabetes since glucagon has an opposing effect to insulin, yet it is unknown whether these medications will be useful in PCOS.

Imeglemin

The innovative class of glucose-lowering medications known as Imeglemin was created to treat T2DM, albeit its exact mechanism of action is yet unknown. Experimental research, however, points to the possibility that it functions



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by obstructing oxidative phosphorylation, a critical stage in hepatic gluconeogenesis [130]. Additionally, it promotes muscle glucose uptake and insulin secretion [131]. Imeglemin can increase insulin sensitivity through a number of cellular pathways, including insulin signaling transduction via stimulating Akt phosphorylation, according to a recent study [132]. Imeglemin may also increase -cell function, decrease gluconeogenesis, reduce insulin resistance, enhance mitochondrial activity, and reduce oxidative stress in order to improve glucose homeostasis [133]. Patients with T2DM have extra advantages from Imeglemin new method of action, which may also support other oral anti-diabetic medications. However, to evaluate its effectiveness and tolerability in PCOS-affected women, clinical trials are required.

micro-RNA therapy

MicroRNAs (miRNAs) may have therapeutic benefits in the treatment of numerous diseases, including issues connected to obesity, according to growing research. Non-coding RNAs called miRNAs, which have a length of about 22 nucleotide and post-transcriptionally affect gene expression, are altered in PCOS compared to healthy controls [134, 135]. They cause mRNA cleavage, translational repression, and mRNA degradation when they attach to the target messenger RNA (mRNA) [136-139]. Adipose tissues release miRNAs, which function as both endocrine and paracrine messengers between different target organs. Additionally, miRNAs are connected to the development of adipocytes and may therefore serve as potential biomarkers for disorders like obesity. Targeting miRNAs in the treatment of obesity and its associated metabolic disorders (T2DM, non-alcoholic fatty liver disease, and non-alcoholic steatohepatitis) is currently the subject of several clinical trials, which may also be helpful for PCOS patients. It is important to note that each of these agents is due to their teratogenic effects, contraindicated during pregnancy and lactation. There is proof that Metformin is safe, does not raise the risk of congenital defects, and is generally well tolerated during pregnancy. Because women with PCOS are often young and of reproductive age, it is important to stress that, aside from metformin, the use of all other pharmaceutical medicines should be restricted to those who have no desire for pregnancy and who are using effective methods of contraception. It should be mentioned that these medications are not approved for usage in PCOS-affected adolescent patients. Currently, the first-line treatment for women with PCOS should concentrate on lifestyle interventions such as dietary changes and physical activity, together with any additional pharmacological medicines that may be necessary.

CONCLUSION

PCOS is a multifaceted hormonal, metabolic, and psychological condition with a wide range of clinical manifestations. One of the most frequent causes of infertility is this. The primary therapy recommendation for PCOS-related infertility should be lifestyle changes before considering any pharmacological choices. PCOS has been proven to increase the risk of endometrial cancer in women of all ages while having no effect on the risk of ovarian or breast cancer. These data suggest that PCOS may increase the risk of developing gynecological cancer. For the treatment of PCOS's metabolic comorbidities, a variety of pharmacological alternatives are available with potential benefits. Despite the fact that metformin lacks a license for this use, it is critical to recognize its effectiveness and safety in improving PCOS-related metabolic outcomes. However, it's also critical to recognize that no single treatment can fully address the range of metabolic disorders in PCOS diagnosed individuals. Metabolic benefits and improvement of metabolic comorbidity markers with a combination of lifestyle changes, metformin, GLP-1 RA, SGLT-2 inhibitors, and bariatric surgery are superior to mono-therapy. Further research is necessary on the treatment drugs SGLT-2 inhibitors and GLP-1 RA, which have great potential benefits in treating metabolic disorders in PCOS-affected women. For some PCOS-affected women, bariatric surgery appears to be a highly successful treatment that can reverse metabolic irregularities and avoid T2DM. Clinical trials are necessary to evaluate the clinical efficacy and safety of a variety of novel medications being developed for the treatment of T2DM that may directly assist the management of the metabolic components of PCOS.





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Bionomics of Four Species of Aquatic Bugs (*Hemiptera: Heteroptera: Nepomorpha*) of Sasthamkotta Lake, A Ramsar Site of Kerala, India

K. Jyothylakshmi^{1*} and S. Nandakumar²

¹Research Scholar, P.G and Research Department of Zoology, N.S.S College, Pandalam, Kerala - 689501, India.

²Associate Professor, P.G and Research department of Zoology, N.S.S College, Pandalam, Kerala - 689501, India.

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*Address for Correspondence

K. Jyothylakshmi

Research Scholar,
P.G and Research Department of Zoology,
N.S.S College, Pandalam,
Kerala - 689501, India.
E.Mail: jyothylakshmik@gmail.com



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ABSTRACT

Predatory aquatic bugs in the infraorder *Nepomorpha* play vital roles in freshwater ecosystems. The present inventory contributes to the bionomics of four species of aquatic bugs, *Diplonychus rusticus* (Fabricius, 1781), *Laccotrephes griseus* (Guerin-Meneville, 1844), *Cercotmetus pilipes* (Dallas, 1850) and *Enithares ciliata* (Fabricius, 1798) collected from Sasthamkotta lake, the largest freshwater lake of Kerala. Possibly, many species including new species and their associated bioecological information is yet to be discovered from the lake. In addition to the taxonomy and diversity of waterbugs, studies on the bioecology are highly crucial for employing better conservation measures. Hence, further studies on the bionomics aspect of water bugs from different wetlands of Kerala, along with its morphotaxonomic description are needed to fill the knowledge gap.

Keywords: Bioecology, Freshwater Lake, Morphotaxonomy, Systematics, Waterbugs.

INTRODUCTION

Aquatic Heteropterans are a group of bugs with distinctive morphological traits and adaptations. They are categorized into three infraorders based on their ecological interests and niches: *Gerronomorpha*, *Nepomorpha*, and *Leptopodomorpha*. Approximately 4940 species of aquatic and semi-aquatic bugs are described globally [1]. With the available checklists, there are 318 species of water bugs under 82 genera and 18 families in India [2-4]. There are only





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fewer studies on aquatic and semi aquatic *Heteroptera* of Kerala. Thirumalai [5] conducted studies on Gerridae and Notonectidae from Silent Valley region, Kerala. Thirumalai and Radhakrishnan [6] studied aquatic Hemiptera of Kasaragod district of Kerala. Nirmala Kumari and Balakrishnan Nair [7] conducted studies on the taxonomy, life history of *Ranatra filiformis* Fabricius and examined the morphotaxonomy of *Cercotmetus pilipes* (Dallas). Due to their abundance in many freshwater ecosystems; they are widely used as bioindicators of water quality [8]. *Nepomorpha* or true bugs is one of the most remarkable groups of Heteropterans, spending complete life cycle within the water. They can be found both in lotic and lentic environments. Most of the bugs are primarily predatory in nature and function as effective bio control agents of risky vectors like mosquito larvae [8]. Systematics and bioecological studies on true bugs in lentic ecosystems of Kerala are sparse. Due to their enormous significance, studies on the morpho taxonomy and bionomics of aquatic bugs are very much essential. The present study deals with the documentation and to build up baseline information on the taxonomy and bionomics of four species of predatory aquatic bugs of Sasthamkotta lake.

MATERIALS AND METHODS

Study area

The present study was conducted in Sasthamkotta lake, the largest fresh water lake of Kerala, in Kollam district. It lies between 9° 00'-9° 05' N and 76° 35'-76° 46' E. It is spreads out in the adjacent villages of Sasthamkotta, Mynagappally and West Kallada of Kunnathur Taluk. The lake covers an area of 3.75 sq. km with an average depth of 6.79 m. Sampling was conducted in four sites of the lake, S1; Vettolikadavu (9°1'56.93" N and 76°37'29.72" E), S2; (9°2'58.4" N and 76°38'24.79"E), S3; Adikkadu Kadavu (9°2'4.01" N and 76°36' 49.36" E) and S4; Punnakkadu Kadavu (9° 2' 41.27"N and 76° 37' 44.17" E) (Fig. 1).

Taxonomy and Bioecological study of Aquatic bugs

Aquatic bugs were collected from the lake, using a hand operated D-frame aquatic insect net with a mesh size of 500µm from July 2021 to June 2022. The collected specimens were preserved in 70% ethanol in the field and transported to the laboratory for detailed morpho-taxonomic analysis. Identification was done using published taxonomic literatures and monograph on aquatic bugs [9-12]. Photographs and measurements were taken using Olympus Tg 6 digital camera and Olympus SZ51 stereomicroscope. During the study period, aquatic bugs from various microhabitats of the lake were surveyed to determine the preferred zone and their presence or absence in the habitat was recorded to investigate their bioecology [13].

RESULTS

SYSTEMATIC ACCOUNT

Class : Insecta
Order : Hemiptera
Suborder : Heteroptera
Infra order : *Nepomorpha* Popov, 1968
Super family : Nepoidea Latreille, 1802
Family : Belostomatidae Leach, 1815
Sub family : Belostomatinae Leach, 1815
Genus : *Diplonychus* Laporte, 1833

Diplonychus rusticus (Fabricius, 1781)

Family : Nepidae Latreille, 1802
Subfamily : Nepinae Latreille, 1802
Genus : *Laccotrephes* Stal, 1866



**Jyothylakshmi and Nandakumar*****Laccotrephes griseus* (Guerin-Meneville, 1844)**

Sub family : Ranatrinae Douglas and Scott, 1865
Genus : *Cercotmetus* Amyot and Serville, 1843

***Cercotmetus pilipes* (Dallas, 1850)**

Super family : Notonectoidea Latreille, 1802
Family : Notonectidae Latreille, 1802
Subfamily : Notonectinae Latreille, 1802
Genus : *Enithares* Spinola, 1837

Enithares ciliata* (Fabricius, 1798)**Diplonychus rusticus* (Fabricius, 1781) (Fig.2A)****Materials examined**

3 exs., Sasthamkotta Lake, Kollam district; S1; Vettolikadavu (9°1'56.93" N and 76°37'29.72" E), 5 exs., S3; Adikkadu Kadavu: 9°2' 4.01" N and 76°36' 49.36" E, 25.xii.2021, Coll. Jyothylakshmi K and S. Nandakumar.

Diagnostic characters

Body flattened and oval shaped. Adult has a size of 15.4-16.5 mm. Colour varies from yellowish brown to dark brown. Lateral margin of head, pronotum and the tip of scutellum are lighter in colour. Vertical border of the abdomen is transparent, broad with dark brownish bands. Ventrally light brown in colour. Legs are yellowish brown in colour with pale brown bands. Mid and hind legs are with dense, long swimming hairs. *Diplonychus rusticus* can be distinguished from other species of this genus by the following combination of characters: wings with spiny patch on corium, fore tarsi single segmented and with a small claw; respiratory straps with cluster of setae in males, Male paramere curved, narrowed at apex.

Bionomics

Diplonychus rusticus was found clinging to rhizomes and leaves of *Salvinia molesta*, a common invasive macrophyte in Sasthamkotta lake. Closer observations revealed that, they move swiftly even under mild disturbance. The species have morphological modifications such as flattened body, curved tarsal claws and respiratory straps for clinging to vegetation. They readily come to light trap. The females of the species forcefully put and attach the eggs on the elytra of males. Male *Diplonychus rusticus* shows parental care, carries the eggs on the dorsal side until it hatches. This amazing behaviour serves to protect the eggs. Wing polymorphism is commonly seen among some Heteropterans. All the collected specimens except the nymphs of the species were macropterous during this investigation. Apterous or brachypterous morphs were not found during this collection. *Salvinia molesta* is the major habitat upon which the bugs move, rest and find shelter in the lake. They extend their body obliquely downward to rest at the surface of aquatic macrophytes. It has been observed that they consume the larvae of chironomids and mosquitoes. The bugs were abundantly during the dry season (December-June) and were few during monsoon (July-November).

Laccotrephes griseus* (Guerin-Meneville, 1844) (Fig. 2B)*Materials examined**

2 exs., S1; Vettolikadavu (9°1'56.93" N and 76°37'29.72" E), 2 exs., S2; Pulikuzhi (9°2'58.4" N and 76°38'24.79"E), 1ex., S3; Adikkadu Kadavu (9°2'4.01" N and 76°36' 49.36" E), 2 exs., S4; Punnakkadu Kadavu (9° 2' 41.27"N and 76° 37' 44.17" E), 18.xii.2022, Coll. Jyothylakshmi K and S. Nandakumar.

Diagnostic characters

Body colour is somewhat similar to dark soil. Length typically ranges from 15-20 mm. Respiratory siphon is 12.9–14.5 mm in length and always shorter than the body. Anterior region of the prosternum is armed with a highly acute tubercle. Abdomen with a faint bluish tint above and abdominal appendages are considerably shorter than the body.



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Base of the front femora is equipped with an obtusely rounded tooth. It can be easily distinguished by the shape of the paramere since male paramere is moderately hook-shaped and symmetrical.

Bionomics

It is a sluggish species. Body colour is bit similar to dark soil. They are frequently found at the edges of the lake, among aquatic weeds or at the bottom of the water. Swimming requires the use of all legs. However they are not proficient swimmers. When they creep or move slowly, abdominal appendages protrude upward. They use front raptorial legs to catch different kinds of small aquatic organisms. It is a voracious feeder on mosquito larvae and hence it can be used as an effective biocontrol agent of mosquito. When handled, they inflict painful bite.

Cercotmetus pilipes* (Dallas, 1850) (Fig. 2C)*Materials examined**

4 exs., S1; Vettolikadavu (9°1'56.93" N and 76°37'29.72" E), 2 exs., S2; Pulikuzhi (9°2'58.4" N and 76°38'24.79"E), 2 exs., S4; Punnakkadu Kadavu (9° 2' 41.27"N and 76° 37' 44.17" E), 25.xii.2021, Coll. Jyothylakshmi K and S. Nandakumar.

Diagnostic characters

Elongated cylindrical body. Size ranges from 40– 41mm; respiratory siphon 10–11 mm in length. Body covered with fine setae. Colour dark brown or yellowish brown. Legs marked with a light brown band. Head is small, subconical and prognate. A sharp tubercle is present on the vertex between the eyes. They have small, three segmented and concealed antenna. Prothorax is narrower than head. Forelegs are highly prehensile without claws. Femur of the anterior leg is heavier, shorter than the pronotum. Mid and hind legs are longer and more specialized for walking. Mid, hind tibiae and tarsi with spinous process and long yellow hairs placed in two rows. Male parameres are bifurcated, symmetrical, stout, and anteriorly narrowed but medially bulged.

Bionomics

Cercotmetus pilipes has elongated and linear in shaped body having similarity to floating sticks and straws. The darker colour, greater size, shorter coxae, stout respiratory tube and shorter fore femora distinguish them from closely similar species *Ranatra filiformis*. They camouflage well with the background of fallen leaves and decaying plant materials. It is a sluggish species. They often attach to the substrate while maintaining close contact with the surface film. In the lake, individuals of this species are commonly found among the aquatic macrophytes near the edges of water. It has been observed that they deposit eggs on the stem of floating aquatic plants. Eggs are collected from inside the stems of floating vegetation. It has two long, thin filaments at its apex. All obtained specimens from the study area were macropterous. They are extremely voracious and found attacking small fishes, tadpoles and mosquito larvae. More individuals of *Cercotmetus pilipes* were obtained during the month of May.

Enithares ciliata* (Fabricius, 1798) (Fig. 2D)*Materials examined**

1 ex., S2; Pulikuzhi (9°2'58.4" N and 76°38'24.79"E) 2 exs., S4; Punnakkadu Kadavu (9° 2' 41.27"N and 76° 37' 44.17" E), 21.x.2022, Coll. Jyothylakshmi K and S. Nandakumar.

Diagnostic characters

Average body length ranges from 8.5 to 9.6 mm. Males are little bit smaller than females. Dorsal side is usually blackish in colour. The vertex is yellow, the anterior half to two thirds of the pronotum is yellow with varying brown patterns and the remaining portion is translucent with blackish underlying sections. Basal portion of the hemelytra and the caudal lobe of the membrane are translucent. Lateral margins of the scutellum have a greenish fluorescent stripe that turns yellow in dead specimens. First mid tarsal segment is concealed, the second mid tarsal segment is broad and nearly triangular in lateral view. The mesotrochanter is rounded with a patch of black spicules along the ventral border. Male paramere is small and round.



**Jyothylakshmi and Nandakumar****Bionomics**

This species comes under the category of "back swimmers" since it swims upside down position. They are often found near to the water surface. Their raptorial forelegs help them to have a grip on the prey. It has been observed that they feed on tadpole larvae, mosquito larvae and the nymphs of other aquatic insects. Only macropterous morphs of the species were obtained from the lake. It was observed that the males of *Enithares ciliata* are smaller than the females. It is very difficult to capture them because of their quick movement. *Enithares ciliata* were abundant in pooled microhabitats which lacked floating macrophytes. Macrophytic association could not be observed for this species.

DISCUSSION

Early studies have systematically classified *Nepomorpha* ns in some of the aquatic ecosystems of the state. However, information on the bioecology of these groups is yet to be documented in detail. Some scientists [7; 5; 6] have made substantial contribution towards the systematics of aquatic bugs in Kerala. The present study would facilitate the knowledge on the bionomics of some Heteropteran species from Kerala which have not yet been described. The present study contributes to the taxonomy and bionomics of four *Nepomorpha* species from the lake. Bionomics of water bugs would definitely help to explore their position and role in the ecosystem. At the same time a comprehensive assessment of the biological significance as well as the biological diversity of these insects are needed to be documented in detail. Systematics of water bugs of Kerala need to be described further to bridge the knowledge gap in the taxonomy of aquatic insects across different regions in India.

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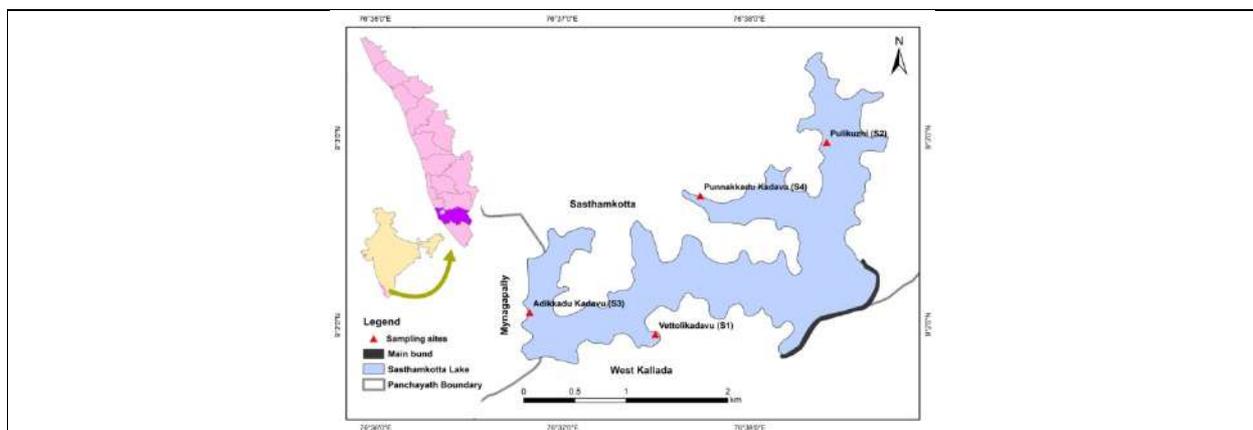


Fig. 1: Location map of the study area with sampling sites.

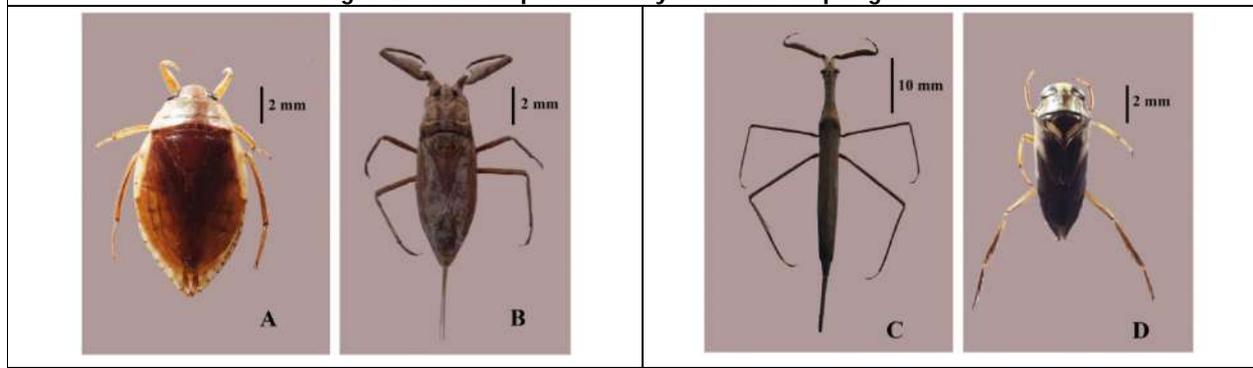


Fig.2: Dorsal habitus images: A- *Diplonychus rusticus* (Fabricius, 1781), B- *Laccotrephes griseus* (Guerin-Meneville, 1844), C- *Cercotmetus pilipes* (Dallas, 1850), D- *Enithares ciliata* (Fabricius, 1798).





Analysis of Text Extraction from Image using Python

Y.Sudha^{1*}, Safia Shaik² and K.Shyama Satya Naga Teja³

¹Assistant Professor, Department of Electrical and Electronics Engineering, Malla Reddy Engineering College, Secunderabad, Telangana, India.

²Assistant Professor, Department of Electronics and Communication Engineering, Maulana Azad National Urdu University, Hyderabad, Telangana, India.

³Assistant Professor, Department of Electrical and Electronics Engineering, St.Martin's Engineering College, Hyderabad, Telangana, India.

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*Address for Correspondence

Y.Sudha

Assistant Professor,

Department of Electrical and Electronics Engineering,

Malla Reddy Engineering College,

Secunderabad, Telangana, India.

E.Mail: yelmareddy.sudha@gmail.com



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ABSTRACT

Text Extraction is the process of putting together groups of optical patterns based on whether they look like letters, numbers, or other figures. The process of Optical Character Recognition (OCR) includes "segmenting," "extracting features," and "classifying." Text capture turns analogue resources with text into digital resources with text. Then, these converted sources can be utilised in different ways, such as making text searchable in indexes so that papers or images can be found. As the first step in capturing text, a picture of a page is scanned. And all the other steps will be based on this scanned copy. The next step is to use Optical Character Recognition (OCR) technology to turn text content into a format that machines can read or understand. OCR analysis takes in a digital image that is printed or written by hand and turns it into a digital text file that a machine can read. The digital image is then broken down into individual pixels by OCR so that individual words or character strings can be searched for. And again the symbol blocks are further divided into elements and are in contrast with definition of characters. Python, a programming language, will make it possible to work on this issue in a controlled setting. It comes with a big library that can be imported and used for OCR processing. Python can be used for a wide variety of purposes, from analysis and method creation to computation and beyond. It's quick and simple solution to our predicament is much appreciated.

Keywords: OCR, digital image, Python, digital text, scanned images



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INTRODUCTION

OCR is the method of sorting optical images into alphanumeric or other characters. The process of optical character recognition contains a number of processes, including segmentation, feature extraction, and classification [1]. The suggested OCR system is used to scan every document, regardless of whether it was written by hand, printed, or typed, and then converts the scanned images into text formats that a machine can read. Scanning and analysing photos of individual characters enables their conversion into the character codes used in data processing and the American Standard Code for Information Interchange (ASCII). The remaining codes are standard optical character recognition systems [2]. It's possible that we'll utilise the OCR software to scan books and articles and save the results as computer files. OCR-enabled digital files are more space- and time-efficient since they provide simple operations like text editing, keyword searching, and phrase searching, among others. The entire check-in and -out process, as well as the storage and organisation of libraries' book holdings, are now conducted entirely online. It can be put to use in the verification of credit card vouchers, the organisation of electronic mail, the digitization of office recording systems, and a variety of other important endeavours [3]. In addition to the foregoing, this OCR can be used to create a protocol for the mechanical exchange of text between the various data processing programmes.

The OCR system can read a wide variety of characters, including letters, numbers, and symbols. In order to prevent the document format from collapsing during the conversion process, many OCR systems perform two primary tasks: page segmentation and decomposition [4]. After the conversion is complete, the OCR system checks the transferred document against the original. The OCR system can recognise a wide range of fonts, styles, and colours, which is a very useful function. However, scripted or hand-written characters might be challenging for OCR systems to decipher because they are typically developed with a certain language in mind. It is challenging to solve the challenge of automatically recognising and segmenting text from document images [5]. OCR is only as good as the input it receives. Current optical character recognition (OCR) technology can only read certain typefaces and languages. However, even the best available OCR systems have substantial drawbacks, some of which can be overcome with well-executed pre-processing of the document picture.

OCR analysis accepts as input a digital image of printed or handwritten text, and then converts that image to a format of digital text that can be read by a machine. After that, the digital image is broken down via OCR into smaller components, which are subsequently investigated in order to identify chunks of text, words, or characters [6]. Once more, the character block is disassembled into its constituent parts, and these parts are contrasted to the character dictionary.

LITERATURE SURVEY

An algorithm for determining the page rotation and skew of a document has been proposed by Le et al., and it uses a projection profile as its input. This approach has the drawback that it is able to determine whether a document is in portrait or landscape orientation, but it is unable to differentiate between the regular orientation of either portrait or landscape documents and the inverted orientation [7]. Aditya et al. propose a method that can identify the orientation of photos that do not contain text. In order to make an estimation of the orientation, they utilised the Bayes classifier; however, this method is not applicable to text pictures. They demonstrated how to estimate the class-dependent density of observed features by making use of a tiny codebook that was retrieved from a Learning Vector Quantizer (LVQ) [8]. This was necessary for Bayesian inference. The subsequent illustration demonstrates how Linear Discriminant Analysis (LDA) and Principal Component Analysis (PCA) can be utilised as feature extraction procedures to reduce redundancy from high-dimensional feature vectors that are utilised for classification. According to Cheetal et al. It is recommended that a support vector machine (SVM) be used to identify and categorise document orientation [9]. The picture of the document's characters has all been extracted, and those extracted have had some valid characters selected for use. Using valid characters, feature extraction can convert a document's picture into a 32-dimensional vector. After extensive training, the SVM classifier is able to correctly identify the orientation of unlabeled document pictures. A novel technique to isolate or eliminate visuals from





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document images, such as emblems and non-text animations, has been put out by Chethan et al. Subsequently, the distortion present in the textual content is rectified, and the characters are identified through the utilisation of readily available optical character recognition (OCR) technology [10]. The fundamental procedure of this approach comprises of a tripartite sequence. Initially, the graphic in the image should be eliminated through the utilisation of vertical and horizontal forecasting techniques. Subsequently, the binary image undergoes the extension operation, which yields an extension image that is comparatively brighter. Ultimately, the Hough transform is employed to ascertain the skew angle. The method under consideration exhibits a high degree of precision in detecting skew at significant angles ranging from 90 degrees to positive 90 degrees. In the case of images with high levels of noise, the performance of the method is inversely proportional to the density of the noise.

The authors Guru et al. (2013) introduced a technique to assess the degree of skew in handwritten documents that contain multiple languages. The aforementioned method involves the utilization of morphological techniques and associated aspect analysis to segment individual phrases from a handwritten fluent record photograph [11]. The skew of each phrase is determined by computing the minimum circumscribing ellipse. The orientation of each phrase is approximated, followed by clustering of the phrases using adaptive k-means clustering to identify the multiple groups present within the document. The average orientation of each group is then calculated.

Proposed System

The OCR system can read a wide variety of characters, including letters, numbers, and symbols. Many OCR systems can convert documents without altering their formatting by handling page segmentation and decomposition. One key distinction between OCR and other methods is how faithfully the perceived text matches the original. OCR also has the handy capability of adapting to various character methods, fonts, and colour schemes. Because they are built for a specific language or script, many systems have a hard time recognising handwritten text. The document's content is crucial, yet it's a challenge to properly recognise and locate embedded text within documents. The size of the characters can range from very little to excessively large. Even within the same text, font size and letter styles can vary. The document's text is rendered in a rainbow of colours and can be read from any angle. Figure 1 depicts a scenario in which text is shown on a background as complicated as the one shown. Therefore, OCR performance is dependent on the type of input provided, making automatic document text recognition and segmentation a challenging challenge.

In Figure 2, the image is crudely segmented into text-containing and text-free regions. Image enhancement is applied to the regions of the image that have been determined to contain text after the non-text regions have been eliminated. The common assumption that each character is made up of a series of connected strokes makes complex component analysis a useful tool for analysing printed text. Character recognition is the process of determining which patterns represent which alphabetic characters. Input into the categorization process is provided by a set of defined character traits. Categorization methods include model comparison and structural categorization. Pattern matching can be used as a starting point for identifying a pattern. The identified pattern is then compared to data stored in the past. In structural or morphological classification methods, structural traits and decision rules are used to categorise characters. Structural features include things like character qualities, character defects, and even things like cavities, which are all aspects of a person's personality. The identification procedure identifies the character primitives, and subsequent rule checks apply to the string primitives.

Tesseract OCR

Tesseract is an open-source, free OCR (optical character recognition) engine that uses the Apache 2.0 licence. The printed text can be read through the input image or API. Several languages are available for use. Tesseract does not come with its own GUI, however there are some available online. It can be used in tandem with preexisting layout analysis to find text in massive texts, or with a different text detector to find text in images embedded in lines of text. A new neural networking subsystem implemented as a group of recognition of text engines is available in Tesseract 4.00. The original OCRopus LSTM implementation was written in Python, whereas this new Tesseract version is





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written in C++. Recognising single-character pictures in photographs requires the use of complex neural networks (CNN). There are several stages to this procedure, which are detailed below:

- Find the word
- Define lines
- Personality

Lines of text are grouped together to form huge binary objects, and then each line and ranges are analysed to determine if the text is fixed-pitch or scaled. Words on a line can be separated in various ways based on the character spacing that was utilised. The method of identification is then carried out twice more. The initial stage involves word-by-word recognition attempts. Each accepted word is used as training data for the dynamic classifier. The bottom of the page text is easier for the adaptive classifier to decipher. It is a free and open source OCR (Optical Character Recognition) programme (Figure 3). It's simple to use and effective in recognizing text in photos. Since it was developed in the public domain, it can be used without cost.

Tesseract's default behaviour is to analyze the input text image as if it were a page of segmented text. A little text box can be cut out of a picture using different Tesseract segments. The `—psm` option allows for this to be accomplished. While Tesseract can completely automate page segmentation, it is unable to identify locations or scripts. Page Segmentation Mode (`--psm`): This option allows you to instruct Tesseract on how to extract text from an image.

OpenCV

OpenCV, short for "Open Source Computer Vision Library," is free and open-source software for computer vision. OpenCV set out to standardise the foundation upon which computer vision programmes may be built. It also hastens the incorporation of mechanical touch into consumer goods. In addition, businesses can benefit greatly from using and customising the code provided by OpenCV, which is distributed under the BSD licence. Over 2500 methods, both old and new, for computer vision and machine learning have been optimised in this package. These algorithms can do a wide range of things, including face identification and recognition, video behaviour classification, object classification, and 3D model extraction. As a preprocessing step, it can help identify text in a picture. OpenCV's imaging operations—such as color-to-binary conversion, contrast enhancement, edge detection, and many others—provide the clean image that Tesseract needs for text detection.

Result Analysis

The Tesseract library was used in the development of this project's Python code. Below Figure 4 and 5 is a screenshot of a typical output screen.

CONCLUSION

Within this system, we've created a mechanism that pulls text out of an image that has been scanned and places it in a predetermined, aligned layout. In addition to providing precise outcomes, the suggested technique is also capable of greatly decreasing image noise. The system requires that the aspect ratio be aligned in a certain way, and if it is not, the process will fail. This system's OCR fails miserably when presented with a picture containing handwritten text. The output is sufficiently precise for the given format.

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Figure 1: Sample Document with different text features

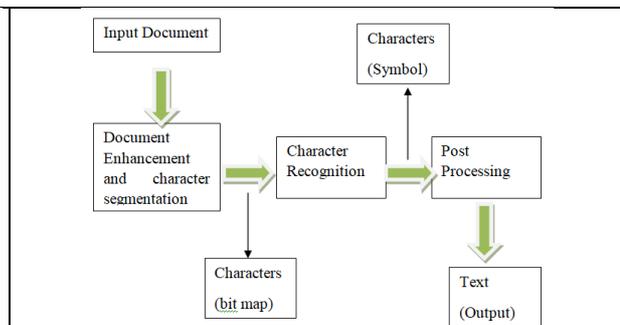


Figure 2: Text processing using OCR





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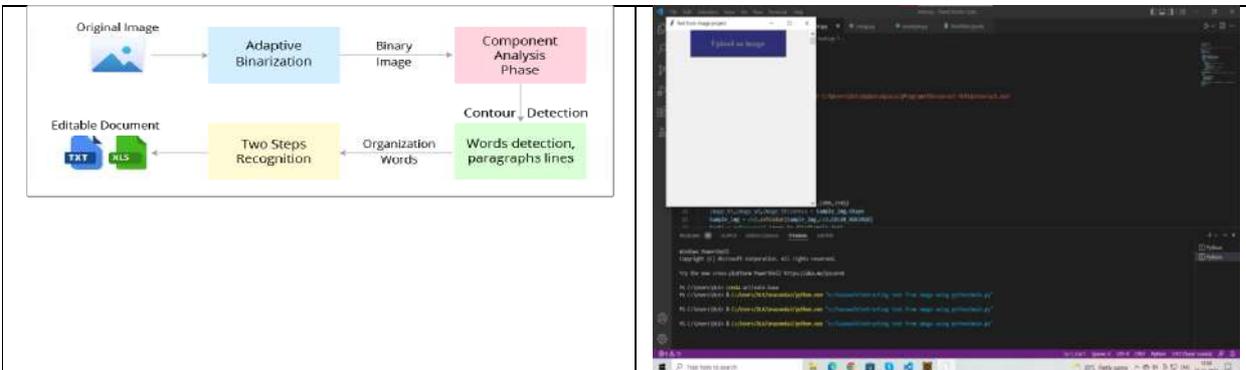


Figure 3: Virtual Representation of OCR

Figure 4 : Output Screen 1

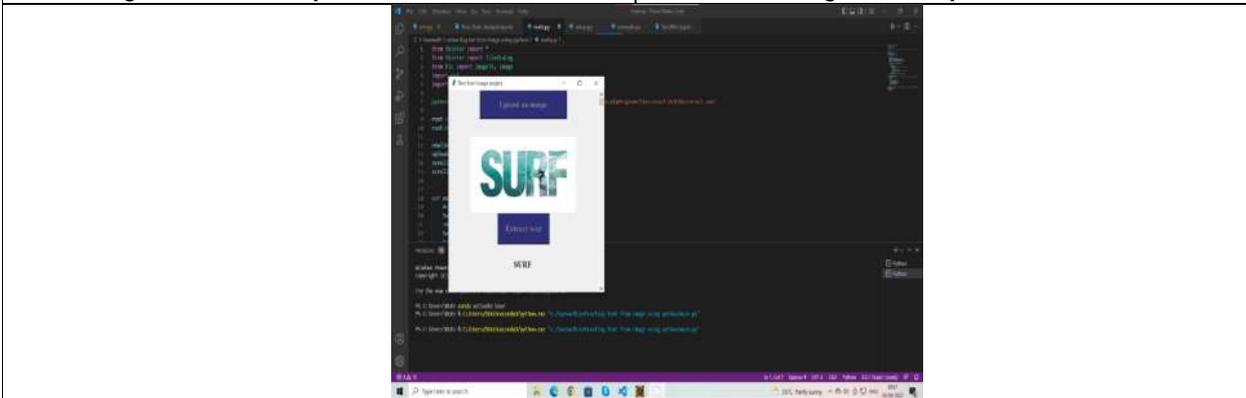


Figure 5: Output Screen 2





Improved Mechanical Behaviour and Strength Metrics in Geopolymer Concrete Made with Fly Ash and Ground Granulated Blast Furnace Slag

Pathi Janardhan^{1*}, R. Venkata krishnaiah² and K.V.B. Raju²

¹Research Scholar, Dept. of Civil Engineering, Bharath Institute of Higher Education and Research (BIHER), Chennai, Tamil Nadu, India

²Professor, Dept. of Civil Engineering, Bharath Institute of Higher Education and Research (BIHER), Chennai, Tamil Nadu, India

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*Address for Correspondence

Pathi Janardhan

Research Scholar,
Dept. of Civil Engineering,
Bharath Institute of Higher Education and Research (BIHER),
Chennai, Tamil Nadu, India
E.Mail: janardhancivilengg@gmail.com



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ABSTRACT

The aim of this study is to determine whether two industrial waste products, fly ash (FA) and ground granulated blast furnace slag (GGBS) powder, may replace typical cement composites in building materials in a sustainable and environmentally responsible manner. Over two distinct curing durations, fly ash and GGBS-based geopolymer concrete is contrasted with traditional concrete. The study looks at both types of concrete's strength characteristics, such as tensile strength and flexural strength, as well as their resistance to acid attack and quick chloride conductivity. This project improve concrete performance by using fly ash and polymers in place of cement. Utilising concrete of the M25 Grade, compression strength tests are performed on geopolymer concrete to evaluate its strength qualities. Controlling the heat of hydration during the manufacturing of concrete is seen to be a significant development. Cement and geopolymer concrete cubes are subjected to water curing and subsequent oven dehydration. According to the test results, geopolymer concrete has potential for a range of construction projects. Its use may help reduce the demand for water and minerals, which are now met through destructive methods like river bed excavation and mineral extraction, which have an adverse effect on ecosystems. Geopolymer concrete offers a sustainable alternative by utilising industrial waste materials.

Keywords: geo-polymer concrete, fly ash, cement, Ground Granulated Blast Furnace Slag (GGBS), M25 Grade





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INTRODUCTION

Environmental preservation and the effects of global warming on the climate have emerged as pressing issues. Producing one huge amount of Portland cement fallouts in the production of about a tonne of carbon dioxide. the production area is often blamed for contributing to the problem of carbon dioxide emissions. Waste and by-product materials must not be dumped in unsafe or unregulated ways if we are to preserve the planet. Three-dimensional structures of aluminosilicate glass, geopolymers are hardened materials. Concrete relies heavily on cement. Cement prices are rising steadily as supply becomes increasingly scarce and demand remains high. Meanwhile, the rate of global warming is accelerating. Carbon dioxide is also produced during the cement-making process. To lessen cement consumption and concrete emissions, an experimental inquiry and a concrete experiment were conducted here. Cement was substituted with Fly ash and GGBS. The effects of using this in place of cement in experimental investigations of ordinary cement concrete were examined. Fly ash, GGBS, were used to create the concrete mix for this research. Concrete grade, chemical inertness, and the RCPT test are all areas where concrete and Geopolymer concrete is compared. Several initiatives are underway to begin fixing these problems. Alternative binders to Portland cement are being developed, and supplemental cementing ingredients are being used. In this regard, geopolymer concrete, which has a substantially less ecological imprint, offers great potential for use in concrete production. The geopolymer technology has the potential to drastically cut the CO₂ production to the troposphere taken on by the adhesive productions, hence lowering the global warming potential of this sector.

By observing the chemical change, one can see that water is released from the mixture as it polymerizes. Although water is necessary for the geopolymer mix to become plastic, it does not add to the material's toughness after it has solidified. In a related manner, more water is required for the same amount of effectiveness as the primary source becomes finer. Easily be accomplished, fly ash granularity, and quarry dust sorting are all taken into account to determine the least amount of water needed to obtain the specified flow ability.

MATERIALS AND METHODS

Constituents of Geopolymer Concrete

The low-calcium fly ash that is collected from coal-burning power plants can be utilized in the production of geopolymer concrete. ASTM Class F fly ash is utilized in this process. Fuel economy is great in coal-fired nuclear reactors; in fact, only hydroelectric plants have a higher energy efficiency ratio. Figure 1 displays the ratio of energy gained to energy spent.

There is evidence it can be successfully produced by reduced fly ash when the silicate and aluminium oxides account for around 90% by volume. In most cases, the percentage of mass that was comprised of iron oxide varied from 10 to 20%, whereas the percentage of mass that was comprised of calcium oxide was less than 5%. Loss of volume upon igniting suggests that the quantity of graphite in the fly ash is a little less than 2%. According to the results of the particle size circulation studies, eighty percent of the fly ash particles were less than fifty micrometers in size. In flake or pellet form, the sodium hydroxide with a cleanliness of 97-98% is available for purchase in commercial settings. In order to produce a solution that has the desired concentration, the solids must first be dissolved in the water. It is possible for the amount of sodium hydroxide solution to range anywhere from 8 Molar to 16 Molar; nevertheless, a solution that is 8 Molar is sufficient for the majority of uses. A solution's effect may be modified by altering its level based on the quantity of NaOH solids that are included in it. For instance, a solution of NaOH that has a concentration of 8 Molar has 8 times 40, which equals 340 grams of solid NaOH, where 40 represents the NaOH. Take note that the frame of water makes up the majority of the ingredient that is alkaline in both of the solutions.

A minor amount of calcium-rich original basis from blast furnaces may be encompassed in the mix to aid curing at room temperature and to expedite the curing process of fresh geopolymer concrete. This may be done by adding a tiny proportion of the material to the mixture. To make the combination more workable, it may benefit from the





addition of supplemental water as well as a rising water superplasticizer. Table 2 provides the proportions of two distinct geopolymer concrete compositions that were employed in laboratory investigations.

Fly Ash

Fly ash is one of the elements that may be found in the greatest quantities on our planet. As a result of the role, it plays in the polymerization process, it is also an essential component in the production of geopolymer concrete. Furthermore. When mixed with calcium hydroxide, a substance can take on the characteristics of a pozzolan due to its cementitious nature. Carbon combustion in power generation that run on coal results in the production of a significant amount of fly ash. Fly ash may be divided into two different classes. The several categories of fly ash each have their own distinct characteristics. Fig.3 provides an investigation of the chemical mechanisms that make up fly ash.

Mix Design

Concrete is the material that underpins the whole field of civil engineering in today's rapidly developing world of contemporary building design. The primary mission of a structural engineer is to design and create a concrete structure that is not only functional and long-lasting but also visually beautiful. The part of a concrete designer has now come into play. In order to construct a building out of concrete that is both functional and long-lasting, it is necessary to adhere to the procedures for the design of concrete mixes that are specified in the IS. It is possible that the mix will not have the necessary strength, durability, or workability if the design of the mix is flawed. This can result in issues like as cracking and a lower resistance to external influences such as weather, fire, and impact. For instance, if the mixture contains an excessive amount of water, it will be weaker than intended since it would be very moist. On the other hand, if insufficient water is utilised, the mixture would be too dry and difficult to deal with because of the lack of moisture. In a nutshell, the mix design is what determines the final quality of the concrete, and it is absolutely necessary to adhere to the procedures and standards for mix design (IS- 10262: 2009 and IS- 456: 2000) in order to make certain that the mix achieves the desired level of strength and functions in the manner that was intended.

Concrete Cement of the Regular M25 Grade

It is necessary for us to formulate a concrete mixture using M25 that can achieve 25 MPa. All of the evaluations pertaining to the concrete's workability and compressive strength have to be carried out on a combination of concrete that is not only fine mixed but also uniform throughout. The performance of concrete hardness could well be determined by testing it more precisely if it is first formed into cubes and then compressed. Specimens cast 7, 14, and 32 days after curing were used to measure the substance's strength property. Table 3 below displays the various experimental data that would be needed to produce the desired blend.

Geo-Polymer Mix Design

The various composite samples of geo-polymer concrete are broken down below in Table 4. The major purpose of carrying out the trial-and-error approach was to achieve a consistent mixture that could be worked with to the required level of satisfaction. It was demonstrated through the use of a mixed design sample that the aggregates make up around 75%–80% of the total mass of the GPCs. The fig.4. shows the mix proportion value

Design of a Geopolymer Conventional Concrete for Use with Fly Ash, M25 Level

Previous research presumed a relentless concrete compactness of 2300 kg/m³, which is unrealistic given that the compactness of concrete fluctuates from mix to mix based on the number of components used. Adding more water or a super-plasticizer to fresh geopolymer concrete can make it easier to shape, but this reduces the amount of concrete that can be used. However, the design for workability, which appears to have an even more significant effect on geopolymer concrete than in conventional concrete, was not considered in their approach. As a result, it has become clear that a systematic approach that is both straightforward and rigorous must be developed for the design of geopolymer concrete mixes as exposed in Fig.5.





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Methods like that are discussed in the following sections. Initially, the method is introduced generally with a flowchart, and then an example is shown in Fig.6 An accurate representation of the alkaline liquid to fly ash ratio is different from the initial ratio because additional aquatic was further to increase the mix's flowability. Figure7 shows that, when comparing different amounts of the same alkaline liquid to fly ash ratio, the optimal compressive strength was attained when the volume of the fly ash content was 340 kg/m³. So, it appears that increasing the percentage of fly ash in the mixture does not improve the concrete's strength. The strength qualities of geopolymer concrete are inversely related to the water-to-geopolymer solids ratio, just as the flexural modulus concrete is inversely related to the water-to-cement ratio. It has been observed that as the alkaline liquid-to-fly ash ratio increases, the water-to-geopolymer particle ratio increases linearly. It is true as long as the moles of NaOH solution and the sodium silicate to sodium hydroxide liquid ratio is constant throughout all mixes.

In Figure 8, we see how the proportion of alkaline fluid to fly ash can affect the water-to-geopolymer particle ratio. When a developer has not yet established a clear and accurate water-to-geopolymer solids ratio, this information can be helpful.

RESULT AND DISCUSSION

In order to manufacture test samples for the test of compressive strength, cubes with dimensions of 150 millimeters on each side and 150 millimeters on top were formed in iron and steel molds. There was a discrepancy in the number of cubes produced for each mixture ratio, and their ages 7, and 28 days after casting, were recorded. Cylinders with a diameter of 100 millimeters and a height of 300 millimeters were employed as the test samples for the split tensile strength test. Cast iron molds were utilized for their production. In order to create the test specimens for the flexural strength test, cast iron steel molds with dimensions of 500 millimeters, 100 millimeters, and 100 millimeters were employed. At 28 days, three dissimilar numbers of prisms were cast for each mix proportion. They were then evaluated.

Molds measuring 15 centimeters on a side are the standard for the vast majority of castings. This really important thing is placed into its mold after being fully cooled, and this helps get rid of any air pockets. Components results are submerged in water for 24 hours before being disposed of and the stress is relieved. Following either seven days of decompression or twenty-eight days of reestablishment and the results are depicted in figure 9. The technique that calls for gradually applying problems at a rate of 140 kg/cm² per second until the specimens crash and burn is the one that is advised. This gives the concrete's compressive strength in pounds per square inch. Comparative elements were coming into awareness as a consequence of tests that were run on conventional M25 Grade concrete and geopolymer concrete. It was decided to conduct these experiments in the same location. The compressive strength test results for a typical M25 grade concrete after 3, 7, 14, 21 and 28 days are 9.56, 15.8, 19.16, 21.24, and 24.12 correspondingly. These findings were obtained from the test. The usual ratio of 0.45 is the minimum amount of water that must be present in this concrete. The provided data allows us to infer the following that the compressive strength testing results for Geo polymer concrete Mix-A are 10.41, 16.25, 19.11, 23.7 and 25.21 accordingly.

CONCLUSION

Overall, this study shows the potential of fly ash and geopolymer concrete based on GGBS as environmentally friendly substitutes for conventional cement composites. It provides a solution to lessen environmental effect and encourage responsible resource utilisation in the construction industry by using industrial waste products.





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Table 1 Proportions of two distinct geopolymer concrete compositions

| Materials | Mass | |
|---------------------------|-----------|-----------|
| | Mixture 1 | Mixture 2 |
| Coarse aggregates -20mm | 268 | 286 |
| Coarse aggregates - 14mm | 367 | 368 |
| Coarse aggregates - 7mm | 747 | 747 |
| Fine sand | 454 | 454 |
| Fly ash | 418 | 418 |
| Sodium silicate solution | 113 | 113 |
| Sodium hydroxide solution | 31 | 31 |
| Superplasticizer | 6 | 6 |
| Extra water | 0 | 22.5 |





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Table 2. Experimental data

| Test data for materials | |
|---|---------|
| Mean, Median, and Mode | 4.2 MPa |
| Fine Aggregate Density Specific Measurement | 2.64 |
| The concentration of Coarse Aggregate | 2.64 |
| The density of Cement Mixture | 3.25 |
| Admixture Density Specification | 1.155 |
| Permeability of Fine Aggregate to Water | 1.0 % |
| Coarse aggregate's capacity to absorb water | 0.5% |
| The Fine Aggregate Surface Wetness under Freeze-Thaw Conditions | 0 |
| Surface Water Content at Rest in Coarse Aggregate | 0 |

Table 3. The Ratio of the Calculation of Cementitious Ingredients During the Manufacture of Geopolymer Concrete

| Mixture id | Fly Ash | GGBS |
|------------|---------|------|
| Mixture 1 | 100 | 0 |
| Mixture 2 | 90 | 10 |
| Mixture 3 | 80 | 20 |
| Mixture 4 | 70 | 30 |
| Mixture 5 | 60 | 40 |

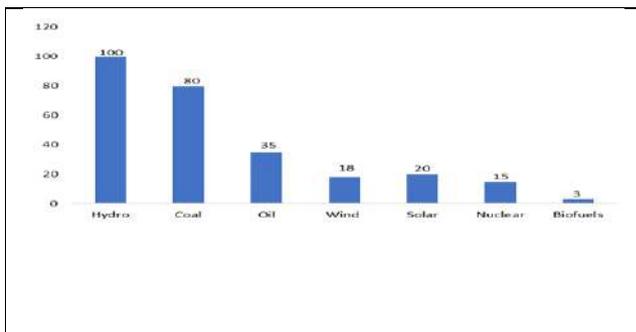


Fig.1. Energy returned/Energy invested ratio

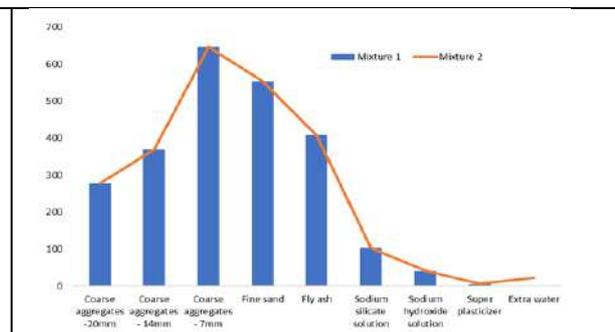


Figure.2. Geopolymer concrete compositions comparison

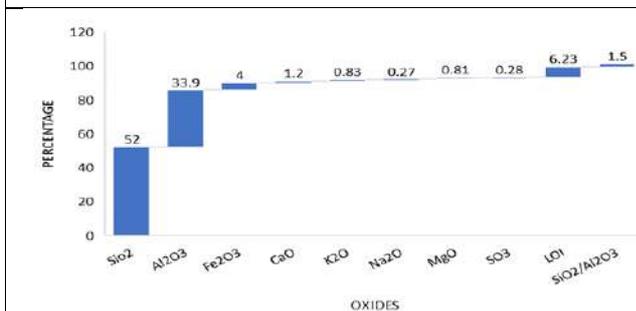


Fig.3. Analysis of the chemical components that make up fly ash

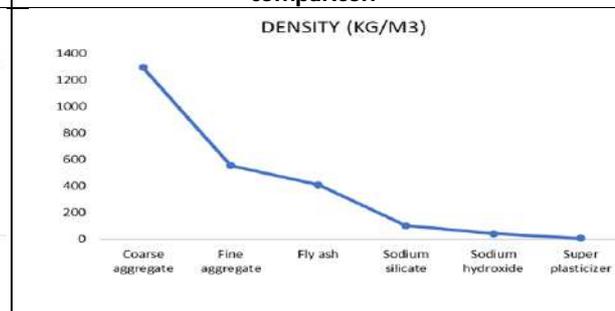


Fig.4. Mix Proportions Value





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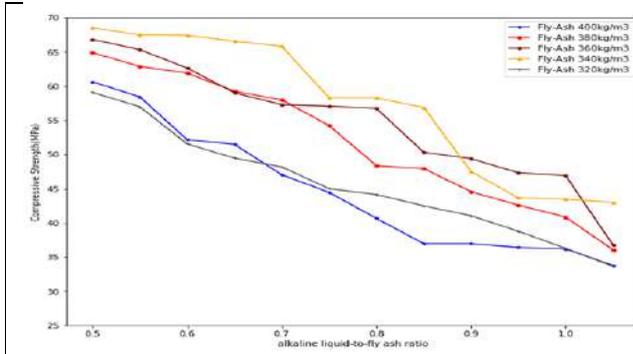


Fig.5. Comparison of different amounts of the same alkaline liquid to fly ash ratio

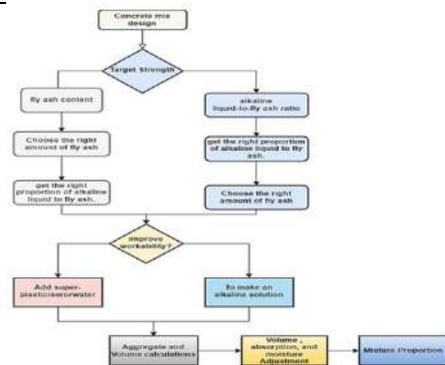


Fig.6. Mix Design Procedure

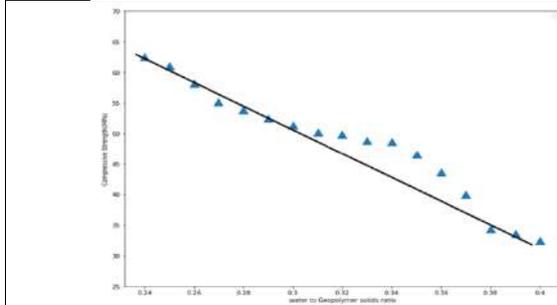


Fig.7. Compressive strength vs W/GS

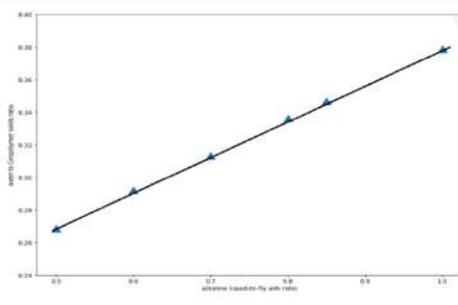


Fig.8. (W/GS) vs alkaline liquid-to-fly ash ratio

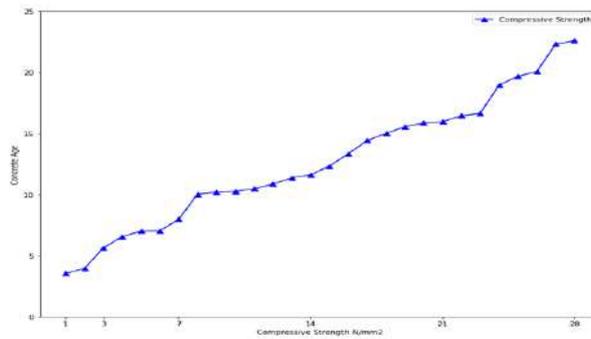


Fig. 9. Compressive Strength





Physical Fitness Training and Psychological Strategies for Improving Swimming Performance

S. Sivamani¹, Kumar. P^{2*} and P.Kasthuri Thilagam³

¹Physical Director, Dept. of Physical Education and Sports, Puducherry Technological University, Puducherry, India.

²Assistant Professor, Department of Physical Education and Sports, Central University of Haryana, Haryana, India.

³Physical Education Teacher, Government Higher Secondary School, Valanadu, Trichy, Tamil Nadu, India.

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*Address for Correspondence

Kumar. P

Assistant Professor,
Department of Physical Education and Sports,
Central University of Haryana,
Haryana, India.
E.Mail: kumarp@cuh.ac.in



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ABSTRACT

The goal of the study was to find the psychological techniques and physical fitness training that improved swimming performance. It is planned and carried out to enable the swimmers to be in an optimum psychic state at the time of competition. So that he can achieve the maximum possible performance. Thirty swimmers from the Pondicherry Aquatics Sports Complex in Puducherry participated in the current investigation. The goal of the current study was to determine how Pondicherry aquatics complex swimmers responded to a 6-week training program in selected psychological techniques. Chosen The allocated training program has a duration of 60 minutes every secession for 3 alternate days. Swimmers that achieved and represented their age group in State, University, and National level swimming competitions were between the ages of 18 and 21. The psychological strategies training program consisted of Attention focus and Mental Imagery. Selected subjects were separated into three equal groups Training Group 1 (Swimming skills with selected psychological strategies training) Training Group 2 (Swimming skills without selected psychological strategies training) and Group 3 then each group consist of 10 subjects considered as autonomous variables. The acquired data has been processed with ANCOVA and Scheffe's post hoc test to assess the study's premise. The measured level of significance is 0.05. In contrast to Training group 2 and Control Group, Training group 1 showed a considerable improvement in swimming performance, according to the results of the current study. Training group 2 showed certain improvement. There has been a



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noticeable improvement in swimming performance among swimmers in the Puducherry region as a result of swimming abilities with and without certain psychological methods.

Keywords: Swimming, Psychological strategies, Attention focus, Mental Imagery, Puducherry, Aquatics Sports, Training.

INTRODUCTION

Swimming is considered by many to be the best form of full-body exercise. The well-documented cardiovascular benefits of swimming focus largely on its healthy effect on the heart and lungs. Swimming uses almost every muscle in the body in a coordinated and repetitive, cycling manner that exposes them to sustained exertion in a medium that is thicker than air. Well-conditioned swimmers, in general, have extremely low resting heart rates, a measure of good fitness, and are known to have one of the most developed capillary systems of all athletes[1]. Swimming is considered by many to be the best form of full-body exercise. The well-documented cardiovascular benefits of swimming focus largely on its healthy effect on the heart and lungs. Swimming uses almost every muscle in the body in a coordinated and repetitive, cycling manner that exposes them to sustained exertion in a medium that is thicker than air. A swimmer's notion of well-being is based on their particular beliefs and ambitions, which are a unique phenomenon in high-performance swimming[2].The process of contemplation and assessment in Swimmers increase the overall quality of training sessions to make them additional productive and efficient[3]. Well-conditioned swimmers, in general, have extremely low resting heart rates, a measure of good fitness, and are known to have one of the most developed capillary systems of all athletes [4].

Psychology is the science of behaviour and mental processes, which studies every aspect of an organism's functioning- overt actions, mental processes, emotional responses, and physiological reactions[5]. The psychological strategies training program will focus on maximal performance by improving attention focus, mental imagery, self-talk, and relaxation exercises [6]. To improve these psychological skills, the athlete will complete sessions using attention focus and imagery. Attention focuses and their role in human performance have been subjects of debate and examination for additional than a century, begging with the following classic description [7]. The term "mental imaginary training" refers to mental exercise that entails picturing a motor movement without also engaging in physical activity, similar to how the individuals practiced their swimming strokes before an actual swim. Imagery is often used to rehearse the competition and competition strategy; to prepare the athlete for the challenge ahead. It can also be used to "take the athlete away" from the stressful environment. Mental Imagery is using all the senses to create or re-create an experience in the mind. An athlete can think of it as "focused daydreaming" "Mental imagery is probably already used imagery both in the athletic training and in the day-to-day life" [8]. The psychological preparation of athletes is an important aspect of the total preparation of athletes for developed their performance and Confident levels [5]. Recent research suggests that different contextual elements connected to sport may have a detrimental influence on top athletes' physical and mental health [2].

MATERIAL AND METHODS

From the Pondicherry Aquatics Sports Complex in the Puducherry region, thirty participants were chosen. The swimmers' ages ranged from 18 to 21 years, and they competed at the State, University, and National levels of swimming events. Thirty swimmers were split into three groups: a control group, a swimming skills group that used specified psychological tactics, and a swimming skills group that didn't. Except for the control group, each group divided the ten swimmers equally before beginning a six-week training regimen. Swimmers participated in preliminary and final 50-meter freestyle swimming tests before and after the training period.The details of the



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training program were described in Tables – 1 to 3 followed by mentioned two training group swimmers. After the end of the training program was concluding the 50 meters final test was taken for the raw score applied in the statistical analysis. Fig. 1. shows the training session of the research participant

STATISTICAL ANALYSIS

ANCOVA was used to compare the effects of specific psychological tactics on the swimming performance of swimmers from the Puducherry region. After data collection, SPSS version 21 was used to statistically assess the final score. The findings of the analysis of covariance on values obtained pre-test and post-test after the training program on swimming performance for the control group and groups with and without chosen psychological tactics are shown in Table - 5.

RESULTS

The outcome shown in the table-V indicates that the pre-test mean scores for swimming performance were 44.96 for control group 3, 37.75 for experimental group 2, and 40.11 for experimental group 1, which included psychological techniques in their swimming performance. Swimming proficiency post-test means for the control group, the group utilizing the selected psychological approaches, 43.58, 37.09, and 33.92, respectively. The table 4 shows the group distinctions before to the experiment. One of the key components of the study's numerical value analysis between the pre-and post-means values of the swimming abilities of regional Puducherry swimmers is shown in table 5 and table 6 indicates that the pre-test and post-test means, Standard Deviation for experimental and control groups. According to Table 7, the adjusted post-test means values for experimental groups 1 and 2 are 36.58 and 37.78, respectively, and 40.24 for the control group. According to Scheffe's posthoc analysis, there were significant changes between the combined mean differences among the three groups. The comparison between the groups using psychological strategies and those not using them was 1.20, and the comparison between the swimming skills groups using psychological strategies and those not using them was 3.66*. Since the results demonstrated a considerable improvement between the experimental groups and the control group in the table values.

DISCUSSION

Swimming is the best exercise for sports and non-sports people for developing their physical structure because when practice exercise on the ground surface the ground reaction force is extra but in water, we have less ground reaction and extra friction /buoyance force helps to lift the body and minimize the knee pain [6]. Increasing the training intensity may cause shoulder pain and lead to severe injuries sometimes called "swimmer's shoulder" the study was conducted during six weeks of a swimming training program[3]. The study result indicates that the swimming performance skills with and without selected psychological strategies were improved by the significant number of changes in their pre-and post-performance as well as when compared to those without selected psychological strategies group them with selected psychological strategies group has nominal changes in their performance. The study showed that the effect of 6 weeks of scientific training was developed anatomical changes and improved performance [9]. Regular swimming practice will strengthen swimming ability, decrease body fat, and improve subject fitness and posture[10].In previously inactive older persons, swimming exercise produces hypotensive effects and improvements in vascular function[11].Anaerobic performance in swimmers was specifically linked to psychological traits[12].Sand and water exercises were used in this research to enhance agility for basketball athletes [13].Improving an athlete's performance in high-intensity sports via psychological skills training [14]. Competitive swimming training is intended to enhance muscular endurance rather than muscle strength or other aspects of body composition[15].Self-regulated learning (SRL), which promotes further effective and efficient learning, comprises motivational beliefs and self-directed metacognitive sub processes [3].Swimming training was administered to stressed rats, and the results show that corticosterone levels were decreased[16].The use of motivational self-talk has helped amateur triathletes' swimmers longer distances performance[17].



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In some cases, such as swimming, this might happen in the pool, as the automatized strokes are done. After a standard warm-up, the swimmer has 50 meters of freestyle at a controllable speed that allows for maximal technique without fatigue. The next 50 meters is swum for four strokes with eyes open and a broad focus of attention, four strokes with eyes closed, practicing imagery of the senses used for the four previous strokes. Once out of the pool, the swimmer repeats the imagery of the full 50 meters free-style swim several times a day. Attention focus is a vital

role and gives extra contributes to increasing a higher level of performance as well as a resource in practical and theories of attention have been exposed to achieve that task performance is constrained by available mental resources [18,19].The majority of participants' post-intervention positive psychological profiles significantly improved for the psychological skills training programme [20].Author Morris[7] in his study represents that the psychological strategies hope to improve the swimming performance and improve the athlete's ability to use imagery and attention focus, it is often effective to have them experience the real sensory input and then immediately re-create it which considerable improvement happened in our study.

CONCLUSION

The current research study tested the hypothesis and came to the conclusion that swimming skills with and without specific psychological tactics associated with the control group significantly improved swimming performance among swimmers in the Puducherry region.

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CONFLICT OF INTEREST

The authors have no conflicts of interest regarding this investigation.

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Table. 1: Swimming Skills with and without Selected Psychological Strategies for week-1 and 3.

| SWIMMING SKILLS WITH SELECTED PSYCHOLOGICAL STRATEGIES (SSWSPS) | | | | SWIMMING SKILLS WITHOUT SELECTED PSYCHOLOGICAL STRATEGIES(SSWOSPS) | | | | |
|---|--|--|---------------------------------|--|-------------|---------------------------------|--|------------|
| Days | Training | | | Days | Training | | | |
| Monday | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | | |
| | | Rest- 1Min | | | Rest- 1Min | | | |
| | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10Min | | 2 x 50 Drill kick with a kickboard, 2 x 50 Drill pull with pull buoy & 100mts freestyle. | 20Min | | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10Min |
| | | Rest- 2Min | | | | | | Rest- 2Min |
| | Drill Week –1 | 20Min | | Drill Week –1 | 20Min | | | |
| | 2 x 50 kick with a kickboard, 2 x 50 pull with pull buoy & 100mts freestyle. | | | 2 x 50 Drill kick with a kickboard, 2 x 50 Drill pull with pull buoy & 100mts freestyle. | | | | |
| | Drill Week –3 | | | Drill Week –3 | | | | |
| | 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull, and 200-meter freestyle. | Rest- 2Min | | 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull, and 200-meter freestyle. | Rest2Min | | | |
| Attention Focus (In the brain, picturing a swimming movement from the starting position to finishing point) | 15 Min | Lead up Activity | 15 Min | | | | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | | | | |
| Wednesday | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | | |
| | | Rest - 1Min | | | Rest - 1Min | | | |
| | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10 Min | | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10 Min | | | |
| | | Rest - | | | Rest - 2 | | | |
| Tuesday | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | | |
| | | Rest- 1Min | | | Rest- 1Min | | | |
| | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10Min | | 2 x 50 Drill kick with a kickboard, 2 x 50 Drill pull with pull buoy & 100mts freestyle. | 20Min | | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10Min |
| | | Rest- 2Min | | | | | | Rest- 2Min |
| Drill Week –1 | 20Min | Drill Week –1 | 20Min | | | | | |
| 2 x 50 Drill kick with a kickboard, 2 x 50 Drill pull with pull buoy & 100mts freestyle. | | 2 x 50 Drill kick with a kickboard, 2 x 50 Drill pull with pull buoy & 100mts freestyle. | | | | | | |
| Drill Week –3 | | Drill Week –3 | | | | | | |
| 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull, and 200-meter freestyle. | Rest2Min | 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull, and 200-meter freestyle. | Rest2Min | | | | | |
| Lead up Activity | 15 Min | Lead up Activity | 15 Min | | | | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | | | | |
| Thursday | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | Explanation & Warm Up | 5Min | Duration of Training 60 Minutes | | |
| | | Rest - 1Min | | | Rest - 1Min | | | |
| | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10 Min | | Basic exercises (Sliding, Pencil float, and Pencil drop) | 10 Min | | | |
| | | Rest - | | | Rest - 2 | | | |





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| | | 2Min | | | Min |
| | Drill Week –1 | 20 Min | | Drill Week –1 | 20 Min |
| | 50 kicks with a kickboard, 50 pull with pull buoy & 200 meters freestyle. | | | 50 kicks with a kickboard, 50 Drill pull with pull buoy & 200 meters freestyle. | |
| | Drill Week -3 | Rest - 2Min | | Drill Week -3 | Rest- 2 Min |
| | 50 kicks with a kickboard,50 pull with pull buoy & 200mts freestyle | | | 50 kicks with a kickboard,50 Drill pull with pull buoy & 200mts freestyle | |
| | Mental Imagery (1 st Properly standing for starting position, 2 nd Hearing for blowing the whistle, 3 rd Dive the swimming pool, 4 th Control the breath in and out, 5 th Focus on arm action, leg action and laneway, 6 th Focus on target finishing point). The subject's mental visualizes the above activity in step-by-step manner. | 15 Min | | Lead up Activity | 15 Min |
| Warm Down | 5 Min | Warm Down | 5 Min | | |
| Friday | | 5Min | Saturday | | 5Min |
| | Explanation& Warm Up | Rest - 1Min | | Explanation& Warm Up | Rest - 1Min |
| | Basic exercises (Sliding, Pencil float, and Pencil Drop) | | | Basic exercises (Sliding, Pencil float, and Pencil Drop) | |
| | | 10 Min | | | 10 Min |
| | Drill Week –1 | 20 Min | | Drill Week –1 | 20 Min |
| | 50 kicks, 50 pull, 2x50meters Streamline freestyle kick, and 100mts freestyle. | | | 50 kicks, 50 pull, 2x50meters Streamline freestyle kick, and 100mts freestyle. | |
| | Drill Week -3 | Rest -2 Min | | Drill Week -3 | Rest- 2 Min |
| | 50 kicks, 50 pull, 2x50 meter Lateral freestyle kick & 100-meter freestyle. | | | 50 kicks, 50 pull, 2x50 meter Lateral freestyle kick & 100-meter freestyle. | |
| Attention Focus (In the brain, picturing a swimming movement from the starting position to finishing point) | 15 Min | Lead up Activity | 15 Min | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | |





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Table. 2: Swimming Skills with and without Selected Psychological Strategies for week-2 and 4.

| SWIMMING SKILLS WITH SELECTED PSYCHOLOGICAL STRATEGIES | | | Duration of Training 60 Minutes | SWIMMING SKILLS WITHOUT SELECTED PSYCHOLOGICAL STRATEGIES | | | Duration of Training 60 Minutes | | | |
|--|---|-------------|---------------------------------|---|-------------|--|--|-----------|--|--------|
| Days | Training | | | Days | Training | | | | | |
| Monday | Explanation & Warm Up | 5Min | Tuesday | Explanation & Warm Up | 5Min | Wednesday | Explanation & Warm Up | 5Min | | |
| | | Rest-1Min | | | Rest-1Min | | | Rest-1Min | | |
| | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10Min | | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10Min | | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | Drill – Week 2 | 20 Min |
| | | Rest-2Min | | | Rest-2Min | | | | | |
| | Drill – Week 2 | 20Min | | Drill – 2 | 20Min | | 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull & 150mts freestyle. | | | |
| | 50mts kick, 2x50 kick, 50-meter pull, 2x50 Pull & 150mts freestyle. | | | | | | | | | |
| | Drill – Week 4 | | | | | | | | | |
| | 200mrt freestyle breath in every 3 strokes, 100mtr 6 kick 6 pull, 100 meters one arm drill 50 change. | Rest - 2Min | | 200mrt freestyle breath in every 3 strokes, 100mtr 6 kick 6 pull, 100 meters one arm drill 50 change. | Rest - 2Min | | Lead up Activity | 15 Min | Warm Down | 5 Min |
| | Mental Imagery (Same as Table -1 Wednesday Training) | 15 Min | | Lead up Activity | 15 Min | | Warm Down | 5 Min | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | | | | | | |
| Wednesday | Explanation & Warm Up | 5Min | Thursday | Explanation & Warm Up | 5Min | Friday | Explanation & Warm Up | 5Min | | |
| | | Rest-1Min | | | Rest-1Min | | | Rest-1Min | | |
| | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min |
| | | Rest -2 Min | | | Rest -2 Min | | | | | |
| | Drill – Week 2 | 20 Min | | Drill – 2 | 20 Min | | 200-meter freestyle | | | |
| | 200-meter freestyle | | | | | | | | | |
| | Drill – Week 4 | | | | | | | | | |
| | 50-meter kick, 50-meter pull, and 12 laps x 50 meters freestyle | Rest- 2 Min | | 50-meter kick, 50-meter pull, and 12 laps x 50 meters freestyle | Rest -2 Min | | Lead up Activity | 15 Min | Warm Down | 5 Min |
| | Attention Focus (In the brain, picturing a swimming movement from the starting position to finishing point) | 15 Min | | Lead up Activity | 15 Min | | Warm Down | 5 Min | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | | | | | | |
| Friday | Explanation & Warm Up | 5Min | Saturday | Explanation & Warm Up | 5Min | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | | | |
| | | Rest-1Min | | | Rest-1Min | | | | | |
| | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | | Basic exercises (Over and under breathing, Flutter kick deck drill, and Vertical flutter kick) | 10 Min | | | | | |





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| | under breathing, Flutter kick deck drill, and Vertical flutter kick) | Rest - 2Min | | | under breathing, Flutter kick deck drill, and Vertical flutter kick) | Rest- 2Min |
| | Drill – Week 2 | 20 Min | | | Drill – 2 | 20 Min |
| | 100-meter kick, 100-pull, 100 - One-arm extended freestyle kick drill. | | | | 100-meter kick, 100-pull, 100 - One-arm extended freestyle kick drill. | |
| | Drill – Week 4 | Rest -2 Min | | | Drill - 4 | Rest - 2 Min |
| | 4x50 kick, 4x50 pull, 4x50 high elbow recovery Drills, 4x50 free style, and 100mrt freestyle. | | | | 4x50 kick, 4x50 pull, 4x50 high elbow recovery Drills, 4x50 free style, and 100mrt freestyle. | |
| | Mental Imagery (Same as Table -1 Wednesday Training) | 15 Min | | | Lead up Activity | 15 Min |
| | Warm Down | 5 Min | | | Warm Down | 5 Min |

Table. 3: Swimming Skills with and without Selected Psychological Strategies for week-5 and 6.

| SWIMMING SKILLS WITH SELECTED PSYCHOLOGICAL STRATEGIES | | | SWIMMING SKILLS WITHOUT SELECTED PSYCHOLOGICAL STRATEGIES | | |
|---|--|---|---|--|------------|
| Days | Training | | Days | Training | |
| Monday | Explanation & Warm Up | 5Min | Tuesday | Explanation & Warm Up | 5Min |
| | | Rest-1Min | | | Rest-1Min |
| | Basic exercises (Streamline front flutter kick, Butterfly kick deck drill, Backstroke recovery deck drill) | 10Min | | Basic exercises (Streamline front flutter kick, Butterfly kick deck drill, Backstroke recovery deck drill) | 10Min |
| | | Rest-2Min | | | Rest-2Min |
| | Drill – Week 5 | 20Min | | Drill – 5 | 20Min |
| | 50 kicks, 50 pull, 2 x 50meter Lateral freestyle kick, Freestyle Start drill 50, 100 freestyles. | | | 50 kicks, 50 pull, 2 x 50meter Lateral freestyle kick, Freestyle Start drill 50, 100 freestyles. | |
| | Drill – Week 6 | Rest-2Min | | Drill – 6 | Rest-2Min |
| | 2x (50 kick, 50 pull, 50 swims), 2 x (50 Breath, kick, slide), Freestyle Finish drill 50, 200 freestyle. | | | 2x (50 kick, 50 pull, 50 swims), 2 x (50 Breath, kick, slide), Freestyle Finish drill 50, 200 freestyle. | |
| Attention Focus (In the brain, picturing a swimming movement from the starting position to finishing point) | 15 Min | Lead up Activity | 15 Min | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | |
| Wednesday | Explanation & Warm Up | 5Min | Thursday | Explanation & Warm Up | 5Min |
| | | Rest -1Min | | | Rest -1Min |
| Basic exercises (Streamline front flutter kick, Butterfly kick deck | 10 Min | Basic exercises (Streamline front flutter kick, Butterfly | 10 Min | | |





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| | drill | Rest - 2 Min | | | kick deck drill, | Rest -2 Min |
| | Drill – Week 5 | 20 Min | | | Drill – 5 | 20 Min |
| | 3×50 kick, 3×50 pull, 3×50 Lateral freestyle breathe and kick, | | | | 3×50 kick, 3×50 pull, 3×50 Lateral freestyle breathe, and kick. | |
| | Drill – Week 6 | Rest - 2 Min | | | Drill – 6 | Rest -2 Min |
| | 2×50 kick (50 fast, 50 slow), 2×50 pull | | | | 2×50 kick (50 fast, 50 slow), 2×50 pull (50 fast 50 slow), | |
| | Mental Imagery (Same as Table -1 Wednesday Training) | 15 Min | | | Lead up Activity | 15 Min |
| | Warm Down | 5 Min | | | Warm Down | 5 Min |
| Friday | Explanation & Warm Up | 5Min | Saturday | Explanation & Warm Up | 5Min | |
| | | Rest - 1Min | | | Rest - 1Min | |
| | Basic exercises (Streamline front flutter kick, Butterfly kick deck drill.) | 10 Min | | Basic exercises (Streamline front flutter kick, Butterfly kick deck drill.) | 10 Min | |
| | | Rest - 2Min | | | Rest- 2Min | |
| | Drill – Week 5 | 20 Min | | Drill – 5 | 20 Min | |
| | 2×50-meter kick 6 kicks 1 breath, 2×50 pull the breath in every 3 strokes, and 200-meter freestyle. | | | 2×50-meter kick 6 kicks 1 breath, 2×50 pull the breath in every 3 strokes, and 200-meter freestyle. | | |
| | Drill – Week 6 | Rest - 2 Min | | Drill – 6 | Rest -2 Min | |
| | 4×50 kick, 4×50 pull, 4×50 over correction drill, 200-meter freestyle. | | | 4×50 kick, 4×50 pull, 4×50 over correction drill, 200-meter freestyle. | | |
| Attention Focus (In the brain, picturing a swimming movement from the starting position to finishing point) | 15 Min | Lead up Activity | 15 Min | | | |
| Warm Down | 5 Min | Warm Down | 5 Min | | | |

Table. 4: Group differences before the experiment

| Groups | Count | Sum | Average | Variance | |
|------------------------------------|---------|--------|---------|----------|-----------|
| Experimental group-1 (Time in Sec) | 10 | 377.54 | 37.754 | 8.11 | |
| Experimental group-2 (Time in Sec) | 10 | 401.1 | 40.11 | 29.47 | |
| Control group (Time in Sec) | 10 | 449.6 | 44.96 | 6.11 | |
| Source of Variation | SS | df | MS | F | P-value |
| Between Groups | 269.999 | 2 | 135.00 | 9.27 | 0.0008609 |
| Within Groups | 393.178 | 27 | 14.56 | | |





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Table. 5: Data for swimming performance of pre and post-test for Experimental and control groups (scores in seconds)

| Subjects | Pre-test value for Experimental group-1 (Time in Sec) | Post-test value for Experimental group-1 (Time in Sec) | Pre-test value for Experimental group-2 (Time in Sec) | Post-test value for Experimental group-2 (Time in Sec) | Pre-test value for Control group-2 (Time in Sec) | Post-test value for Control group-2 (Time in Sec) |
|----------|---|--|---|--|--|---|
| 1 | 31.01 | 28.02 | 30.51 | 29.5 | 38.56 | 37.51 |
| 2 | 34.55 | 30.12 | 35.13 | 33.21 | 44.11 | 42.58 |
| 3 | 38.11 | 34.17 | 35.23 | 34.26 | 44.53 | 42.59 |
| 4 | 38.16 | 34.21 | 36.21 | 35.29 | 45.04 | 43.18 |
| 5 | 38.37 | 34.27 | 42.15 | 37.39 | 45.09 | 43.56 |
| 6 | 38.5 | 35.24 | 42.22 | 37.44 | 45.38 | 44.58 |
| 7 | 39.32 | 35.33 | 43.21 | 38.2 | 46.28 | 45.26 |
| 8 | 39.53 | 35.42 | 44.26 | 39.31 | 46.51 | 45.51 |
| 9 | 39.57 | 35.44 | 45.02 | 41.1 | 47.02 | 45.44 |
| 10 | 40.42 | 37.02 | 47.16 | 45.2 | 47.08 | 45.59 |
| Total | 377.54 | 339.24 | 401.1 | 370.9 | 449.6 | 435.8 |
| Mean | 37.75 | 33.92 | 40.11 | 37.09 | 44.96 | 43.58 |
| S.D | 2.85 | 2.74 | 5.43 | 4.37 | 2.47 | 2.45 |

*Significant at 0.05 level Table F-ratio at 0.05 level of confidence for 2 and 27 (df) =3.35.

Table. 6: Data on swimming performance of Experimental and control groups (scores in seconds)

| Test | EXP GR-1 | EXP GR-2 | CG | Source of variance | Sum of squares | Degree of Freedom | Mean squares | F- ratio | P-value |
|-------------------------|----------|----------|-------|--------------------|----------------|-------------------|--------------|----------|---------|
| Pre-Test mean | 37.75 | 40.11 | 44.96 | Between | 269.99 | 2 | 134.99 | 9.27* | 0.001* |
| | | | | Within | 393.18 | 27 | 14.56 | | |
| Post-Test mean | 33.92 | 37.09 | 43.58 | Between | 484.61 | 2 | 242.30 | 22.28* | 0.000* |
| | | | | Within | 293.59 | 27 | 10.87 | | |
| Adjusted Post-Test mean | 36.58 | 37.78 | 40.24 | Between | 41.33 | 2 | 20.67 | 24.78* | 0.000* |
| | | | | Within | 21.69 | 26 | 0.83 | | |
| Mean Diff | 3.83 | 3.02 | 1.38 | | | | | | |

Significant at 0.05 level Table F-ratio at 0.05 level of confidence for 2 and 27 (df)= 3.35





Table. 7: Scheffe's post-hoc test for experimental groups and control groups

| Model | Swimming Skills with Selected Psychological Strategies Group | Swimming skills without selected psychological strategies Group | Control Group | Mean Difference |
|-----------------------|--|---|---------------|-----------------|
| 50-Mts Swimming (Sec) | 36.58 | 37.78 | - | 1.20 |
| | 36.58 | - | 40.24 | 3.66* |
| | - | 37.78 | 40.24 | 2.46* |



Fig. 1. Swimming Training session.





A High Engagement of Teacher's Personalities and their Aptitudes other than Engineers, and Lawyers in Tamil Nadu

A. Antony Lawrence¹ and R. Jeyanthi^{2*}

¹Research Scholar, School of Education, Vels Institute of Science, Technology, and Advanced Studies (VISTAS), Pallavaram, Chennai-600 117, Tamil Nadu, India.

²Associate Professor, School of Education, Vels Institute of Science, Technology and Advanced Studies (VISTAS), Pallavaram, Chennai-600 117, Tamil Nadu, India

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*Address for Correspondence

R. Jeyanthi

Associate Professor, School of Education,
Vels Institute of Science, Technology and Advanced Studies (VISTAS),
Pallavaram, Chennai-600 117, Tamil Nadu, India



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ABSTRACT

The term "aptness," "schmancy," "suitability," "readiness," "tendency," "natural or acquired disposition or capacity for a particular activity," or "innate component of a competency" are all different ways to define "aptitude." It is the combination of acquired abilities and innate abilities. A person's unique and relatively stable traits that define behavior patterns over time and in various contexts are referred to as their personality. The person's personality is the intricate arrangement of their thoughts, feelings, and actions that give their life direction and pattern (coherence). The purpose of this study is to investigate the differences between the three sets of respondents, namely: A sample size of 131 including lawyers, engineers, and teachers. The personality and aptitude of teachers are high other than that of lawyers and engineers. This shows the positive response of teachers.

Keywords: Aptitude, Personality, Teachers, Engineers, Lawyers.

INTRODUCTION

APTITUDE

The term "aptness," "schmancy," "suitability," "readiness," "tendency," "natural or acquired disposition or capacity for a particular activity," or "innate component of a competency" are all different ways to define "aptness." It is the combination of acquired abilities and innate abilities. The first aspect of aptitude is out of one's control—we are simply born that way—and the second is a dependent attitude! None of us can influence our own aptitude (with the exception of when we acquire learned skills) shown in Figure 1.





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Capability is a person's maximum level of effectiveness with the best training.

A loose synonym for ability or even aptitude, capacity frequently implies innateness.

A high level of aptitude or ability is referred to as talent.

The popular terms "gift" and "endowment" refer to a high ability that is mostly innate.

Competence is fitness, whether for a specific task or fitness as a whole.

DIFFERENCE BETWEEN APTITUDE AND ABILITIES

This is unquestionably more significant than a desire to succeed in one's career. The IT companies' aptitude tests before hiring freshmen from college campuses have made the term "aptitude" well-known. According to the dictionary, "ability to perform a particular type of work at a particular level is referred to as aptitude." According to research, successful individuals have chosen careers that maximize their inherent potential. The following examples will demonstrate the various types of intelligence and the successful individuals associated with them.

LINGUISTIC

Arundhati Roy, Salman Rushdie, RK Narayanan, and Sujatha, all successful novelists, would be highly linguistic.

LOGICAL

People with a high level of intelligence can comprehend concepts, analyze and resolve issues, think in sequence, and solve mathematical problems. Careers that involve scientific work and require conceptual thinking, such as technical jobs, scientific research, and mathematicians, require this ability more than any other. Ramanujam Homi Bhabha, Abdul Kalam, C.V. Raman, and others are successful individuals.

MECHANICAL

GD Naidu, our very own, exemplifies high mechanical aptitude beautifully.

SPATIAL:

Ravi Varma, MF Hussain, actors Kalal Hassan, Mani Ratnam, Sundar, and others

BODILY-KINESTHETIC

Dhoni, Sachin Tendulkar, Prabhu Deva, Shyamak Davar, and others

MUSICAL

Lata Mangeshkar, SP Balasubramanian, and P. Suseela.

PERSONALITY

We can organize the diversity that we have observed in human behavior by using labels and putting personality traits into categories. Based on similarities, personality types are used to communicate certain expected behaviors. Since ancient times, such endeavors have been made. The original treatise known as the Charak Samhita of Ayurveda, or the Indian science of medicine, divides people into three groups known as doshas: Vata, pitta, and Kapha. Vata is created when Akasha (ether) and Vayu (air) interact, pitta is created when Vayu and Agni (fire) interact, and Kapha is created when Ala (water) and Prithvi (earth) work together. They are biophysical components that have some inherent forces that charge them. The Vata is dry, cold, light, subtle, clear, and rough in terms of its properties. The pitta is hot, abrasive, fluid, acidic, mobile, and slightly unctuous (oily). The Kapha is thick, cold, soft, unctuous, sweet, stable, and Viscous. It is also soft. The person's Prakriti (nature) is referred to as a type of temperament in each of these examples.

The term "personality" is used in psychology to describe a person's distinctive and relatively stable characteristics that are reflected in their behavior patterns over time and in a variety of contexts. The person's personality is the intricate arrangement of their thoughts, feelings, and actions that give their life direction and pattern (coherence). Personality is made up of both structures and processes, reflecting both nature (genes) and nurture (experience), just



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like the body. Personality also includes the effects of the past, such as memories and constructions of the present and future.

Temperament: the characteristic way of reacting based on biology.

Trait: Permanent, particular, and consistent behavior.

Type: the distinct class that is given to people who share a pattern of characteristics.

Disposition: a person's tendency to respond in a particular way to a given situation.

Character: total pattern of behavior that occurs regularly.

Habit: the adopted way of acting.

Values: goals that are thought to be important.

DEFENSE MECHANISMS:

Everybody probably experiences the need to defend themselves from unpleasant feelings from time to time. He might be ashamed or feel guilty about something he does, has done, or doesn't do. He might feel defeated (depressed) by the accomplishments of another person. He might be snubbed (hurt) by someone he values's positive opinion. In such situations, it is normal to look for justifications and explanations that lessen the hurt. Everyone is motivated by a strong need to feel good about themselves. Nobody likes to think that he is to blame, that he is better than other people, or that they don't like him.

Self-defenses of this kind may at times be justified. There might be valid justifications or justifiable explanations. However, there are times when people fabricate reassuring justifications to feel better about themselves, ignoring or distorting reality. Some people have inner flaws that cause them to be especially quick to feel threatened by something. They are unable to acknowledge the truth about themselves. As a result, they might come up with ways to counteract these facts. This method of self-defense, known as defense mechanisms, becomes so ingrained that whenever the time is right, they automatically spring into action.

Although defense mechanisms are frequently viewed as negative reactions, some of them can be beneficial. Using humor, for instance, to get through a stressful or anxious situation can be an adaptive defense mechanism.

EGO DEFENSE MECHANISMS:

The psychoanalytic theory of personality developed by Sigmund Freud states that personality is made up of three parts. The id, ego, and superego are the three personality components that collaborate to produce complex human behaviors.

OBJECTIVES:

To study the Correlation between aptitude and Personality among working Executives (Engineers, Teachers, and Lawyers)

The distribution of the 131 respondents is shown in Table No. 1, with teachers making up the majority of the total number. Engineers make up 34.4 percent of the 45 respondents' total no. of 49, or 37.4%, and lawyers make up the total number. of 37, or 28.2 percent, of the total number of respondents (131). For engineers, the data comes from private schools for teachers, government agencies, and practitioner lawyers. The demographic characteristics of respondents, such as category, age, gender, qualification, category, years of service or work, and family type, are included in the socioeconomic profile that is presented in Table 2.

SAMPLE CHARACTERISTICS:

From Table 2, it can be deduced that the entire study is based on a proportionately selected sample. There are three categories of respondents: engineers (C2), lawyers (C3), and teachers (C1). 131 people responded in total. Teachers make up 45.4% of the sample, engineers make up 49.4%, and lawyers make up 37.2%. The sample's age distribution indicates that Age group A3 accounts for the majority of respondents or 35.11 percent. Age groups A1 and A2 make up 25.10% and 20.61 percent, respectively. 19.08% of people fall into age group A4. As a result, it is deduced that



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35.11 percent of respondents fall into the age range of 41-50. The respondents were categorized according to the length of time they had worked in their field. A maximum of 58.01 percent of the 131-person sample falls into the age groups E1 (those between the ages of 5 and 15), E2 (those between the ages of 16 and 25), and E3 (those between the ages of 26 and 35). According to table 4.3, 52.67 percent of respondents belong to group G1 (male) and 47.32 percent to group G2 (female). This is inferred from the gender of the respondents. The respondents were divided into groups according to their qualifications. 58.77 percent of respondents are graduates and belong to group Q2, while 28.2 percent are postgraduates and belong to group Q3. Only 12.97% of respondents fall into the group Q1 (diploma) category. Respondents were also categorized according to the type of family they were a part of to examine their way of life. Additionally, 62.59 percent of respondents live in a nuclear family, while 37.4 percent live in a joint family, as shown in Table 2.

RESEARCH METHODOLOGY**PROCEDURE OF ANALYSIS OF DATA**

All data are analyzed using statistical measures like mean, median, standard deviation, and the correlation between two personality and aptitude scales.

TOOLS

The following are the tools chosen in this study to accomplish the goals:

DAVID'S BATTERY OF DIFFERENTIAL ABILITIES (DBDA)

Sanjay Vohra had revised David's arsenal of different abilities. The growing realization that, even though the majority of the primary abilities traits had been isolated and studied, the available standardized test batteries of intelligence and abilities did not reflect currently accepted views of the number and nature of the psychological constructs involved was largely what prompted the development of David's battery of differential abilities (DBDA). The overall goal of the development of the DBDA was to offer researchers a cost-effective method for evaluating a wide range of significant ability constructs. It measures seven aspects of a person's mental abilities, such as David's Battery of Differential Abilities (DBDA)-a revised version is being developed to have an accurate measure of an individual's various mental abilities. CA stands for closure ability, CL for clerical ability, MA for mechanical ability, NA for numerical ability, RA for reasoning ability, SA for spatial ability, and VA for verbal ability. In light of the aforementioned idea, the DBDA-revised version is being developed.

THE EYSENCK PERSONALITY QUESTIONNAIRE

Eysenck's Personality is the result of extensive development work over many years. It was created to provide a rough measurement of four crucial personality dimensions: The Lie Scale, Extraversion, Neuroticism, and Psychoticism. The structure of human personality reviews these three traits and provides a comprehensive description of the actual derivation of the scale here presented. Each of these four traits is measured utilizing 100 questions that have been carefully selected after extensive item analysis.

ABOUT THE LIE SCALE

It also seems obvious that some people will dissimulate or camouflage under certain conditions, such as during a selection process, and that some people will dissimulate even under normal experimental test conditions. This makes it crucial to attempt the construction of scales for the dissimulation measurement. The construction of the Lie Scales has unquestionably been the most significant approach in this regard, though other approaches have been considered.



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RESULTS

The mean values of the aptitude dimensions in each of the three categories, C1 (teachers), C2 (engineers), and C3 (lawyers), are shown in Table 3. Four of the seven dimensions - A4 (numerical), A5 (rational), A6 (spatial), and A7 (verbal) - have a significant impact on each of the three categories, as follows: In (C1-teachers) - Numerical (A4)3.69, Reasoning (A5)3.62, Spatial (A6)3.42, Verbal (A7)2.24. In (C2- Engineers) - Numerical(A4)3.02, Spatial(A6)2.93, Reasoning(A5)2.60, Verbal(A7)2.38. In (C3- Lawyers)- Spatial(A6)2.81, Numerical(A4)2.38, Verbal(A7)2.19, Reasoning(A5)2.05. On the other aspect, A1(Closure) in Category C1 and C2 and A2(Clerical) in Category C3 are the least influential dimensions of Aptitude. The second variable in this study is personality. The Lie scale, Extraversion, Neuroticism, and Psychoticism are the four personality dimensions. The C1, C2, and C3 Lie scales are the most significant dimensions in all three categories; psychoticism and neuroticism come in second and third, respectively as shown in Table 4. Table 6 reveals little correlation between the aptitude and personality of engineers in this second group. At significance levels of .05, only A6 correlates with moderately positive P2. The C3 (lawyers) respondent group also has a weak relationship between aptitude and personality. At significance levels of 0.05, there is only a moderately negative correlation between P3 and A7 as shown in Table 7.

INTERPRETATION AND DISCUSSION

The majority of respondents in this study were engineers, who made up category 2 (37.4%), who work in the core disciplines of electrical and architecture, followed by teachers, who made up category 1 (34.4%), and lawyers, who made up category 3 (28.2%). When compared to Psychoticism (mean=6.41), Extraversion (mean=5.64), and Neuroticism (mean=5.02), descriptive statistics indicate that the lie scale of personality is the most effective factor in the entire respondent (mean=8.06). When compared to other factors, the Lie scale is the most crucial component of any personality psychological questionnaire. According to Eysenck (1976), the lie scale that is included in the Eysenck Personality Questionnaire makes it possible to diagnose lying when the respondent endorses a set of rarely performed acts as being habitually done and denies frequently performing non-desirable acts.

The Numerical (Mean=3.07) and Spatial (Mean=3.07) dimensions of the independent variable Aptitude have a significant impact on all respondents in this study (131). According to the Lie scale of the independent variable, personality has the greatest impact on all respondents (131). According to the findings of the current study, teachers have the highest aptitude other than engineers and lawyers (Table No. 3). Teachers exhibited the highest Lie scale scores, followed by engineers and lawyers, in personality analyses of all three categories.

The facts are being concealed. This conclusion, which is supported by Dicken (1959), has three plausible reasons for the high L scale score:

- 1) Deliberate "faking" to mislead the user;
- 2) Response in terms of an ideal self-concept rather than a candid self-evaluation; and
- 3) Response in terms of an "honest" self-evaluation that is inaccurate and lacking in insight.

In addition to the three possibilities listed above, a high Lie scale score could also be attributable to genuine conformity to social norms. In this study, the researcher also found that female executives scored higher on the Score of lie scale (70% vs. 65%) than male executives, indicating that female executives are highly conformist toward the socially desirable direction. Hartshorne and May had already made this point (1928). Girls had higher L scores than boys, they discovered; They bravely suggested that this might be because girls were more conformist, which allowed them to answer more questions honestly in a socially acceptable way rather than because they were more dissimulated. It is possible to determine whether the respondent's L score reflects conformity or dissimulation based on the context in which they are. Because the majority of respondents in this study are experienced executives between the ages of 41 and 50, the results tend to be more dispersed.



**Antony Lawrence and Jeyanthi****FINDINGS**

The researcher came to many useful conclusions with the assistance of statistical techniques and analysis following the goals. The researcher was compelled by the study's various findings to draw a specific conclusion and make appropriate recommendations. To draw a systematic conclusion, the study's findings are divided into two categories: general findings and specific findings.

1. Teachers are among the 131 respondents who answered no. Engineers make up 34.4 percent of the 45 respondents' total no. of 49, or 37.4%, and lawyers make up the total number. of 37, or 28.2 percent, of the total number of respondents (131).
2. Numerical Abilities (3.07) and Spatial Abilities (3.07) were found to have the greatest impact on the aptitude battery.
3. The Lie scale has the greatest influence out of the four personality dimensions—extraversion, neuroticism, psychoticism, and the Lie scale—with a score of 8.06.
4. According to the analysis, female executives have a higher score on the lie scale (70 percent) than male executives (65%).
5. A4 (numerical), A5 (rational), A6 (spatial), and A7 (verbal) were found to have a significant impact on each of the three categories.
6. Psychoticism and neuroticism were found to be least important in teachers, engineers, and lawyers' personalities.

CONCLUSION

1. In the category of teachers, there is some evidence of a correlation between aptitude and personality, but engineers and lawyers do not demonstrate a strong correlation.
2. Teachers receive the highest possible score on the Lie scale, while lawyers receive the lowest possible score.

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Table 1: Frequency of Working Executives (Teachers, Engineers, and Lawyers)

| Respondents | Frequency | % |
|------------------|------------|------------|
| Teachers(Cat-1) | 45 | 34.4 |
| Engineers(Cat-2) | 49 | 37.4 |
| Lawyers(Cat-3) | 37 | 28.2 |
| Total | 131 | 100 |

Table 2: Demographic Data (N-131)

| Variables | Category | Frequency | % |
|------------------------|---------------------|-----------|-------|
| Age | 20 to 30 years (A1) | 27 | 20.61 |
| | 31 to 40 years (A2) | 33 | 25.10 |
| | 41 to 50 years (A3) | 46 | 35.11 |
| | 51 to 60 years (A4) | 25 | 19.8 |
| | Total | 131 | 100 |
| Gender | Male (G1) | 69 | 52.67 |
| | Female (G2) | 62 | 47.32 |
| | Total | 131 | 100 |
| Qualification | Diploma (Q1) | 17 | 12.97 |
| | Graduation (Q2) | 77 | 58.77 |
| | Post Graduate (Q3) | 37 | 28.2 |
| | Total | 131 | 100 |
| Category | Teacher (C1) | 45 | 34.4 |
| | Engineer (C2) | 49 | 37.4 |
| | Lawyer (C3) | 37 | 28.2 |
| | Total | 131 | 100 |
| Work Experience | 5 to 15 years (E1) | 76 | 58.01 |
| | 16 to 25 years (E2) | 31 | 23.66 |
| | 26 to 35 years (E3) | 24 | 18.32 |
| | Total | 131 | 100 |
| Family Type | Joint (F1) | 49 | 37.41 |
| | Nuclear (F2) | 82 | 62.59 |
| | Total | 131 | 100 |





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Table 3: Category Wise and Dimension Wise Comparison of Mean and SD of Respondents Mean and SD of Aptitude (Teacher, Engineer, and Lawyers)

| Aptitude Dimension | Category | Teachers (C1) | Engineers (C2) | Lawyers (C3) |
|--------------------|----------|---------------|----------------|--------------|
| A1 Closure | Mean | 1.29 | 1.00 | 1.38 |
| | SD | 0.84 | 0.35 | 0.89 |
| A2 Clerical | Mean | 1.58 | 1.20 | 1.16 |
| | SD | 1.25 | 0.68 | 0.55 |
| A3 Mechanical | Mean | 1.44 | 1.88 | 1.32 |
| | SD | 0.81 | 1.27 | 0.78 |
| A4 Numerical | Mean | 3.69 | 3.02 | 2.38 |
| | SD | 1.83 | 1.96 | 1.497 |
| A5 Reasoning | Mean | 3.62 | 2.60 | 2.05 |
| | SD | 2.43 | 1.87 | 1.68 |
| A6 Spatial | Mean | 3.42 | 2.93 | 2.81 |
| | SD | 2.20 | 2.05 | 1.82 |
| A7 Verbal | Mean | 2.24 | 2.38 | 2.19 |
| | SD | 1.46 | 1.79 | 1.39 |

Source: (Primary Data and analysis SPSS 19.0)

Table 4: Mean and SD of Personality (Teacher, Engineer, and Lawyers)

| Personality Dimension | Category | Teachers (C1) | Engineers (C2) | Lawyers (C3) |
|-----------------------|----------|---------------|----------------|--------------|
| P1 Extraversion | Mean | 6.07 | 5.27 | 5.62 |
| | SD | 1.68 | 1.69 | 1.23 |
| P2 Neuroticism | Mean | 4.80 | 5.08 | 5.22 |
| | SD | 1.69 | 2.23 | 1.83 |
| P3 Psychoticism | Mean | 6.80 | 6.27 | 6.11 |
| | SD | 2.09 | 2.01 | 1.66 |
| P4 Lie Scale | Mean | 8.13 | 8.35 | 7.59 |
| | SD | 1.29 | 1.33 | 1.82 |

Source: (Primary Data and analysis SPSS 19.0)

Table 5: Correlations of Aptitude and Personality (Teachers) N-45

| Dimensions | P1 | P2 | P3 | P4 |
|------------|--------|--------|--------|--------|
| A1 | 0.226 | .473** | .396** | -0.245 |
| A2 | 0.197 | .303* | 0.28 | -0.077 |
| A3 | -0.138 | 0.215 | 0.161 | -0.231 |
| A4 | 0.117 | 0.178 | 0.019 | 0.085 |
| A5 | 0.184 | -0.013 | -0.118 | 0.168 |
| A6 | 0.035 | 0.189 | 0.187 | 0.044 |
| A7 | -0.191 | 0.075 | -.349* | 0.103 |

Source: (Primary Data and analysis SPSS 19.0)

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed).





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Table 7: Correlations of Aptitude and Personality (lawyers), N-37

| Dimensions | P1 | P2 | P3 | P4 |
|------------|--------|--------|--------|--------|
| A1 | -0.018 | -0.086 | 0.159 | -0.177 |
| A2 | -0.233 | 0.184 | -0.05 | 0.095 |
| A3 | -0.071 | -0.05 | 0.079 | -0.159 |
| A4 | -0.011 | 0.213 | 0.139 | -0.187 |
| A5 | 0.117 | 0.276 | 0.137 | -0.038 |
| A6 | -0.132 | -0.071 | -0.121 | -0.25 |
| A7 | 0.011 | 0.016 | -.333* | 0.064 |

Source: (Primary Data and analysis SPSS 19.0)

** . Correlation is significant at the 0.01 level (2-tailed).

* . Correlation is significant at the 0.05 level (2-tailed)

Table 8: Comparison Table showing Categorization of Variables with Categorization of Respondents

| Variables | Category | Teachers (C1) | Engineers (C2) | Lawyers (C3) | Total / (%) |
|--------------------|-------------------|-----------------|---------------------------|--------------------------|-------------|
| Aptitude | Low | 37 | 44 | 37 | 118/90.07 |
| | Average | 8 | 5 | 0 | 13/9.92 |
| | High | 0 | 0 | 0 | 0 |
| Total | | 45 | 49 | 37 | 131 |
| Personality | Low on Lie Scale | 11 | 14 | 17 | 42/32.06 |
| | High on Lie Scale | 34 (75%) (F) | 35 (71%) 31 (M), 4 (F) | 20 (54%) 14 (M), 6(F) | 89/67.93 |
| Total | | 45 | 49 | 37 | 131 |

(Source: Primary Data)

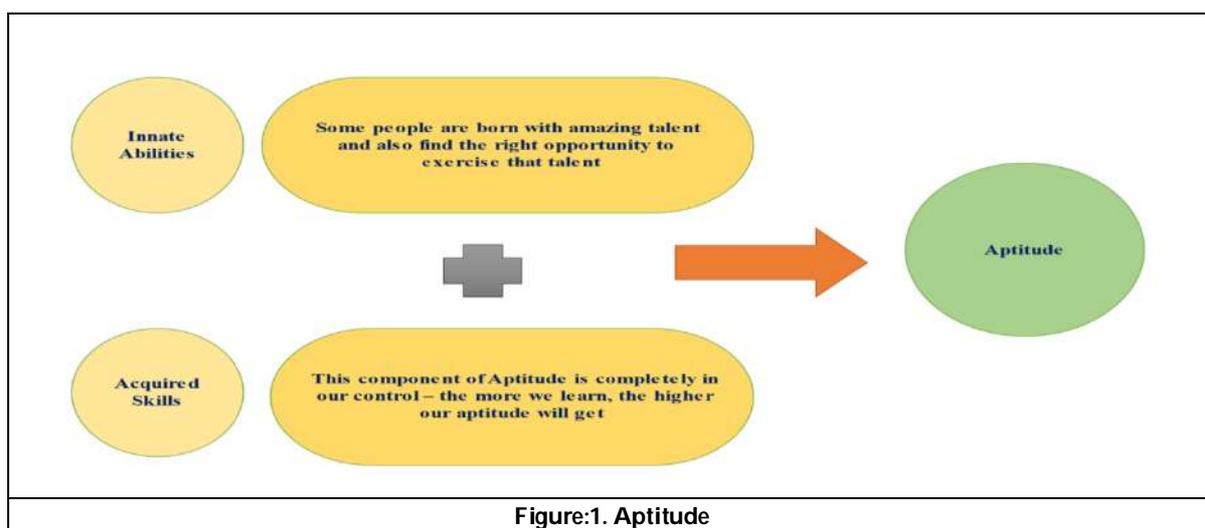


Figure:1. Aptitude





Sustainable Management of Environment through Ancient Literature

Jyoti Pathak¹ and Thakur Dev Pandey^{2*}

¹Assistant Professor, Department of Communication Skill, Marwadi University, Rajkot, Gujarat -360001, India

²Assistant Professor, Department of Economics, Faculty of Management Studies, Marwadi University, Rajkot, Gujarat 360001 India.

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*Address for Correspondence

Thakur Dev Pandey

Assistant Professor,
Department of Economics,
Faculty of Management Studies, Marwadi University,
Rajkot, Gujarat 360001 India
E.Mail: thakurdev009@gamil.com



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ABSTRACT

The natural world is called Prakriti in ancient Indian literature. The Vedas, the Bhagwat Geeta, the Ramayana, and the Upanishads all contain narratives about the care that was taken of the natural environment in ancient times. Writers and philosophers have also voiced their concerns regarding the environment, its relationship with human life, and the severe punishments that ancient rulers meted out to those who polluted the earth's natural beauty. In this paper, an attempt is made to investigate the management of the environment from the perspective of the earliest culture that existed within Indian society. The findings of this research shed light on the significance of the environment in ancient times as well as the actions that people took during that time period. It also looks at our traditional cultural values about the environment, which are becoming less important in modern society, and talks about how to take care of the environment in a way that doesn't harm it.

Keywords: Environment, Ancient culture, India, Environmental tending, Literature.

INTRODUCTION

The natural world, including all of its living things, is referred to as the environment. The value of life is surrounded by the environment, and the environment is valuable for all beings, living and non-living, in order to maintain a running and sustainable process. However, the environment is in danger at the present time. The quality of the environment has been altered by humans, who have also distanced themselves from the natural world. As a result, efforts to conserve the natural world are becoming less successful. This is one of the most obvious reasons why



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environmental problems are becoming more severe: economic growth and a rising population. It is a matter that should be of concern to environmental management because, somewhere on the planet, humans are acting selfishly by exploiting the environment for the sake of their economic goals, which has begun to damage the ecology. This should be a cause for concern. Management of the environment is more concerned with the management of activities carried out by humans. It refers to having knowledge of both the ecosystem and how people interact with it. In a broader sense, environmental management entails locating the environmental problem, determining its solution, making use of natural resources, minimising the effects of extreme natural disasters, and achieving the highest possible level of efficiency in the utilisation of natural resources [1]. Taking care of the environment means not only making sure that the environment doesn't suffer too much and that tragedies don't happen, but also focusing on the problems that have come up because of environmental disasters.

Environment Management in the Ancient Literature

Literature and environmental studies, which are often called "environmental criticism" as an analogy to the more general term "literary criticism," are a diverse, multifaceted, and cross-disciplinary effort to study the environmental aspects of literature and other inventive means in a way that isn't limited to any one method [2]. Environmental studies are based on the idea that the arts of study of them with their understanding of how stories can strengthen, and direct environmental concern also can help us understand environmental problems, such as the many kinds of environmental degradation that affect Earth today. In this way, environment studies agree with other areas of the ecological humanities that environmental events need to be understood and that today's growing number of environmental concerns need to be dealt with both quantitatively and qualitatively. At least as important to fixing these problems as scientific breakthroughs and better ways of putting policies into action are creative ideas, and faith. Creative pictures of environmental damage probably won't be enough to get people to give up lifestyles that depend on drastically changing ecosystems. On the other hand, thinking about ancestral culture may make us more concerned about how it will affect the coming generation and what we can do instead. Since the beginning of time, literature and the arts have been interested in showing how people interact with their environments. The modern environmentalist has led to a wide range of fictional and nonfictional writings about how people's relationships with the natural world are changing. Studies has been interested in these issues for a long time.

The environmental management in India can be traced back to the ancient culture [3]. In the ancient culture environment management is associated with a wide variety of topics. However, the knowledge associated with this culture was frequently looked down upon, and people preferred to learn from modern techniques rather than ancient knowledge. This ancient know-how of caring for the environment needs to be brought into power, and it needs to be included with modern education in order to bring prosperity and happiness to society. Dr. Abdul Kalam, the former President of India, wrote in his book *Ignited Minds: Unleashing the Power Within India* (2010) that ancient India was a society that was very knowledgeable for its time. Its institutions were obliterated, and its fundamental capabilities were taken away as a result of invasions and colonial rule. The quality of life for its inhabitants has been systematically reduced to lower levels over time. By the time the British left, the aspirations of our younger generation had been lowered, and they were content with a mundane means of subsistence. India needs to reacquaint itself with its heritage as a land of learning in order to fully realise its potential. Once this rediscovery has been made, achieving the quality of life, strength, and sovereignty of a developed nation won't require nearly as much of a struggle as it would otherwise (p 66) [4].

During the ancient period, people believed that life, knowledge, peace, and prosperity were all intertwined with the natural world, which is why the ancient philosophy of India is so environmentally conscious. The Harappan culture began in the north-western part of the Indian subcontinent. It is thought to be India's first civilization. From what has been found, it is clear that it was a city-based culture that thrived mostly in cities and towns. The people of the civilization in the Indus valley cared a lot about cleanliness and the environment. Some of the unique things about the Harappan culture are the materials used to build houses, the way houses were laid out, and the most advanced urban water supply. This shows that the Harappans not only knew a lot about hygiene and sanitation but also cared



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a lot about keeping it up and running well. This civilization grew mainly because of the river Indus and the fertile soil it brought up [5].

Further, in the Vedas, Puranas, and Upanishads, as well as the Mahabharata, Bhagavad-Gita, and the Ramayana, the value of the environment was discussed in terms of knowledge, harmony, peace, and fortune. They referred to the environment as “Prakriti” and related to humans who plant trees that produce fruits and flowers for the welfare of humankind are assisted by those trees in the afterlife, in the same way that a son assists his father and saves his father from the suffering of the other world (*The Mahabharat, Anusasan Parv, Section 58, Shloka 30*) [6].

The natural world, including forests, trees, and animals, was revered in ancient cultures. People were instructed to have a deep affection for the natural world and to strive to live in harmony with all of its constituent parts, including space, air, fire, water, and earth. The idea that a tree sustains an ecosystem and that the destruction of a tree through cutting or burning results in the loss of the ecosystem is stated more explicitly. It is astounding to learn that ancient texts discuss the idea of preserving the natural world and living in a way that is compatible with sustainable living in relation to ecology. This reflects the high level of knowledge possessed by the people living during that era. According to the Vedas, plants and trees are the embodiment of cosmic beings and hold significant value for future generations. Other sacred texts of Sanatan Dharma, such as the Vedas, Upanishads, Puranas, and Sutras, as well as the Bhagavad-Gita, the Ramayana, and the Mahabharata, all contain a variety of references to the adoration and veneration of the natural world [7]. The lotus (*Nelumbo nucifera*) and the Banyan tree (*Ficus benghalensis*) were both revered as sacred flowers and were among the many plants and flowers that were utilised in religious rituals. Both the Indian Basil (*Ocimum basilicum*) and the Peepal Tree (*Ficus religiosa*) were revered because they were thought to be divine beings [8]. In a similar fashion, in order to preserve the environment during the ancient period, significant values were placed on the earth, the water, the air, the animals, and the forests [9]. The ancient Hindu text known as the Manusmriti condemns cruel treatment of animals and states that the person who kills a cow is destined to die a sum of times equivalent to the total numbers of hairs that are found on the cow's skin. This statement makes it clear that the value of human life should not be given undue weight (Bithin 1075) [5]. Ancient people held trees in high religious esteem because they wanted to protect the natural environment.

During the phases of Jainism and Buddhism represented by Gautama Buddha and Vardaman Mahavir, respectively, the relationship with the natural world was maintained. They had a close connection to the pacifist philosophy that was applied to environmental issues [10]. These practises were not only prohibited with regard to plants, but also with regard to animals and humans. It's also interesting that both people reached their highest level of enlightenment while sitting under a tree. This suggests that nature and the process of becoming enlightened are linked.

Mahavira attained kevalagyana (supreme knowledge) under a Sal tree (*Shorea robusta*) and Lord Buddha became enlightened one under a Peepal tree (*Ficus religiosa*), known as Bodhi tree on the banks of the river Rajupalika and Niranjana respectively. The Bodhi tree in the Buddhist religion is regarded as the embodiment of enlightenment. Not only Mahavira, all the twenty-four Tirthankaras of Jainism were closely associated with environment in one way or other. (Patra 48) [11]. Both Gautama Buddha and Vardaman Mahavir held the belief that there ought to be freedom for nature and that there ought to be appropriate coherence between humans and their natural surroundings [11]. Later on, Indian treaties and Indian emperors were responsible for the management of the environment, which included encouraging the planting of forests, ensuring their protection, and the construction of water reservoirs [12]. The Indian treatise *Arthashastra*, (3rd BCE) which was written by Kautilya, contains information about the preservation of the natural environment and the preservation of forestry [13]. The *Arthashastra* is where one can find information regarding agricultural land, the distribution of water, the significance of seeds, and rivers [14].

The rule of the Indian Emperor is widely regarded as the earliest instance of effective environmental protection in antiquity. They used to give orders to the public regarding the plantation in the same way that Emperor Ashoka the Great evoked people regarding plantations for the welfare of society [15]. His passion for shrubs is the reason why his name is connected together with the Ashoka tree (*Saraca asoca*). His pursuits included the planting of trees and



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the gathering of wild herbs for use in the treatment of both human and animal ailments. Additionally, he managed a Banyan tree (*Ficus benghalensis*) plantation that was adjacent to a road. In later years, during the fifth century, the Chaliapata forest was planted by the Gupta empire. This forest has survived to the present day and is a popular destination for tourists. This thick forest can be found in close proximity to the Jaldpara National Park and all around the Brahmaputra valley in Dooars, Alipurduar district, West Bengal, India. Whether they were the Indian Emperor or a Rajput Maharaja, it is clear from the historic architecture and literature that they did their best to preserve nature through their one-of-a-kind architectural style [16]. On the other hand, strict punishment was given for degrading the environment. This is something that can be seen. The state of the environment was kept in check by doing things in this manner.

Other conquerors from the West did the same thing; they built monuments, parks, and gardens along the banks of rivers so that people could appreciate the splendour of nature and spend their free time there. In addition, they spent their time conquering new lands. People in that era had a very different mentality when it came to how they related to nature. It was regarded as a source of delight by those who used it. The historical gardens of Jammu and Kashmir, such as Shalimar Bagh in Srinagar, Jammu and Kashmir, and Verinag Garden in Anantnag, Jammu and Kashmir, give us a glimpse of the enchanting splendour of the natural world [17]. These gardens were commissioned by emperors, who then gave the orders to the artists and designers who created the gardens and monuments [18]. Both Baber's *Babernama* (1589) and Jahangir's *Tuzuk-I Jahangiri* (1609) provide us with an analysis of the flora and fauna of India as well as a geographical description of the country [19]. When the Mughal Emperor Jahangir travelled to Kashmir, he made some comments about the beautiful scenery in India: "Agar firdaus bar ru-ye zamin ast Hamin ast-o hamin ast-o hamin ast, "If there is a paradise on the face of the earth," It is this, it is this, it is this" (*Khusro Amir*) [20].

During the ancient period, environmental management was a collaborative effort between the common people and their emperors. Every single text place a strong emphasis on the protection of the natural world and describes how it is against the rules of the religion for humans to exploit nature [21]. The current generation of humans are becoming more materialistic, and for the sake of their desires, they are not concerned about nature and the blessings it bestows upon them. As a consequence of this, a significant amount of this ancient knowledge has been lost because it has not been passed on to the subsequent generations. The only pieces of the memory that remain are fragments. The environment is in danger, so we need to protect and preserve it.

CONCLUSION

The surrounding environment is an important component of everything, living and non-living alike. Additionally, it satisfies every human being, and, by virtue of the myriad ways in which the environment is composed, it brings peace to humans. It is essential for humans to show concern for the surrounding environment, and they should also keep balance in mind. We are able to enhance environmental values in the modern era by maintaining a healthy relationship with the natural world and by following the practises of environmentally responsible indigenous cultures. However, in the modern day and age, various efforts are being made by the government to save the natural world. However, mankind, in its desire, is not concerned about nature and is creating more disasters for the environment and is destroying the harmony of the earth [22]. The modern human race has a responsibility to treat the earth with the same level of care and concern that a mother would, as well as to put an emphasis on traditional cultures. We have seen that the ancient India cared about the balance of the environment. However, there were no problems like global warming or water, air or soil pollution back then. The ancient Indians knew a lot about the world around them. It helps solve some environmental problems, and at that time, the modern ideas of sustainability were put into practise. But sadly, we have forgotten the golden rules they set out for us. It is very important that we keep what little of this knowledge is still around and revive this lost knowledge so that it can be used again in the future to manage environmental resources.





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Inter Relationship of Determinants of Service Climate in District Central Cooperative Bank, Coimbatore among the Farmers in Rural Location of Coimbatore

M.Sampath Nagi^{1*}, R.Vanathi², Prabavathi Venugopal³, Suganya.B⁴ and G. Sathya⁵

¹Assistant Professor, Department of Management, Pondicherry University, Port Blair Campus, Andaman and Nicobar Island, India.

²Assistant Professor (SS) & Head, Department of Business Management, Erode Arts and Science College, (Autonomous), Erode - 638 009, Tamil Nadu, India.

³Associate Professor, School of Management Studies, Jai Shriram Engineering College, Tirupur - 638660, Tamil Nadu, India.

⁴Head and Assistant Professor, Department of Business Administration, St. Joseph's College for Women, Tirupur, Tamil Nadu, India.

⁵Assistant Professor, Department of Commerce, Government Arts College, Gudalore, Tiruvannamalai, Tamil Nadu, India.

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*Address for Correspondence

M.Sampath Nagi

Assistant Professor,
Department of Management,
Pondicherry University, Port Blair Campus,
Andaman and Nicobar Island, India.
E.Mail: sampathnagi@gmail.com



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ABSTRACT

Banking is a key industry in the service sector and it will not be travesty of truth to call it the financial nerve center of the economy of a country. In the past, customers were simple persons and were happy at whatever banks dished out to them. Over a period of time with the competition and technological improvements, customers have become fully aware of their rights. In this study the researcher was interested in evaluating the service climate prevailing at District Central Cooperative Bank, Coimbatore. The research is descriptive research in nature. The questionnaire for the survey about the service climate comprises of two parts. The first part consists of 31 questions distributed under five dimensions of "HR Practices". The second part consists of 30 questions distributed under five dimensions of "Service Encounter". The researcher has used 5-point Likert scales. The pilot study was conducted with the help of the structured questionnaire which was administered among the sample of 35 respondents. The reliability and validity of the questionnaire were also tested. The Cronbach alpha value was found to be above 0.8 and proved that the questionnaire is good fit for the current research. The population of the

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study comprises of employees working in District Central cooperative banks at Tamil Nadu (Annual Report 2020 - National Federation of State Cooperative Banks Ltd). There are 23 head office and 878 branches including head office in Tamil Nadu. There are 4512 employees are working in all the branches including head office at Tamil Nadu. The researcher has adopted the Demorgan's sample size estimator for consolidating the samples. The researcher has identified for a population of five thousand, 357 sample size is need with a confidence level of 95 % and 5 % margin of Errors for collecting the data. The researcher has adopted two stage stratified random sampling, The researcher has distributed 400 questions and was able to received back 361 properly filled in research instrument and the remaining 39 were found to be biased. The collected data were analyzed using Statistical Package for Social Sciences (SPSS), Statistics for Windows, Version 20.0. The Bi Variate Pearson's Correlation was used to identify the relationship among the variables of Service Climate. The results clearly shows that the training and the personal development does not have any relationship with the service climate. This clearly shows that the training and development given to the employees by the DCCB management has to be improved a lot or new hands-on training related to E-commerce has to be definitely provided to the employees, because many employees are facing difficulties in handling the E-commerce based application. The requirements of the customers towards the banking sector have been increasing day by day. To meet this requirement the district central cooperative bank management has to focus on fulfilling the employees needs of training and development also to enable them to have a positive service encounter effectively. The monetary has to be focused with much concentration by the management. Then management has to provide proper training, while handling the customers on communication, customer focus, service delivery and empathy.

Keywords: Service Climate, Determinants, Rural and District Central Cooperative Bank

INTRODUCTION

Banking is a key industry in the service sector and it will not be travesty of truth to call it the financial nerve center of the economy of a country. In the past, customers were simple persons and were happy at whatever banks dished out to them. Over a period of time with the competition and technological improvements, customers have become fully aware of their rights. In this study the researcher was interested in evaluating the service climate prevailing at District Central Cooperative Bank, Coimbatore.

Service Climate and Banking

Service Climate is the shared perceptions of the practices, procedures and behaviour that are rewarded, supported and expected with regard to customer service and service quality (Schneider et al 1998 a &b) (Sampath et.al, 2021). People integrate hundreds, even thousands, of their experiences into a theme, and that theme is the climate of an organization. When the theme connotes service, they conclude that a climate for service exists. Customers' requirements and demands towards the banking industry services are increasing consistently. To meet this demand, the banking sector requires workforce with a new mindset to ensure high quality service delivery and instill greater professionalism in service provision (Sinclair and Zairi, 2001). When employers establish an Service climate that is perceived as positive by their employees, this will result in better organizational performance and higher levels of commitment, motivation and job-satisfaction. Thus, service climate as a whole will shows its impact towards the banking sector in all the aspects of its excellence.



**Sampath Nagi et al.,****Aim and Purpose of the Study**

The main purpose of the study is to find out the service climate prevailing in the context of District Central Cooperative Banks. This would be done by distributing a measuring instrument to the employees of the bank, where the policies, principles and practices followed by the bank and how it has been perceived by the employees of the bank. This study aims to determine the various dimensions that would bring the best outcomes from the employees, in order to serve the customers, the best.

Statement of Problem

India's Credit-to-Gross Domestic Product (GDP) ratio is 56 % to nation's economy and comparatively it shows its growth over a period. The pace of development for the Indian banking industry has been tremendous over the past decade. The expectation of the customers forms the banking industry has increased rapidly. The banks are trying to sustain themselves for this demand using the modern technologies, skilled employees and rigidly framing the policies. This would become the main stimulator for their performance. In controversy, the mushrooming of many nationalized private as well as public sector banks creates a competition between themselves. To sustain this competition, the banks have transformed in all the aspects to render best to their customers. The employees of the bank, technology and the policies followed by the bank would be able to serve the customers the best with the help of management support. The main contributor of human capital is the workforce, which has to be competent, innovative, motivated and capable of high productivity in order for an organization particularly services sector to achieve organizational excellence. Hence, organizations must encourage their employees to be more knowledgeable, customer focused, capable of team working (Wright and Brauchle, 1994), flexible and have the ability to work independently. As an organization, it needs to create a new approach for the work system to place greater emphasis on performance that is comprehensive, market driven, customer focused and multi-skilled workforce. From the employee perspective, multi skilling helps to improve service encounter by understanding the customer needs and expectations with reasonable prices and on-time service delivery to retain existing customers and win new ones.

Johnston (2004) argues that the service researchers and practitioners specifically recognised that when the employees are recognised and rewarded, communicated about the facts prevailing in the bank, trained to handle any kind of situations, provide with enough facilities and feasible environment to work, by perceiving these supports, they would feel happy in delivering the service, where a peaceful service climate prevails and in turn service climate will lead to increased job satisfaction. (Lytle and Timmerman (2006); Walker (2007)). There have been many changes in the fundamental banking reforms, still Cooperative banks are lagging behind on many fronts compared with commercial banks with wide range of capitalization, overseas network, modern management expertise, experience and technological advancement, hence the climate for service delivery, which prevailing at the cooperative banks are at a weak status. To help the bank management to overcome the problem, it becomes imperative for researchers to identify the factors which are influencing the performance of the study banks and its antecedents. If management treats employees well, therefore employees will treat customers well. Management's perception has been considered as more accurate than other employees' perceptions. This Management and employee's perception combined together to create a favourable service climate. The employees were more satisfied and felt committed in delivering service as they highly agreed with the statement staying long period for the same organisation. Thus, it resulted in high degree of organisational performance Powell and Butterfield (1978).

LITERATURE REVIEWS

Sughra Bibi, Asif Khan, Hongdao Qian, Achille Claudio Garavelli, Angelo Natalicchio and Paolo Capolupo (2020), in their study "Innovative Climate, a Determinant of Competitiveness and Business Performance in Chinese Law Firms: The Role of Firm Size and Age". The study was conducted in 408 Chinese law firms. This study assesses the effects of the firm's innovative climate on organizational learning and employees' innovative behavior as well as its consequences on the firm's competitiveness and business performance. The results indicate that the firm's innovative climate has a significant positive relationship with organizational learning and employees' innovative behavior. It is





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also found that organizational learning has a significant positive influence on employees' innovative behavior. Meanwhile, organizational learning and employees' innovative behavior have a significant positive influence on firm competitiveness and business performance. Another important finding is that contextual factors, i.e., firm size and age, strengthen these relations.

According to Navon, Vashdi, and Naveh (2020), the service climate emphasizes its benefits for customers, employees and organizational outcomes. The purpose of study is to examine the relationship between the service climate and employees' use of emotional labour strategies and investigate how service employees' service knowledge, skills, abilities and other attributes (KSAOs) affect this relationship. The researcher has conducted the study with two different samples i.e. 100 nurses working in 15 wards and the other is 244 luxury hotel chain employees working in 39 departments. The results of the study showed a positive association between the service climate and the use of surface emotional labour strategies for employees who had limited service KSAOs. The researcher was able to conclude in accordance with the basic definition of the service climate like service climate depicts the core values and beliefs of the organization about service, and helps employees to translate them into behaviours that promote high service performance, the current paper points to a potential toll it may have on employees well-being due to their use of surface emotional labour strategies.

Kang, Hee & Busser, James. (2018), This paper introduces a new conceptual model that incorporates internal service quality (Sampath et.al, 2021) as a mediator between service climate and employee performance and two personal cultural orientations (independence and interdependence) as the moderators of these relationships. Design/methodology/approach – An online survey of 353 employees representing 19 different nationalities, working in 18 branches and offices of a multinational Business-to-business (B2B) civil engineering services firm, spread across 14 countries. Findings – All the hypotheses are supported. Specifically, internal service quality (Sampath et.al, 2021) mediates the influence of service climate on employee performance and these relationships are stronger for employees with relatively higher interdependent (vs. independent) cultural orientations. Research limitations/implications – This paper uses data collected from the employees in a single B2B firm in one industry (Civil Engineering Services) and focuses on a few key variables, which may restrict the generalizability of its findings. Practical implications – Our findings highlight the importance of cultural factors in building a service climate in multinational service organizations to help their employees work more effectively and efficiently with their colleagues from diverse cultural backgrounds. Originality/value – This study clarifies the relationships among service climate, internal service quality and employee performance, by showing that internal service quality (Sampath et.al, 2021) mediates the influence of service climate on employee performance. Keywords – business-to-business services, independence, interdependence, internal service quality (Sampath et.al, 2021), service climate, service-profit chain.

Walker, (2007) in his study at English Language Centre (ELC) said that the staffs are receiving good levels of support from their management, particularly in areas such as encouragement, fostering mutual cooperation and training and development. However, when it comes to giving reward and recognition for a job well done, they sensed that the management does not do such a good job. The management practices said by the respondents are barriers to superior service e.g. resourcing, planning, organising, leadership, communication and staffing. The major findings from the study were that the background information of the respondents that might contribute to service climate are area of responsibility and personality which seemed to play an important role in shaping individual employees' service behaviour, regardless of the level of service climate.

Research Gap

After carefully analyzing various research studies conducted so far using the service climate, the researcher realizes that many research works have been carried out in different service industries (Sampath et.al, 2022) such as telecommunication, education, restaurants, banking, health care, and so on, but still there is a few empirical studies have been conducted on the aspects of service climate in District Central Co-operative Banks in India. Therefore, the researcher has proposed this study. The present study pays its attention on various service climate dimensions of



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banking services of district central cooperative banks. The research commences with policies, principles and practices of HR and service encounter which would ultimately motivate the employees in fulfilling their job and also it determines the positive service climate of district central cooperative bank.

Research Question

This study seeks to answer the following research questions: **(a)**.What is the perception of the employees towards human resource practices and service encounter (Sampath et.al, 2022) prevailing in District Central Cooperative Bank? and **(b)**.What is relationship between human resource practices and service encounter (Sampath et.al, 2022)faced by the employees of District Central Cooperative Bank?

Objectives of Study

Based on a thorough study of previous literature related to the service climate of service sector especially banking sector, the researcher aims to measure employee's perception towards human resource practices and service encounter and its relationship among the above-mentioned dimensionalities with respect to District Central Cooperative Banks.

Research Proposed Model

The theoretical framework is a model of logical relationship among the variable of Service Climate.The self-developed theoretical model consists of a set of dimensions relating to service climate from the outlook of the employees working in the co-operative bank such as HR practice and Service encounter dimensions has been developed by the researcher, which is depicted in **Figure 1**. Based on this model, the research hypotheses are also formulated.

RESEARCH METHODOLOGY

The research is descriptive research in nature, where the descriptive research (Mc Daniel & Gates, 2008) helps to measure and report in detail a situation or relationship of the specific variables occur in the sample.

Research Instrumentation

The questionnaire for the survey about the service climate comprises of two parts. The first part consists of 31 questions distributed under five dimensions of "HR Practices". The second part consists of 30 questions distributed under five dimensions of "Service Encounter". The researcher has used 5 point Likert scales, where the respondents are asked to select the most appropriate number that correspondent to the extent to which they agree with a statement.

Pilot study

The pilot study was conducted with the help of the structured questionnaire which was administered among the sample of 35 respondents. The results of the pilot study are discussed in the following section.

Sampling

The population of the study comprises of employees working in District Central cooperative banks at Tamil Nadu (Annual Report 2020 - National Federation of State Cooperative Banks Ltd). There are 23 head office and 878 branches including head office in Tamil Nadu. There are 4512 employees are working in all the branches including head office at Tamil Nadu. The researcher has adopted the Demorgan's sample size estimator for consolidating the samples. The researcher has identified for a population of five thousand, 357 sample size is need with a confidence level of 95 % and 5 % margin of Errors for collecting the data. The researcher has adopted two stage stratified random sampling, where in the first stage the 23-district head office has been segmented in to strata's, including branch offices under district. Then in the second stage the researcher has distributed the question on random basis to the employees of banks and collected the data. The researcher has distributed 400 questions and was able to received back 361 properly filled in research instrument and the remaining 39 were found to be biased.





Data Analysis

The collected data were analyzed using Statistical Package for Social Sciences (SPSS), Statistics for Windows, Version 20.0; The Bi Variate Pearson's Correlation was used to identify the relationship among the variables of Service Climate.

RESULTS

The results of the hypothesis are drawn with the help of Bi Variate Pearson's Correlation. The Pearson's correlation coefficient (r) is a measure of the linear association of two variables. The values of correlation coefficient vary from -1 to $+1$, the results are categories under three heads "Positive", "Negative" and "No" correlation.

Positive Correlation - The independent variable "Service Policies & Practices" has positive correlation with the independent variables like "HR Practices" ($r = 0.342$); "Service Role" ($r = 0.844$); "Service Encounter" ($r = 0.293$) and "Service Climate" ($r = 0.385$). The independent variable "Infrastructure & Facilities" has positive correlation with the independent variables like "HR Practices" ($r = 0.155$); "Products & Service" ($r = 0.528$) and "Service Climate" ($r = 0.193$). The independent variable "Internal Communication" has positive correlation with the independent variables like "Benefits, Rewards & Recognition" ($r = 0.612$); "HR Practices" ($r = 0.297$); "Service Delivery" ($r = 0.137$); "Service Role" ($r = 0.136$); "Empathy" ($r = 0.267$); "Service Encounter" ($r = 0.121$) and "Service Climate" ($r = 0.267$). The independent variable "Training & Development" has positive correlation with the independent variables like "HR Practices" ($r = 0.170$). The independent variable "Benefits, Rewards & Recognition" has positive correlation with the independent variables like "HR Practices" ($r = 0.474$); "Empathy" ($r = 0.136$) and "Service Climate" ($r = 0.297$). The independent variable "HR Practices" has positive correlation with the independent variables like "Service Delivery" ($r = 0.208$); "Service Role" ($r = 0.275$); "Empathy" ($r = 0.215$); "Service Encounter" ($r = 0.256$) and "Service Climate" ($r = 0.526$). The independent variable "Products and Service" has positive correlation with the independent variables like "Service Encounter" ($r = 0.192$) and "Service Climate" ($r = 0.161$). The independent variable "Customer Focus" has positive correlation with the independent variables like "Service Delivery" ($r = 0.154$); "Empathy" ($r = 0.110$); "Service Encounter" ($r = 0.246$) and "Service Climate" ($r = 0.164$). The independent variable "Service Delivery" has positive correlation with the independent variables like "Service Role" ($r = 0.119$); "Empathy" ($r = 0.678$); "Service Encounter" ($r = 0.340$) and "Service Climate" ($r = 0.346$). The independent variable "Service Role" has positive correlation with the independent variables like "Service Encounter" ($r = 0.264$) and "Service Climate" ($r = 0.475$). The independent variable "Empathy" has positive correlation with the independent variables like "Service Encounter" ($r = 0.302$) and "Service Climate" ($r = 0.335$). The independent variable "Service Encounter" has positive correlation with the independent variables like "Service Climate" ($r = 0.594$).

Negative Correlation - The independent variable "Training & Development" has negative correlation with the independent variables like "Products & Service" ($r = -0.130$). The independent variable "Products and Service" has negative correlation with the independent variables like "Customer Focus" ($r = -0.146$).

No Correlation - The independent variable "Service Policies & Practices" has no correlation with the independent variables like "Infrastructure & Facilities"; "Internal Communication"; "Training & Development"; "Benefits, Rewards & Recognition"; "Products & Service"; "Customer Focus"; "Service Delivery" and "Empathy". The independent variable "Infrastructure & Facilities" has no correlation with the independent variables like "Internal Communication"; "Training & Development"; "Benefits, Rewards & Recognition"; "Customer Focus"; "Service Delivery"; "Service Role"; "Empathy" and "Service Encounter". The independent variable "Internal Communication" has no correlation with the independent variables like "Training & Development"; "Products & Service" and "Customer Focus". The independent variable "Training & Development" has no correlation with the independent variables like "Benefits, Rewards & Recognition"; "Customer Focus"; "Service Role"; "Empathy"; "Service Encounter" and "Service Climate". The independent variable "Benefits, Rewards & Recognition" has no correlation with the independent variables like "Products & Service"; "Customer Focus"; "Service Delivery";



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“Service Role” and “Service Encounter”. The independent variable “HR Practices” has positive correlation with the independent variables like “Products & Service” and “Customer Focus”. The independent variable “Products and Service” has no correlation with the independent variables like “Service Delivery”; “Service Role” and “Empathy”. The independent variable “Customer Focus” has no correlation with the independent variables like “Service Role”. The independent variable “Service Role” has no correlation with the independent variables like “Empathy”.

DISCUSSION AND CONCLUSION

The results clearly shows that the training and the personal development does not have any relationship with the service climate. This clearly shows that the training and development given to the employees by the DCCB management has to be improved a lot or new hands-on training related to E-commerce has to be definitely provided to the employees, because many employees are facing difficulties in handling the E-commerce based application. The requirements of the customers towards the banking sector have been increasing day by day. To meet this requirement the district central cooperative bank management has to focus on fulfilling the employees needs of training and development also to enable them to have a positive service encounter effectively. The monetary has to be focused with much concentration by the management. Then management has to provide proper training, while handling the customers on communication, customer focus, service delivery and empathy.

Limitation and Future Implications

The cross-sectional nature of the study is certainly a limitation. The functioning of organizations involves a dynamic process that changes over time. Future researchers should elaborate the study by conducting longitudinal studies. In particular, aggregate perceptions of interpersonal and informational climate could have a variety of beneficial effects in departments, branches or organizations. As for industry specific recommendations in India, future research should examine the relationships of service climate and service quality at the industry level (including different type of industries). Potential researchers should also examine other ways of nurturing service climate and service quality in line with what scholar have proposed in their study.

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Conflict of Interest

The authors certify that, they have had no affliction with or involvement in any organisation or entity with any financial interest, or non-financial interest in subject matter, or material discussed in the manuscript.

Authors' Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Table 1. Reliability Test for HR Practices Dimensions

| | Number of Items | Cronbach Alpha |
|-------------------|-----------------|----------------|
| HR Practices | 31 | 0.8286 |
| Service Encounter | 30 | 0.8456 |
| Service Climate | 61 | 0.8371 |

Table 2. H₀: No Significant Relationship between the Determinants of Service Climate

| Variables | SPP | IF | IC | TD | BRR | HRP | PS | CF | SD | SR | EMP | SE | SC |
|-----------|--------|--------|--------|--------|--------|------|----|----|----|----|-----|----|----|
| SPP | 1 | | | | | | | | | | | | |
| IF | .010 | 1 | | | | | | | | | | | |
| IC | .060 | .010 | 1 | | | | | | | | | | |
| TD | -.079 | -.095 | -.079 | 1 | | | | | | | | | |
| BRR | .023 | -.033 | .612** | .094 | 1 | | | | | | | | |
| HRP | .342** | .155** | .297** | .170** | .474** | 1 | | | | | | | |
| PS | .046 | .528** | -.025 | -.130* | -.073 | .100 | 1 | | | | | | |





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| | | | | | | | | | | | | | |
|--|--------|--------|--------|-------|--------|--------|---------|----------------------------------|--------|--------|--------|--------|---|
| CF | -.019 | -.008 | .022 | .038 | .051 | .001 | -.146** | 1 | | | | | |
| SD | .097 | -.063 | .137** | .135* | .078 | .208** | -.056 | .154** | 1 | | | | |
| SR | .844** | .075 | .136** | -.065 | .100 | .275** | .032 | .031 | .119* | 1 | | | |
| EMP | .042 | -.004 | .267** | .096 | .136** | .215** | -.028 | .110* | .678** | .086 | 1 | | |
| SE | .293** | .078 | .121* | .012 | .074 | .256** | .192** | .246** | .340** | .264** | .302** | 1 | |
| SC | .385** | .193** | .267** | .086 | .297** | .526** | .161** | .164** | .346** | .475** | .335** | .594** | 1 |
| ** Correlation is significant at the 0.01 level (2-tailed). | | | | | | | | HRP - HR Practices | | | | | |
| * Correlation is significant at the 0.05 level (2-tailed). | | | | | | | | PS - Products and Service | | | | | |
| Pearson Values are displayed in table | | | | | | | | CF - Customer Focus | | | | | |
| SPP- Service Policies and Practices | | | | | | | | SD - Service Delivery | | | | | |
| IF - Infrastructure and Facilities | | | | | | | | SR - Service Role | | | | | |
| IC - Internal Communication | | | | | | | | EMP - Empathy | | | | | |
| TD - Training and Development | | | | | | | | SE - Service Encounter | | | | | |
| BRR - Benefits, Rewards and Recognition | | | | | | | | SC - Service Climate | | | | | |

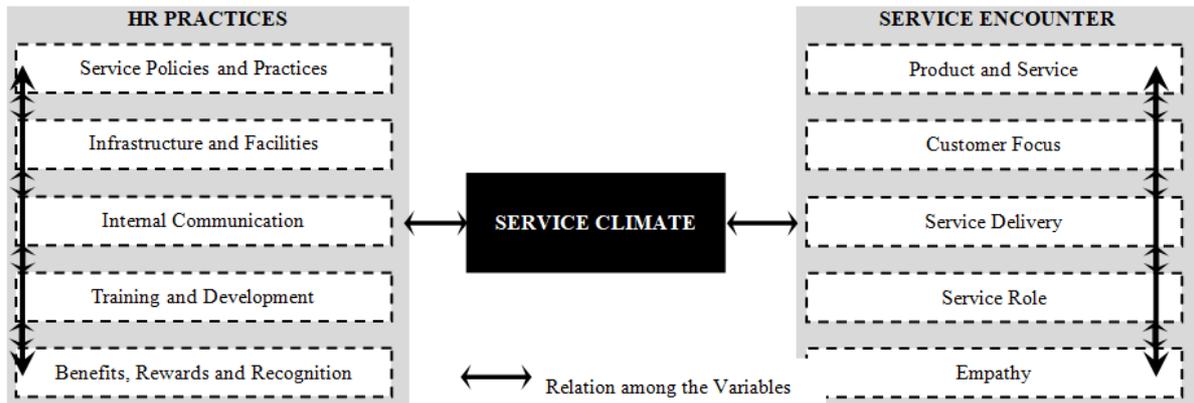


Figure 1. Relationship Among the Variable of Service Climate





Avifaunal Diversity in JAC Campus in Periyakulam, Theni District, Tamil Nadu

Pushpa P* and Iruthaya Kalai Selvam

Assistant Professor, Department of Zoology, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Theni, Tamil Nadu, India

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*Address for Correspondence

Pushpa P

Assistant Professor,
Department of Zoology,
Jayaraj Annapackiam College for Women (Autonomous),
Periyakulam, Theni, Tamil Nadu, India
E.Mail: pushpazoo@annejac.ac.in



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ABSTRACT

JAC campus plays a significant role in the conservation of avifaunal diversity. Understanding the diversity and structure of bird communities is essential to delineate the importance of regional or local landscapes for avian conservation. Birds are very sensitive to environmental changes and are used as “bioindicator.” The study was carried out to explore the diversity and status of avifauna in the JAC campus, Periyakulam, Theni District, from January to June 2021. Total count method was used to cover most of the study area. A total of 21 species of birds belonging to 15 families and 8 orders were identified. The diversity of the birds was high during the month of January followed by February and March and low in June. The abundance was high during the month of January and February and low in May and June. The highest number of species richness were seven sisters, house crow, common myna and little egret and the lowest number of species richness were parrot, purple rumped sunbird, white breasted kingfisher and quail. Thus, the JAC campus provides good supports for avifaunal diversity. Its proper management will not only improve the situation for its resident species, but will also attract more migrants in the future. This study provides baseline data for monitoring the avifauna in the JAC campus, and demonstrates the importance of JAC campuses in bird conservation.

Keywords: JAC campus, Diversity of birds, Abundance and Richness.





INTRODUCTION

The health of the forest ecosystem depends on the avian population, density and species diversity which in turn directly reflects the changes in their habitat conditions. The extent of change determines the long term conservation of biodiversity (Canterbury et al., 2000) [4]. The population studies have traditionally been used to monitor large scale, long term changes in avian population and to assess both habitat quality and the responses of birds to both natural and human caused environmental changes (Weins, 1989) [11]. Preservation of global species diversity has emerged as one of the most important issues today. Ali (1979)² opined that the study of birds in relation to habitats would give valuable data for forest management. Although the vegetation determined the distribution and abundance of most terrestrial bird species, correlation analysis of various communities, resource and habitat parameters provide no such guidance to underlying causes community pattern (Gilbert *et al.*, 1984) [7]. Many factors are thought to play a secondary role in determining the community structure (MacArthur, 1972) [10]. (Cody, 1968) [5] explored the resource division in grassland bird communities and found that the influx of migratory bird population was due to the availability of arthropods (Greedberg, 1995) [8]. Therefore the present work is a documentation of the distribution of birds in the JAC campus, based on their migratory status, feeding habit, guild and threats of avifauna. Birds are used as a tool for environmental monitoring. Birds are good biological indicators. They are good indicators of the general state of our biodiversity. When they start disappearing, it means something is wrong with the environment and is a signal that action must be taken. They are suitable for detecting changes in the environment like environmental contaminants and air pollution. Birds detect changes in the environment which cannot be detected or observed by physical parameters. Bird extinction and population reduction can result to disruption of ecosystem processes that are of great importance to the society. (Anderson et al., 2006) [3] the ecological roles of birds often disappear with them due to habitat loss, over exploitation and increased predators are the causes of decline in population. The broad objective of the study was to determine the species composition, relative abundance and species diversity of avifauna found in and around the JAC campus.

MATERIALS AND METHODS

Study Area

Periyakulam (PKM) is located at 10.07°N 77.33°E. It has an average elevation of 282 metres (925 feet). It is located at the foothills of the Western Ghats bordering the neighbouring state of Kerala. It is one of the most fertile places in the state of Tamil Nadu. Periyakulam Town has an area of 21 km², within an Semi urban area now extending over as much as 55 km². The climate is dry and hot, with Northeast monsoon rains during October–December. Temperatures during summer reach a maximum of 40 and a minimum of 26.3 °C, though temperatures over 43 °C are not uncommon. Winter temperatures range between 29.6 and 18 °C. The average annual rainfall is about 135 cm. Jayaraj Annapackiam College situated at periyakulam in Theni District and it is 2 kms distance from periyakulam. Jayaraj Annapackiam College located on Mt. St. Anne, nestling in lush green, facing the kodaikanal hills, which is 2 kms. away to the West of Periyakulam, Theni District.

Bird Census

The bird census was taken twice in a month from July to December 2021. The method of total count was employed to survey the bird population. In this method, the blocks were counted using (7X50) pentax binocular and identified using features with the help of field guild (Ali and Ripley, 1983)¹ Inskipp *et al.*, 1998)⁹.

Data Analysis

Species Abundance

Species abundance was measured by (number of water birds in each recorded on the habitat) during the monthly census (Verner, 1985).





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Species Diversity

Species diversity was calculated using the Shannon – Weaver index (Shannon Weaver, 1964).

$$H1 = -\sum P_i \times \ln(P_i)$$

Where, P_i = The proportions of individuals found in the 1st species

\ln = Log Normal

Species Richness

The richness of bird species was calculated using the margalef (1958) index.

$$R1 = (S - 1) \ln(n)$$

Where,

S = The total number of species

N = The number of individuals

RESULT AND DISCUSSION

A study was conducted from January to June 2021 to enumerate the bird species composition in Jayaraj Annapackiam College Campus in Periyakulam at Theni District. . A total of 21 species represented, 8 orders of 15 families were observed. The result showed that the most dominant bird species were seven sisters, house crow, common myna and little egret. The rarest bird species were parrot, purple rumped sunbird, white breasted kingfisher and quail. The bird diversity was high in January followed by February, March and April and minimum during May and June. This showed that this species is equally distributed in January and February and low in May and June with lowest equitability. Bird abundance was high in January and February. The abundance was very low in May and June due to food availability and the migrant bird species in JAC Campus.

The highest number of species richness were seven sisters, house crow, common myna and little egret. The rarest bird species were parrot, purple rumped sunbird, white breasted kingfisher and quail. because of the availability of prey categories. The same result was reported as Geoffrey Soka (2013)⁶. So, it may be suggested that drastic steps must be taken to preserve and maintain these types of wetlands and to save the wetland birds.

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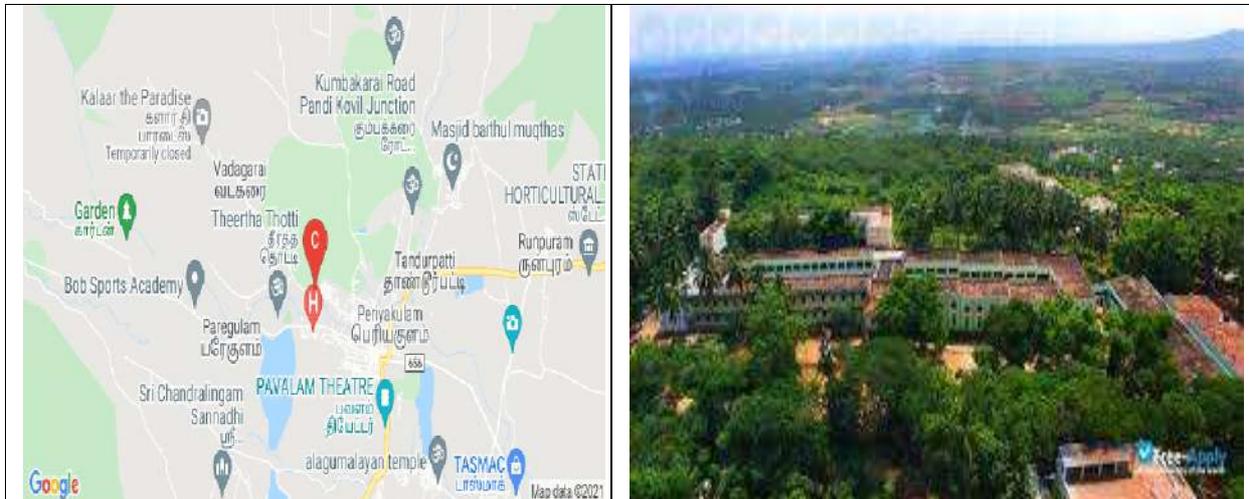


Fig.1. Study Area

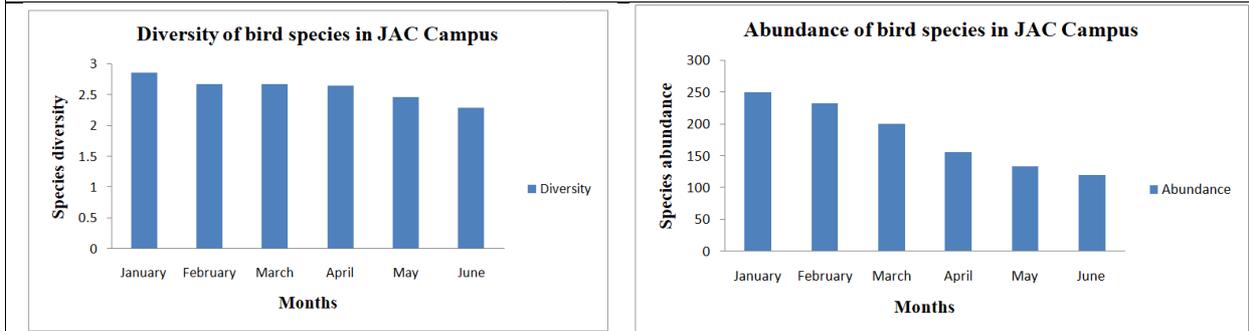


Fig.2. Diversity of bird species in JAC Campus

Fig.3. Abundance of bird species in JAC Campus

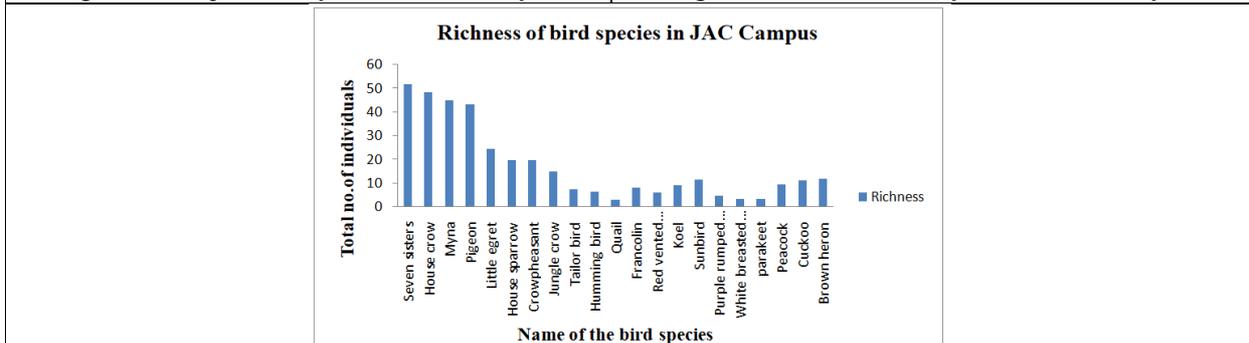


Fig. 4. Richness of bird species in JAC Campus





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| | | |
|--|---|--|
|  |  |  |
| <p>Fig. 5. Crow Pheasant</p> | <p>Fig. 6. Seven sisters</p> | <p>Fig. 7. Red vented bulbul</p> |
|  |  |  |
| <p>Fig. 8. Peacock</p> | <p>Fig. 9. Myna</p> | <p>Fig. 10. Tailorbird</p> |





The Silent Killer : A Comprehensive Review on Hepatocellular Carcinom

Dinesh Raut¹, Priyatama Powar² and Devendra. Shirode^{3*}

¹M.Pharm Student, Dr. D. Y. Patil College of Pharmacy, Akurdi - 411044, Pune, Maharashtra, India.

²Assistant Professor, Dr. D. Y. Patil College of Pharmacy, Akurdi - 411044, Pune, Maharashtra, India.

³Associate Professor, Dr. D. Y. Patil College of Pharmacy, Akurdi - 411044, Pune, Maharashtra, India

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*Address for Correspondence

Devendra. Shirode

Associate Professor,

Dr. D. Y. Patil College of Pharmacy,

Akurdi - 411044, Pune,

Maharashtra, India

E.Mail: devendrashiode@dyppharmaakurdi.ac.in



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ABSTRACT

This article provides a brief overview of Hepatocellular Carcinoma, its epidemiology, etiology, and diagnosis, and various conventional and modern treatment approaches for hepatocellular carcinoma. Also, this article will validate the curtain potential of different plants as anticancer activity. This review article gives an idea for the ideal treatment of hepatocellular carcinoma using various phytomedicinal plants, their active constituents and various formulations using it gives significant relief from the disease and, to some extent, it will give prevent form further growth of the disease. In the modern approach to treatment, we can go with the various phytomedicine combination that gives much more effective than the conventional ones. Phytomedicines have fewer side effects than conventional ones. In the treatment not only do phytomedicines play an important role but also the various spices, and fruits play an important role. In addition to phytomedicines, various therapies have a significant role in treatment. The modern approach using phytomedicines is one of the best solutions to deal with hepatocellular carcinoma. Understanding the role of phytomedicines is a very important aspect of the treatment. The active chemical constituent in the medicinal plant has the significant ability to fight certain diseases and disorders, in this, the various plants have the role of the fight with hepatocellular carcinoma. The various therapies are also useful in the treatment. Along with the herbal formulation, there are fruits that are discussed in this for the treatment.

Keywords : Hepatocellular Carcinoma, Phytomedicines, Diagnosis of Hepatocellular Carcinoma, Modern treatment approaches, Future prospects for the treatment of HCC.

INTRODUCTION



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Hepatocellular carcinoma is one the malignant tumour that affects the liver cells and has the highest rate of morbidity, and mortality in the world[1, 2]. The hepatocellular has various characteristics that show its uniqueness among other cancer like fast progression, recurrence, and a highly malignant property with a high rate of metastasis[1, 3]. Hepatocellular carcinoma is a complex type of disease with the comparison to the other type of cancer [4]. Due to various factors, there is a genetic alteration in the body that genetic alteration leads to hepatocarcinogenesis [2]. Hepatocellular carcinoma has another abbreviated common name “The Silent Killer” this is because there is a long-term progression of the disease, it takes around 3-5 years for progression [5, 6]. In the early stage of hepatocellular carcinoma, it should be detected earlier as possible. For early treatment, there are some methods by which we can cure hepatocellular carcinoma which is as local ablation, resection, and the transplantation of the liver[3]. In addition to this, there are some methods like hepatectomy, and embolization, are also help to cure hepatocellular carcinoma[1]. In some patients, hepatocellular carcinoma remains untreated because it is diagnosed very late. Nowadays conventional agents are not giving satisfactory results against disease and they have side effects to some extent. To overcome the side effect and systemically treat disease we are shifted towards phytochemicals. Various plants possess anticancer activity that will be helpful for the treatment of hepatocellular carcinoma.

Epidemiology

Hepatocellular carcinoma has a major burden on the world, according to (Global Cancer Observatory) Cancer Today in 2020 there were 905677 incident cases and 830180 deaths cases are reported, contributing to around 988687 cases world wide[7]. In males, it is accounting for 632320 cases of incidence and 577522 cases of mortality were reported in 2020, in a similar manner 273357 cases of incidence and 252658 cases of mortality were reported [7]. This clearly indicated that there is more prevalence in males than females. Over the past decades, we can see there is number of cases is increasing day by day. According to various research shows that most significant cause of hepatocellular carcinoma is the Hepatitis B virus (HBV) which accounts 33% of liver cancer deaths in the world[2]. Also, various factors like alcohol, Hepatitis C other cause have some effect on cause of hepatocellular carcinoma[2]. In India hepatocellular carcinoma incidence case were 691635 and mortality case were 642691 were reported in 2020[7].

Etiology and Pathogenesis

The etiological factor for the development of hepatocellular carcinoma includes Hepatitis B, Hepatitis C, various alcoholic liver diseases, non – alcoholic fatty liver diseases, and other factors like inflammation, fibrosis that affects the cause of hepatocellular carcinoma. In addition to this due intake of excessive hot, saline, poisons food and drinks also responsible for the development of hepatocellular carcinoma[4]. The majority of hepatocellular carcinoma cases are caused by the chronic liver diseases. Condition of hepatocellular carcinoma is very complex. Hepatocarcinogenesis is a lengthy process in which the hepatocellular phenotype is gradually altered by genetic alterations to give rise to cellular intermediates that develop into hepatocellular cancer.

Hepatitis B and Hepatitis C

Development of cirrhosis due to the Hepatitis B and Hepatic C makes the hepatocellular carcinoma more complicated. The risk with the cirrhosis is higher to convert into hepatocellular carcinoma[2]. Along with the HVB/HCV chronic alcohol abuse, obesity diabetes mellitus its risk is at higher rate. The major pathophysiology for oncogenesis in HBV is the integration of the hepatitis B viral genome into the host genome. Hepatitis B virus (HBV) is a member of a DNA virus family and is an enclosed, partially double standard virus with a circular DNA genome [8]. A DNA virus called HBV causes a chronic neuro inflammatory illness, that encourages liver cell abnormalities and result in HCC. HCC is caused by the viral genome insertion in the human genome at telomerase reverse transcriptase (TERT) promoter region[9]. In addition to this there is micro-RNA which play an important role in the development of HBV induced HCC. basically, micro-RNA is small non coding RNA which has function of regulatory cellular processes like proliferation, differentiation, an apoptosis. Due to changes in their function it leads to formation of hepatitis B which further leads to formation of hepatocellular carcinoma[10]. the hepatic steatosis-causing potential of the HCV core proteins, an oxidative stress-mediated pathway is most likely associated with HCC caused by HCV[11].





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Alcohol

The alcoholic cirrhosis is the world's second most cause of development of hepatocellular carcinoma[12]. The chronic alcohol consumption leads to development of various diseases, it enhance the certain Oncogene expression, thus they increase the chance of changes in Oncogenic Mutation and increase in reactive oxidative stress[13]. In addition to that .Chronic alcohol exposure increases TNF- sensitivity, which causes stellate cell activation, chronic hepatocyte destruction-regeneration, cirrhosis, and ultimately HCC[12].The various study stated that due to the chronic ethanol consumption there is 2 fold chance of development of hepatocellular carcinoma[2]. The incidence of hepatocellular carcinoma associated with alcoholic cirrhosis quite lower than other.

Diabetes mellitus and Obesity

Diabetes mellitus and Obesity are one of the important factors for the development hepatocellular carcinoma. Presence of this diseases head should increase in the risk of cancer[2]. By diabetes mellitus risk of HCC is 2-3fold and by obesity it is 4 times.

Non - alcoholic fatty liver diseases

Non- alcoholic fatty liver disease is characterized by an excessive fat in a hepatocyte without a history of alcohol consumption. The primary environment for the non- alcoholic fatty liver disease is to develop metabolic syndrome. The syndrome is the condition that increase the risk of cardiovascular disease in patient who also have abdominal obesity, hypertension, hypertriglyceridemia , and insulin resistance[8]. The consumption of red meat and low intake of fruit and vegetables are linked to cellular damage and the development of tumors[14].TNF- specifically promotes pro-oncogenic pathways including c-Jun amino acid-terminal kinase and the mammalian target of rapamycin complex (mTOR) (JNK).

Chemicals

Aflatoxin

Aflatoxin are nothing but mycotoxins which is potent hepatocarcinogenic agent[12, 15].The Aspergilli *Aspergillus flavus* and *Aspergillus parasiticus* create the mycotoxin known as aflatoxin B1[9]. Most cancers are caused by mutation in the tumor suppressor gene in (p53)[16, 17]. Aflatoxin B1 (AFB1) is the most potent of the four main aflatoxin, along with aflatoxin B2 , G1 and G2[18].

Diethylnitrosamine

There is various compound that result in the production of synthetic aflatoxin that cause cancer in the lab animals.The diethylnitrosamineis awell-known hepatocarcinogenic substance that transforms ethyl radicals in liver. That active radicals interact with DNA to generate mutagenesis, which result in the development of cancer[19].It exists in environmental components such as commercially available cheese, fried meats, alcoholic drinks, tobacco products, soybean, cosmetic products, and agricultural fertilizers plays a vital role in carcinogenesis in human beings[20].

N-Nitroso dimethylamine

The chemical compound N-nitrosodimethylamine (NDMA), also known as diethylnitrosamine (DMN), dimethylnitrosoamine (DMNA), N-dimethylnitrosoamine, N-methyl-N nitrosomethanamine, and N-nitroso-N, N-dimethylamine, is a strong carcinogen. A powerful hepatotoxin, carcinogen, and mutagen, dimethyl nitrosoamine[19, 20]. Along with this factor, some drugs cause hepatotoxicity that further leads to the hepatocellular carcinoma directly or indirectly. Example of this agents are Paracetamol, chlorpromazine, diclofenac, erythromycin, isoniazid, and various oral contraceptives, Amoxicillin, Ciprofloxacin, Fluconazole[19, 21].

Diagnosis of hepatocellular carcinoma

It is essential for the correct therapy of individuals with hepatocellular carcinoma to confirm the diagnosis and determine the degree of the disease (HCC). The diagnosis and staging of HCC depend heavily on imaging studies. For each patient, choosing the best course of treatment requires accurate staging of their HCC in order to establish their prognosis. The prevention of cancer must include screening. Dynamic CT and MRI are suggested as the first-

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line diagnostic modalities for HCC in cirrhotic livers. A dynamic (tri-phasic or four-phasic) computed tomography (CT) or magnetic resonance imaging (MRI) scan should be performed, ideally at facilities with the necessary tools and knowledge, if a nodule of size >1 cm is found in the cirrhotic liver during surveillance or random ultrasonography. When a nodule is found in a cirrhotic liver, these imaging modalities are advised as the first-line diagnostic methods for HCC.[2].

Diagnostic and prognostic biomarkers

Tumor markers

A biomarker with improved diagnostic precision and high reliability is therefore required. Numerous tumour biomarkers have recently been found in HCC research. Modern analytical methods including Next Generation Sequencing (NGS), mass spectrometry, proteomics, and metabolomics can help identify new molecular biomarkers for the diagnosis of HCC and provide crucial information for medical oncology[22]. These biomarkers are discovered by genomic platforms and other genetic analyses of blood, tissue, urine, feces, and saliva, and they may help in the creation of tailored treatments based on a patient's genetic makeup and exposure to environmental risk factors. Biomarkers can be employed for HCC clinical staging, prognosis, and diagnostic purposes[23].

In the diagnosis of HCC by serum, alpha-fetoprotein (AFP) has been employed as a biomarker.

AFP

AFP has been regarded as the most helpful biomarker for assessing HCC. It is typically produced by the liver, yolk sac, and, to a lesser extent, the gastrointestinal system during fetal and neonatal development[15, 17]. The estimation of serum AFP is still a helpful test for the management of HCC patients. The prognosis of HCC patients is negatively affected by high tumour marker levels[2]. A glycoprotein called AFP, which has a molecular weight of roughly 70 kDa, transports a wide range of compounds, including bilirubin, fatty acids, retinol, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and probably different medicines[23].

Des-γ-carboxy prothrombin

Des-carboxy prothrombin (DCP), a prothrombin variant that is more prevalent in HCC, is prothrombin generated by vitamin K absence II (PIVKA II). The vitamin K-dependent carboxylase system deteriorates with the malignant transformation of hepatocytes. Numerous investigations have demonstrated that patients with benign and malignant liver disorders have considerably different serum DCP levels from normal, and that DCP may have a higher diagnostic sensitivity than AFP[23, 24].

AFP-L3

There are three distinct glycoforms of FP, each with a different ability to bind to the lectin *Lens culinaris* agglutinin (LCA): AFP-L1 (non-binding fraction), AFP-L2 (weak binding fraction), and AFP-L3 (strong binding fraction) (binding fraction). While AFP-L3 is specifically enhanced in HCC, AFP-L1 is elevated in both liver cirrhosis and chronic hepatitis. It has been thought that AFP-L3 is a more specific biomarker for HCC because it is only produced by cancer cells[15].

Osteopontin

The integrin-binding glycoprotein osteopontin (OPN), also known as the transformation-related protein phosphatase, is overexpressed in a variety of cancers, including lung, breast, and colon cancer. The protein has been discovered to be involved in a variety of physiological cellular processes, including as metastasis, invasion, and migration[25]. OPN is typically not expressed in hepatocytes but is found in the bile duct epithelium, stellate cells, and Kupffer cells. However, compared to people with normal livers, liver cirrhosis, or chronic hepatitis, higher expression of serum OPN has been observed in HCC patients[25–27].



**Dinesh Raut et al.,****Imaging diagnosis**

Imaging is essential for detecting and diagnosing HCC. Since serum alpha-fetoprotein was previously utilised as a screening test for HCC. Ultrasonography (US), multiphase computed tomography (CT), and magnetic resonance imaging (MRI) with contrast are the imaging procedures most frequently used to diagnose HCC [28]. When compared to the liver's surrounding tissue, typical HCC lesions on CT and MRI show enhanced arterialization and a lower presence of contrast agents during portal vein and/or equilibrium phase imaging[29].

Ultrasound (US)

In the HCC diagnostic method, US plays two roles. Contrast-enhanced US not only serves as a primary screening tool in individuals at risk, but it also has the potential to provide a conclusive diagnosis. The use of ultrasound examination to find HCC is still important since it can find even the smallest lesions in the liver. The effectiveness of ultrasound as an HCC screening method has been well studied. Its sensitivity and specificity in this situation have been observed to be rather high. Although CT and magnetic resonance imaging have essentially superseded it in the diagnostic process, it is still useful in some circumstances, such as the evaluation of vascular invasion by HCC[30, 31].

Computed tomography (CT)

Due to its widespread availability and quick examination times, computed tomography is the imaging technique that is most frequently utilised to diagnose HCC [30]. Volume perfusion imaging is the most recent advancement in CT applications for liver imaging. Perfusion CT offers quantitative information on perfusion parameters, may be able to distinguish between different types of tumour tissue based on perfusion behavior, and might therefore improve the ability to grade tumours and monitor therapy. In comparison to normal tissue, HCCs have been shown to have high perfusion values (high blood flow, blood volume, and permeability, and reduced mean transit time)[30, 32].

Magnetic resonance imaging

Recent improvements in liver MRI technology include volumetric imaging, parallel acquisition imaging, a powerful gradient system with greater speed, and a new three-dimensional gradient-echo (3D GRE) sequence[33, 34]. Compared to computed tomography, magnetic resonance imaging offers better lesion-to-liver contrast, which is a considerable advantage.

Staging

There are various liver cancer staging methods. Staging is used to forecast a patient's likelihood of recovery and organize care. HCC must be staged in order to inform management choices, forecasts, and research protocol standardization[12]. The HCC staging is crucial for choosing a treatment plan. The Barcelona clinic liver cancer (BCLC) criteria are one of the most widely used HCC staging systems because they link each tumour stage to a management strategy, ranging from curative intent to supportive care, by integrating pertinent imaging findings and clinical factors like underlying liver function and patient functional status. Tumors are staged based on their radiologic appearance determined on CT or MRI, which is mostly dependent on the size, amount, and presence of macrovascular invasion. The Organ Procurement and Transplantation Network (OPTN) also establishes stringent imaging requirements connected to cancer stage in order to determine eligibility and priority assignment for liver transplantation[29, 34, 35].

Treatment on Hepatocellular Carcinoma.

People of any age can develop primary liver cancer. However, pediatrics treatment is not the same as adult treatment. Since most treatments for HCC might aggravate underlying liver disease, the management of HCC includes a complex decision-making process that takes into account not only the amount of the tumour and the patient's comorbidities but also the severity of liver dysfunction. Curative and noncurative therapies make up the majority of the therapy options. LR, thermal ablation, and LT are curative treatments, but TACE, TARE, stereotactic body radiation therapy, and systemic chemotherapy are noncurative treatments that aim to prolong survival by slowing the growth of the tumour[12, 36]. If detected early enough, HCC can be cured by surgical resection or liver

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transplantation; however, as the majority of HCC patients present with advanced illness and underlying hepatic failure. The various treatment approaches are given in (fig 1)

Conventional treatment

Tumor Resection

A treatment that has the potential to be curative, is surgical resection. Patients with resectable illness who do not have clinically substantial portal hypertension are advised to get it. If the tumour is restricted to the liver, its size and location, and whether the underlying liver function will permit resection without increasing morbidity and mortality are the main factors considered in the assessment of the prospective resectability of HCC. For individuals with isolated liver tumours that are not radiographically demonstrated to have invaded the vasculature and have preserved liver function, resection is the preferred course of action[12, 28, 36].The availability of the surgical histopathology specimen, which can aid in estimating the risk of recurrent HCC, is one of the benefits of surgical resection.

Ablation

The use of ablation as a potentially curative alternative has grown significantly during the past ten years. The ideal treatment for HCC is radiofrequency ablation (RFA), which has now superseded percutaneous ethanol injection as the most widely utilised ablative therapy[36].One of the most popular and efficient treatment techniques for very early and early HCC is image-guided percutaneous ablation. It includes cryoablation, irreversible electroporation (IRE), irreversible microwave ablation (MWA), radiofrequency ablation (RFA), and percutaneous ethanol injection (PEI). Ablation is simply reproducible and less invasive for recurrence.When a tumour nodule is close to big intrahepatic arteries or bile ducts, ethanol injection may be the preferable therapeutic option in high-resource nations to prevent thermal damage to these organs during radiofrequency ablation or microwave ablation.[2, 36].

Transplantation

The most effective form of treatment for people with HCC is liver transplantation, which is recognized on a global scale. Because cirrhotic livers account for the majority of HCC cases, curative procedures including partial hepatectomy and tumour ablation are not acceptable alternatives due to the existence of decreased liver function. Given that it removes the tumour with the widest margin possible and replaces it with a noncirrhotic liver, LT is regarded as the best therapeutic option for cirrhotic patients with HCC[2, 12].

TACE. (Trans arterial Chemoembolization)

TACE is advised as a first-line, noncurative treatment for BCLC stage B multinodular asymptomatic tumours without vascular invasion or extrahepatic dissemination among patients with large multifocal HCC or those whose tumour features are not suitable for surgical or ablative therapy. There is no set criterion for the selection of chemotherapeutic drugs, but many have been utilised, including doxorubicin, cisplatin, mitomycin, and epirubicin[12, 28, 36]. Future studies should look into the possibility of using newer systemic treatments rather than conventional chemotherapeutic agents for TACE given the efficacy and approval of newer systemic treatments for advanced-stage HCC.

TARE. (Trans arterial radioembolization)

TARE, often referred to as selective internal radiation therapy (SIRT), is a type of radiation treatment that involves injecting an embolic agent together with a radiotherapy agent into the arteries that supply the HCC. In circumstances where TACE cannot be performed or is generally contraindicated, TARE is probably more appropriate[37]. TARE is therefore recommended for a small number of patients with advanced HCC, such as those who have portal vein thrombosis[2, 12, 12, 38].

Various therapies for treatment of hepatocellular carcinoma

Chemotherapy is typically used as a palliative treatment for HCC patients who are not candidates for possibly curative procedures like resection, transplantation, or ablation. Chemotherapeutic drugs of many kinds have been



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tested against HCC. Tyrosine kinase inhibitors (TKIs) such as sorafenib, lenvatinib, regorafenib, and cabozantinib, monoclonal antibodies such as ramucirumab and bevacizumab, and immune checkpoint inhibitors (ICI) such as nivolumab, pembrolizumab, and atezolizumab are all included in systemic therapy.

Sorafenib

Sorafenib is a small-molecule multikinase inhibitor that targets the Raf family of kinases, platelet-derived growth factor receptors (PDGFR), and the vascular endothelial growth factor receptors (VEGFR1, VEGFR2, and VEGFR3) (predominantly C-Raf rather than B-Raf)[2, 39]. sorafenib was the first medication approved for use as the first-line systemic therapy for patients with advanced-stage HCC. But due of its side effects, it is frequently necessary to cut doses or stop taking the medication; for this reason, sorafenib should be begun at a lower dose. According to published data from other nations, Indians reported a greater prevalence of liver dysfunction and hand-foot syndrome-rash in a prospective real-world investigation of sorafenib[2, 10, 36].

Lenvatinib

Lenvatinib is a tyrosine kinase inhibitor (TKI) of the RET, KIT, PDGF, FGF, VEGF, and FGF receptors. Hand-foot syndrome, low RBC, skin rashes, fatigue, abdominal pain, and mouth sores are side effects.[2, 10].

Regorafenib

Regorafenib, a multikinase inhibitor with a structure resembling that of sorafenib, was the first systemic medication to be authorized for use as a second-line therapy[2]. Fever, chills, flu-like symptoms, excessive perspiration, blood in the stools or urine, and heavy menstrual bleeding[10].this are the side effect of regorafenib.

Cancer Immunotherapy

Although the immune system is a powerful tool for rejecting microbiological infections or transplanted organs, effective spontaneous immunologically driven cancer remissions are extremely uncommon. Much has been learned about the methods through which the immune system detects and reacts to malignancies in recent years. In many instances, the exact antigens at play have now been identified. There are upgraded adjuvant available. It is possible Oncolytic virus therapy, a promising novel therapeutic strategy for cancer treatment, has recently received increased focus and research efforts. This course of treatment entails using a genetically modified or naturally occurring virus that can destroy cancer cells by triggering the immune system, which then fights the malignant cells without causing damage to healthy organs[42]. When injected into malignant melanoma lesions, a second-generation oncolytic herpes simplex virus-type 1 with granulocyte macrophage colony-stimulating factor inhibits tumour development and progression, which has been linked to improved overall survival[43, 44].

Oncolytic virus therapy, a promising novel therapeutic strategy for cancer treatment, has recently received increased focus and research efforts. This course of treatment entails using a genetically modified or naturally occurring virus that can destroy cancer cells by triggering the immune system, which then fights the malignant cells without causing damage to healthy organs.[43] When injected into malignant melanoma lesions, a second-generation oncolytic herpes simplex virus-type 1 with granulocyte macrophage colony-stimulating factor inhibits tumour development and progression, which has been linked to improved overall survival[43, 44].

Proton Beam Therapy

In untreated individuals with localized HCC that could be treated within the irradiation field, proton beam therapy demonstrated great long-term efficacy and good safety. Our survival statistics agree with the BCLC staging report's findings. PBT may be advantageous for patients with advanced HCC that is inoperable as well as operable HCC who are at risk for complications after surgery[37].





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Modern treatment approaches using medicinal Plants**Anti-cancer Drug of Ayurveda**

The anti-cancer drugs of ayurveda have significant use in treatment of liver cancer. The list of drugs which are used in treatment of hepatocellular carcinoma has given in (Table1)

***Andrographis paniculata* (Kalmegh)**

Through stimulation of hepatic regeneration, activation of the reticuloendothelial system, and inhibition of protein biosynthesis, andrographolide, the active constituent isolated from *Andrographis paniculata*, was found to be more potent than silymarin, a standard hepatoprotective agent. It significantly increases the viability percentage of the hepatocytes and counteracts the toxic effects of paracetamol[45], CCl₄, and galactosamine[46]. By controlling the antioxidant defence system and microsomal drug metabolism, the hydroalcoholic extract of *A. paniculata* improves carcinogen detoxification. As a result, *A. paniculata*'s anti-hepatocarcinogenic capabilities are well established[46].

***Eclipta alba* (Bhringaraj)**

When rats were exposed to CCl₄-induced toxicity, the hydroalcoholic extract of *E. alba* reduced GSH depletion, controlled drug metabolizing enzymes, and restored the activity of lysosomal acid and alkaline phosphatases during hepatic injury[46, 47]. Wedelolactones from this plant source shown anti-hepatotoxic efficacy in cultured rat hepatocytes as well as substantial stimulatory effects on liver cell regeneration[46].

***Picrorrhizakurroa* (Katuki)**

As a general liver tonic for liver cleansing, liver poisoning, hepatitis, and alcohol-induced hepatotoxicity, *P. kurroa* has been used traditionally in ayurveda for millennia. Aflatoxin B₁, a potent hepatocarcinogenic mycotoxin, caused increased lipid peroxidation, and it shown antioxidant properties against this[46, 48]. Picroliv, an efficient inhibitor of hepatocarcinogenesis, decreases liver enlargement and hepatic nodular development and lowers increased bilirubin and other marker values in hepatocarcinoma circumstances brought on by N-nitrosodiethylamine[46].

***Podophyllum hexandrum* (Giriparpata)**

P. hexandrum is a powerful blood purifier, anticancer medication, and hepatic stimulant that is recommended for liver problems. By virtue of its qualities of mitotic arrest, nuclear fragmentation, and poor spindle formation dispersing the chromosomes, podophyllotoxin, its active principle, is recognised for its cytotoxic effects and affects both dividing and non-dividing cells[49]. Necrosis has been proposed as the mechanism of action and results directly from its cytotoxic effect on tumour tissues. In addition, a rapid and noticeable drop in cytochrome oxidase was also noticed in animal tumours [46].

***Tinospora cordifolia* (Guduchi)**

T. cordifolia inhibits liver fibrosis, a significant HCC consequence, by promoting hepatic tissue regeneration in rats with liver damage brought on by CCl₄ through activation of kupffer cells. When treatment for obstructive jaundice is given prior to surgery, there is a reduction in morbidity and death from sepsis and liver failure[50]. The active ingredients in *T. cordifolia* are potent immunomodulators that greatly improve both cell- and humoral-mediated immunity. It raises WBCs and bone marrow cells and encourages the proliferation of stem cells[46].

Broussonetia luzonica

The Philippines is home to an endemic strain of *B. luzonica*, often known as himbabao or alukon. The indigenous Filipinos use its leaves and blooms in veggie preparations. Edible plants provide therapeutic as well as nutritional value. *B. papyrifera* shown significant cytotoxicity against breast, lung, and liver cancer cell lines, displayed antioxidant properties, and had the capacity to inhibit specific TNF- (tumour necrosis factor-) and IgB subunits

Treatment using herbal ayurvedic formulation

It has been noted that the majority of traditional Ayurvedic medicines are used to treat liver diseases, including Jaundice, Hepatitis, Alcoholism, Cirrhosis, and Ascites problems. These goods are a blend of minerals and herbs.



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Commonly used traditional remedies for liver disorders include Draksadiarkom, Gudapippali, Kumari Asava, Punarnava Mandoora, and Udramrit Vati.

Arogyavardhinivati

It is a traditional mineral-polyherbal combination. The well-known Arogyavardhinivati's protective efficacy against CCL 4's hepatotoxic effects in rats was well proven in an experimental study[52]. AVR has demonstrated hepatoprotective activity against rats with liver damage brought on by PCM. In past investigations, the safety of Arogyavardhini Vati on the liver, kidney, and brain was assessed[4].

PhalatrikadiKwatha (PTK)

It is a useful and efficient formulation for treating patients with hepatitis B. Due to an excess of free radicals, the sick liver may benefit from protection[4].

Vasaguduchyadi Kwatha

It is a chemical formulation that is intended to treat liver conditions, particularly (jaundice) and (anemia). Up to a dose of 5 g/kg, vasaguduchyadi kwatha are comparatively safe[4].

Liv 52

Utilizing Liv.52 can assist control enzyme concentrations, maximise absorption, and enhance the liver's functional effectiveness. By defending the hepatic parenchyma and assisting in stimulating hepatocellular regeneration, it aids in restoring the liver's functional efficiency[4].

Fruits in the Treatment of HCC**Pomegranate**

One of the first known edible fruits, the pomegranate (*Punica granatum L.*) has positive impacts on health, fertility, and longevity. It has anti-inflammatory, antioxidant, antiparasitic, antibacterial, and anti-atherogenic properties. It is abundant in bioactive molecules such as anthocyanins, 3-glucosides, cyanidin, pelargonidin, ellagitannins, delphinidin 3,5-diglucosides, and other phenolic compounds, all of which have powerful antioxidant and anti-inflammatory activities. Through antioxidant pathways mediated by nuclear factor E2-related factor 2 (Nrf2), these chemicals have shown to have a notable inhibitory effect on the prevention and treatment of many cancers, including prostate cancer[10].

Apple

The primary antioxidant action of apples is attributed to polyphenols such as rutin, proanthocyanidin B2, epicatechin, catechin, chlorogenic acid, and phloretin. A naturally occurring substance called phloretin (Ph), which has glucose transporter (GLUT) inhibitory action, has been shown to have anticancer benefits. High levels of GLUT2 are expressed by human HCC and hepatoma cells, and Ph treatments led to apoptosis in HepG2 cells that was GLUT2 expression dependent. In a mouse model with xenograft liver tumours, phloretin inhibited tumour growth[10].

Strawberry and Grapes

Fisetin, a crucial bioactive molecule found in strawberries and grapes, is chemically a flavonol (3,3',4',7-tetrahydroxyflavone). Fisetin is able to normalise antioxidant enzyme levels in a rat model of HCC caused by AFB1, indicating that it may act as a tumour suppressant for HCC. A dietary flavonoid called fisetin inhibits the activity of HSF1 and prevents it from binding to the promoters of the hsp70 gene in order to exert its anticancer effects. In consequence, HSF1 inhibition reduced the production of HSP70 and BAG-3, preventing the growth of cancer cells and encouraging death[10].

Treatment using Medical Plants

The various plant possesses the different medicinal properties in their fruits, stems, barks, etc. sometime whole plant possess the medicinal properties. Natural substances that have hepatoprotective properties include *Andrographis*

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paniculata, Silymarine, Pirorrhiza, Azadirachta indica, Curcuma longa, Eclipta alba, Fumaria officinalis, Phyllanthus amarus, Phyllanthus niruri, Phyllanthus embellica, Solanum nigrum, Tinospora cordifolia, Uncariagambir[19, 21]. The list of medicinal plants are given in (Table 2)

Acanthus ilicifolius

Common name - Acanthus with holly-like leaves, sea holly, and holy mangrove.

The "Holy Mangrove," *Acanthus ilicifolius*, is widely distributed in Indian mangroves. The Indian medicinal herb *A. ilicifolius* exhibits damaging effects on the development of liver cancer cells, rheumatism, neuralgia, and poison arrow wounds (in Malaysia) and snake bites[19, 53]. Plant extract has been shown to have considerable pharmacological effects in vitro, including reducing oxidative stress, fighting cancer, treating osteoporosis, and preventing hepatic injury.[19]

Allium sativum

Common name - Lasan (English) and garlic (Hindu and Gujarat).

The rat hepato-carcinoma caused by N-nitrosodiethylamine (NDEA) was treated with *Allium sativum* or garlic oil (GO) because of its hepatoprotective effects. NDEA produces oxidative stress, which increases hepatic nodulation, serum indicators such as alkaline phosphatase, gamma-glutamyl transpeptidase, serum glutamate pyruvate transaminase (SGPT), and serum glutamate oxaloacetate transaminase (SGOT), as well as liver inflammation. Additionally, Bcl-2, Bcl-x1, and -arrestin-2 protein and mRNA levels significantly decreased while Bax and caspase-3 levels significantly increased. According to scientific studies, GO may be able to prevent NDEA-induced hepatocellular cancer by increasing antioxidant activity and inducing apoptosis[19, 54].

Artemisia princeps

Common name - Korean wormwood, Korean mugwort.

Artemisia princeps var. traditional herbal medicine from Asia. utilised to treat hepatocellular cancer is orientalis (APME). HepG2 and Hep3B cells were treated to APME to gauge its anticancer potential. Apoptosis occurred in HepG2 and Hep3B cells exposed to APME, and the cell cycle was halted[19, 30].

Artemisia vulgaris

Common name - Mugwort

Potentially acting as an anticancer agent is *Artemisia Vulgaris*. Methanolic leaf extract was tested in vitro against HepG2 cells to see if it had any apoptotic effects that would work well with etoposide and a common chemotherapy medication. Recent research on *Artemisia* species has isolated coumarins, monoterpenes, sterols, sesquiterpenes, sesquiterpene lactones, flavonoids, and polyacetylenes. In vitro and in vivo studies on the anticancer activity of many *Artemisia* species have been reported. Previous studies on select *Artemisia* species have indicated that most species possess therapeutic qualities like antibacterial and anti-cancer effects[19, 55].

Amorphophallus campanulatus

Common name - Elephant foot yam

Amorphophallus campanulatus is a tuber crop grown in the Indian plains for its edible corm (bulb). It originates from South East Asia and is a member of the Araceae family. Its common name is elephant foot yam. *A. campanulatus* tuber is mostly employed in Ayurvedic preparations and is approved to treat piles disorders caused by vitiated blood and rheumatic swellings, as well as liver infections, elephantiasis, bronchial infections, asthma, abdominal pain, dysentery, and spleen enlargement. The methanolic tuber extract of *A. campanulatus* (ACME) has hepatoprotective and strong antioxidant properties.[19, 56]

Broussonetia luzonica

Common name - Himbabao or Alukon

Philippines-endemic *Broussonetia luzonica* is also known as himbabao or alukon. Local Filipinos consume its leaves and flowers as ingredients in vegetable dishes. However, further studies on the *B. spec.* of *Broussonetia*, the other



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species, have been conducted. *Papyrifera* exhibited a number of biologic processes. *B. Papyrifera* also showed anti-oxidant properties and shown substantial cytotoxicity against breast, lung, and hepatocellular cancer cell lines. The leaves of *B. indica* were extracted with ethyl acetate. *Luzonica* may be effective in combating hepg2 cell lines.[19, 57]

Dracocephalum kotschy

Common name - No common name has been associated with this taxon yet

One of the Labiatae family's fragrant and therapeutic plants, *Dracocephalum kotschy* is found naturally in mountainous and high terrain. A native Iranian medicinal plant is *D. kotschy*. -pinene, neral, geraniol, -citral, terpinene-4-ol, linalool, carveol, myrcene, germacrene-D, isopinocarveol, and -terpineol were the main ingredients identified in the essential oil. Due to its antispasmodic and analgesic qualities as well as effects on the mitochondria of liver cancer cells, *D. kotschy* has been utilised for a number of years in Iranian folk medicine.[19, 58]

Graptopetalum paraguayense

Common name -Mother-of-pearl-plant and Ghost plant.

Graptopetalum paraguayense (GP), a traditional Chinese herb, has several health benefits, including the improvement of hepatic infections, blood pressure irregularities, skin whitening, pain relief, infection treatment, inflammation suppression, and cognitive function recovery. According to Chen et al. (2008), when GP stem extracts are grown with the HCC HepG2 cell line, they demonstrate antioxidant and anti-proliferative effects. Their aqueous extracts also have antioxidative and anti-inflammatory properties. Because GP extracts and HH-F3 (isolated active GP extract components) have been shown to exponentially reduce cancer growth, HH-F3 is thought to be responsible for treating HCC.[19, 59]

Nigella sativa

Common name Black seed or black cumin

The Ranunculaceae family includes *Nigella sativa* (NS), often known as black seed, black cumin, or "Al-Habba Al-Sauda" or "Habbet Al-Barakah" (in Arabic). The thymoquinone (TQ), α -cymene, carvacrol, α -anethole, and 4-terpineol are the active components of NS. There is hepatoprotective action in *nigella sativa*. [19, 60]

Petasites japonicas

Common name - Japanese butterbur

The *Petasites japonicus* (PJ) plant is cultivated in Eastern Asia and used as a vegetable and traditional medicine. A PJ methanolic extract of roots showed cancer-suppressing efficacy toward Hep3B, according to literature review. In both in vitro and in vivo settings, PJE reduces the growth of HCC cells by inhibiting the Akt/mTOR and Wnt signalling pathways. The Akt/ mTOR and Wnt signalling pathways, which are suppressed by PJE in Hep3B cells, are responsible for the proliferation and survival of HCC cells.[19, 61]

Silybum marianum

Common name - *Cardus marianus*, Milk thistle, Marian thistle and Mary thistle etc.

For many centuries, *Silybum marianum* (thistle plant) has been regarded as a "hepatic elixir." The Milk Thistle plant (*Silybum marianum*), exert effects on liver to inhibit oxidative stress. Silymarin offers hepato-protective effects by reducing ROS free radicals and lipid peroxidation and maintaining redox status. Because it inhibits oxidative stress and proliferation, the bioactive component silibin found in *S. marianum* has been described as an antioxidant, hepatoprotective, and antitumorogenic effects both in vitro and in vivo.[19, 62]

CONCLUSION

The hepatocellular carcinoma is one of the biggest problems in world wide. The cancer management and their treatment are the difficult task in front of the us. The recent therapies like virus therapy and the immunomodulatory therapy with the systemic approaches it can be cure. The flavonoid containing drugs can be used the treatment of



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hepatocellular carcinoma. The late diagnosis and the different pathways make the disease complicated. The hepatocellular carcinoma can be prevented if it diagnosed early. Preventive measures such hepatitis B vaccination lifestyle changes, alcohol restriction, and HCC surveillance may somewhat lower the incidence of HCC. The pathways-oriented treatment is necessary and the for the early diagnosis there should be more research is needed. In addition to this there should the change in the lifestyle that will effect on the prevention of it. The present therapy strategy also requires a lot of work. A high rate of illness recurrence makes it difficult for treatments to give an initial cure, and there is a need for efficient adjuvant medicines.

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Table 1 - Anti-Cancer drugs of Ayurveda for treatment of Hepatocellular Carcinoma

| Drugs | Name | Action/Properties | Reference |
|------------------------------|---|--|-----------|
| Anti-cancer Drug of Ayurveda | <i>Andrographis paniculata</i> (Kalmegh) | Stimulation of hepatic regeneration, activation of the reticuloendothelial system, and inhibition of protein biosynthesis | [44-45] |
| | <i>Eclipta alba</i> (Bhringaraj) | Reduced GSH depletion, controlled drug metabolizing enzymes, and restored the activity of lysosomal acid and alkaline phosphatases during hepatic injury | [45-46] |
| | <i>Picrorrhiza kurroa</i> (Katuki) | Efficient inhibitor of hepatocarcinogenesis, decreases liver enlargement and hepatic nodular development and lowers increased bilirubin | [45-47] |
| | <i>Podophyllum hexandrum</i> (Giriparpata) | Blood purifier, anticancer medication, and hepatic stimulant Drop in cytochrome oxidase | [48] |
| | <i>Tinospora cordifolia</i> (Guduchi) | Promoting hepatic tissue regeneration, Potent immunomodulators | [49] |
| | <i>Broussonetia luzonica</i> | Inhibit specific TNF- (tumor necrosis factor-) and IG-b subunits (IL1B) | [50] |

Table 2 – Medicinal Plants for treatment of Hepatocellular Carcinoma

| Plants | Name | Action/Properties | Reference |
|------------------------------|------------------------------------|--|-----------|
| Medical Plants for treatment | <i>Acanthus ilicifolius</i> | reducing oxidative stress, fighting cancer, treating osteoporosis, and preventing hepatic injury | [19,52] |
| | <i>Allium sativum</i> | increasing antioxidant activity and inducing apoptosis | [53] |
| | <i>Artemisia princeps</i> | Apoptosis | [30] |
| | <i>Artemisia vulgaris</i> | apoptotic effects | [54] |
| | <i>Amorphophallus campanulatus</i> | hepatoprotective and strong antioxidant properties | [19,55] |
| | <i>Broussonetia luzonica</i> | anti-oxidant properties and shown substantial cytotoxicity | [56] |
| | <i>Dracocephalum kotschy</i> | antioxidant | [19,57] |
| | <i>Graptopetalum paraguayense</i> | antioxidative and anti-inflammatory | [58] |
| | <i>Nigella sativa</i> | hepatoprotective | [19,59] |
| | <i>Petasites japonicas</i> | inhibiting the Akt/mTOR and Wnt signalling pathways. | [19,60] |
| | <i>Silybum marianum</i> | inhibit oxidative stress reducing ROS free radicals and lipid peroxidation, antioxidant | [61] |





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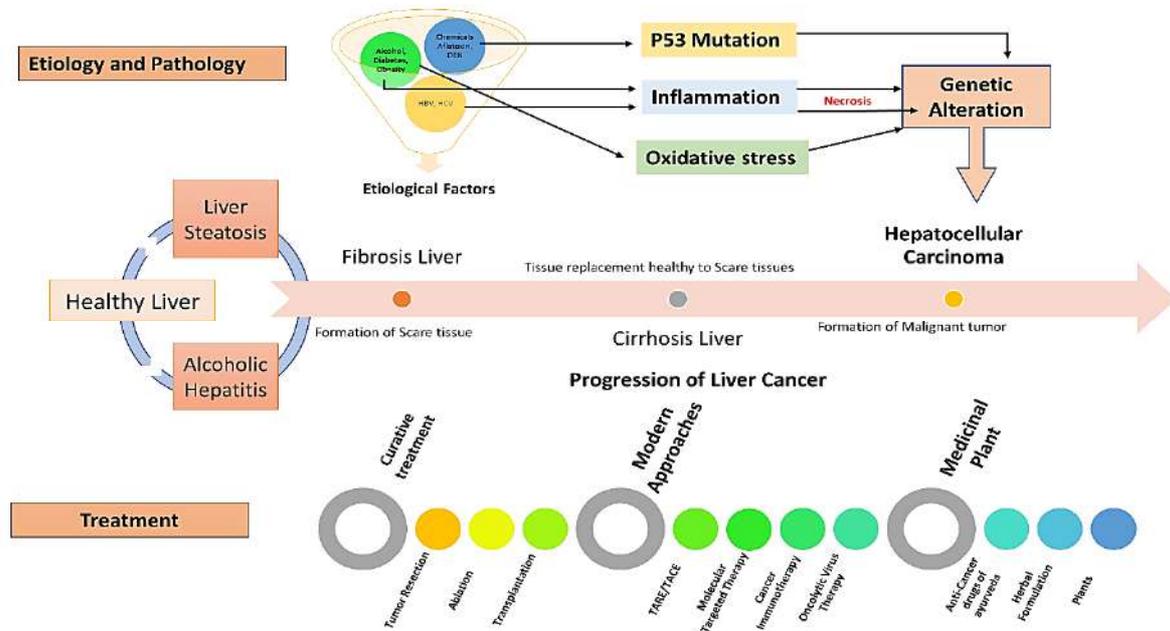


Fig 1 – Etiology, pathology and Treatment aspects of Hepatocellular Carcinoma[11, 19, 41]





A Disease Detection for Tomato Leaves using Machine Learning with Google Colab

Divisht Jaiswal^{1*}, Ashok Kumar², Rajesh Singh² and Sanjay Joshi²

¹M.Tech Scholar, Department of IT, GBPUA and T Pantnagar, US Nagar, Uttarakhand - 263145, India.

²Assistant Professor, Department of IT, GBPUA and T Pantnagar, US Nagar, Uttarakhand-263145, India.

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*Address for Correspondence

Divisht Jaiswal

M.Tech Scholar,
Department of IT,
GBPUA and T Pantnagar,
US Nagar, Uttarakhand - 263145, India.
E.Mail: ashu.gbpec@gmail.com



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ABSTRACT

Effective disease detection in tomato plants is essential for maintaining crop health and optimizing yield. This paper based on a disease detection system for tomato leaves using Google Colab, an online development environment, and incorporates an alert generation mechanism to notify farmers using Telegram, a popular messaging platform. The study aims to leverage machine learning techniques to accurately classify healthy and diseased tomato leaves based on leaf images. A dataset of tomato leaf images, comprising both healthy and diseased samples, was collected and preprocessed. Utilizing TensorFlow and Convolutional Neural Networks (CNNs) within the Google Colab environment, a model was trained to identify specific patterns and features indicative of different diseases. Upon successful training, the model was implemented to classify incoming leaf images into healthy or diseased categories. When a disease is detected, an alert is automatically generated using Telegram, providing farmers with timely information about the presence of diseases in their tomato crops. This allows for prompt intervention and preventive measures, reducing potential crop losses and improving overall plant health. Experimental results demonstrate the effectiveness of the proposed approach, achieving accurate disease classification and timely alert generation. The integration of Google Colab and Telegram offers a user-friendly and accessible solution for disease monitoring in tomato plants. By combining machine learning with instant alert mechanisms, this system provides a valuable tool for farmers, enabling them to make informed decisions and take immediate action to mitigate the spread of diseases.

Keywords: Disease Detection, Tomato leaf disease, Machine learning for disease detection, Alert system, Google colab for plant disease analysis



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INTRODUCTION

We cannot exist without plants because they provide us with nourishment and protect us from harmful radiation. No life is imagined without plants. The tomato is a nutritious plant that is a popularly grown edible vegetable [1]. An estimated 160 million tonnes of tomatoes are consumed annually worldwide [2]. The tomato is regarded as a source of revenue for farm households and makes a substantial contribution to the reduction of poverty [3]. One of the most nutrient-dense crops in the world, tomatoes have a big influence on the agricultural economy through production and cultivation. The tomato is nutrient-rich, but it also has pharmacological qualities that can help prevent conditions including hypertension, hepatitis, and gingival bleeding [1]. As a consequence of its extensive use, tomato need is also rising. Over eighty percent of agricultural output is produced by small farmers, yet roughly fifty percent of their crops are lost to diseases and pests. It is vital to do research on field crop disease diagnostics since parasitic insects and diseases are the main variables affecting tomato growth. Pest and pathogen manual identification is time-consuming and expensive. Therefore, it is essential to offer farmers automated AI image-based solutions. They use image processing, a smart picture identification technique that boosts image recognition effectiveness while lowering costs and enhancing recognition accuracy [3].

A suitable treatment must be used to address the problem of leaf disease [4,5]. Controlling tomato disease is a difficult procedure that constantly accounts for a sizable portion of seasonally incurred production costs [6,7,8,9]. Diseases of the vegetables are common and include bacteria, late mildew, leaf spot, tomato mosaic, and yellow curvature. Their negative effects on plant growth result in decreased product quality and quantity [10]. 80–90% of plant diseases, according to prior studies, manifest themselves on leaves [11]. It takes a lot of time to locate the farm and identify the many disease strains with afflicted plants. Farmers' assessments of the kind of plant disease may be inaccurate. This choice can result in the plant implementing insufficient and ineffective defenses. Early identification can minimize loss risk, lower processing costs, and lessen the adverse environmental effects of chemical inputs [12,13,14]. The major goal is to emphasize the lesion further than other picture regions. Picture contrast, greyscale alteration, picture scaling, image cropping, and image filtering are a few pre-processing processes [15,16,17]. The partition of an image to objects is the following phase. These items are utilised to identify areas of interest in the image as infected regions [18].

Identifying which class the sample belongs to is the next stage in the classification process. The procedure's input variables are then examined, either one or several at a time. The technique is occasionally used to identify a certain input type. The most difficult classification problem is without a doubt increasing classification accuracy. Finally, datasets different from the training set are created and validated using the actual data. Moreover, the integration of the disease detection model with alert generation systems allows for real-time notifications and updates to be sent to farmers. One prominent platform for such alert generation is Telegram, an instant messaging platform that facilitates the creation of programmable bots. Through the integration of the disease detection model with Telegram, farmers can receive immediate alerts and information regarding the presence of diseases in their tomato plants, empowering them to take swift actions to manage and mitigate the spread and impact of these diseases. The remainder of the paper is structured as follows: The second part reviews the current body of work. After that, Section 3 describes the procedure and material method. After that, Section 4 explains the analysis of the results. Section 5 serves as the discussion.

RELATED WORK

Numerous researchers have built automated disease detection systems using various technology. These employ extremely precise techniques to detect plant illness in tomato leaf tissue. With 94–95% accuracy, a previously trained network model has been presented for identifying and categorizing tomato disease [19,20]. With a dataset of 300 photos, the Tree Identification Model and Segmentation are utilized to identify and categorize six different forms of tomato leaf disease [21]. With a success rate of 93.75%, a method has been put out to identify and categorise plant leaf disease [22]. Better detection and classification of plant leaf disease is now possible thanks to computational





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technology and classification algorithms [23]. Here, sample data is gathered and split into fifty percent healthy and fifty percent unhealthy groups using an 8-megapixel smartphone camera. Three steps make up the image processing process: enhancing comparison, segmenting, and extracting features. A multi-layer neural network with feed-forward function is used to execute classification procedures, and two different network architectures are contrasted. The outcome was superior to that of the Radial Basis Function (RBF) and Multilayer Perceptron (MLP) networks. The search categorises the plant blade's image as healthy or sick; it is unable to identify the disease's type. Colour space analysis, colour time, histogram, and colour coherence were utilised to diagnose and classify leaf diseases with an accuracy of 87.2% [24]. Using a frame size of 13,262, illnesses impacting tomato crops have been identified using AlexNet and VGG 19 models. To obtain 97.49% precision, the model is utilised [25]. With a 95% accuracy rate, the transfer learning and CNN Model is utilised to identify illnesses affecting dairy crops. The accuracy of a neural network that used transfer learning as a deep learning algorithm based on AlexNet to identify and categorise tomatoes plant leaf conditions was 95.75 percent [27,28]. The first convolution layer's kernel size has been modified to 11 11, and the Network Activation function for comparability has been changed to Leaky-ReLU. After numerous iterations, the model identifies the class of diseases with a success rate of 98.30% and an accuracy of 98.0% [29]. To identify and categorise tomato leaf disease, a streamlined eight-layered CNN model was developed [30]. The circumstances of a tomato plant were determined using a straightforward CNN model with eight hidden layers. In comparison to other traditional models, the suggested strategies produce the best results [32,33,34,35].

METHODS AND METHODOLOGY

Detecting diseases in tomato leaves using machine learning involves a combination of data collection, preprocessing, feature extraction, model training, and disease prediction. Here is methodology for disease detection in tomato leaves using machine learning:

Dataset Collection

A comprehensive dataset of tomato leaf images is collected, encompassing both healthy leaves and leaves affected by various diseases. This dataset serves as the foundation for training and evaluating the disease detection model.

Image Preprocessing and Labeling

The raw images that were to be analyzed by the CNN classifier were altered or improved before to training the model. It is necessary to examine both the network's design and the input data's format in order to build a good model. In order for the suggested model to extract the appropriate features from the image, we pre-processed our dataset. The image was first resized to 256 256 pixels in order to normalize its size. After that, the photos were made grey. The explicit acquisition of the training data features necessitates an enormous amount of training data at this stage of pre-processing. The next stage was to sort the photographs of tomato leaves into different categories before labeling each image with the appropriate illness term. Five classes were present in the dataset's test collection and training in this particular scenario.

Training Dataset

The initial step in processing the current dataset was to prepare the dataset. This step involved entering image data into the Convolutional Neural Network procedure, which eventually created a model that evaluated performance. Figure illustrates the normalization procedures used on tomato leaf pictures. Figure 1 displays the normalization procedures on tomato leaf pictures.

Convolutional Neural Networks

CNN is a type of neural network technology that is frequently used today to train or analyse visual data. Convolution's matrix format is intended to filter the images. The Convolution Neural Network used for data training. The input test data in each layer can be mapped to a number of calculations.





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Convolution Layer

A convolutional layer is a fundamental building block of convolutional neural networks (CNNs), a type of deep learning architecture commonly used for image and video processing tasks. Convolutional layers are designed to automatically learn and extract meaningful features from input data. In a convolutional layer, the input is typically a multidimensional array or tensor, often representing an image or a feature map from a previous layer. The layer consists of a set of filters, also known as kernels or convolutional kernels. Each filter is a small window or matrix of weights that is applied across the input using a convolution operation.

The formula for a 2D convolutional layer can be described as follows:

Output width = $((\text{Input width} - \text{Filter width} + 2 * \text{Padding}) / \text{Stride}) + 1$

Output height = $((\text{Input height} - \text{Filter height} + 2 * \text{Padding}) / \text{Stride}) + 1$

Number of filters in the layer = Number of output channels

Where:

- Input width and height refer to the dimensions of the input feature map.
- Filter width and height represent the dimensions of the filter/kernel.
- Padding refers to the number of additional border pixels added around the input. Padding is usually used to ensure that the spatial dimensions of the output feature map are the same as the input feature map.
- Stride represents the step size used when sliding the filter over the input. A stride of 1 means the filter moves one pixel at a time, while a stride of 2 means the filter moves two pixels at a time.
- Number of output channels refers to the number of filters applied in the layer, which determines the depth of the output feature map.

The convolution operation is performed by sliding the filter over the input feature map, computing the element-wise multiplication between the filter weights and the corresponding input values within the receptive field, and summing up the results. This process is repeated for each location in the input, producing a 2D activation map for each filter.

Pooling Layer

A pooling layer is a common component in convolutional neural networks (CNNs) that follows one or more convolutional layers. The pooling layer reduces the spatial dimensions (width and height) of the input feature maps, thereby decreasing the computational requirements and providing a form of spatial invariance. The pooling operation aggregates information from local regions of the input, capturing the most prominent features.

Max pooling divides the input feature map into non-overlapping regions (often squares) and outputs the maximum value within each region.

The formula for max pooling is as follows:

Output width = Input width / Pool size

Output height = Input height / Pool size

The number of channels remains the same as the input where:

- Input width and height represent the dimensions of the input feature map.
- Pool size refers to the size of the pooling window.
- The number of channels remains unchanged since max pooling operates independently on each channel.

The pooling layer's primary purpose is to reduce the spatial dimensions of the input, reducing the model's sensitivity to translations and spatial variations



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In practice, pooling layers are often inserted between convolutional layers to progressively down sample the feature maps, reducing the spatial dimensions while preserving the essential features. This down sampling also helps reduce the number of parameters in the subsequent layers and control over fitting.

Fully Connected Layer

A fully connected layer, also known as a dense layer, is a type of layer commonly used in convolutional neural networks (CNNs) for tasks like image classification, object detection, and semantic segmentation. Unlike convolutional and pooling layers, which operate on local patches of the input, fully connected layers connect every neuron in the previous layer to every neuron in the current layer.

The formula for a fully connected layer is as follows:

Output size = Neurons in layer

Each neuron in the fully connected layer receives input from all the neurons in the previous layer. The input to each neuron is multiplied by a weight, and the results are summed up along with a bias term. This process is repeated for every neuron in the fully connected layer, resulting in a vector of outputs.

The output of a fully connected layer can be computed using the following formula:

Output = Activation($W * Input + B$)

Where:

- Input represents the input vector from the previous layer.

The weight matrix, or W , contains two dimensions: the Neurons in layer

- At the moment and the before. The weights linking the neurons of the present layer to the neurons of the preceding layer are represented by each row of the weight matrix.
- B is the bias vector with dimensions (Number of neurons in the current layer). Each element of the bias vector is added to the corresponding weighted sum.

Activation is an activation function that introduces non-linearity to the output of the fully connected layer. Common activation functions used in fully connected layers include ReLU (Rectified Linear Unit), sigmoid, and tanh. The fully connected layer is typically the last layer in CNN architecture and is responsible for mapping the high-level learned features from the convolutional layers to the desired output format, such as class probabilities or regression values. The weights and biases of the fully connected layer are learned during the training process using gradient-based optimization algorithms, adjusting them to minimize a predefined loss function. The output of the preceding layers (often convolutional and pooling layers) is frequently flattened or reshaped into vector data before being fed into the fully connected layer since the fully linked layer requires a fixed-size input.

Dropout

Dropout is a regularization technique commonly used in convolutional neural networks (CNNs) to prevent overfitting and improve generalization performance. It randomly "drops out" a fraction of the neurons in a layer during training, forcing the network to learn more robust and diverse representations. The dropout technique works by setting a fraction of the neuron activations to zero at each training iteration. The fraction of neurons to be dropped out is determined by a hyperparameter called the dropout rate, typically set between 0.2 and 0.5. During inference or testing, all neurons are used, but their activations are scaled down by the dropout rate to compensate for the higher number of active neurons.

The formula for applying dropout is as follows:

Output = Dropout(Input, Dropout rate)





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Where:

Input refers to the input tensor or activation map to the dropout layer.

Dropout rate is the probability of dropping out a neuron, typically ranging from 0.2 to 0.5.

Dropout is a stochastic operation that sets a fraction of the input activations to zero. The neurons to be dropped out are selected randomly at each training iteration.

Dropout helps in reducing overfitting by preventing the network from relying too heavily on a specific subset of neurons. By randomly dropping out neurons, dropout forces the network to learn more robust features that are not overly dependent on any single neuron. It effectively simulates an ensemble of multiple subnetworks, where each subnetwork is trained with different subsets of neurons being active. This ensemble behavior improves the model's generalization ability, as it learns to make accurate predictions even when some neurons are missing. Dropout can be applied to any layer in a CNN, including fully connected layers, convolutional layers, and recurrent layers. It is most commonly applied after the activation function in fully connected layers or as a masking layer in convolutional layers. Dropout has been shown to be particularly effective in deep neural networks, where overfitting can be a significant challenge.

Performance evolution matrices

Performance evaluation metrics in CNNs are used to assess the accuracy and effectiveness of the model's predictions. Here are some commonly used performance evaluation metrics with their formulas:

Accuracy:

Accuracy measures the overall correctness of the model's predictions, i.e., the percentage of correctly classified samples in the test set.

Formula: Accuracy = (Number of correctly classified samples) / (Total number of samples)

Precision:

Precision measures the model's ability to correctly identify positive samples out of the total samples predicted as positive. It focuses on minimizing false positives.

Formula: Precision = (True Positives) / (True Positives + False Positives)

Recall (sensitivity, or genuine positive rate):

Recall measures the model's ability to correctly identify positive samples out of the total positive samples. It focuses on minimizing false negatives.

Formula: Recall = (True Positives) / (True Positives + False Negatives)

F1 Score: The F1 score provides a balanced assessment of the model's efficiency by combining accuracy as well as recall into a single statistic. It is the precision and recall harmonic mean.

F1 Score is calculated as follows: $2 * ((\text{Precision} * \text{Recall}) / (\text{Precision} + \text{Recall}))$.

Specificity (also known as True Negative Rate):

Specificity measures the model's ability to correctly identify negative samples out of the total negative samples. It focuses on minimizing false positives.

Formula: Specificity = (True Negatives) / (True Negatives + False Positives)

Area under the ROC Curve (AUC-ROC):

AUC-ROC measures the model's ability to discriminate between positive and negative samples across different classification thresholds. It provides an aggregate measure of performance, regardless of the chosen threshold.

Formula: AUC-ROC is calculated by plotting the True Positive Rate (TPR or Recall) against the False Positive Rate (FPR) at various classification thresholds and calculating the area under the curve.



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Mean Average Precision (mAP):

mAP is commonly used in object detection tasks and evaluates the average precision of the model across multiple classes or categories.

Formula: mAP is the mean of Average Precision (AP) values calculated for each class. AP is calculated by plotting precision against recall for various classification thresholds and calculating the area under the precision-recall curve.

Steps in the Proposed Algorithm for Detecting Disease

Step 1: Input colour for the IRGB image of the leaf that was obtained via the Plant Village dataset in step one.

Step 2: Using CNN-based segmentation to create the mask Mveq from the input IRGB.

Step 3: To obtain Mmask, encrust IRGB with Mveq.

Step 4: Create smaller areas Ktiles (square tiles) within the image Mmask.

Step 5: Consider Ktiles of Mmask into Tomato in step five.

Step 6: The leaf component used to diagnose illness is Ktiles.

Step 7: Come to a complete stop.

The inputted picture IRGB from the multiple classes dataset serves as the foundation for disease detection. Using CNN, the mask is segmented Mveq after input picture IRGB. Ktiles has separated the mask image into many regions. The area of Interest (RoI) is then chosen, and the same process is utilized to identify leaf disease.

Set up a Telegram Bot

Create a Telegram account.

Search for the "BotFather" in Telegram and start a conversation.

Follow the instructions to create a new bot and obtain the API token for the bot.

Install Required Libraries:

In Colab notebook, install the python-telegram-bot library using the following command:

```
!pip install python-telegram-bot
```

Import Libraries:

Import the necessary libraries in Colab notebook:

```
import telegram
```

Set Up Telegram Bot Instance:

Create an instance of the Telegram Bot using the API token obtained from the BotFather:

```
bot = telegram.Bot(token='YOUR_API_TOKEN')
```

Define Alert Function

Define a function that takes the message or alert text as input and sends it to the farmers via Telegram. You can use the bot.send_message method to send messages to the users or a specific chat/group:

```
def send_alert(message):  
    chat_id = 'CHAT_ID' # Replace with the chat ID of the farmers or group  
    bot.send_message(chat_id=chat_id, text=message)
```

Trigger Alerts

Depending on your disease detection algorithm, when a disease is detected in tomato leaves, you can call the send_alert function and provide the appropriate alert message.

```
# Disease detected
```

```
alert_message = "Disease detected in tomato leaves. Please take necessary action."
```

```
send_alert(alert_message)
```



**Divisht Jaiswal et al.,****Obtain Chat ID:**

To send alerts to specific farmers or groups, you need to obtain their chat IDs. One way to do this is by creating a dedicated Telegram group for the farmers and adding the bot to the group. Then, use the following code snippet to obtain the chat ID:

def get_chat_id():

```
updates = bot.get_updates()
chat_id = updates[-1].message.chat_id
return chat_id
```

Execute the get_chat_id function after the bot has been added to the group, and it will return the chat ID to be used in the send_alert function.

RESULT ANALYSES

On Google Colab, the entire experiment was carried out. In the following, the outcome of the suggested strategy is discussed along with examples using test epochs and learning rates. Epoch 5 was used in this study however, learning rates were just 0.001. The contrast of accuracy and loss (training and validation)

CONCLUSION AND DISCUSSION

The integration of technology, specifically Google Colab and Telegram, offers a promising solution for disease detection in tomato leaves and timely alert generation for farmers. By leveraging the power of deep learning and cloud-based infrastructure, researchers and developers can build accurate and efficient disease detection models using Google Colab. The use of pre-trained convolutional neural network (CNN) architectures enables transfer learning, allowing the model to leverage knowledge learned from vast datasets like ImageNet. Through the collection of a comprehensive dataset and data augmentation techniques, the model can learn to differentiate between healthy and diseased tomato leaves with high accuracy. The training and evaluation process ensures the model's performance is optimized, providing reliable disease detection results. By integrating the trained model with Telegram, farmers receive real-time alerts and notifications regarding the disease status of their tomato plants. The Telegram bot acts as a bridge, enabling farmers to easily upload images of tomato leaves for analysis. The disease detection model deployed on Google Colab processes these images and promptly sends alerts to farmers, empowering them to take swift and informed actions to mitigate disease spread and manage their crops effectively.

The article proposed a deep learning model for identifying and categorising leaf diseases on tomato plants. It also took into account morphological characteristics of the plant, such as colour, texture, and leaf margins. Standard profound learning models with variations were introduced in this article. This article covered biotic illnesses brought on by bacterial and fungal pathogens, notably tomato leaf blight, bacterial spot, and septoria leaf spot. The accuracy of the proposed model's detection rate was 89.42%. The suggested model was contrasted with versions of VGG and ResNet using the same dataset. The suggested method for locating tomato illness is a novel idea.

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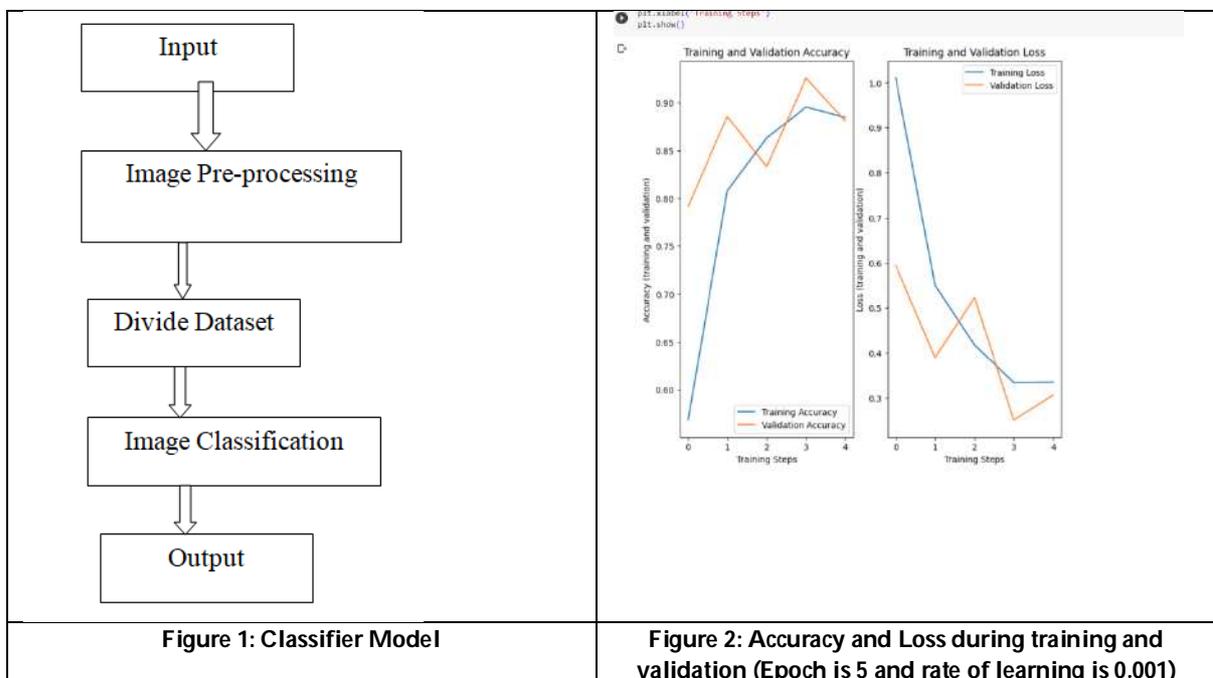
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Comparison of Nutrient Intake and Sleep Status among Selected Early Adults

K.Aswini¹, N. Preetha^{2*} and A.J. Hema Malini³

¹PG Student, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai - 600116, Tamil Nadu, India.

²Assistant Professor, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Chennai - 600116, Tamil Nadu, India.

³Professor and Head, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai - 600116, Tamil Nadu, India

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*Address for Correspondence

N. Preetha

Assistant Professor,
Department of Clinical Nutrition,
Sri Ramachandra Faculty of Allied Health Sciences,
Sri Ramachandra Institute of Higher Education and Research (DU),
Chennai - 600116, Tamil Nadu, India.



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ABSTRACT

Early adults often undergo sleep fragmentation and sleep deprivation due to physical and psychological problems. The reduced sleeping time affects the dietary intake, weight gain and performance of the studies. Some studies have investigated relationships among sleep disturbance and nutrient intake in late adults. The lack of research in the relationship between sleep status and nutrient intake among other age groups motivated the present study. To study the association between sleep quality and nutritional status of early adults. Comparative study design was adopted to find an association of sleep status and nutrient intake in early adults. This study was conducted among 70 early adult subjects who were divided into two groups Group 1- normal sleepers (≥ 19 to ≤ 36), Group 2-people with sleep disturbance (≥ 1 to ≤ 18). Sleep score was obtained by using the sleep quality questionnaire, BMI and three day dietary recall was used to assess the nutritional status. From the study, it was observed that the nutrient intake among good quality sleepers is better when compared to poor quality sleepers. Higher number of good quality sleepers met more than 50 % of the nutrients requirement but their vitamin B6 intake was low. This study concludes that there is positive correlation between nutrient intake and sleep status. People with poor quality sleep were counseled on the importance of good sleep and its correlation with



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nutritional status. Therefore this study aimed to create a better insight among early adults in the relationship between nutrition and sleep.

Keywords: nutrient, sleep, study, adults, vitamin

INTRODUCTION

Sleep is a highly complex state that arises from an interaction between multiple brain regions, neurotransmitter pathways, and hormones. Small changes in brain function can have a big effect on sleep, and disrupted sleep leads to many health problems (1). Disrupted sleep is closely linked to an increased susceptibility to a broad range of disorders includes, reduced motivation, depression, insomnia, metabolic abnormalities, obesity, immune impairment, and even a greater risk of cancer(2). Disturbances in sleep status generally described as difficulty in falling asleep, waking up frequently during the night with difficulty returning back to sleep, waking up too early in the morning, and un-refreshing sleep are collectively referred to as insomnia. Good quality sleep is important for optimal neurocognitive and psychomotor performance as well as physical and mental health. In general, following four fundamental sleep characteristics influence academic performance; sleep quality, sleep quantity, sleep regularity and sleep phase scheduling(3). Among college-aged students, one of the most common causes of daytime sleepiness is sleep deprivation, i.e. students get inadequate sleep because they go to bed late and wake up early(4). Frequent use of cell phones around bedtime is associated with difficulties falling asleep, repeated awakenings, or waking up too early(5). The effect of nutrition on sleep status has been recognized recently. Vitamin B deficiency results in poor sleep status by shortening the length of the sleep-wake rhythm and affects the circadian aspect of sleep propensity (6).

MATERIALS AND METHODS

The sample size was fixed to 70 by statistical consultant using sample size calculation. Both genders 70 early adult from the age group of 19-23 years were included and subjects consuming regular medication for any medical or psychiatric problem were excluded. The study was conducted for a period of four months from July 2019-october 2019. The subjects of the study were the students of Sri Ramachandra institute for higher education and research (Deemed to be university). Written consent was obtained from the study participants and the details and the purpose of the study was explained. The proforma was used to collect the demographic data such as name, age, sex, type of diet(non vegetarian/vegetarian). Details of anthropometric parameters, such as Height, Weight and Body Mass Index of the subjects were documented. Nutrient intake was assessed using three day dietary recall (two week days and one week end) method for macro nutrient Calorie, Protein, Fat, Carbohydrate and micro nutrients Vitamin B2, Vitamin B6, Vitamin B9, Calcium and Iron. The intake was collected through telephone. The intake were compared between the subjects and categorized into two groups on sleep score obtained by the sleep quality questionnaire with the interpretation as Group 1- normal sleepers(≥ 19 to ≤ 36), Group 2-people with sleep disturbance(≥ 1 to ≤ 18). The data collected was subjected to statistical analysis such as calculation of percentage, arithmetic mean, standard deviation and analysis using 't' test. Institutional Ethical Committee For Students of Sri Ramachandra Institute of Higher Education and Research (Deemed to be University) has approved the project.

RESULTS AND DISCUSSION

A total number of 70 early adults were divided into two groups, group 1 (n= 35) belonged to good sleep quality category and their sleep quality questionnaire score ranges from ≥ 19 to ≤ 36 and group 2 (n=35) belonged to poor sleep quality category and their sleep quality questionnaire score ranges from ≥ 1 to ≤ 18 . Sleep deprivation can arise from poor sleep behaviors; sleep hygiene encourages habits conducive to restorative sleep and avoidance of substances or





behaviors. Good sleep hygiene includes a regular sleep–wake schedule, quiet sleep environment, and avoidance of caffeine after lunch and stimulating activities before bed (6). It was found that, 14% subjects belonging to age 19 years, in age 20 years 26%, in 21 years 37%, in 22 years 23% and none (0%) in 23 years belonged to poor quality sleep category. Majority of the subjects belonging to age 21 years (37%) was found to have poor quality of sleep (figure 1). Majority of the subjects enrolled for study were female. It was found that 29% male and 71% female belonged to good quality sleep, 23% male and 77.1% female belonged to poor quality sleep (Figure 1). It was found that among the good sleepers 71.4% and 80% of the subjects in poor quality were non vegetarian. According to (7), inadequate sleep hygiene is common, as college students often use technology and substances that compromise sleep quality and quantity. This chronic sleep deprivation may impair academic performance, mood regulation, and driving safety.

It was found that the BMI of, 31.4% subjects in good quality and 14.3% subjects in poor quality sleep were found to be in underweight ($< 18.4 \text{ kg/m}^2$) category, 42.9% subjects in good quality and 60% subjects in poor quality sleep were found to be in normal weight (≥ 18.5 to $\leq 24.9 \text{ kg/m}^2$) category, similarly 20% in good quality and 22.9% in poor quality sleep category subjects BMI were found to be overweight (≥ 25 to $\leq 29.9 \text{ kg/m}^2$), also six percent in good quality and three percent in poor quality sleep category subjects BMI were found to be obese ($\geq 30 \text{ kg/m}^2$) (Table 1). Based on Sleep Quality Questionnaire Score, it was found that 17.1% of subjects belonged to severe sleep problem, 83% of the subjects belonged to moderate sleep problem, 34.3% of the subjects belonged to good sleep, 66% of the subjects belonged to great sleep. (8) concluded that both short and long time spent in bed and poor sleep quality are associated with overweight/obesity status in young adults.

In the study, for both good quality and bad quality sleepers, their consumption of macro and micro nutrients were compared with the RDA. The above table 2 represents the association between Energy, Protein, Fat, Carbohydrate, Vitamin B2, Vitamin B6, Vitamin B9, Iron, Calcium intake and sleep status among early adult subjects. The percentage of macro nutrients includes Energy, Protein, Fat, Carbohydrate and following micro nutrients Vitamin B2, Vitamin B6, Vitamin B9, Iron, Calcium intake of good quality sleepers-Male were found to be 85.6, 96.5, >100 , >100 , 84.2, <10 , 41.5, >100 , 88 respectively and good quality sleepers-Female were found to be 98.7, >100 , >100 , >100 , 95, <10 , 39.3, 91, 87 respectively.

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While considering the comparison of RDA and percentage of consumption of poor sleep quality-Male subjects Energy, Protein, Fat, Carbohydrate, Vitamin B2, Vitamin B6, Vitamin B9, Iron, Calcium were found to be 64, 56.6, >100 , >100 , 35, <10 , 27.6, 74.8, 66 respectively and poor sleep quality – Female subjects were found to be 67, 55.4, >100 , >100 , 50%, <10 , 26.6, 55, 65.4 respectively. From the above table, when comparing both the nutrients includes, Energy, Protein, Fat, Carbohydrate, Vitamin B2, Vitamin B6, Vitamin B9, Iron, Calcium intake and sleep status, it was found that nutrient intake among good quality sleepers were better than the subjects with poor quality sleep. is positively correlated with good quality sleep. This indicates, if nutrients consumption increases the quality of sleep will also increase. Among the good quality sleepers Vitamin B6 intake was very low. Similar study done by (9) states that sleep quality and quantity were associated with characteristics of a healthy dietary pattern in oldest-old men living in the community. Studies have reported that B-12 shortens the length of the sleep-wake rhythm and affects the circadian aspect of sleep propensity. Also identified vitamin B complex as a helpful treatment of nocturnal leg cramps (10).

DISCUSSION

A total number of 70 early adults were divided into two groups, group 1 (n= 35) belonged to good sleep quality category and group 2 (n=35) belonged to poor sleep quality category. Majority of the subjects belonging to age 21 years (37%) was found to have poor quality of sleep. The subjects were found to have poor quality whose weight falls between 61-70 kg indicating that as weight increases the quality of sleep decreases. Majority of the subjects 60 % in poor sleep quality and 42.9% good sleep quality category has normal BMI. It is found that 17.1% of subjects





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belonged to severe sleep problem, 83% of the subjects belonged to moderate sleep problem, 34.3% of the subjects belonged to good sleep, 66% of the subjects belonged to great sleep. It was also observed that the macro and micro nutrient intake of good quality sleepers were better when compared with the poor quality sleepers.

CONCLUSION

From the study it can be concluded that good sleep quality has a positive influence in nutrient intake (Energy, protein, fat, carbohydrate, vitamin B2, vitamin B6, vitamin B9, iron, calcium). BMI is weakly and negatively associated with good sleep quality (≥ 19 to ≤ 36) while it was positively associated with poor sleep quality (≥ 1 to ≤ 18).

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Table 1: Distribution of Subjects Based on BMI

| BMI (kg/ m ²) | Good quality sleep (N=35) | | | Poor quality sleep (N=35) | | |
|--------------------------------|---------------------------|-------------|------------|---------------------------|-------------|------------|
| | Male n=10 | Female n=25 | % | Male n=8 | Female n=27 | % |
| (< 18.4) Underweight | 2(6%) | 9(26%) | 31.4 | 1(3%) | 4(11%) | 14.3 |
| (≥18.5 to ≤24.9) Normal weight | 5(14%) | 10(29%) | 42.9 | 6(17%) | 15(14%) | 60.0 |
| (≥25 to ≤29.9) Overweight | 3(9%) | 4(11%) | 20.0 | 1(3%) | 7(20%) | 22.9 |
| (≥30 kg) Obese | - | 2(6%) | 6 | - | 1(3%) | 2.9 |
| Total | | 35 | 100 | | 35 | 100 |





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Table 2: Comparison and Association of Nutrient Intake in Normal Sleepers and People with Sleep Disturbance Among Selected Earlyadults.

| Nutrients | Recommended dietary allowance for female adults | | Good sleep quality | | Poor sleep quality | | p value |
|-----------------|---|-------------------|--------------------|--------------|--------------------|--------------|--------------------|
| | MALE | FEMALE | Mean±Stddev | | Mean±Stddev | | |
| | | | MALE | FEMALE | MALE | FEMALE | |
| ENERGY (kcal) | 2320 [#] | 1900 [#] | 1987±142.5 | 1877.4±138.5 | 1487.3±132.7 | 1282.3±115.7 | 0.000 [*] |
| PROTEIN(g) | 60 [#] | 55 [#] | 57.9±15.3 | 55.79±15.3 | 34±8.92 | 30.51±8.929 | 0.000 [*] |
| FAT(g) | 25 [#] | 25 [#] | 54.23±85.12 | 50.2±85.12 | 37.3±3.63 | 35.3±3.83 | 0.000 [*] |
| CARBOHYDRATE(g) | 130 [^] | 130 [^] | 268.3±60 | 242.3±60 | 188.70±42.4 | 168.70±42.4 | 0.000 [*] |
| VITAMIN B2(mg) | 1.4 [#] | 1.1 [#] | 1.18±0.464 | 1.14±0.464 | 0.50±.456 | 0.56±.456 | 0.000 [*] |
| VITAMIN B6(mg) | 2.0 [#] | 2.0 [#] | 0.093±0.010 | 0.085±0.010 | 0.068±0.010 | 0.065±0.010 | 0.000 [*] |
| VITAMIN B9(µg) | 200 [#] | 200 [#] | 83.6±31.6 | 78.6±30.6 | 55.2±19.83 | 53.2±19.53 | 0.000 [*] |
| IRON(mg) | 17 [#] | 21 [#] | 20.3±4.454 | 19.3±4.454 | 12.72±2.70 | 11.72±2.70 | 0.000 [*] |
| CALCIUM(mg) | 600 [#] | 600 [#] | 532.8±128.29 | 523.8±125.29 | 398.8±8.4 | 392.8±80.4 | 0.000 [*] |

[^]-RDA for (carbohydrate)adults established by THE INSTITUTE OF MEDICINE(IOM)

[#]-RDA for Indians suggested by ICMR 2010

^{*}p value <0.05

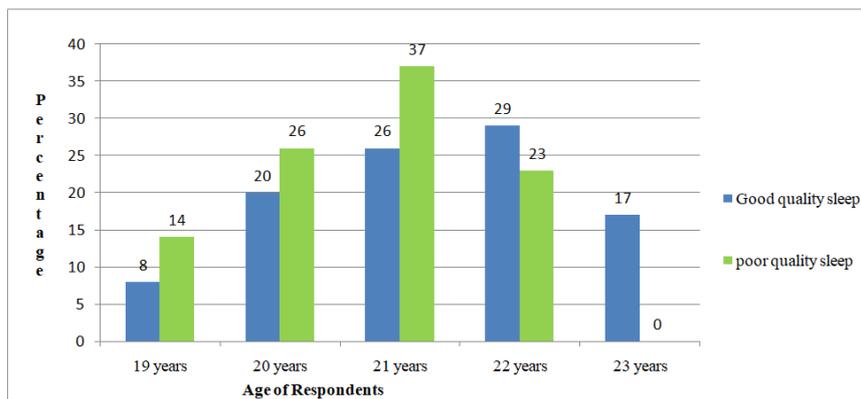


Figure 1: Distribution of Subjects Based on Age





Crop Shield Protection System

K.Shyama Satya Naga Teja^{1*}, Y.Sudha² and G Ravi Kishore³

¹Assistant Professor, Department of Electrical and Electronics Engineering, St.Martin's Engineering College, Hyderabad, Telangana, India.

² Assistant Professor, Department of Electrical and Electronics Engineering, Malla Reddy Engineering College, Secunderabad, Telangana, India.

³ Assistant Professor, Department of Electronics and Communication Engineering, Vidya Jyothi Institute of Technology, Aziznagar Gate, Chilkur Balaji Road, Telangana State, India.

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*Address for Correspondence

K.Shyama Satya Naga Teja

Assistant Professor,
Department of Electrical and Electronics Engineering,
St.Martin's Engineering College,
Hyderabad, Telangana, India.
E.Mail: kssnteja@gmail.com



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ABSTRACT

The Crop Shield device with Lot Technology is a cutting-edge device designed to increase crop output while lowering the likelihood of crop failure due to adverse environmental conditions. The system combines IOT technologies with farming methods to build a platform for tracking and managing the crop environment. The system is made up of sensors that are positioned around the farm in various locations to keep track of environmental variables including humidity, temperature, and soil moisture. Sent through the internet to a central server, where it is processed and analysed, is the data that the sensors have collected. According to the information gathered from the sensors, the system may also be designed to automatically modify the ambient parameters. Increased crop yields, decreased water use, and improved farming practises are just a few advantages of the Crop Shield System with lot technology. The technique can also lessen the chance that crops would perish because of climatic conditions like drought or extremely high temperatures. Farmers' profitability may rise significantly as a result of this. In conclusion, the Crop Shield System with IOT technology provides a contemporary answer to the problems encountered by farmers in maintaining a high yield of crops while reducing chances of crop failure. With the help of the device, farmers might operate more profitably and use less water.

Keywords: Arduino, Motor drive, Lcd display, PIR sensor, Rain sensor, Buzzer.





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INTRODUCTION

Agriculture, which is the main source of food, is one of the most important parts of human survival. Unfortunately, the majority of farmers in our nation still practise traditional farming, which requires laborious manual analysis of data pertaining to soil and crops. The use of contemporary farming techniques can solve this. It is essential to introduce automation into agriculture since it contributes significantly to crop yield and the development of an economy. The agriculture sector is one of the key factors in a nation's economic growth. Agriculture automation allows for efficient crop monitoring without the need for on-site labour. Since sensors and microcontrollers can't be directly connected to the internet, the internet of things is a network of real-world items that have sensors, software, and electronic parts like them built in. A robust irrigation system is the cornerstone of crop productivity.

The field is equipped with a sensor that monitors the soil's need for water and automatically activates the irrigation system to keep it functioning properly. The farmer can use IOT technology to monitor sensor data on a computer or mobile device. For monitoring soil moisture, rain sensor, and rain detection, we employ a variety of sensors. Using IOT technology, all data is sent to the cloud. Anyone can watch by opening a server from anywhere at any time. Agriculture, which is the main source of food, is one of the most important parts of human survival. Unfortunately, the majority of farmers in our nation still practise traditional farming, which requires laborious manual analysis of data pertaining to soil and crops. The use of contemporary farming techniques can solve this. It is essential to introduce automation into agriculture since it contributes significantly to crop yield and the development of an economy. The agriculture sector is one of the key factors in a nation's economic growth.

Agriculture automation allows for efficient crop monitoring without the need for on-the-ground labour. Since sensors and microcontrollers cannot be directly connected to the internet, the internet of things is a network of real-world items that have sensors, software, and electronic parts like microcontrollers implanted in them. A good irrigation system is the foundation for crop productivity. A sensor is placed in the field to detect the soil's need for water and automatically activate the irrigation system as needed to maintain it properly. Using IOT technology, the farmer will be able to monitor the sensor data on a computer or mobile device. For detecting soil moisture, we employ a variety of sensors, including a rain sensor and a rain detection sensor. IOT technology is used to transport all data to the cloud. Anyone can watch a server by opening it from anywhere at any time. Agriculture, which is the main source of food, is one of the most important parts of human survival. Unfortunately, the majority of farmers in our nation still practice traditional farming, which requires laborious manual analysis of data pertaining to soil and crops. The use of contemporary farming techniques can solve this. Since the agriculture sector contributes significantly to a nation's economic development, automation in this sector is essential to increasing crop yields and fostering economic progress. Agriculture automation allows for efficient crop monitoring without the need for on-the-ground labour. Due to the fact that sensors and microcontrollers cannot be directly connected to the internet, the internet of things is a network of actual physical items that have been embedded with sensors, software, and electronic parts like these. Good irrigation systems are the cornerstone of crop productivity. The field is equipped with a sensor that monitors the soil's need for water and automatically activates the irrigation system to keep it functioning properly. IOT technology will enable the farmer to monitor sensor data on a computer or mobile device. For monitoring soil moisture, rain sensor, and rain detection, we employ a variety of sensors. Using IOT technology, all data is sent to the cloud. Anyone can watch by opening a server from anywhere at any time.

LITERATURE SURVEY

The processor, IC-S8817BS, and wireless transceiver module with Zigbee protocol are utilized in a system using sensors to monitor various environmental variables, such as water level, humidity, temperature, etc. The specialists send the farmer the field condition by email and text messaging sent to their mobile devices. This system manages energy efficiency and sensor node failure. Zigbee technology, which occasionally has limited communication range, is employed.



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On the basis of Zigbee technology, a system for intelligent agriculture greenhouse monitoring is proposed. The system handles the functions of data collecting, processing, transmission, and reception. The aim is to develop a greenhouse environment system that can efficiently regulate the environment while saving money, lowering farming costs, and conserving energy. IOT technology employed in this instance is based on the BS framework, with the CC2530 acting as a processing chip for wireless sensor nodes and coordinators. The Linux operating system and the cortex A8 processor are both found in the gateway. Overall, the design makes it possible to remotely monitor and regulate greenhouses intelligently, as well as to switch from old wired technology to wireless, which also lowers the cost of labour.

A system that uses infrared imaging to track plant growth is suggested. Here, the irrigation temperature distribution measurement (ITDM) method has been allusive. Better irrigation is provided in real time by thermal images that include both low and high temperature ITDM readings. Unlike thermometry, which only provides an average value, thermal imaging may provide the temperature of each individual pixel in the area. Thermal imaging produces erroneous information at very close temperatures, making it difficult to distinguish between the objects.

A technique for assessing the effectiveness of wireless sensor networks used in irrigation automation and data transmission across wireless networks to a web server. The sensors are used to monitor crops by detecting moisture, humidity, and temperature. When the sensor reading drops below the predetermined threshold levels, watering is automatically.

Proposed System

The Arduino-mega 2560, PIR sensor, Rain sensor, and Buzzer are the components of the suggested system. This technology employs a motion sensor to identify approaching wild animals close to the field. In this circumstance, the sensor instructs the micro controller to operate. Currently, the microcontroller calls the farmer and emits an alarm to entice the animals out of the field. In doing so, the farmer is protected from loss and the crops are completely safe from animals. Here is a rain sensor that detects the presence of rain and sounds an alarm. Therefore, we can save water so that we can utilise it later for different uses. There are numerous strategies for water conservation, including harvesting, etc. By employing this technique, we can raise the level of subsurface water. These sensors are primarily employed in fields such as automation, irrigation, vehicles, etc.

RESULT ANALYSIS

The sensor will detect movement when a human or animal body comes within range of it because they both release heat energy in the form of infrared radiation. The sensor is called a passive infrared sensor for this reason. Additionally, the term "passive" denotes that the sensor only detects the energy emitted by other things, not by using its own energy for detection. A switching device that is used to detect rainfall is a rain sensor. The sensor operates on the same principle as a switch, with the switch being generally closed whenever it rains. A switching device that is used to detect rainfall is a rain sensor. This sensor operates similarly to a switch, with the basic premise being that the switch will always be open in the absence of rain.

CONCLUSION

An effective technique for agricultural field crop monitoring. Data may be saved and retrieved from anywhere with the help of IOT applications. The sensor portion of the proposed work is only capable of monitoring crops; however, in the future, watering may be automated, and the system might be improved with security of farmland under video surveillance, preventing infiltration.

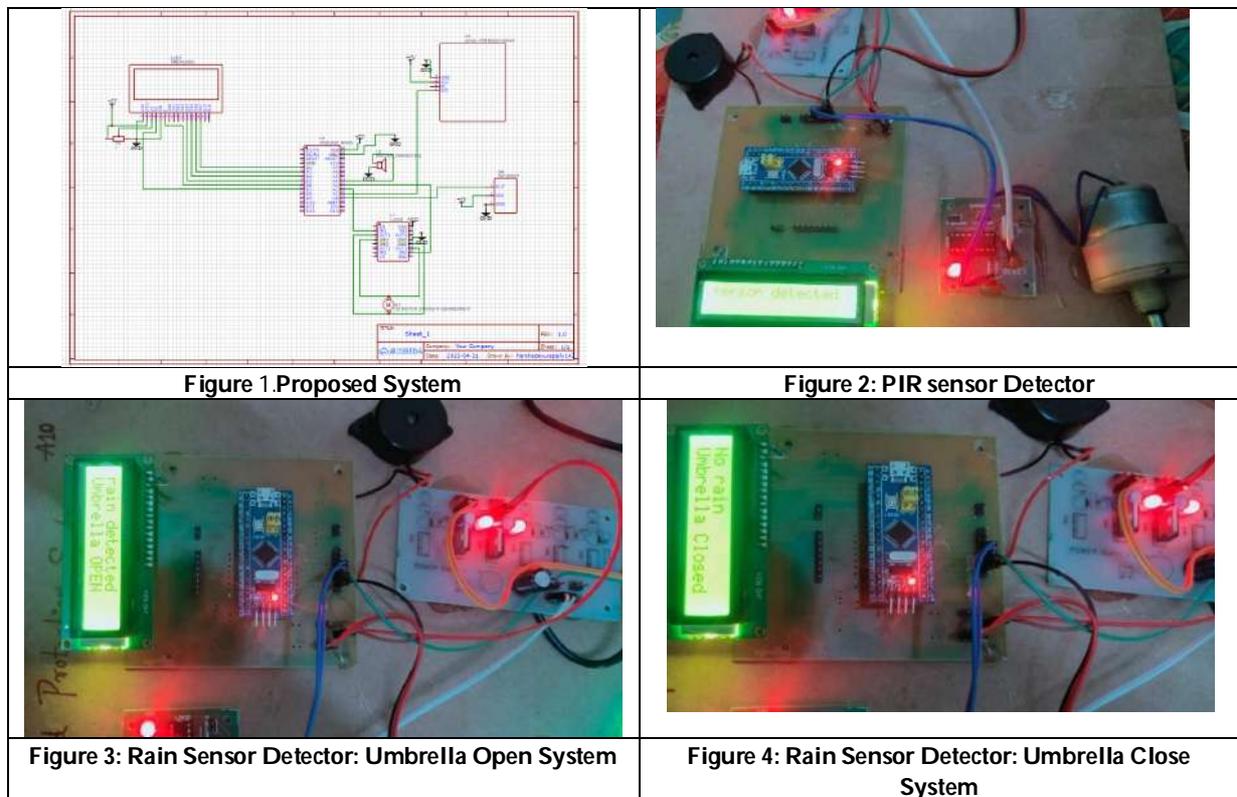




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Pedagogical Approach towards Story Telling based on Bloom's Taxonomy

S.Stalin^{1*} and Yazhnee Stalin²

¹Assistant Professor, Department of Tamil Madras Christian College Tambaram, Chennai - 600059, Tamil Nadu, India.

²Counselling Psychologist, Shalom Ayurvedic Clinic, Agaram then, Chennai - 600 126, Tamil Nadu, India.

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*Address for Correspondence

S.Stalin

Assistant Professor,
Department of Tamil Madras Christian College,
Tambaram, Chennai - 600059,
Tamil Nadu, India.
E.Mail: stalin.s@mcc.edu.in



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ABSTRACT

Stories carry information from generation to generation and travel throughout decades. Stories in the classroom make the people more interactive. Simple stories with embedded information that fulfil the pedagogical strategies facilitate learning in classrooms and in the places where story is read or performed. Science that taught on a blackboard or as a passage in textbook when merged with the literature form and their own cultural background are carefully perused and reviewed for pedagogical standards. Stories are picked up and fractionated under bloom's taxonomy in pedagogy system. The text is fixed under psychological, functional memory systems, linguistic and phonetic approach, setting, plot and character analysis method of short stories. The formative and interventional evaluation is done over the stories. The written text explores the cultural essence of the people and places where setting of the story is fixed. Cultural elements of the particular region and some regional vocabularies specific to particular landscape is seen. The stories relate science with the surrounding environment and helps to understand the science as sub-culture. The stories accomplishes bloom's pedagogy disseminate science, mathematical, ecological and social awareness to the children who are about to know the details in higher age and avail aids to children with learning, writing difficulties and the rural children who lack the regular schooling.

Keywords: bloom's Taxonomy, storytelling, Pedagogy, sub-culture, storytelling psychology, science stories, cognition.



**Stalin and Yazhnee Stalin****INTRODUCTION**

Learning should not only take us somewhere; it should allow us to grow go further more easily. The best way to create interest in the subject is to render it worth knowing, which means to make the knowledge gained usable in one's thinking beyond the situation in which learning has occurred [1]. Narrative text, which is easier to understand is usually the genre of choice of beginning instruction. Before they start school, children are exposed to great deal of narration, through bedtime stories as well as adult conversation [2]. Reading and explaining the text does not actually help the students, to make familiar with it. Communication is not only about mastery of language (English or mother tongue) but it is concerning articulation of given content through appropriate blending of organisation and dissemination.(Paul Wilson)[3].National reading panel 2000 reported that focus on cognitive strategies. The information or text is conceptualized in the way student can deliberately approach a text in order to uncover its meaning[4]. Stories pave way for to raising questions and finding answers for those. Stories also help in passing the information. F. Boutros conceded in 1842 that "we instruct them in nothing but general scienceand literature, as if we expected them to have a choice of either as a profession, or as if they were to be a body of literates spending their lives in intellectual enjoyment" [5].

Any information/ science can be synthesised in the way to reach the target groups. The pre requisites are imagination (artistic, intuition), critical observation, personal experience (Meta cognition), prior knowledge. (Unique selling proposition of 21st century chemist, Dr.Paul Wilson). Considering those elements narrative text is written and communicated to the students. Bloom's pedagogical taxonomy is applied over the text, compared the text under that learning strategy results are analysed and outcome is brought out. Stories for educational learning purposes should be comprised of pedagogical modes. Stories with pedagogy enhances the multimodal learning process. It is possible to find out pedagogical strategies in a simple story. A full statement fledged paragraph regarding science or ecology would not attract students towards the concept. A mathematical problem solved on a blackboard in a classroom could simply attract only few students towards it. But wrapping those concepts with the known story or a new story would show affinity towards learning among them.

MATERIALS AND METHODS**Remembering**

Process by which we recall experience information and skills can be simply defined as remembering. Remembering is the recall of information terms or sir time facts remembering is a factual knowledge and done under describe define and label categories. By describing defining or labelling a particular information it can be remembered by the readers. According to Uri Hassan and Princeton a story is the only way to activate parts in brain so that listeners listen turns story into own Idea and experience. Among the sensory memory, echoic memory retains each individual syllable. Spoken language recognises words and stored in memory and transferred to short term memory [6]. As the story is narrated, some Phonics sounds that are included reaches the echoic memory and stored in the brain. It is important for the echoic memory should be converted into short term memory for the later use. Hence any such sound or Phonics used in stories would help the readers to convert them into short term memory and the story is remembered for the time. In the story of explaining the lifecycle of *mosquito* the sound "Buzz...." is included. This sound here acts as a quick memory processing unit and transformed into short term memory and the real aim of the story is remembered as a long term memory. Sensory cortex of the brain helps to process information that is picked up by the five senses. In general by reading the story visual sense is triggered and by hearing audition sense is triggered. Certain techniques are used in the story to help in the stimuli of such senses.

Metaphors used in the story helps in remembering pedagogy. Again in the *mosquito* story, the mosquito states directly the humans are the parents and creators of them. So this metaphor usage here helps in recalling the facts expected by the author.



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Elaborate encoding is another stage of memory. And this works under the information already known to the Brain. The perceived item is converted and constructed under the base of the older information present in the long term memory. From the long-term memory the stored information is recalled and merged with the new information and to be stored permanently. In the story, that reveals about the information of Tortoise and Turtle the very well-known story (i.e. rabbit and tortoise) is used as a base. The winner Tortoise and the lost Rabbit in the race is used by the author. The story here begins with the crying Rabbit who lost the race and the further conversation helps in storage and retrieval memory. This results in easy learning of the features if tortoise and turtle.

Brain of the participants are scanned. This helps to reveal activity in the motor cortex which coordinates the body movements. Once the movement of the character is explained The Reader can some moments and correlate the movement with their body on mood and remembers the text. For example, in the story regarding Methane Gas it is written as

“Marx now clutched his Grandpa’s arm”. The word clutched would help to scan the mood of the characters to the readers. Use of the certain powerful verbs are also helps in triggering certain memory points of brain.

The monkey ushered the fly- The firefly story
Grandpa succoured them-Devil of the wild story.

These powerful words here serves as remembering tool in the story. The story are created where “The firefly” speaks about the luminous effect and “Devil of the wild” teaches about the methane gas[7]. The remembrance also in initializes mental process in the act of acquiring knowledge about the history of the specific place. In a story of explaining waggie dance through the school excursion historical information are revealed simultaneously to the readers. Frontal cortex is the area of the brain implicated in planning complex cognitive behaviour, personality expression, decision making and moderating social behaviour[8].

The boy with trembling in the fear.

Here the emotional area is lit up and information is passed then stored in long-term memory. Any experience narrated in the story activate the part called Insular cortex which is involved in the acquisition of pain, love, emotion and craving etc...in the story that describe the importance of Palmyra tree the grandpa shares his emotional experience regarding the destruction of the Palmyra trees. This lit up the not only is a cortex area of the characters that are involved but also the lit up readers of the story. Any previous experiences regarding the destruction of the sources would be recalled and remembered from the memory cell.

Cause and effect relationship also help to remember and recall the stories. Cause is something happened an effect is the result of such as action. These type of happening in the story would help the target readers to achieve the aim of the story. The readers whenever they are exposed to the cost in their own environment, would remember and recall the story and its purpose. Again in the *Palmyra tree* story the destruction of Palmyra tree is the cause and groundwater level goes down is the effect. In the mosquito story unclean environment is the cause and a mosquito replication and the disease is the effect. Stories written in persuasive way would convince the reader and changes someone's mind. The persuasive has the power of making to believe text written, so they could be remembered for the long time. In the story of abacus the grandson who rely on the calculator realises the power of the mental ability. The examples of the events that happened to be persuasive and also convince the readers to accept the fact [9] Usage of simple language and low complexity helps in activating the brain regions making truly relate to your situation and happenings. Simple sentences are the familiar words activate the frontal cortex part of the brain. In the *Rabbit and Tortoise story* the following is a sample conversation between the characters.

“Will you accept me as your friend?” the rabbit gave a friend request to tortoise.

“With pleasure”, the tortoise accepted its friend request.



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This usage of words very familiar in social media would help us the readers to involve and remember that text which activates the frontal cortex.

Explicit and implicit memory

Episodic memories are formed in hippocampus region of temporal lobe. Any specific events or incidents, formulas or scientific facts are generally indexed for later access. Neo cortex of the brain is involved in sensory perception, spatial reasoning, and conscious, thought and in human's language. Amygdala is brain process emotion. So the emotional contents is stored there in the brain and helps and remembering pedagogy. Especially, the emotion of fear in the story which is misunderstood as devil or demon, here the emotion (fear) plays as a key in remembering the facts delivered regarding the Methane gas in the story. Some other most emotions like love, compassion are expressed in other stories for activate the brain through the stimuli given to the amygdala region of the brain.

Implicit memories have the impact that activities and experience can have on the behaviour. This can be unconsciously adapted [10]. Basal ganglia helps in the cognition and learning process. By the help of the basal ganglia and new cortex the information learnt through the perception are processed and the habit formation occurs in the readers. After reading the *Mosquito* story the habit of conscious towards the environment clean would be the desired outcome. Components of the story and its techniques are activating the cognitive and memory area of the brain. By making emotional connections, observations the stories helps in remembering the implicit facts involved in the story and also helps in the development of optimistic skills of learning.

Understand

Understanding is one of the important pedagogy for a valid outcome of purpose. It is the capability of the readers or the target group to grasp the real meaning and acquiring the information in their own way. Understanding can be elaborated or elicited by the other verbs such as discussions, test, summarisation, explanation, illustration, presentation, match, classify [11]. It can be also clarified that understanding can be acquired through the actions of the verbs mentioned above. When the inner meaning is summarised, explained, presented, illustrated the understanding of the particular concept can be bought out to the target groups. It is the mastery of decoding information. When the word used in the text is of high vocabulary content children would exhaust their mental efficacy in searching meaning for the words. Understanding becomes the tedious process and the aim of the text gets dimmer and vocabulary gets focused on. Using the language easily accessible for their family in the science communication children would exhaust their mental efficacy and searching meaning for the words. Using the language easily accessible or familiar to the children brings out the desired outcome. In the science communication stories the main aim is its outcome i.e. scientific literacy. As the stories focus on the introducing or familiarizing the concept the language is important strategy to be considered.

Conversation mode involves more reader participation and more entertaining to the Reader. It makes the story more advanced story moves forward faster than the author's explanation with the example. It opens up the floor for the further exploration. A conversation between mosquito and the boy makes the story faster. As this is themed on environmental awareness the dialogues used by the mosquito make story more interesting. Here the conversation between mosquito and the boy turns to pedagogical tool to drive the story to the desired outcome. *The mosquito asks the boy, "Why did you hit me idiot?"* .The reader could make the story in advance by guessing the answer.

It develops the character:

The character would lost their effect or more of the situation if simply narrated. The dialogue between them visualises the mode of the situation to the readers. In the story of methane gas, the catfish would hit the water.

"What?" Engels asked with timid.

The catfish hit the water, smiled grandpa.

Owls hooted in deep woods of darkness. They also could hear the howling of fox.

Marx in trembling voice asked, "Is there devil in Lake?"





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The above conversation helps to understand the mode that is described in the situation the boys' mood of the fear scare can be felt by the readers to better understanding the situation of the story. The conversation part of the grandpa is handled as that he is not scared and he explains the facts to his grandsons. Thus dialogue defines and develops the character for a better understanding. Student changes comprehension into simple symbol for understand bees eight (8) shaped dance.

It provides realism:

The characters turn and exhibits lively performance. The scene can be visualised by the readers. A suitable dialogue pattern make the reader engage with the story. Even the non-living things or materials can show some characteristic features through their pattern.

It defines the character:

Dialogue is used to depict the characters and their relationship in the story. It portrays image of the particular character involved into the story. When the character is understood by the Reader the aim of the story is nearly fulfilled. In the story that explains about the waggle dance of the honeybee the boys dialogue itself shows that he is naughty and searched all the ways to hit the comb.

"Beware!" The place is dangerous you will be punished severely if anything goes wrong says the teacher.

"Ok..... Sir."

The prolonged *ok* and caution of the teacher reveals mischievous nature of the boys' Provides information:

Information process starts from external stimuli such as sound, and touch then transferred to sensory organs and activate the brains. Both cultural and child's experience are necessary to understand children's cognitive development. According to Vygotsky, society shaped child's mind through transmission of tool according to the culture [15]. Transmission of information from one generation to another constitutes cultural language tradition. In the story named *Treasure of the earth* the information regarding the culture and tradition of Tamilnadu (India) is transformed from the grandpa to his grandson. The dialogue between worried grandpa and grandson about uprooted Palmyra tree reveals the cultural and ethnicity of the particular tree. The old historical even scientific facts are exposed in following manner.

"It is necessary to know better about the tree and grandpa started explaining the facts. Your father and I enjoyed all the benefits of the Palmyra tree. You at least saw the tree at your age. However, your fore comers will not even see this. All these Palm trees were planted by our ancestors two or three Generations before.

Our grandfather told, "The King who formed all this lake planted all the Palmyra trees over the shore", expounded Grandpa."

Culture represents an excellent amalgamate of creativity, intellectual and human intelligence along with old tradition. Also, when child uses mother tongue for thinking and reasoning the understanding of any concepts become easy. Thus Language also helps in expanding cognitive abilities of individual.

Application

Use, execute, implement, manipulate role-playing are the verbs connected with application pedagogy.

Paraphrase from outer sources

The environment around us show you a greater influence or impact towards a Lifestyle and habitat of the readers. In the stories observation of the external environment act as a paraphrase for the original text. Exploring the environment and social condition around the readers/children provides floor for the implementation of learned text. In the story of waggle dance the boy notices the honeycomb during his school excursion and again when he notices the similar comb in the forest the story expands from the location and grabs information that is required to be delivered to the students. This incident stays as an example how the application strategy works. The same outcome was expected among the readers for different situation and application of different scientific methods and facts. A strong knowledge base is needed for the application of conceptual knowledge. At the edge of application is the imagining about what we are not aware of [16]. Any written context should show a specific impact has changed over





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the readers. Innovative problem solving is the idea of developing new solution to the problem when the error, failure, interpretation or a space found in the conventional way a new solution can be applied[17]. Through the story pedagogical tools to new solution can be created to the existing problem. Considering Methane Gas as a devil is a traditional belief. After the exposure to the scientific solution to the existing problem (fear) is solved.

Invention is the application where the new solution is applied to the new problem. Instead of trying old solution to a new problem the readers are expected to innovate new solution to the new problem. World and knowledge are the one which keep changing on according to times. The stories encourage the readers and teaching new approach to solve the environmental and societal problems. The story Abacus which deals with Mathematics and mind calculation, the boy is encouraged to use mental calculations. Some mathematical puzzles are introduced in the story followed by the formulas and the solutions in the same text. Whenever the readers are introduced to the new problem or any numerical skills the story, context is applicable and aids them in inventing new solution to the newer problems.

Routine problem solving is application of known solution to the known problem. Through the story certain questions are presented before the readers. The problems presented in the story may be known by the readers previously and the text allows the mind of the readers to frame some doubts and problems regarding the setting, plot and characters of the story. Some questions and doubts are raised by the characters themselves answers are revealed by the other characters in the story. Some answers or solutions are directly presented in the text and their serve as the framed formulas or the facts and they are used for solving their issues when they are under certain circumstances narrated in the text. It is done by the direct application of the gained information to their lifestyle. In the story about habit and habitat of tortoise the shell of tortoise is compared to that of the material *keratin* which is similar to the human nail. This direct application of the information would assist in applying known solution to the known problem.

Non-routine problem solving is the application through pedagogical method, where it need certain degree of creativity and originality to solve the problem. It may not provide direct solution but improve the ability to solve issues in multiple ways. These solving skills help in increasing the reasoning ability. One or more solution for the same problem is implemented and more strategies are applied in solving the problems. In the story of mosquito the incomplete information is provided in the text. Information regarding reproduction and replication of Mosquitoes or its preventive measures are not provided. All these nested issues are left to the application method to be adapted by the readers. One or more conflicts are proposed and pathways are created for multiple solution. Behavioural modification can also be considered as the application outcome of the readers. When certain behavioural changes believe changes occur within the readers of the particular text the characters in the and finally after knowing about ants' unique nature the boy's behaviour is modified and he started showing compassion towards them and accepted the world is common to everyone and all the creature have right to live in the world.

Analysis

Analysis of a story is in the way of interpreting the text in the view of the readers. Author gives insight of growing up, human nature relationship, and other experiences in the story. In the Science communication stories, it is essential to analyse the plot, character, theme of the story and the point of view of the readers. As the children of the target readers, analysis should be done in the way of passing the required information to them and analysis done to fulfil the requirements of the pedagogical strategy.

Plot analysis

Freytag pyramid serves as a basis for analysing a story. Beginning of the story where characters and setting are introduced shows great impact over the readers to enter into the story. Faith, awareness, belief are taken as the plot where it could create essential strength to the knot of the story.





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Character analysis

The characters play a vital role in the stories. A hypocritical character is depicted in a story of mosquito where the mother blames the society for keeping the environment unclean and at the climax of the story, she throws her trash into the street. This hypocritical depiction of the character enhances awareness among the readers. A self-deception character is also predicted in the stories. In the story of introducing meteors and meteorites to the readers, the plot is based on the superstitious belief that prevails among the people of rural areas. They believe that the falling star is a bad sign for family and diminishes the intelligence of the people who gaze it. The grand daughter would try to convince her grandma's belief as false and introduce meteors and meteorites to her. Though grandma knows her granddaughter's intelligence she stubbornly regrets the truth.

Action of the main characters

The actions which are performed by the characters also show a great influence in the pedagogical strategies. Depicted characters are deliberate to some extent. Grandpa and grandma characters constitute the major role in the story. This is done intentionally to create a bond among them to the readers and also explicate their intelligence to the future generation.

Action of other characters in the story

These characters help to drive the story and they show their impact over the conflict and climax of the story [18]. *Dynamic character* is posed over the boy of Ant story. He learns a lesson and changes his behaviour. *Flat characters* introduced in Rabbit and Tortoise story where the another Tortoise pulls his limb inside the shell and the story drives forward from the action performed, and an information is revealed to the readers. *Ethical character* includes honesty and proves moral values depend on the situation they are introduced in the text. In the story of Firefly the monkey shows compassion towards the Firefly and help them to escape from the Weaver bird nest. In the story of ant, by accepting all the facts regarding the ant's boy shows an ethical transformation in his behaviour. Characters have huge impact over the readers as the people like to live their lives through the character it becomes more excited and impressive to the readers [19].

Setting:

Setting of a story also help to set the mood, influence and behaviour of the character invoke emotional response, reflects the society of the characters involved in the story [20]. Primary setting is a place where the majority of the story occur, it helps in the involvement of characters with the readers. It also acts as a backdrop of the mood of the story. In the *firefly* Story the setting is as "A big jamun tree, stood by the shore of beautiful pond. Palm tree grow along with the jamun tree. The woods over the field gleam the even in the dark. This setting helps in the perception of the darkness to the readers.

Setting also helps in assessing the atmosphere. The setting set stage for conflicts and solution of the story [21]. In the story of *Devil of the wild-* a story based on belief, setting is the hut over the lake shore. *A sudden dashing sound emitted from the lake. Owls hooted in the deep woods and they could hear the howling of the fox.* The scare created by the author results in finding solution for the conflict. Society setting aids in examining the cultural aspects of the particular character involved in the story. Some hidden details are used in the setting and relates the readers to the cultural and social engagement of the story. In the story of Palmyra tree the dried lake and the demolished Palmyra shore are used as setting which helps readers to pay attention towards the weather rain, water scarcity of the of the region.

EMIC and ETIC point of view

The story emphasizes the observer than the native's explanation. They are decided and analysed previously what be studied by the readers [21]. Established theories and proven scientific fact serve as the mainstream in the story. The non-members of the group was chosen to interpret the culture and behaviour. In the story of *Rabbit and Tortoise, The firefly* the characters of the story are animals i.e. readers the non-members is a given opportunity to read their cultural behaviour and scientific facts. Emic perspective is the members of the same culture perceive their world. In such perspective the awareness stories regarding Honey Bee, Palmyra tree the characters and the readers belong to





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the same culture and view through the same glasses provided to the characters of the story. Through these perspectives effective analysis of the story is done.

Evaluation

The outcome evaluation is based on assumption and techniques used in text. Formative evaluation is one that occurs during the process/story is going on. Evaluation is the pedagogical practice in a language as special manner of influence and special instrument used in societal, group or cultural context. . It is the cognitive reasoning process that exists in order to determine the text value using explicate and implicate criteria. The plot of the story shows explicit characters at one time and implicit characters in another time. The learning objective that meant for the story is describing the chemical reaction that occurs in the body of the butterfly. The plot implicitly drives towards the concept of Bio-luminescence. The character chosen here is the monkey and it is justified under the basis that monkey can climb up the tree and it can hold the object with his hand. The conflict is the ambivalent monkey climbs the tree by watching the light streak from the nest. The desired information is passed to the readers through theme setting, justifying the characters defining the plots. This enables readers to perform knowledge or skill. The cognitive domain receive more attention. The objectives related to knowledge or influence naming, solving predicting and other intellectual aspects. Cognitive evaluation of coherence, linguistic features make the story comprehensible[23]. Beginning of the story *Mosquito "kabadi...kabadi..."* a traditional game word is used. As this a familiar and popular gaming word it receives attention of the target group.

Interventional evaluation

Intervention should be relevant to the context. It should be coherent with other intervention. Achieve their object, deliver in effective way and have positive impact at last [24]. Interventions provide assistance to raise the reading and achievement. In a story of describing the honeybee the story begins within School excursion. The school exhibition context acts as the intervention. This is relevant to the story as the main character notices the honeycomb in the fort and the story begins. The interventions should be coherent to other interventions used in context. In the second part of a story a forest setting is introduced where he gazes the honeycomb similar to one which tempted him over the Gingee Hills. These interventions assists the boy to achieve his knowledge towards the honeycomb. These contextual interventions also help the author to deliver the theme of the story in an effective way. Whenever Gingee Hills or forest context is perceived through auditory or visual senses the Reader would be able to remember the text. Affective domain deals with attitudes appreciations values and emotions. The levels start from simple is simple awareness to acceptance to internalization and become part of practicing value system [25]. Student interprets social and cultural context that create meaning in text. Evaluating by the affective means it is categorised under certain criteria. Receiving that context the reader being aware or sensitive to ideas facts or phenomena. The Reader would be able to identify or aware of something in their environment. Through the photosynthesis story, the process of photosynthesis is sensed by the character and passed through the readers .In another story of introducing *Meteors and Meteorites* the evaluation is to differentiate the solar elements.

Respond is a fine way of exhibiting a lower level actions to the awareness received. In the story of devil of the wild, once the grandfather created awareness towards the methane gas, the scared boys' were out of the fear and started enjoying their stay, the evaluation mode here is the boys' responds to the awareness created by their grandpa. Value is the willing of spreading the gained knowledge to others and involving them in certain ideas and exhibiting involvement. The evaluation strategy is identified in the story of grandfather creating awareness towards his grandson and he responds to it. With his friends and he started planting seedlings of Palm trees over the Shore of the lake.

Organisation

Relating the values and accepting it and bring internal harmonics. New values are added to general values. In the story of Tortoise and Rabbit on hearing the traits and uniqueness of Tortoise the Rabbit internally harmonizes Tortoise and accept their creation.





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Characterization

The added new value through the story becomes their lifestyle. Once the awareness is created the reader responds to it and internally accepts and organises the value and further make them as their lifestyle. It is expected that from the mosquito story would serve as an awareness campaign for the environment. The expected evaluation is that readers would ensure for clear environment. Evaluation in the cognitive domain would be expected as the knowledge and development of intellectual skills. Evaluation in affective domain includes manners which we deal with things emotionally such as feeling, values, appreciation, motivation and attitudes[26].

Creation

Knowledge is always becoming. Knowledge is changed to the extent that reality moves and changes. It is not stabilized, immobilized [27]. Collaborative creation of exchanging ideas or information from the text to the other outer sources. With the piece of information grasped from the text the readers are encouraged to create new collaborative ideas. Critical thinking against the characters and the facts written on the story would help them in collaborative creation. In the *Ant* story farming technique of the ants is exposed to the students the expected outcome would be able to collaborate the information with agricultural farming techniques. Structural creation is understanding the resources back and forth and comprehend the meaning and the structural component of the text. For example in the honey bee story life cycle of a honey bee explained so it will help the students to process the perceived information back and forth and they create own art forms based on some other ecological connections. The students are expected to exhibit imaginative ways in their work related and academic related activity. Skills are developed in the way as they could create their own stories with the school lesson connecting it with their own life incidents or experiences. And even the incidents that occur that happen in their daily activities can be questioned and examined under scientific views. For example, why meteors are alone falling and not the sun? This question can be answered in their schools and wholesome knowledge of solar system is created among them. Social responsibility is also considered as another important outcome of the stories. The students are able to make to believe that science is actually the subculture that we adapt in our every day to day life. Everything is examined under scientific approach. Critical thinking and logical thinking is created among the students. There expected to express their ideas in coherent and logical way.

Develop Awareness of cultural difference it involves the ability of standing back from ourselves and becoming aware of cultural values beliefs and perception [28]. Cultural sensitivity was defined as being aware of cultural differences without assigning them in the value which means without believing that one culture is superior to the other. It is important to be informed about cultures and beliefs. typically a person who is well informed about various cultures tend to be open-minded and respect to other groups because they are less likely to form and founded opinion about other culture [29]. Incidents that takes place in the stories and the contextualization represent the culture prevailing in the particular region. In the story of *Palmyra tree*, the story expresses the culture of Tamilnadu and relationship between the country people and Palmyra tree. It also helps in understanding different perceptions of the world and their values. Every country and region have its own unique customs beliefs, traditions, religion and Lifestyle. So it is necessary to bring out the cultural awareness in order to bring the honour and respect to their own cultural identities. Over the racing and fastening Science and Technology and in speed of cosmopolitanism and globalization of their cultures, students should be aware of own cultural significance and traditions. Children and grandparents travel throughout the story which transport the cultural richness from generation to generation. Culture and own traditions can be characterized by visible and invisible factors which include culture personality and work style [30].

Material culture references are the iconic feature show some cultural significance. Day to day objects, things or materials that are unique to particular region is given in the story. In the *Devil of the wild story*-the chimney lamp, in *Palmyra tree story*-the palm fruit, Palm jaggery, in *photosynthesis story*- bullock cart are identified as material culture. And equally language between the regions also shows significant cultural identities and they are quoted throughout the texts. Collective intelligence is another expected creation out of the stories. It is believed that individuals share their own experience and information can progress more powerful and the story is narrated in a group or in teams. In the classroom the emergence of various other information from their own is possible. Some students would have





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gone through experience similar to that of described in the stories. So different views can be popped out for a single story or the information. Connectivity usually acts as a driving force in collective learning. Connecting themselves or their relatives, friends, neighbours with the characters of the story would facilitate learning strategy. Connecting their own life with the setting of the story would also help in attaining the knowledge.

Story Telling In Science Classroom

A good story will stay alive in mind of the student. Stories enhances the educational curriculum. Stories can provide enjoyable way for all the students to grasp the scientific principles. A chemistry professor wanted to make the students to remember the symbols uses the technique of story behind every element. The symbol of lead this Pb, it is originated from the word "Plumbum", which is hard to remember. The professor uses the story strategy. The story is, during the Roman times people working in the lead pipes are called "plumbers" the word is that is used even today. As plumber is well known word, Pb becomes easier to remember.

And for the Copper, he had another story. Copper has got the symbol Cu derived from the word Cuprium. Copper is mined from the Cyprus Islands and called as Cyprium and overtime called as Cuprium and got the symbol. He noticed every student was listening intently and the outcome was also very good and reflected in their exams [31].

Stories are used as starting point to teach the lessons. For the concept of gravity the Story of at Newton who was sitting under the tree was surprised to see why the Apple is falling down?The teacher asks the question and follows the gravity lessons. Stories are used to explain the ideas (Wells 1986) argued that the story makes meaning, it is an activity that pervades all aspects of meaning. Anthropomorphism is the technique used in the classroom for the concept teaching. This technique was opposed by some educators but Tamir and Zohar (1991) was against this concern and they believe the students are able to distinguish between anthropomorphic and factual concept.

The great journey of William water is the story developed by Banister and 2001 the story was about the water cycle and William was the name given for the water. Water was given with human attributes such as feelings and gender. The story says, *when he was evaporating in the air he said "whee" as he was flying.* Adding Analogues to the stories is another technique used. Making elements as actors in the story. Casting of protagonist that must be taken into existence (ogborn et.al 1996, p.14) role and action of Fungi in ringworm disease can be explained in dramatic way pretending fungi as an actor and explaining its action in the disease. Action of a Corona virus, its replication and mutation can be dramatized. The way how the immune system body is also like a story explained in the science classroom [32].

RESULTS AND DISCUSSION

Stories servers a major tool in memorizing new word and phrases. Memory mechanism such as Visual, auditory, emotion and exaggeration are stimulated in the story telling. The combination of Teaching and storytelling made the classroom fun, interesting and everlasting. The teaching of English is still haphazard in many countries. Teaching of English lies under the category foreign language teaching. Universidad Cooperative Columbia decided to implement a pedagogical form for teaching the language. The main objectives of that is to create stories of the student's interest, teaching the language in context around the stories. Through these methods they made the language learning a fun and a lifelong process for the young learners. Learning of second language goes through five predictable stages. Preproduction, early production, speech emergence, intermediate fluency and advanced fluency (Karsen and Teller 1983) in the book (The natural approach) the book also speaks about the five the language learning hypothesis. Among that input hypothesis seem to be very important in the elementary stage of the student. Qualitative input is the base are the most important step to the rest of the other stages of the hypothesis. Listening and reading are the most important input that is fed to the children. Here comes a story telling and the reading strategy which is the most powerful tool in the learning process. Stories help to improve the ability to understand the language and thinking skills. Stories helps the students to know the cultural values different from their own. The ability of prediction and inference is developed. As human mind is always wired to the stories, it would provide a better





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outcome. More than learning the grammar books the learner is able to come across new words in the story. When the guest are the plot of the story becomes interesting, children automatically starts following the story. While the learners are engaged in the story they actively participate in the session and results in the better retention of the words and context. Enrichment of vocabulary is the major take away. By hearing the stories the student is exposed to new vocabulary and also learns the answers to the questions of how? Where? When? and Where? The words are to be used. An emotional response connection with the language for beyond the words is built in this case. Exposure to virtues, culture tradition and helps to feel the empathy and compassion about other culture, language and people. The cognitive skills such as think, perceive, remember and recognize are achieved through lingual development. Through the story the skills are advanced and it makes the sense of world seen in the stories. Divergent thinking, problem solving skills are the major outcome of the story telling strategy. Story telling reduces and anxiety towards the language. As the focus shifts from correctness to the content it boosts the self-confidence. It shows the positive impact in the classroom. Tsou pointed out that “student willing to exchange opinion and work in team” (Tsou 2004) a story telling classrooms allows for more peer to peer learning connection.

CONCLUSION

Self-motivation it is an internal force that drives towards achievement and human development. The students are intrinsically motivated to create interest in particular fields such as literature, Science, Mathematics and ecology. The outcome of the scientific stories is expected in the way of both inspiring them and being inspired by those facts and exploring with other unfocused scientific or literary works. Creating a desire to study is the most valued strategy for any pedagogical method. These stories are written or focussed for the rural areas of the developing country who are unfocussed to science and hard vocabularies. All these stories are written from their own cultural background which creates a desire to study. These stories would definitely help in improving the life standards and also some extent of social awareness. Finally, it is concluded that creating affinity towards the science and persuading science as the sub-culture is the utmost goal of the text.

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Hesitant Anti Fuzzy Subrings and Ideals

Anitha.B^{1*} and Vidhya.M²

¹Assistant Professor, Department of Mathematics, Annamalai University, Annamalai Nagar - 608002, Chidambaram, Tamil Nadu, India

²Research Scholar, Annamalai University, Annamalai Nagar-608002, Chidambaram, Tamil Nadu, India.

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*Address for Correspondence

Anitha.B

Assistant Professor,
Department of Mathematics,
Annamalai University,
Annamalai Nagar -608002,
Chidambaram, Tamil Nadu, India
E.Mail: anibhavan05@gmail.com



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ABSTRACT

This article discusses the hesitant anti fuzzy set over subring and ideal, establishes a characterisation as well as discusses certain features. Then, the homomorphism characteristics of hesitant anti fuzzy subring and ideal investigated.

Keywords: Hesitant fuzzy set(HFS), Hesitant fuzzy subring(HFSR), Hesitant fuzzy ideal(HFI), Hesitant anti fuzzy subring(HAFSR), Hesitant anti fuzzy ideal(HAFI), Homomorphism.

INTRODUCTION

Our descriptions of the world are not always accurate. Everywhere we look, there is some uncertainty. But there are certain topics that mathematics covers. This indicates a difference between the mathematical model and actual experience. Zadeh [13] developed the fuzzy set notion as a solution to this problem, marking the beginning of a paradigm change. This paradigm shift will open up a new horizon to researchers all over the world. Later, different types of uncertainties were introduced. Fuzzy subgroups and fuzzy ideal are investigated by Martinez [5]. Later, in 1982, fuzzy subrings and fuzzy ideal are defined by Liu [8]. In 2014, Hesitant fuzzy subgroup and obtained some results regarding homomorphism defined by Deepak and John [3]. In 2010, In order to reduce the amount of information lost during fuzzification, Torra [9,10] presented the idea of a hesitant fuzzy set [6] in 2010. This concept further characterises an element by a collection of membership values. The hesitant fuzzy set has been applied to decision-making by xia and xu [11]. Then, Deepak and John [2] explored hesitant fuzzy relations through hesitant





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fuzzy rough set. Anti-fuzzy ideal defined by Azam [1]. Subnarring of anti hesitant fuzzy introduced by Poornima & Shanmugapriya [7].

The following is about the document set up. The hesitant fuzzy set's foundational ideas are reviewed in the second part, along with some of its operations. In the third part, we present the ideas behind hesitant anti fuzzy subrings before establishing that they are union. Then provides an illustration to demonstrate that a hesitant anti fuzzy subring need not exist at the intersection of two such subrings. The term of the hesitant anti fuzzy ideal is presented in the fourth part, which also gives an example of a hesitant anti fuzzy subring that is not a hesitant anti fuzzy ideal. This section gives a characterization for hesitant anti fuzzy ideal and discusses some of its algebraic properties. The fifth part explains the hesitant fuzzy set extension principle before demonstrating that the homomorphism

PRELIMINARIES

Definition:2.1.[12] Consider \tilde{X} as a fixed set, a HFSH: $\tilde{X} \rightarrow [0,1]$ is defined by

$$A = \{ \langle x, H_A(x) \rangle / x \in \tilde{X} \}$$

denoting the possible membership degrees of a element $x \in \tilde{X}$.

Definition:2.2.[10] Consider H_1, H_2 as HFS, The accompanying actions are then specified:

- a) $LB : H^-(x) = \min H(x)$
- b) $UB : H^+(x) = \max H(x)$
- c) $\alpha-LB : H_{\alpha}^-(x) = \{ H \in H(x) / H \lesssim \alpha \}$
- d) $\alpha-UB : H_{\alpha}^+(x) = \{ H \in H(x) / H \gtrsim \alpha \}$
- e) Complement: $H^c(x) = \tilde{\gamma}_{\gamma \in H(x)} \{1-\gamma\}$
- f) Union: $(H_1 \tilde{\vee} H_2)(x) = \{ H \in (H_1(x) \tilde{\vee} H_2(x)) / H \gtrsim \max (H_1^-, H_2^-) \}$ or equivalently $(H_1 \tilde{\vee} H_2)(x) = (H_1(x) \tilde{\vee} H_2(x))_{\alpha}^+$ for $\alpha = \max (H_1^-, H_2^-)$.
- g) Intersection: $(H_1 \tilde{\wedge} H_2)(x) = \{ H \in (H_1(x) \tilde{\wedge} H_2(x)) / H \lesssim \min (H_1^+, H_2^+) \}$ or equivalently $(H_1 \tilde{\wedge} H_2)(x) = (H_1(x) \tilde{\wedge} H_2(x))_{\alpha}^-$ for $\alpha = \max (H_1^+, H_2^+)$.

Definition:2.3.[4] Consider \mathbb{R} as a ring and H as a HFS of \mathbb{R} . Then H is called a HFSR(\mathbb{R}) if $\forall x, y \in \mathbb{R}$,

- a) $H(x+y) \gtrsim H(x) \tilde{\wedge} H(y)$.
- b) $H(-x) \gtrsim H(x)$
- c) $H(xy) \gtrsim H(x) \tilde{\wedge} H(y)$.

Definition:2.4.[4] Consider \mathbb{R} as a ring and H as a HFSR(\mathbb{R}). Then H is called

- a) a HFLI if $H(tx) \gtrsim H(x), \forall t, x \in \mathbb{R}$,
- b) a HFRI if $H(xt) \gtrsim H(x), \forall t, x \in \mathbb{R}$,
- c) a HFI if it is both HFLI and HFRI.

HESITANT ANTI FUZZY SUBRING

Definition:3.1. Consider \mathbb{R} as a ring and H as a HFS of \mathbb{R} . Then H is called a HAFSR (\mathbb{R}) if $\forall x, y \in \mathbb{R}$

- a) $H(x+y) \lesssim H(x) \tilde{\vee} H(y)$.
- b) $H(-x) \lesssim H(x)$
- c) $H(xy) \lesssim H(x) \tilde{\vee} H(y)$.

Example:3.2. Consider $Z_3 = \{0,1,2\}$ as the ring. Consider H as a HFS is defined by

$$H(x) = \begin{cases} \{0.6\} & \text{if } x = 0 \\ \{0.7, 0.75\} & \text{if } x = 1 \text{ and } 2 \end{cases}$$

Then, H is a HAFSR(Z).

Proposition:3.3. Consider H as a HFS in \mathbb{R} . Then $H \in \text{HAFSR}(\mathbb{R})$ iff





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- a) $H(x-y) \lesssim H(x) \tilde{\vee} H(y)$
- b) $H(xy) \lesssim H(x) \tilde{\vee} H(y)$.

Proof: \Rightarrow Trivial.

Conversely, assume (a) and (b).

Putting $y = x$ in (a), we get

$$H(0) \lesssim H(x) \tilde{\vee} H(x) = H(x)$$

(i.e.) $H(0) \lesssim H(x), \forall x \in \mathbb{R}$.

Putting $x=0$ in (a),

we get $H(-y) \lesssim H(0) \tilde{\vee} H(y) = H(y)$.

Putting $y = -y$ in (a), we get, $H(x+y) \lesssim H(x) \tilde{\vee} H(-y) \lesssim H(x) \tilde{\vee} H(y)$.

$\therefore H \in \text{HAFSR}(\mathbb{R})$.

Lemma:3.4. Consider $H \in \text{HAFSR}(\mathbb{R})$. Then $H(0) \lesssim H(x), \forall x \in \mathbb{R}$.

Theorem:3.5. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$ then $H(-x) = H(x)$ and $H(x) \gtrsim H(0)$ for x in \mathbb{R} ,

Proof: Consider $x \in \mathbb{R}$.

$$\text{Then } H(x) = H(-(-x)) \lesssim H(-x) \lesssim H(x)$$

$\therefore H(-x) = H(x) \forall x \in \mathbb{R}$.

$$\text{Then } H(0) = H(x-x) \lesssim H(x) \tilde{\vee} H(-x) \lesssim H(x)$$

$\therefore H(0) \lesssim H(x) \forall x \in \mathbb{R}$.

Theorem:3.6. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$ and $H(x-y) = H(0)$. Then $H(x) = H(y) \forall x$ and $y \in \mathbb{R}$,

Proof: Consider x and $y \in \mathbb{R}$,

$$\text{Now } H(x) = H(x-y+y)$$

$$\lesssim H(x-y) \tilde{\vee} H(y)$$

$$= H(0) \tilde{\vee} H(y),$$

$$= H(y)$$

$$= H(x-(x-y))$$

$$\lesssim H(x-y) \tilde{\vee} H(x)$$

$$= H(0) \tilde{\vee} H(x)$$

$$= H(x)$$

$\therefore H(x) = H(y) \forall x$ and $y \in \mathbb{R}$.

Theorem:3.7. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$ then $H = \{x/x \in \mathbb{R}: H(x) = 0\}$ is either \emptyset or a subring of \mathbb{R} .

Proof: If $x \in H$,

$$H(x) \neq 0 \Rightarrow H = \emptyset$$

If x and $y \in H$ then

$$H(x-y) \lesssim H(x) \tilde{\vee} H(y) \lesssim (0) \tilde{\vee} (0) = 0$$





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$\therefore H(x-y) = 0$ so get $x-y$ in H .

$$H(xy) \lesssim H(x) \tilde{Y} H(y) = (0) \tilde{Y} (0) = 0$$

$\therefore H(xy) = 0$ so get $xy \in H$

$\therefore H$ is a subring of \mathbb{R} . Hence $H = \emptyset$ or a subring of \mathbb{R} .

Theorem:3.8. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$ then $H = \{x \in \mathbb{R} : H(x) = H(0)\}$ is a subring of \mathbb{R} .

Proof: Consider x and $y \in H$

$$\text{Then } H(x-y) \lesssim H(x) \tilde{Y} H(y) = H(0) \tilde{Y} H(0) = H(0)$$

$$\therefore H(x-y) \lesssim H(0)$$

Then by theorem:3.5

$$H(0) = H(x-y)$$

$\therefore (x-y)$ in H .

$$H(xy) \lesssim H(x) \tilde{Y} H(y) = H(0) \tilde{Y} H(0) = H(0)$$

$\therefore H(xy) \lesssim H(0)$.

Then by theorem:3.5

$$H(0) = H(xy)$$

$\therefore (xy)$ in H . Hence H is a subring of \mathbb{R} .

Theorem:3.9. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$. Consider $H(x-y) = 0$ then $H(x) = H(y) \forall x$ and $y \in \mathbb{R}$.

Proof: Consider x and $y \in \mathbb{R}$

$$H(x) = H(x-y+y)$$

$$\lesssim H(x-y) \tilde{Y} H(y)$$

$$= (0) \tilde{Y} H(y)$$

$$= H(y)$$

$$= H(-y)$$

$$= H(-x+x-y)$$

$$\lesssim H(x) \tilde{Y} H(x-y)$$

$$= H(x) \tilde{Y} (0)$$

$$= H(x)$$

$\therefore H(x) = H(y) \forall x$ and $y \in \mathbb{R}$.

Theorem:3.10. Consider H as a HAFSR of $(\mathbb{R}, +, \cdot)$. Consider $H(x-y) = 1$ then $H(x) = 1$ or $H(y) = 1 \forall x$ and $y \in \mathbb{R}$.

Proof:

Consider x and $y \in \mathbb{R}$.

$$H(x-y) \lesssim H(x) \tilde{Y} H(y) (\because H \text{ is a HAFSR}(\mathbb{R}))$$

$$1 \lesssim H(x) \tilde{Y} H(y)$$

$\therefore H(x) = 1$ or $H(y) = 1$.





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Definition:3.11 [2] Consider H as a fuzzy subset of X , then we denote $H_{(\alpha)} = \{x \in X / H(x) < \alpha\} \forall \alpha \in [0,1]$.

Theorem:3.12 Consider H as a fuzzy subset of a ring R . Then the following are equivalent:

- a) H is a HAFSR(R).
- b) $H_{(\alpha)}$ is a subring of R , for any $\alpha \in [0,1]$.

Proof: (a) \Rightarrow (b)

Consider H as a HAFSR(R).

For any $\alpha \in [0,1] \exists H_{(\alpha)} \neq \emptyset$,

(a) $x - y \in H_{(\alpha)}$ and (b) $xy \in H_{(\alpha)} \forall x, y \in H_{(\alpha)}$.

Let $x, y \in H_{(\alpha)}$, then $H(x) < \alpha$ and $H(y) < \alpha$,

$$H(x - y) \lesssim H(x) \tilde{\vee} H(y)$$

$$< \alpha \tilde{\vee} \alpha = \alpha.$$

$$\therefore H(x - y) < \alpha$$

$$\therefore (x - y) \in H_{(\alpha)}.$$

Consider $H(xy) \lesssim H(x) \tilde{\vee} H(y) < \alpha \tilde{\vee} \alpha = \alpha$.

$$\therefore H(xy) < \alpha$$

$$\therefore xy \in H_{(\alpha)}.$$

$\therefore H_{(\alpha)}$ is a subring of R .

(b) \Rightarrow (a)

Conversely, let $H_{(\alpha)}$ is a subring of R for any $\alpha \in [0,1]$.

We've to prove $H(x - y) \lesssim H(x) \tilde{\vee} H(y)$ and

$$H(xy) \lesssim H(x) \tilde{\vee} H(y) \forall x, y \in R.$$

Then choose $\alpha \ni H(x - y) > \alpha > H(x) \tilde{\vee} H(y)$

$$\therefore H(x) < \alpha \text{ and } H(y) < \alpha$$

Thus $x, y \in H_{(\alpha)}$

$$H(x - y) > \alpha$$

$$x - y \notin H_{(\alpha)} \text{ for } x, y \in H_{(\alpha)}$$

which is a contradict to that $H_{(\alpha)}$ is a subring of R .

$$\therefore H(x - y) \lesssim H(x) \tilde{\vee} H(y)$$

Then choose $\alpha \ni H(xy) > \alpha > H(x) \tilde{\vee} H(y)$

$$\therefore H(x) < \alpha \text{ and } H(y) < \alpha$$

Thus $x, y \in H_{(\alpha)}$

$$H(xy) > \alpha$$

$$x - y \notin H_{(\alpha)} \forall x, y \in H_{(\alpha)}$$

which is a contradict to that $H_{(\alpha)}$ is a subring of R





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$$\therefore H(x_y) \lesssim H(x) \tilde{\vee} H(y)$$

Thus, H is a HAFSR.

Proposition:3.13. Consider H_1 and H_2 as two HAFSR(\mathbb{R}). Then $H_1 \tilde{\vee} H_2$ is a HAFSR(\mathbb{R}).

Proof:

It is enough to prove the following:

a) $(H_1 \tilde{\vee} H_2)(x-y) \lesssim (H_1 \tilde{\vee} H_2)(x) \tilde{\vee} (H_1 \tilde{\vee} H_2)(y)$

b) $(H_1 \tilde{\vee} H_2)(x_y) \lesssim (H_1 \tilde{\vee} H_2)(x) \tilde{\vee} (H_1 \tilde{\vee} H_2)(y)$

Let x and $y \in \mathbb{R}$

a) $(H_1 \tilde{\vee} H_2)(x-y) = H_1(x-y) \tilde{\vee} H_2(x-y)$

$$\lesssim (H_1(x) \tilde{\vee} H_1(y)) \tilde{\vee} (H_2(x) \tilde{\vee} H_2(y))$$

$$= (H_1(x) \tilde{\vee} H_2(x)) \tilde{\vee} (H_1(y) \tilde{\vee} H_2(y))$$

$$= ((H_1 \tilde{\vee} H_2)(x)) \tilde{\vee} ((H_1 \tilde{\vee} H_2)(y))$$

$$\therefore (H_1 \tilde{\vee} H_2)(x-y) \lesssim ((H_1 \tilde{\vee} H_2)(x)) \tilde{\vee} ((H_1 \tilde{\vee} H_2)(y)) \forall x \text{ and } y \text{ in } \mathbb{R}.$$

b) $(H_1 \tilde{\vee} H_2)(x_y) = H_1(x_y) \tilde{\vee} H_2(x_y)$

$$\lesssim (H_1(x) \tilde{\vee} H_1(y)) \tilde{\vee} (H_2(x) \tilde{\vee} H_2(y))$$

$$= (H_1(x) \tilde{\vee} H_2(x)) \tilde{\vee} (H_1(y) \tilde{\vee} H_2(y))$$

$$= ((H_1 \tilde{\vee} H_2)(x)) \tilde{\vee} ((H_1 \tilde{\vee} H_2)(y))$$

$$\therefore (H_1 \tilde{\vee} H_2)(x_y) \lesssim ((H_1 \tilde{\vee} H_2)(x)) \tilde{\vee} ((H_1 \tilde{\vee} H_2)(y)) \forall x \text{ and } y \text{ in } \mathbb{R}.$$

Theorem:3.14. If H_1, H_2, \dots, H_n are HAFSR(\mathbb{R}) then $\cup_i H_i$ where $i = \{1, 2, 3, \dots, n\}$ also HAFSR(\mathbb{R}).

Proof: It is obvious.

Remark:3.15. The intersection of two HAFSR does not have to be considered a HAFSR. The following example shows this.:

Example:3.14. Consider $Z_5 = \{0, 1, 2, 3, 4\}$ as the ring. Consider H as a HFS is defined by

$$H_1(x) = \begin{cases} \{0.2, 0.3\} & \text{if } x = 0, 2 \\ \{0.8, 0.9\} & \text{if } x = 1 \\ \{0.6, 0.4\} & \text{if } x = 3 \\ \{0.1, 0.3\} & \text{if } x = 4 \end{cases} \quad H_2(x) = \begin{cases} \{0.3, 0.4\} & \text{if } x = 0, 2 \\ \{0.7, 0.9\} & \text{if } x = 1 \\ \{0.3, 0.5\} & \text{if } x = 3 \\ \{0.1, 0.4\} & \text{if } x = 4 \end{cases}$$

Consider $H_1, H_2 \in \text{HAFSR}(Z)$.

Taking $x = 4$ and $y = 3$

we Have $((H_1 \tilde{\wedge} H_2)(4 - 3)) = ((H_1 \tilde{\wedge} H_2)(1)) = \{0.8, 0.7, 0.9\}$

But $((H_1 \tilde{\wedge} H_2)(4)) \tilde{\wedge} ((H_1 \tilde{\wedge} H_2)(3)) = \{0.5, 0.6\}$.

$$\therefore ((H_1 \tilde{\wedge} H_2)(4 - 3)) \not\supset ((H_1 \tilde{\wedge} H_2)(4)) \tilde{\wedge} ((H_1 \tilde{\wedge} H_2)(3))$$

As a result, the first requirement of the HAFSR is violated.

Hence, $H_1 \tilde{\wedge} H_2 \notin \text{HAFSR}(Z)$





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Definition:4.1. Consider \mathbb{R} as a ring and H as a HAFSR(\mathbb{R}). Then, H is called

- (i) a HAFLI if $H(ir) \lesssim H(x)$, $\forall r, x \in \mathbb{R}$
- (ii) a HAFRI if $H(xr) \lesssim H(x)$, $\forall r, x \in \mathbb{R}$,
- (iii) a HAFI if it is both HAFLI and HAFRI.

Lemma:4.2. Consider H as a HFS(\mathbb{R}), Then $H \in \text{HAFI}(\mathbb{R})$ iff $H \in \text{HAFSR}(\mathbb{R})$ and $H(xy) \lesssim H(x) \tilde{\wedge} H(y)$.

Proof: It is straightforward.

Theorem:4.3 Consider H as a fuzzy subset of a ring \mathbb{R} . Then the following are equivalent:

- (a) H is a HAFI(\mathbb{R}).
- (b) $H_{(\alpha)}$ is an ideal of \mathbb{R} , for any $\alpha \in [0,1]$.

Proof: (a) \Rightarrow (b)

Consider H as a HAFI(\mathbb{R}).

We have to prove $H_{(\alpha)}$ is an ideal of \mathbb{R} .

Let $x, y \in H_{(\alpha)}$, then $H(x) < \alpha$ and $H(y) < \alpha$,

Then $H(x-y) \lesssim H(x) \tilde{\vee} H(y) < \alpha \tilde{\vee} \alpha = \alpha$.

$H(x-y) < \alpha$

Thus $(x-y) \in H_{(\alpha)}$.

Consider $x \in H_{(\alpha)}$, $r \in \mathbb{R}$.

Now $H(xr) \lesssim H(x) \tilde{\wedge} H(r) < \alpha \tilde{\wedge} H(r) < \alpha$.

$\therefore H(xr) < \alpha$

Thus $rx \in H_{(\alpha)}$. Similarly, $rx \in H_{(\alpha)}$.

Hence $H_{(\alpha)}$ is an ideal of \mathbb{R} .

(b) \Rightarrow (a)

Conversely, consider $H_{(\alpha)}$ as an ideal of $\mathbb{R} \forall \alpha \in [0,1]$.

Suppose $\exists x, y \in \mathbb{R} \exists, H(xy) > H(x) \tilde{\wedge} H(y)$

Choose $\alpha \exists H(xy) > \alpha > H(x) \tilde{\wedge} H(y)$

Hence $H(x) < \alpha$ and $H(y) < \alpha \Rightarrow x, y \in H_{(\alpha)}$

Now $H(xy) > \alpha > H(x) \tilde{\vee} H(y)$

$\Rightarrow H(xy) > \alpha$

$\Rightarrow xy \notin H_{(\alpha)} \forall x, y \in H_{(\alpha)}$

which is a contradiction to that $H_{(\alpha)}$ is an ideal of \mathbb{R}

Hence H is a HAFI(\mathbb{R}).

Remark:4.4. Every HAFI is a HAFSR, but a HAFSR does not need to be a HAFI. This will be illustrated by the following example:

Example:4.5. Consider $Z_4 = \{0,1,2,3\}$ as the ring. Consider $H \in \text{HFS}(Z)$ as defined by





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$$H_u(x) = \begin{cases} \{0.6,0.67\} & \text{if } x = 0 \\ \{0.15,0.23\} & \text{if } x = 1,2 \\ \{0.4,0.5,0.56\} & \text{if } x = 3 \end{cases}$$

Taking $x = 3$ and $y = 0$, we have $H_u((3)(0)) = H_u(0) = \{0.6,0.67\}$

But $(H_u(3))\tilde{\wedge}(H_u(0)) = \{0.4,0.5,0.56\}$

$\therefore H_u((3)(0)) > (H_u(3))\tilde{\wedge}(H_u(0))$

$H_u \in \text{HAFSR}(Z)$ but $H_u \notin \text{HAFI}(Z)$.

Proposition:4.6. Consider $H_1, H_2 \in \text{HAFI}(\mathbb{R})$ Then $H_1 \tilde{\vee} H_2 \in \text{HAFI}(\mathbb{R})$.

Proof: Clearly, by Proposition 3.13, $H_1 \tilde{\vee} H_2 \in \text{HAFSR}(\mathbb{R})$.

It is sufficient to show the following:

$$(H_1 \tilde{\vee} H_2)(x,y) \lesssim ((H_1 \tilde{\vee} H_2)(x))\tilde{\wedge}((H_1 \tilde{\vee} H_2)(y))$$

Let x and $y \in \mathbb{R}$

$$(H_1 \tilde{\vee} H_2)(x,y) = H_1(x,y) \tilde{\wedge} H_2(x,y)$$

$$\lesssim (H_1(x) \tilde{\vee} H_1(y))\tilde{\wedge}(H_2(x) \tilde{\vee} H_2(y))$$

$$= (H_1(x) \tilde{\vee} H_2(x))\tilde{\wedge}(H_1(y) \tilde{\vee} H_2(y))$$

$$= ((H_1 \tilde{\vee} H_2)(x))\tilde{\wedge}((H_1 \tilde{\vee} H_2)(y))$$

$\therefore H_1 \tilde{\vee} H_2(x,y) \lesssim ((H_1 \tilde{\vee} H_2)(x))\tilde{\wedge}((H_1 \tilde{\vee} H_2)(y)) \forall x$ and y in \mathbb{R} .

Remark:4.7. The intersection of two HAFI need not be a HAFI. The following example explains this:

Example:4.8. Consider $Z_5 = \{0,1,2,3,4\}$ as the ring Consider H as a HFS is defined by

$$H_1(x) = \begin{cases} \{0.8,0.9\} & \text{if } x = 0,2 \\ \{0.6,0.7,0.8\} & \text{if } x = 1 \\ \{0.3,0.4\} & \text{if } x = 3 \\ \{0.2\} & \text{if } x = 4 \end{cases} \quad H_2(x) = \begin{cases} \{0.5,0.7\} & \text{if } x = 0,2 \\ \{0.4,0.5\} & \text{if } x = 1 \\ \{0.1,0.3\} & \text{if } x = 3 \\ \{0.1\} & \text{if } x = 4 \end{cases}$$

Consider $H_1, H_2 \in \text{HAFI}(Z)$.

Taking $x = 4$ and $y = 3$, we have $(H_1 \tilde{\wedge} H_2)(4 - 3) = (H_1 \tilde{\wedge} H_2)(1) = \{0.4,0.5\}$

But $((H_1 \tilde{\wedge} H_2)(4))\tilde{\vee}((H_1 \tilde{\wedge} H_2)(3)) = \{0.1,0.3\}$.

$\therefore (H_1 \tilde{\wedge} H_2)(4 - 3) > ((H_1 \tilde{\wedge} H_2)(4))\tilde{\vee}((H_1 \tilde{\wedge} H_2)(3))$

As a result, the first requirement of the HAFI is violated.

$\therefore H_1 \tilde{\wedge} H_2 \notin \text{HAFI}(Z)$.

HOMOMORPHISM OF HESITANT ANTI FUZZY SUBRING AND IDEAL

Definition:5.1 [3](Extension principle). Consider f as a function from \tilde{X} into \tilde{Y} , and consider $H_1 \in \text{HFS}(\tilde{X})$ and $H_2 \in$

$\text{HFS}(\tilde{Y})$. Define the Hesitant fuzzy subsets

$f(H_1) \in \text{HFS}(\tilde{Y})$ and $f^{(-1)}(H_2) \in \text{HFS}(\tilde{X})$ by $\forall y \in \tilde{Y}$,

$$f(H_1)(y) = \begin{cases} \tilde{\wedge} \{H_1(x) / x \in \tilde{X}, f(x) = y\} & \text{if } f^{(-1)}(y) \neq \emptyset \\ \{0\} & \text{otherwise} \end{cases}$$





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$\forall x \in X, f^{-1}(H_2) = H_2(f(x))$. Then, $f(H_1)$ is called the image of H_1 under f and $f^{-1}(H_2)$ is called the pre-image of H_2 under f .

Theorem:5.2.Consider H as a HAFSR(\mathbb{R}) and f as a homomorphism from \mathbb{R} to a ring \mathbb{S} . Then $f(H)$ is a HAFSR over \mathbb{S} .

Proof:Consider $y_1, y_2 \in \mathbb{S}$.

If $f^{-1}(y_1) = \emptyset$ or $f^{-1}(y_2) = \emptyset$, Then we have

$$(f(H)(y_1)) \tilde{\cap} (f(H)(y_2)) = \emptyset.$$

$\therefore f(H)(y_1 - y_2) \lesssim (f(H)(y_1)) \tilde{\cap} (f(H)(y_2))$ and

$$f(H)(y_1, y_2) \lesssim (f(H)(y_1)) \tilde{\cap} (f(H)(y_2))$$

$\therefore f(H)$ is a HAFSR of a ring \mathbb{S} .

If $f^{-1}(y_1) \neq \emptyset$ and $f^{-1}(y_2) \neq \emptyset$, Then $f^{-1}(y_1 - y_2) \neq \emptyset$ and $f^{-1}(y_1, y_2) \neq \emptyset$.

Let us assume that $\exists x_1, x_2 \in \mathbb{R} \exists x_1 \in f^{-1}(y_1)$

And $x_2 \in f^{-1}(y_2)$,

$$f(H)(y_1 - y_2) = \tilde{\lambda}_{f(x)=y_1-y_2} H(x)$$

$$\lesssim H(x_1 - x_2)$$

$$\lesssim (H(x_1)) \tilde{\cap} (H(x_2)).$$

which holds for $x_1, x_2 \in \mathbb{R}$ satisfying $f(x_1) = y_1$ and $f(x_2) = y_2$, we have

$$f(H)(y_1 - y_2) \lesssim (\tilde{\lambda}_{f(x)=y_1} H(x)) \tilde{\cap} (\tilde{\lambda}_{f(x)=y_2} H(x))$$

$$= (f(H)(y_1)) \tilde{\cap} (f(H)(y_2))$$

Similarly, $f(H)(y_1, y_2) \lesssim (f(H)(y_1)) \tilde{\cap} (f(H)(y_2))$.

$\therefore f(H)$ is a HAFSR of ring \mathbb{S} .

Theorem:5.3.Consider H as a HAFSR over a ring \mathbb{S} and f as a homomorphism from a ring \mathbb{R} to \mathbb{S} . Then $f^{-1}(H)$ is a HAFSR over \mathbb{R} .

Proof: We 've

$$f^{-1}(H)(x) = H(f(x)), \text{ for all } x \text{ belongs to } \mathbb{R},$$

$$\forall x_1, x_2 \in \mathbb{R},$$

$$f^{-1}(H)(x_1 - x_2) = H(f(x_1 - x_2))$$

$$= H(f(x_1) - f(x_2))$$

$$\lesssim (H(f(x_1))) \tilde{\cap} (H(f(x_2)))$$

$$= f^{-1}(H)(x_1) \tilde{\cap} f^{-1}(H)(x_2).$$

Similarly, $f^{-1}(H)(x_1, x_2) \lesssim f^{-1}(H)(x_1) \tilde{\cap} f^{-1}(H)(x_2)$.

$\therefore f^{-1}(H)$ is a HAFSR of ring \mathbb{R} .

Theorem:5.4.Consider H as a HAFI of a ring \mathbb{R} and f as a surjective homomorphism from \mathbb{R} to a ring \mathbb{S} . Then $f(H)$ is a HAFI of \mathbb{S} .





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Proof: Consider $y_1, y_2 \in \mathbb{S}$.

Let us assume that $\exists x_1, x_2 \in \mathbb{R} \ni x_1 \in f^{(-1)}(y_1)$ and $x_2 \in f^{(-1)}(y_2)$,

$$f(\mathbb{H})(y_1, y_2) = \tilde{\lambda}_{f(x)=y_1, y_2} \mathbb{H}(x)$$

$$\lesssim \mathbb{H}(x_1, x_2)$$

$$\lesssim (\mathbb{H}(x_1)) \tilde{\lambda} (\mathbb{H}(x_2)).$$

which holds for $x_1, x_2 \in \mathbb{R}$ satisfying $f(x_1) = y_1$ and $f(x_2) = y_2$, we 've

$$f(\mathbb{H})(y_1, y_2) \lesssim (\tilde{\lambda}_{f(x)=y_1} \mathbb{H}(x_1)) \tilde{\lambda} (\tilde{\lambda}_{f(x)=y_2} \mathbb{H}(x_2))$$

$$= (f(\mathbb{H})(y_1)) \tilde{\lambda} (f(\mathbb{H})(y_2))$$

Similarly, $f(\mathbb{H})(y_1 - y_2) \lesssim (f(\mathbb{H})(y_1)) \tilde{\gamma} (f(\mathbb{H})(y_2))$.

$\therefore f(\mathbb{H})$ is a HAFI of ring \mathbb{S} .

Theorem:5.5. Consider \mathbb{H} as a HAFI of a ring \mathbb{S} and f as a homomorphism from a ring \mathbb{R} to \mathbb{S} . Then $f^{(-1)}(\mathbb{H})$ is a HAFI of \mathbb{R} .

Proof: We 've

$$f^{(-1)}(\mathbb{H})(x) = \mathbb{H}(f(x)), \text{ for all } x \text{ belongs to } \mathbb{R},$$

$$\forall x_1, x_2 \in \mathbb{R},$$

$$f^{(-1)}(\mathbb{H})(x_1, x_2) = \mathbb{H}(f(x_1, x_2))$$

$$= \mathbb{H}(f(x_1), f(x_2))$$

$$\lesssim (\mathbb{H}(f(x_1))) \tilde{\lambda} (\mathbb{H}(f(x_2)))$$

$$= (f^{(-1)}(\mathbb{H})(x_1)) \tilde{\lambda} (f^{(-1)}(\mathbb{H})(x_2)).$$

Similarly, $f^{(-1)}(\mathbb{H})(x_1 - x_2) \lesssim (f^{(-1)}(\mathbb{H})(x_1)) \tilde{\gamma} (f^{(-1)}(\mathbb{H})(x_2))$.

$\therefore f^{(-1)}(\mathbb{H})$ is a HAFI of ring \mathbb{R} .

CONCLUSION

In this study, we offer the concepts of hesitant anti fuzzy subrings and ideals. It is proved that hesitant anti fuzzy subrings and ideals are in fact a union rather than an intersection. It is also discussed how hesitant anti fuzzy subrings and ideals exhibit homomorphic features. We anticipate that our findings will advance the field of hesitant structures research. Field structure can as analysed using hesitant fuzzy sets.

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Geographical Distribution of *Frerea indica* Dalz.: A Critically Endangered, Palaeoendemic and Monotypic Genus of the Western Ghats of India

Balasaheb Shantilal Kale^{1*} and Mangesh Shankar Bhale²

¹Assistant Professor, Department of Botany, S.S.S.M. Arts, Science and Commerce College, Saikheda, Tal. Niphad, Dist. Nashik, M.S, India.

²Assistant Professor, Department of Chemistry, Arts, Commerce and Science College, Jawhar, Palghar, (M.S.) India.

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*Address for Correspondence

Balasaheb Shantilal Kale

Assistant Professor,
Department of Botany,
S.S.S.M. Arts, Science and Commerce College,
Saikheda, Tal. Niphad, Dist. Nashik, M.S, India.
E.Mail: kaleunipune@gmail.com



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ABSTRACT

The Western Ghats are one of the super biodiversity problem areas at the worldwide level because of their large number of endemic plant species. That plant species, particularly the endemics, have been accounted for and distributed routinely in the Red Data Book. *Frerea indica* Dalz. belongs to the plant family Apocynaceae found in rocky slopes and hill cliffs at high altitudes. It is an adequate data critically endangered (CR), monotypic, and endemic genus of the Western Ghats of Maharashtra (India). The International Union for Conservation of Nature (IUCN) gave the situation with this plant, which is available in the Western Ghats. This is going under adequate threatened data as critically endangered (CR) in the Western Ghats. The present distribution of *F. indica* is only in India, and it is limited to the Maharashtra state as it were. The locale circulated are Pune, Satara, Raigad, Ahmednagar, and Nashik. For primary data collection used repeated survey of the some area under study using GPS. For secondary data collection used different literature such as Floras, database and herbarium consultation from BSI and ARI, Pune. Their taxonomy will be reinvestigated to confirm their taxonomic distinctness. Identification and classification of *F. indica* using different Floras and other available literature. During the study, 34 unique *F. indica* GPS regions were noted in the Western Ghats of Maharashtra, India. The population distribution of this specie covered the Northern Western Ghats of India. The concentrated on plant populace covered Pune (85%), Satara (6%), Raigad (3%), Ahmednagar (3%), and Nashik (3%) regions of Maharashtra. Examined essential and optional data sets of *F. indica* species populace dispersion: most elevated from Pune, moderate from Satara, least from Raigad, Ahmednagar, and Nashik

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areas of Maharashtra State. In the present study, I'm producing essential and optional information to rank an species categories in the IUCN classification. Created primary and secondary information utilized for preservation of this species. As of now, this exceptional plant species is assessed under threatened data as critically endangered (CR).

Keywords- Critically endangered, palaeoendemic, Geographic distribution, *Frerea indica* Dalz.

INTRODUCTION

The Western Ghats are one of the 34th biodiversity hot spots areas at the worldwide level because of their large number of endemic plant species. The plant family Apocynaceae (Oleander family) contains 410 genera and 5,556 species [1]. This taxon was first reported to Shivneri Fort from the Northern Western Ghats of Maharashtra by Nicol Alexander Dalzell. *Frerea* is a monotypic genus that contains the species *Frerea indica* Dalz., which was distributed only in the Northern Western Ghats [2, 3,4]. Worldwide distribution of *F. indica* endemic concern for Northern Western Ghats of Maharashtra, India (Pune, Satara, Raigad, Ahmednagar, and Nashik regions of Maharashtra) [5,6,7,8]. It is endemic to Western Ghats of India, specially Northern Western Ghats of Maharashtra (India) [9]. This taxon found in rocky steep slopes and hill cliffs at high altitudes. *F. indica* coming under adequate data critically endangered (CR), monotypic, and endemic genus of the Western Ghats of Maharashtra (India) [3,10]. The succulent stem and leaves of *F. indica* are eaten as vegetables by native tribal communities [11]. A few ancestral people groups utilized this plant leaf paste to promote hair development in the Pune district of Maharashtra [12]. *F. indica* populations are always grown beneath *Euphorbia nerifolia* L. as a complete association in the microhabitat [10].

The taxonomic citation is *Frerea indica* Dalz. in J. Linn. Soc. Lond. 8:10, t. 3. 1865; Hook.f. Fl. Brit. India 4: 76. 1883; Cooke, Fl. Pres. Bombay 2: 243. 1958 (Repr.); Sant. & Irani in Univ. Bombay Bot. Mem. no. 4: 44. 1962; Jagtap et N.P. Singh in Fasc. Fl. India 24: 243. 1999. Taxonomical description: Herbs, trailing or pendulous, sap watery; stems and branches green, fleshy, quadrangular, marked with scars of fallen leaves. Leaves shortly petiolate, 3-5 x 1.8-2.5 cm, oblong or elliptic, obtuse or mucronate at apex. Flowers solitary, extra axillary; pedicels curved; corolla 2.0-2.5 cm across, fleshy, yellowish green on outer side, deep purple on inner, with an irregularly shaped yellow spot at centre of each lobe, divided less than 1/2 way down, fringed with fine, deep purple hairs on the edges; corona staminal, outer bowl shaped, with five, short, broad, sinuate truncate lobes, inner arising from inner margin of outer corona, 5 linear, truncate lobes, incurved at apex. Flowering: September to December. Fruiting : December to January [13].

MATERIALS AND METHODS

Primary data collection

Repeated survey of some areas under study using the Global Positioning System (GPS).

Secondary data collection

Using different literature such as Floras, Monographs, Research articles, India Biodiversity Portal etc. This plant database gathered from Western Ghats of India (Fig. 2).

Taxonomy & Morphology

Identification and classification of *F. indica* Dalzell using different Floras and other available literature [13]. Collection of samples and preparation of herbarium specimens are not possible because of conservation purposes and taxon that are critically endangered (CR), monotypic, and endemic (Table 1 and Fig. 1).



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Herbarium consultation: The *F. indica* identified by using different deposited herbarium voucher specimen No. 17540, K000911102, 27510.000, 33586.000, and 62981.000 of BSI, Western Circle, Pune herbaria [14].

RESULT

During the study, 34 distinct GPS regions were noted in the Western Ghats of Maharashtra, India. The concentrated on plant populace covered Pune (85%), Satara (6%), Raigad (3%), Ahmednagar (3%), and Nashik (3%) regions of Maharashtra (Fig.3). Examined essential and optional data sets of *F. indica* species populace dispersion: most elevated from Pune, moderate from Satara, least from Raigad, Ahmednagar, and Nashik areas of Maharashtra State (Fig. 2). This plant populace is appropriated above sea level at altitudes ranging from 320 to 1360 meters. Here is the variable range of height levels noticed. This plant has a novel perception noticed, these are patchy population developing on the rocky slopes and hill cliffs at high altitudes in the mountain range of the Northern Western Ghats. The present study carries a critical finding to the population dispersion of this species: that irregular examining was finished at Pune, Satara, Raigad, Ahmednagar, and Nashik locales in Maharashtra. During the survey, one of the significant perceptions noted was that these plant populations are constantly grown underneath *Euphorbia nerifolia* L. as a total relationship in the microhabitat. This patchy plant population was seen during the study (Table 2.). The succulent stem and leaves of *F. indica* are eaten as vegetables by native tribal communities. A few ancestral people groups utilized this plant leaf paste to promote hair development in the Pune district of Maharashtra. In the future, immense loss of normal natural habitat in some regions of the Northern Western Ghats will result in the plant becoming extinct in the wild (EW) IUCN category.

DISCUSSION

In the review of the north-west India during the year 1990 by Gilbert, M. G, they reported Monotypic genus and species from the Northern Western Ghats of Maharashtra, India [4]. *F. indica* Dalz. is endemic to Maharashtra State. The IUCN has recognized this taxon as critically endangered (CR). This study could assist with recognizing compelling strategies for preserving the species and saving it from extinction in its natural habitat [15,16]. *Frerea* is a monotypic genus that contains the species *Frerea indica* Dalz., which was distributed only in the Northern Western Ghats of Maharashtra. During the survey, we likewise detailed that this plant species' conveyance range is in the Northern Western Ghats of Maharashtra, India. Map showing the dispersion of *F. indica* Dalz. in the Northern Western Ghats; however, during the current study, in our outcome, this plant population covered some regions of the Northern Western Ghats of Maharashtra; those regions are Pune, Satara, Raigad, Ahmednagar, and Nashik. During the survey, this plant population conveyance was noted, involving both area and GPS locality. They are currently concentrated on vegetation destinations with fewer detailed regions; however, they didn't report with a GPS map. During the review, we get two kinds of information, including more areas and GPS maps. [2, 3,4].

This taxon was first reported to Shivneri Fort from the Northern Western Ghats of Maharashtra by Nicol Alexander Dalzell. The type specimen of *F. indica* Dalz. was firstly reported by Nicol Alexander Dalzell. J. Linn. Soc. 8: 10 (1865) from India. After that's many of authors reported other localities, they mentioned this plant population distribution range is only in the Northern Western Ghats of Maharashtra, India (Ahmednagar (Randha falls), Pune (Junnar: Shivneri fort; Purandhar; Vajirgad; Mulshi: Dongarwadi, Pimpri lake), Nashik (Kalmuste hills), Raigad (Shivthar ghal), and Satara (Mahabaleshwar: Kate's point; Sajjangad)), but they did not mention other localities mentioned in Table no. 2. During the survey, we reported some new localities and previous old localities is approximately 34 localities distributed in the northern ranges of the Western Ghats [5,6,7,8]. On the basis of the tropical plants database- *F. indica* Dalz. is distributed only in the Northern Western Ghats of Maharashtra, India. It is endemic to the Northern Western Ghats ranges of India [3,10].



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The present survey of this taxon will give future systems to further develop protection procedures. Utilizing different plans (government and non-government) of protection, this plant's IUCN status will change from critically endangered (CR) to endangered (EN) status.

CONCLUSION

In the present study, I'm producing essential and optional information to rank an species categories in the IUCN classification. Created primary and secondary information utilized for preservation (in situ & ex situ conservation) of this species. As of now, this exceptional plant species is assessed under threatened data as critically endangered (CR). Because of habitat loss, this species will become extinct in the wild (EW) in the future. The major goal of the species recovery program will be to re-establish populations within their natural habitats or man-made habitats through in-situ and ex-situ conservation. Taking into account these realities, the study region is critical as for floristic diversity in the Western Ghats.

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Table 1. Taxonomy hierarchy of *F. indica* Dalzell

| | |
|---------------|--|
| Kingdom- | Plantae |
| Phylum- | Tracheophyta |
| Class- | Magnoliopsida |
| Order- | Gentianales |
| Family- | Apocynaceae |
| Genus- | <i>Frerea</i> |
| Species- | <i>F. indica</i> Dalzell |
| Common names- | Shindal-Makadi, Shiv Suman, and Makad Meva |

Table 2. Geographic distribution of *F. indica* Dalzell

| Sr. No | Locality | Altitude (Meter) | G.P.S. location |
|--------|---|------------------|----------------------|
| 1. | Shivneri fort, Junnar, Pune | 940 | 19.198586, 73.858804 |
| 2. | Shivneri fort, Junnar, Pune | 920 | 19.200757, 73.859765 |
| 3. | Godre, Junnar, Pune | 1160 | 19.276182, 73.848957 |
| 4. | Lenyadri Caves, Junnar, Pune | 960 | 19.245514, 73.884240 |
| 5. | Manganewadi, Junnar, Pune | 980 | 19.265331, 73.822226 |
| 6. | Shivechiwadi, Junnar, Pune | 920 | 19.248042, 73.813410 |
| 7. | Hadsar, Junnar, Pune | 920 | 19.269214, 73.814239 |
| 8. | Hadsar, Junnar, Pune | 940 | 19.268127, 73.804065 |
| 9. | Hadsar, Junnar, Pune | 1060 | 19.270070, 73.800263 |
| 10. | Kotamwadi, Hadasar | 1030 | 19.284096, 73.798503 |
| 11. | Sitewadi, Junnar, Pune | 1070 | 19.288119, 73.796163 |
| 12. | Nimgiri Fort, Junnar, Pune | 950 | 19.294070, 73.774199 |
| 13. | Hatkeshwar Mandir, Junnar, Pune | 1240 | 19.284451, 73.868730 |
| 14. | Nirgude, Junnar, Pune | 950 | 19.204692, 73.833508 |
| 15. | Nirgude, Junnar, Pune | 940 | 19.207702, 73.831461 |
| 16. | Khangaon, Junnar, Pune | 930 | 19.214359, 73.815392 |
| 17. | Manikdoh, Vandev Mandir, Junnar, Pune | 950 | 19.214539, 73.825140 |
| 18. | Chawand Fort, Junnar, Pune | 880 | 19.233873, 73.748915 |
| 19. | Muthalane, Junnar, Pune | 980 | 19.332096, 73.934399 |
| 20. | Muthalane, Junnar, Pune | 980 | 19.325486, 73.942745 |
| 21. | Muthalane, Junnar, Pune | 985 | 19.331378, 73.948325 |
| 22. | Muthalane, Junnar, Pune | 970 | 19.333013, 73.955370 |
| 23. | Ambedara, Junnar, Pune | 1060 | 19.319653, 73.939163 |
| 24. | Jambhulshi, Junnar, Pune | 1360 | 19.361291, 73.906932 |
| 25. | Pimpalgaon Joga, Junnar, Pune | 945 | 19.326330, 73.899495 |
| 26. | Pimpalgaon Joga, Junnar, Pune | 1020 | 19.332049, 73.886502 |
| 27. | Purandar fort, Pune | 1245 | 18.278254, 73.973236 |
| 28. | Vajirgad fort, Pune | 1200 | 18.282540, 73.988106 |
| 29. | Dongarwadi, Mulshi, Pune | 980 | 18.725249, 73.615744 |
| 30. | Mahabaleshwar: Kate's point, Satara | 1200 | 17.941416, 73.708062 |
| 31. | Sajjangad Fort, Satara | 940 | 17.648099, 73.910353 |
| 32. | Shivthar ghal, Raigad | 320 | 18.146735, 73.622205 |
| 33. | Randha Waterfall, Bhandardara, Ahmednagar | 680 | 18.278254, 73.973236 |
| 34. | Kalmuste, Trimbak, Nashik | 883 | 19.931599, 73.478387 |





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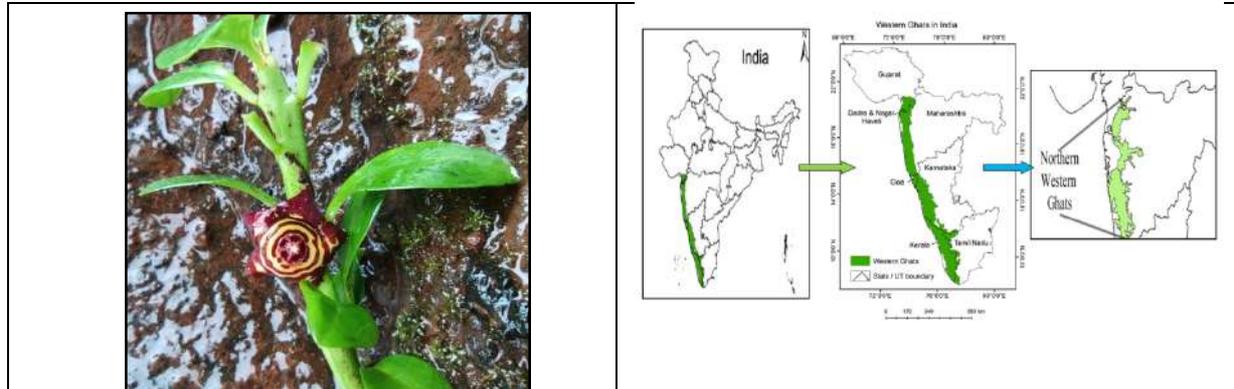


Figure 1: Morphology of *Frerea indica* Dalzell

Figure 2. Distribution of *F. indica* Dalzell in Western Ghats of India.

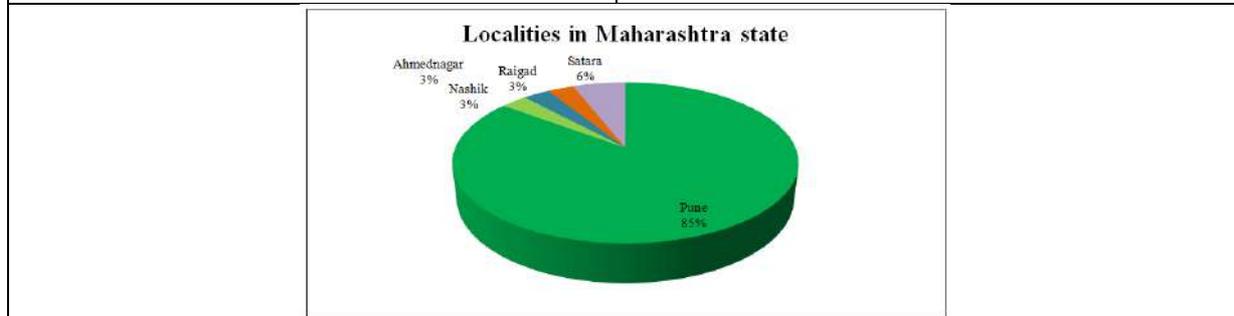


Figure 3. GPS localities of *F. indica* Dalz.





Immediate Effects of Moist Heat Pack with Effleurage Massage and Yoga Poses in Primary Dysmenorrhea

Shruti Panchal^{1*}, Jolly Patel² and Harini Patel²

¹Assistant Professor at Parul Institute of Physiotherapy, Parul University, Gujarat, India.

²Internee at Parul Institute of Physiotherapy, Parul University, Gujarat, India

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*Address for Correspondence

Shruti Panchal

Assistant Professor at Parul Institute of Physiotherapy,
Parul University,
Gujarat, India.



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ABSTRACT

Primary Dysmenorrhea characterized by cramping and radiating pain to lumbar and groin area. Effleurage massage and Yogasana are non-pharmacological, non-invasive methods considered effective in decreasing the pain. To compare the immediate effects of effleurage massage and Yoga poses in primary dysmenorrhea. It was a Quasi experimental study in which 60 females with dysmenorrhea divided in to 2 groups. Group A received yoga poses with moist heat pack and group B received effleurage massage with moist heat pack during menstruation period over 4 days. Pre and post intervention VAS and WaLIDD score has been taken. pre and post parameters compared using paired t-test which showed significant reduction in VAS (3.13 ± 0.77 , 2.00 ± 0.74) and WaLIDD (5.93 ± 0.9 , 5.8 ± 0.71) cumulative in both groups. Where as unpaired t-test compared within group differences showing effleurage is more effective in reliving pain (3.13 ± 2.00 , 0.77 ± 0.74) over Yoga poses. But, location of pain was reduced more on WaLIDD in group B who performed yoga poses (5.93 ± 0.9 , 5.8 ± 0.71). From the results, it has been concluded that both interventions were beneficial in relieving pain during menstruation but massage manipulation was more effective in relieving pain during menstruation

Keywords: Dysmenorrhea, Yogasana, menstruation, parameters, yoga poses.

INTRODUCTION

Menstrual dysfunction is the most common problem in the population of reproductive age group [1]. These disorders are common problems faced by women which contribute to physical and psycho-biological issues. Dysmenorrhea is a supreme complication of menstruation with painful cramps[2].The term dysmenorrhea comes from the Greek word meaning painful monthly pain[3]. The condition is characterized by cramping radiating pain to



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lumbar region and groin area[4]. Symptoms such as diarrhoea, headache, bloating, stress, nausea, vomiting and tiredness correspond with dysmenorrhoea [5]. Menstruation is the visible manifestation of cyclic physiologic uterine bleeding due to shedding of the endometrium.

Primary Dysmenorrhoea is one in which there is no identifiable pelvic pathology. The pain is usually in the pelvis or lower abdomen which radiates into upper back and thigh. Pain begins few hours before and lasts for 24-48 hours after the onset of menstruation[6]. The pain is more in the first day and rarely continues on the second day[7]. It is distinguished from secondary dysmenorrhoea as it refers as painful menses resulting from pelvic pathology such as endometriosis [8]. It is difficult to establish the mechanism of initiation of uterine pain in primary dysmenorrhoea. However, following are too often related; such as mostly confined to adolescents, almost always confined to ovulatory cycles, pain is related to dysrhythmia uterine contractions and uterine hypoxia.

Outcome measures**WALIDD**

This dysmenorrhoea scale was designed to diagnose dysmenorrhoea and to predict medical leave. Scale contains working ability, location, intensity, days of pain. In this tool score is given between 0-3 and total score range between 0-12 points. If total score is 0 it indicates that the individual is without dysmenorrhoea. Score 1-4 is mild dysmenorrhoea, score 5-7 is moderate dysmenorrhoea and score 8-12 is severe dysmenorrhoea. The scale's first component working ability describes frequency of pain in which the ability to perform activity is never, almost never, almost always or always. Its second component location is decided by number of anatomical body parts in which it describes no pain, lower abdomen, lumbar region, inguinal region. Its third component intensity is described by does not hurt, hurt a little, hurts a little more, hurt even more, hurt a lot. Its last component days of pain describes 1-2, 3-4, >=5[9]

VAS

It is an instrument which is used to measure a characteristic or attitude that is believed to range across values and cannot be measured directly. It is usually a horizontal line with 100mm length; descriptors are present at each end. Patient marks on the line the point which they feel their pain in the current state. The score is determined by measuring in millimetres from the left end of the line till the patient marks.

Yoga

Yoga is a non-invasive method which is easily accessed for pain relief. Regular practice of yoga has positive effect on menstrual cycle[10]. Practicing yoga is helpful in patients with hormonal imbalance [11]. Yoga benefits physical and emotional health by downregulating the hypothalamic pituitary-adrenal axis and the sympathetic nervous system [12]. Simple home based yoga program also reduce menstrual pain and improve overall health[13]. Three yoga poses(cobra, cat, fish poses) reduces severity and duration of pain in women with primary dysmenorrhoea[14].

Heat therapy

The analgesic effect of local heat is similar like electrical cutaneous stimulation of nerves. Heat therapy has been used as a traditional way to treat dysmenorrhoea. They are used in forms of hot water bottles, hot towels, electric pads and hot bags [15]. The superficial heat ranges from 40-45 degree Celsius which treats the application site to 1cm depth. Studies have shown that heat is a common method for coping with dysmenorrhoea. It reduces muscle tension and relaxes the abdominal muscles. It can also increase pelvic blood circulation to eliminate local blood and body fluid retention which thereby enables to reduce pain [16].

Massage

Effleurage massage is a non-pharmacological method which is considered effective in decreasing pain. It is a technique in which calm rhythmic gentle pressure is applied distally or downwards. It increase blood circulation, applies pressure, warm the abdominal muscles and also promotes physical and mental relaxation. It is a safe, easy





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massage technique and does not require many tools. It has no side effects and can be done alone or with others help. Effleurage massage can also be done on back [17].

AIMS AND OBJECTIVES

Aim of the study

To compare the immediate effects of Moist heat pack with effleurage massage and Yoga poses in primary dysmenorrhea

Objectives of the study

- To compare the effectiveness of moist heat pack with effleurage massage in primary dysmenorrhea.
- To compare the effectiveness of moist heat pack with yoga poses in primary dysmenorrhea.
- To compare the effectiveness of moist heat pack with effleurage massage and yoga poses in primary dysmenorrhea.

METHODOLOGY

Study design: Quasi Experimental study design.

Study setting: Parul Institute Of Physiotherapy, Parul Institute Of Nursing, Parul Institute Of Pharmacy, Samaras Girls Hostel Vadodara.

Sample Size: 60 subjects were divided into two groups- Group A and Group B; 30 subjects in each group.

Sampling: convenient sampling was done. Subjects fulfilling the inclusion criteria were alternatively assigned to Group A and Group B respectively.

Inclusion criteria

- Age group 16 to 30 years
- Having moderate to severe menstrual pain
- With normal gynaecology
- Having score of more than 4 in VAS

Exclusion criteria

- Having PCODs
- Having any gynaecological condition
- Having any medical condition (e.g. Diabetes, Hypertension)

Materials used in studies: pen, paper, hot pack, watch etc...

Outcome measures:

- Visual analogue scale
- WaLIDD

All the outcome measures were taken before the expected menstrual date and after the treatment are completed.

Procedure

Prior to the beginning of the study, the subjects were asked to fill out the questionnaires and the post data was collected at the end of the study. The duration of intervention was decided on the basis of total days of pain during menstruation. The subjects were allotted into two groups in which one group had to perform yoga poses and the



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other was given effleurage massage. All the subjects in both the groups were given moist heat pack prior to the intervention.

Intervention protocol

Group A: The subjects of this group were given two Questionnaires and were made to perform five yoga poses which were Cobra pose, Fish pose, Thunderbolt pose, Hare pose and crocodile pose. Before yoga they were given moist heat pack. They were asked to perform the poses. Total duration of treatment was 20 minutes in which the moist heat pack was given for 10 minutes prior to actual intervention. Poses were to be held for at least 30 seconds and performed for 3 repetitions with the rest of 30 seconds between each pose.

Group B: 30 patients were enrolled according to the inclusion criteria. They were given questionnaires before the intervention and post intervention. Moist heat pack was given prior to the intervention. Effleurage was given on back (sacrum) by long stroking movements performed using flat hands smoothly over entire lower back continuously. Total duration of treatment was 20 minutes in which heat pack was given for 10 minutes first. Effleurage was given for 10 minutes continuously.

STATISTICAL ANALYSIS

Statistical analysis was done by using SPSS software version 27. In which comparison of pre and post parameters (WaLIDD and VAS) for Group A and Group B has been done using paired t-test. Whereas, within group comparison between post intervention parameters of group A and group B was done using unpaired t-test. Graphical presentations and tables have been made using Microsoft excel version 2019.

RESULTS

Results would be obtained by comparing pre post intervention values of outcome measures (WaLIDD. Visual Analogue Scale) by using unpaired sample t-test and between group comparison of post data mean and SD is done by using independent t-test.

Within group comparison for WaLIDD pre and post intervention for group A

When compared WaLIDD scale in group A with pre mean \pm SD values of working ability 2.53 ± 0.51 , location 1.5 ± 0.51 , intensity 2.3 ± 0.50 , days 2 ± 0.0 and post mean \pm SD values are 1.73 ± 0.45 , 1.17 ± 0.38 , 1.23 ± 0.43 , 1.8 ± 0.4 respectively. Whereas, t-values are 5.76, 3.8, 9.1, 2.6 in working ability, location, intensity and days respectively with p-value significant at <0.001 for working ability location, intensity and for days it was <0.012 . When compared WaLIDD scale in group B with pre mean \pm SD values of working ability 2.53 ± 0.5 , location 1.7 ± 0.53 , intensity 2.4 ± 0.53 , days 2 ± 0.26 and post mean \pm SD values are 1.73 ± 0.47 , 1.36 ± 0.61 , 0.96 ± 0.18 , 1.5 ± 0.5 respectively. Whereas, t-values are 5.75, 3.34, 3.81, 4.87, in working ability, location, intensity and days respectively with p-value significant at <0.001 for working ability, days, intensity and for location it was <0.002 .

When compared VAS scale in group A with pre mean \pm SD value 5.66 ± 3.13 ; post mean values \pm SD values 3.13 ± 0.77 , respectively whereas t-values is 27.34 with p-value significant at <0.001 . When compared VAS scale in group B with pre mean \pm SD value 5.93 ± 0.733 ; post mean values \pm SD values 2.00 ± 0.74 , respectively whereas t-values is 36.93 with p-value significant at <0.001 . When compared WaLIDD scale in group A with pre mean \pm SD value 8.4 ± 1.25 ; post mean values \pm SD values 5.93 ± 0.9 , respectively whereas t-value is 6.9 with p-value significant at <0.001 .

When compared WaLIDD scale in group B with pre mean \pm SD value 8.4 ± 0.86 ; post mean values \pm SD values 5.8 ± 0.71 , respectively whereas t-value 16.52 with p-value significant at <0.001 . When compared WaLIDD scale with Group A mean \pm SD values of working ability 1.73 ± 0.4 , location 1.16 ± 0.38 , intensity 1.23 ± 0.43 , days 1.8 ± 0.4 and Group B mean \pm SD values are 1.73 ± 0.7 , 1.36 ± 0.61 , 0.96 ± 0.18 , 1.56 ± 0.5 respectively. Whereas, t-values are -1.68, -1.516, 3.12, 1.97 in working ability, location, intensity and days respectively with p-value significant at <0.001 for working ability location, intensity and for days it was <0.012 .



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When compared VAS scale with group A mean \pm SD value 3.13 ± 2.00 ; group B mean values \pm SD values 0.77 ± 0.74 , respectively whereas t-value 5.778 with p-value significant at <0.625 . When compared WaLIDD scale with group A mean \pm SD value 5.93 ± 0.9 ; group B mean values \pm SD values 5.8 ± 0.71 , respectively whereas t-value 0.617 with p-value significant at <0.32 .

DISCUSSION

In the present study, the effects of moist heat pack with effleurage massage and yoga poses in primary dysmenorrhea. This study was also assessing working ability, intensity of pain, sites of pain and days of pain in adolescent females having primary dysmenorrhea which was influenced by the post interventions. From results of WaLIDD in group A the mean from 5.66 ± 0.75 reduced to 3.13 ± 0.77 and in group B the mean from 5.93 ± 0.73 reduced to 2.00 ± 0.74 in which there was significant improvement in working ability in both the groups. There was improvement in location in Group A as compared to Group B and intensity was decreased more in group B as compared to Group A. For days there was no as such significant change. From results of VAS in group A mean from 5.66 ± 0.75 reduced to 3.13 ± 0.77 and in group B mean from 8.4 ± 0.86 reduced to 5.8 ± 0.71 but more effect was seen in Group B as compared to Group A.

Shraddha Prabhu et.al in found that yoga asanas reduces pain. They explained that yoga play an important role in reducing stress and sympathetic activity, increasing parasympathetic activity and thereby improving one's quality of life. It also shows beneficial effects on physical and mental health problems through down regulation of the hypothalamic pituitary adrenal axis and the sympathetic nervous system which is also useful for managing primary dysmenorrhea by reducing menstrual pain. Postures help to stimulate the nervous system with sensory output from all over the body. This mechanism is consistent in presenting study by which locations of pain during menstruation has been reduced in group A, who performed yogasana poses [10]. Froozan Bakhtshrin et.al identifies mean severity reduced after the intervention of massage. According to him the massage therapy results in pain relief by stimulating endorphins. It also relaxes and reduces tension in the muscles [5]. Slow pressure can increase the elasticity of muscles and therefore increase the blood flow there by it reduces the intensity of pain in group B receiving Effleurage massage. On the other hand, Nur Rahmati Sholihah observed reduction in pain before and after giving effleurage massage, it is a non-pharmacological method that is considered effective in reducing pain. It is a distraction effect that increases the formation of endorphins in the descending control system so that it makes it more comfortable because of muscle relaxation. Giving effleurage Massage makes pain fibers that bring stimulus to the brain slower than broad tactile fibers and the brain slower than the broad tactile fibers and the sensation goes faster so that when touch and pain are stimulated together [18]. Wulan et.al in his study said that massage therapy affect motor nerve and cardiovascular systems which trigger the body's resting and relaxation phases. It is an effort to restore venous and lymph flow and stimulate sensory receptors in the skin and sub skin to reduce pain [19]. However these effects were consistent in the presented study where group B received effleurage massage has significantly reduced pain intensity on VAS and Wong-Baker scale over group A.

In our results the improvements was seen in both the outcome measures from both the groups after receiving the interventions but the improvement was better in Group B then in Group A. according to VAS, and in WaLIDD the location of pain reduced much in Group A compared to Group B whereas intensity was much reduced in Group B this may be due to positive thoughts, reduction of muscular tension through relaxation, production and release of hormones and physical health.

CONCLUSION

From the results there was significant reduction in pain and WaLIDD score in both the groups. However, intensity of pain on WaLIDD and on VAS has been reduced more in group B who received massage along with moist heat pack whereas, location of pain has been reduced more on WaLIDD in Group A who performed yoga poses with moist





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heat pack. Furthermore equal reduction of working disability has been found in both the groups. So, it has been concluded that both interventions can be beneficial but massage manipulation was more effective in relieving pain during menstruation.

LIMITATIONS

- Study performed on small sample size
- It was done only on young adult female students
- Effectiveness of treatment is limited during menstruation and no further follow up intervention and assessment made.

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Table No. 1 Within group comparison for WaLIDD pre and post intervention for group A

| Pre post comparison of WaLIDD scale in Group A | | | | | |
|--|------|------|------|---------|---------|
| | | Mean | SD | t-value | p-value |
| Working Ability | Pre | 2.53 | 0.51 | 5.76 | <0.001 |
| | Post | 1.73 | 0.45 | | |
| Location | Pre | 1.5 | 0.51 | 3.8 | <0.001 |
| | Post | 1.17 | 0.38 | | |
| Intensity | Pre | 2.36 | 0.50 | 9.1 | <0.001 |
| | Post | 1.23 | 0.43 | | |
| Days | Pre | 2 | 0 | 2.69 | 0.012 |
| | Post | 1.8 | 0.4 | | |

Table No. 2 Within group comparison for WaLIDD pre and post intervention for group B

| Pre post comparison of WaLIDD scale in Group B | | | | | |
|--|------|------|------|---------|---------|
| | | Mean | SD | t-value | p-value |
| Working Ability | Pre | 2.53 | 0.5 | 5.75 | <0.001 |
| | Post | 1.73 | 0.47 | | |
| Location | Pre | 1.7 | 0.53 | 3.34 | 0.002 |
| | Post | 1.36 | 0.61 | | |
| Intensity | Pre | 2.4 | 0.49 | 3.81 | <0.001 |
| | Post | 0.96 | 0.18 | | |
| Days | Pre | 2 | 0.26 | 4.17 | <0.001 |
| | Post | 1.5 | 0.5 | | |

TableNo.3 Within group comparison for VAS pre and post intervention for group A and B

| Pre post comparison of VAS scale in Group A | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Pre | 5.66 | 0.75 | 27.34 | <0.001 |
| Post | 3.13 | 0.77 | | |

Table no.4

| Pre post comparison of VAS scale in Group B | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Pre | 5.93 | 0.73 | 36.93 | <0.001 |
| Post | 2 | 0.74 | | |





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Table no. 5 Pre- Post comparison of cumulative WaLIDD scale in Group A and B

| Pre post comparison of cumulative WaLIDD scale in Group A | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Pre | 8.4 | 1.25 | 6.96 | <0.001 |
| Post | 5.93 | 0.9 | | |

Table No.6

| Pre post comparison of cumulative WaLIDD scale in Group B | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Pre | 8.4 | 0.86 | 16.52 | <0.001 |
| Post | 5.8 | 0.71 | | |

Table.no 7 Within group comparison of WaLIDD

| Within Group comparison of WaLIDD | | | | | |
|-----------------------------------|---------|------|------|---------|---------|
| | | Mean | SD | t-value | p-value |
| Working Ability | Group A | 1.73 | 0.4 | -1.68 | <0.001 |
| | Group B | 1.73 | 0.7 | | |
| Location | Group A | 1.16 | 0.38 | -1.516 | <0.001 |
| | Group B | 1.36 | 0.61 | | |
| Intensity | Group A | 1.23 | 0.43 | 3.125 | <0.001 |
| | Group B | 0.96 | 0.18 | | |
| Days | Group A | 1.8 | 0.4 | 1.973 | 0.012 |
| | Group B | 1.56 | 0.5 | | |

Table no 8 Within group comparison of VAS and WaLIDD

| Within group comparison of WaLIDD- cumulative | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Group A | 5.93 | 0.9 | 0.617 | 0.32 |
| Group B | 5.8 | 0.71 | | |

Table no 9. Within group comparison of WaLIDD- cumulative

| Within group comparison of WaLIDD- cumulative | | | | |
|---|------|------|---------|---------|
| | Mean | SD | t-value | p-value |
| Group A | 5.93 | 0.9 | 0.617 | 0.32 |
| Group B | 5.8 | 0.71 | | |





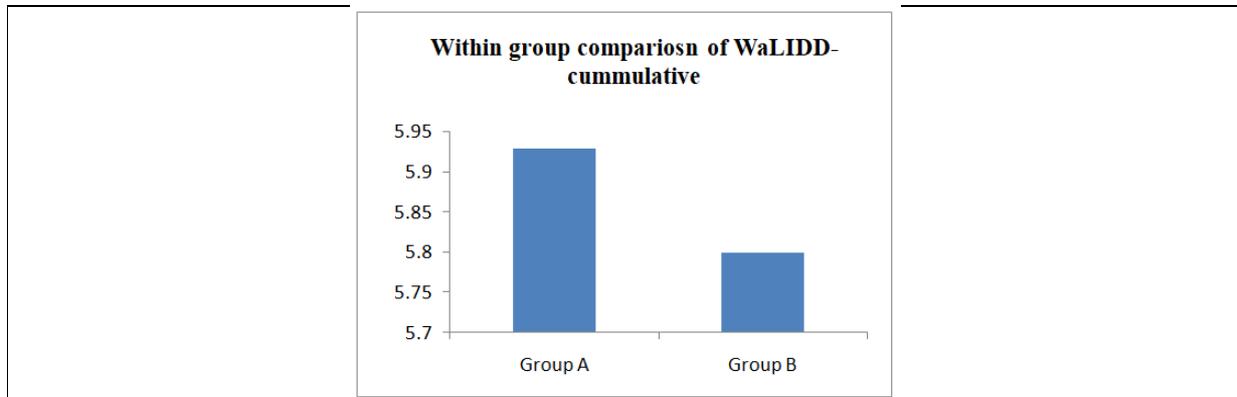
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| <p>Graph 1. Pre and Post comparison of WaLIDD in group A</p> | <p>Graph 2. Pre and Post Group B</p> |
| | |
| <p>Graph 3. Pre and Post Group A</p> | <p>Graph 4. Pre and Post Group B</p> |
| | |
| <p>Graph 5. Pre Post Comparison of Cumulative WaLIDD Scale in Group A</p> | <p>Graph 6. Pre Post Comparison of Cumulative WaLIDD scale in Group A</p> |
| | |
| <p>Graph 7. Pre Post Comparison of Cumulative WaLIDD scale in Group B</p> | <p>Graph 8. Within Group comparison of VAS</p> |





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Graph 8. Within group comparison of WaLIDD- cummulative





Evaluation of Osteogenic Differentiation Capability and Antimicrobial Effect of Graphene - Dexamethasone Coated PMMA Craniofacial Implants : Literature Review and Analysis

Ishita Jakhanwal^{1*}, Sweta Kale Pisulkar², Preeti Prakash Kale³, Mridul Ayush⁴ and Rashmi Rajendra Pagare⁵

¹Assistant Professor, Department of Prosthodontics and Crown and Bridge, D.Y.Patil Dental School, Pune, Maharashtra University of Health Sciences, Maharashtra, India.

²Professor, Department of Prosthodontics, Sharad Pawar Dental College and Hospital, Datta Meghe Institute of Medical Sciences, Deemed to Be University, Sawangi (Meghe), Wardha, Maharashtra, India.

³Lecturer, Department of Periodontology, Pravara Rural Dental College and Hospital, Loni: 413736, Dist: Ahmednagar, Maharashtra, India.

⁴Consultant, Radiodiagnosis Ayucare Diagnostic Centre and Dental Clinic, Viman Nagar, Pune, Maharashtra, India.

⁵Consultant, Oral Medicine, Diagnosis and Radiology, Ayucare Diagnostic Centre and Dental Clinic, Viman Nagar, Pune, Maharashtra, India.

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*Address for Correspondence

Ishita Jakhanwal

Assistant Professor,
Department of Prosthodontics and Crown and Bridge,
D.Y.Patil Dental School, Pune,
Maharashtra University of Health Sciences,
Maharashtra, India.
E.Mail: isitapublication12345@gmail.com



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ABSTRACT

Craniofacial defects represent alternations in the anatomy and morphology of the cranial vault and the facial bones as a result of trauma, tissue necrosis associated with infections, or a sequel following surgical procedures. Graphene is a single layer of graphite where sp² bonded carbon atoms are arranged in a 2D hexagonal frame first reported in 2004.⁶ Studies have shown that graphene and GO are highly biocompatible and characterized by low toxicity levels, thus, allowing their use in tissue regeneration, cell growth and cell differentiation. Polymethyl methacrylate (PMMA) is a lightweight, economical, synthetic polymer that is used in reconstructive surgeries where extremely high strength is not necessary. It is a non biodegradable polymer utilized in applications that require permanent, mechanically stable structures such as bone tissue regeneration. Osseointegrated implants were placed secondarily in the fibula for complete dental rehabilitation. Since both autogenous tissue and alloplastic implant were used ,

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it was named as “hybrid reconstruction. Being highly prone to bacterial adhesion and infection, along with its hydrophobic nature, the long term success of PMMA implants are still being debated.

Keywords: PMMA, construction, Graphene, Craniofacial, tissue.

INTRODUCTION

Craniofacial defects represent alternations in the anatomy and morphology of the cranial vault and the facial bones as a result of trauma, tissue necrosis associated with infections, or a sequel following surgical procedures [1]. Nowadays the most used alloplastic material by surgeons for this rehabilitation is the polymethyl methacrylate (PMMA), which is an acrylic based resin, biocompatible, non degradable material. PMMA prosthesis can be prefabricated, thus bringing advantages such as reduction of surgical time, easy technical handling and good esthetic results [2]. Furthermore, such polymeric implants allows better evaluation of bone-to-implant contact by micro computer tomography (μ CT) with light microscopy, which otherwise cause streaking artifacts around full metallic implant [3]. It is well known that mechanical and chemical features of such polymer based materials can be improved by doping the polymer with a suitable material, in order to produce a significant increase in their features for clinical application [4]. Graphene is regarded as a new carbon-based nanomaterial and its potential in various fields has been widely acknowledged, including chemical, biomedical, environmental and electrical fields [5]. Graphene is a single layer of graphite where sp^2 bonded carbon atoms are arranged in a 2D hexagonal frame first reported in 2004 [6]. Studies have shown that graphene and GO are highly biocompatible and characterized by low toxicity levels, thus, allowing their use in tissue regeneration, cell growth and cell differentiation [7].

Moreover, biological surface modification methods using bone-forming drugs or growth factors have been attempted to improve the osseointegration at the interface between implants and host bone. Dexamethasone, amongst such drugs, have seen to induce osteogenic differentiation in vitro and increases the alkaline phosphatase (ALP) activity, the expression of osteocalcin (OC) and bone sialoprotein (BSP) levels [8]. Consequently, the current study is being performed to evaluate the antibiofilm and antimicrobial properties along with the osteogenic potential of PMMA implants modified with Graphene-Dexamethasone (G-DEX) surface coatings.

REVIEW OF LITERATURE

Polymethyl methacrylate (PMMA) is a lightweight, economical, synthetic polymer that is used in reconstructive surgeries where extremely high strength is not necessary. It is a non biodegradable polymer utilized in applications that require permanent, mechanically stable structures such as bone tissue regeneration [9]. PMMA was first developed by German chemist Otto Rohm in 1901 and in its earliest health applications, it was primarily used in the field of dentistry. Furthermore, Sir John Charnley suggested that PMMA adds an elastic value between prosthesis and bone which allows for increased shock absorption, distribution of contact forces, and rigid fixation [10]. The first clinical application of PMMA was to repair cranial defects in monkeys in 1938. Further, with greater understanding of PMMA, surgeons moved towards using these materials in reconstructive surgery on humans [11]. Both Titanium and PMMA are the most widely used materials for implants in the correction of large bony defects, however, PMMA, because of its reduced surgical time, use of simple technique and excellent long term esthetic results along with being less expensive, more readily accessible and easier to handle and contour for specific craniofacial defects, have still kept it as a more reliable and acceptable option. Samiran Ghosh et al, in 2017, have used Polymethyl methacrylate implant for Fronto-orbital reconstruction. The contour and esthetic correction of the deformity in right supraorbital region was achieved by overlaying the defect with a PMMA implant fabricated over a three-dimensional stereo lithographically printed rapidly prototyped model [12]. Reconstruction of postmaxillectomy defect has always been challenging. Sanjay Mahendru et al, in his case report presented a patient with left



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zygomatoco maxillary complex reconstruction with a combination of autogenous tissue (free fibula flap) and alloplastic implant (patient specific template PMMA) . Osseointegrated implants were placed secondarily in the fibula for complete dental rehabilitation. Since both autogenous tissue and alloplastic implant were used , it was named as “hybrid reconstruction” [13]. Furthermore, owing to its property of being well tolerated without significant inflammatory foreign body reactions and the property to create PMMA customized implants unique to each patient’s need, its use also have been reported in esthetic reconstruction of midfacial defects which includes repair of orbital and malar defects [14].

Despite the excellent PMMA bulk properties, its surface inherited hydrophobic nature limits its compatibility when used as implant bio-material. Thus, surface modification of PMMA is necessary to improve its biocompatibility and to reduce implant complications [15]. Also, although PMMA allows a fast primary fixation to the bone, it does not gurantee a mechanically and biologically stable interface with bone, mainly because it is prone to bacteria adhesion and infection development [16]. Studies demonstrate that PMMA – based bone cements can be mixed with inorganic ceramics or bioactive glass to modulate curing kinetics and enforce mechanical properties. Additions of antibiotics within the cement have shown to reduce the risk for prosthesis – related infection [17]. In the recent past, graphene has raised wide interest due to its thermal, mechanical, electrical and biological properties. It is characterised by a carbon-based material with mono-atomic layer thickness , considered as the first two-dimensional crystal. This structure offers graphene an exceptionally high mechanical stiffness and extraordinary high thermal and electrical conductivity. It is used as coating of materials and biomaterials that usually lack these characteristics, hence, is ideal for improving the performance of autopolymerising acrylic resins [4].

Graphene modified PMMA implant has become a core area of research in the recent past mainly because of its ability to modify thermal, mechanical and biological properties of the implant. One of its highly studied properties include the osseoblastic differentiation and tissue regeneration capability around the bone-implant interface. Antonio Scarano et al, in 2021, evaluated osseointegration of Graphene Doped (GD) PMMA compared with PMMA as potential material for dental implant, and concluded that GD-PMMA surfaces enhanced osseointegration in rabbit femurs.4 Roberta Di Carlo et al, in his study, confirmed that the graphene oxide functionalization provides a non cytotoxic biomaterial that is able to stimulate cell viability and induces osteogenic differentiation better with respect to titanium discs [7]. Furthermore, the study on the effect of graphene substrate on osteoblast cell adhesion and proliferation by Ashkan Aryaei et at, showed that graphene does not have any toxic effect on osteoblasts and also cell adhesion is improved with graphene coated substrate than the substrate alone. Moreover, the study concluded that a layer of graphene on bone implants will be beneficial for osteoblastic attachment and proliferation [18].

However, reviewing other articles, it is evident that there is a school of thought which believes that with the increase in osteoblastic (cell) attachment property of the implant, the bacterial cell adhesion also increases. Thus, any initiation of peri-implantitis might lead to an increase in bacterial cell adhesion and aggravate the inflammatory process around such implants. Hence showing the need of thorough research and better understanding of the inter-relation between the Cell-adhesion and antibacterial properties of the surface modified implants.

Davies proposed an ideal osseointegration period of 3-6 months after a successful implant operation. However, the soft tissue and bone are more susceptible to periodontitis during this time, which stimulates inflammation and bone loss, requiring antibiotic treatment. Lindhe and Meyle revealed that the risks of peri-implant mucositis are about 80% in patients and the risks of peri-implantitis are about 28-56% in patients, following dental implant surgery [5]. Different protocols have been proposed in the literature to reduce the bacterial load and remove biofilm from the surfaces of infected implants, including mechanical, chemical photodynamic and laser treatments. However, none of these methods have been proven to be effective in achieving satisfactory decontamination of the implant surface, and there is no consensus about the most advantageous treatments in recovering peri-implant health. In addition, the widespread use of antibiotics may lead to adverse events and even select antibiotic-resistant bacteria. Sunho Park et al, developed a graphene-chitosan (GC) hybrid dental implant using various concentrations of graphene and concluded that the GC hybrid implant under optimal condition (1% GC hybrid implant) could significantly promote

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osteoblast proliferation while reducing biofilm formation and bacterial activity [5]. Apart from chitosan, various other drugs such as simvastatin, vitamin D, ascorbic acid and amethasone have been proved to be good options for improving biocompatibility and osteogenic activity. Among them, dexamethasone (DEX) has already been widely used to upgrade the osteogenic activity and biocompatibility of biomaterials [8]. It has also been proved that active bone formation and strong osseointegration occurs at the interface between DEX/ Growth and Differentiation Factor-5 Surface modified titanium implants and the host bone, as evaluated by micro computed tomography analysis, by Dae Hyeok Yang et al [8]. Importantly, Dex is also a kind of potent steroidal, anti-immune and inflammatory-response suppressing drug, and could inhibit inflammatory response around implant surfaces [20].

Research Gap Analysis

Current State : PMMA is a widely used material for implants in corrective and reconstructive surgeries of large bony defects, maxillofacial and craniofacial defects. Various studies are being undertaken to improve its surface inherited hydrophobic nature and bacterial prone surface to increase its compatibility when used as implant biomaterial.

Desired State : To obtain surface modified PMMA implants to improve its biocompatibility and anti-biofilm effect, thus reducing implant complications. Thus, the study aims to provide a novel surface modified PMMA implant for large bony defects, maxilla-facial and craniofacial defects.

Identified Gap Analysis : Thorough and systematic literature review suggests that only limited data have been published regarding inclusion of graphene in PMMA [21] implants for improving its osteoblastic differentiation capability, which can thereby improve its osseointegration and biocompatibility. Moreover, it is still a challenge to develop an ideal PMMA implant that can effectively promote osseointegration while suppressing bacterial biofilm formation and activity.

Implications Of the Gap : Being highly prone to bacterial adhesion and infection, along with its hydrophobic nature, the long term success of PMMA implants are still being debated. Moreover, PMMA implants although provides a fast primary bone fixation, does not guarantee a long term mechanically and biologically stable interface with the host bone and require studies to fill the gap regarding the lack of data on surface modification of PMMA implants to improve its osseointegration and biocompatibility while suppressing bacterial biofilm formation and activity.

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AUTHOR'S CONTRIBUTION

1. Ishita Jakhanwal: Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review, Guarantor.
2. Sweta Kale Pisulkar :Concepts, Design, Definition of intellectual content, Literature search, Clinical studies, Data acquisition, Data analysis, Statistical analysis, Manuscript review
3. Preeti Prakash Kale: Concepts, Design, Data acquisition, Data analysis, Statistical analysis, Manuscript preparation, Manuscript editing, Manuscript review
4. Mridul Ayush: Concepts, Design, Data acquisition, Data analysis, Statistical analysis
5. Rashmi Rajendra Pagare: Concepts, Manuscript preparation, Manuscript editing, Manuscript review.



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Teaching of Chemistry through Eclectic Method; Need and Importance

R. Suresh^{1*} and G. Arumugam²

¹Ph.D Research Scholar, Department of Education, Annamalai University, Annamalai Nagar - 608002, Tamil Nadu, India.

²Associate Professor, Department of Education, Annamalai University, Annamalai Nagar -608002, Tamil Nadu, India.

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*Address for Correspondence

R. Suresh

Ph.D Research Scholar,
Department of Education,
Annamalai University,
Annamalai Nagar -608002,
Tamil Nadu, India.



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ABSTRACT

Teaching is a Noble profession by which children are trained at all Educational levels. Students learn so many things and grow physically, mentally and morally. Teacher use various Approaches and Techniques to teach their students at every level. when it comes to a science subject particularly chemistry, there are certain methods and Technique followed by Teachers to teach Eclectic method is one of kind that provides and is a method that integrates in teaching chemistry. This approach depend learners. This research paper Explores the advantages, characteristic, and steps followed in Eclectic method. And Examines various Aspect of Eclectic Approaches in teaching chemistry at higher secondary schools.

Keywords: Eclc, Elt, Eclectic Approach, Eclectic Method Paradigam, Blended Learning, Chemistry.

INTRODUCTION

As its Name indicates. Eclectic method is not an ESL teaching method. but an approaches According to the British council approach we follow as Teacher is going to determine what method activities and techniques we will apply in our classes. There was and probably Never will be a method for all.(D.Nunan). As indicated by Atkinson who tried to recommend ECLC in teaching through his research (1988), no one who is aware of psychology and art of teaching would recommend a "monolithic model" of teaching. One of the main reasons for experimenting on the use of ECLC is the revelation that various students learn and understand concepts by different means, as students can be visual, auditory or kinesthetic learners. Consequently a teacher has to adopt a combination of methods to cater to all types of learners and to maintain interest in the lesson. With this kind of an understanding researchers have developed

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different Eclectic Approaches highlighting several methodologies. Cruz's ECLC consists a combination of the DM, ALM, S and TPR, utilizing all the benefits these teaching methodologies offer (Acevedo 2006). However, ECLC cannot be thus restricted, when numerous methods which can be artistically combined in a classroom are available. Thus, going beyond the research format of Cruz, the present research attempts to suggest the effectiveness of combining a variety of methods, following a coherent procedure. Here, the ideas of Freeman and Mellow become vital as they indicate the importance of combining structural and communicative approaches in teaching.

Freeman and Mellow (2000) who refer to "principled eclecticism", have proved the significance of mixing structural approaches with communicative use of language (Shakir 2011). For instance structure of the language, traditional reading, writing and conversational activities are included in one lesson while the learner's role is cooperative and the teacher acts as a facilitator. Thus, as Li has also correctly mentioned, "monotonous activities can never keep students highly motivated and only various activities catch their attention", hence; identifying and recommending the best combination for an effective use of high value. Consequently, following Freeman, Mellow, Li and *et. al.* the current research attempts to examine the effectiveness of using ECLC which combines both structural and communicative methods of teaching.

Few Eclectic method for learning chemistry (June Venelee A. Delgado *et al.* (2017), Fateme SamieiLari (2014), Ozaslan&Maden (2013), Zhao (2007), KubilayKaptana Ozden Timurlenk 75 (2012), Emendu, N.B. P, Okoye, C.M (2015), Soewarno S, Hasbi Ali *et al.* (2013), QaiserSuleman *et al.* (2016), Suleman, Qaiser and Hussain, Ishtiaq (2016), Honebein, Peter C.; Sink, Darryl L. (2012), Price, Matthew; Rogers, Michael (2016), Coskun, Hilal; Dogan, Alev and Uluay, Gulsah (2017), Demir, Yusuf; Ertas, Abdullah (2014), Kim, Mijung (2011), Cavagnetto, Andy R. *et al.* (2011), Duru, Adem (2010), George, Linda A.; Brenner, Johanna (2010), Buxton, Cory A. and Provenzo, Eugene F., (2010), Inel, Didem and Balim, Ali Gunay (2010), Dandy jan, Mongfing (2007), Raes, Annelies; Schellens, Tammy; De Wever, Bram (2014), Edwin (1991), Molina Mercado (1984), Gerido,Leona; Curran, Mary Carla (2014), Roehling, Patricia V and Trent-Brown, Sonja (2011)).

The Eclectic Approach

The main goal here is to create a new method that combines all of the existing methods and incorporates them into ELT while maximising the benefits of each method, approach, and technique. In fact, the main idea is to employ all available methods, approaches, and techniques in varying proportions depending on the circumstances of the classroom. The Eclectic Approach incorporates all teaching methods based on the classroom circumstances and the learners' abilities.

Advocated strengths and weaknesses context. Taking the drawbacks of the various methods into consideration, Eclecticism is a conceptual approach that does not adhere to a single paradigm or set of assumptions, but instead draws on a variety of theories, styles, or ideas to gain complementary insights into a subject or applies different theories in specific cases."Eclecticism is defined as a type of methodology that employs a variety of language learning approaches rather than relying on a single standard approach" (A L Hamash, 1985:22).

According to Weidman (2001:2), "The Eclectic Approach has been widely accepted in classrooms that many good teachers today use it proudly as a tag to describe to their teaching, wearing it almost like a badge of honour". Kumar (2013) supports the Eclectic Approach And Declares, "The purpose of advocating eclectic method is to connect life experiences to the ideas presented in learning of the language. The types of learning activities teachers select are often directly related to their experiences in the real world." Brumfit (1984) emphasized that eclecticism was a right approach for teaching and he further commented on learning program should provide a balance of activities such as accuracy and fluency. Accuracy focuses on form and fluency on exchanging meaning, accomplishing tasks and reaching outcomes." Thus, many teaching experts have supported the Eclectic Approach for teaching methods, particularly ELT.



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The Eclectic Approach's multiple lines embody teachers to choose wisely and assists them in their work. Eclecticists strive for stages, with an emphasis on early aural-oral skills development. To keep lessons varied and interesting, an eclectic teacher must be imaginative, energetic, and willing to experiment. He went on to say that eclecticism was the right approach for teaching, and that learning should provide a balance of activities such as accuracy.

What are the main characteristics or advantages of eclectic approach?

Advantage or characteristic is no aspect of language skill ignored.(Crandal,2003)[10] but also encourage student pride in their heritage, language, communication.(Alizadeh & *et al*, 2016) [11] states that different teaching methods are borrowed and adopted to suit the requirement of the learners. It breaks the monotony of the class. In addition, it is conceptual approach that does not merely include one paradigm or a set of assumptions. Such variety and combinations of methods and techniques which are used in this method it maintain keeping the learning process interesting and no boring can be touched during the whole learning and teaching process to gain the main object of the class. Then communicative competence will be developed in a unique way.

Safety

The use of a variety of ideas and procedure from different existing Approaches and methods will increase the chances of learning taking place.

Interest

Teacher Need to use different techniques to hold the learners attention.

Diversity

Different learning/teaching contexts require different methodologies.

Flexibility

Awareness of a range of available Techniques will help teachers exploit materials better and manage an expected situation.

Salient features of the Eclectic Approach

The Eclectic Approach is regarded as one of the best approaches in ELT. Teachers will be successful in teaching if they implement this approach in chemistry learning classrooms. Let us examine the salient features of Eclectic Approach.

- Eclectic Approach (EA) makes teaching innovative and enjoyable. The learning objectives are easily achieved.
- The learner participates in a lesson actively and interacts with teacher and other students.
- EA brings capabilities among the students and caters for individual needs.
- The EA makes the teaching of main subject-chemistry practical as it facilitates retention and build confidence in learners.
- This approach enables teachers attain the objectives of learning easily as the learners understand the concepts and acquire knowledge because learning becomes fun and innovative.
- It further strengthens effective learning of chemistry as more information of knowledge is retained for a long time that enables learners to developing knowledge.
- It breaks boredom and creates a room for acquiring more concepts.
- Depending on the aims and objectives of the lesson and the learners in the group, the teacher decides what methodology or approach to use.
- It is a problem-based approach to teaching chemistry that solves the problems faced by the learners in the classroom.
- Testing is a part of this method and not a separate entity.
- Teachers can attain good results without much pressure on the learner.
- Learners can have a clear vision what they are learning.
- The features of this method include multiple tasks, lively learning, high interaction, objective correlative and fast results.
- This approach connects life experiences to the ideas presented in learning the subject.



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- This approach is very popular now-a-days because the learners are heterogeneous and versatile level intelligent in the classroom.
- EA supports natural order of learning, first listening, followed by speech and then reading and writing.
- This approach gives equal importance to the four language skills, i.e., listening, speaking, reading and writing.
- The students should be active learners instead of being mere recipients of information.
- Teacher should act as facilitators and provide opportunities for students to express their ideas, without any fear, in chemistry.
- Activities and resources should be adapted as much as possible to use chemistry in natural contexts.
- Various activities are introduced in the chemistry classroom by using the innovative technology available.
- There is flexibility for teachers to choose any method or approach that suitable for teaching inside the classroom.
- EA saves both time and effort in the presentation of subject activities.
- The teacher introduces exercises of guided discovery for new rules.
- The teacher proposes a variety of exercises, both written and oral, in order to improve the learners' communicative abilities with accuracy and fluency.
- EA focuses on motivation and learner autonomy, selecting techniques based on the learner and context.
- The Eclectic Approach is the juxtaposition of both the inductive and deductive strategy to teaching science.
- The concept of the Eclectic Approach is that learners learn not only from teachers but also from fellow learners by working together or as a group.
- Thus, the salient features of Eclectic Approach are discussed thoroughly and in a comprehensive way. There are more advantages than disadvantages of the Eclectic Approach

Advantages of Eclectic Approach

Change is unavoidable in any field, including education. As the saying goes, "Novelty is the spice of life," and every human being desires change and strives to find solutions and make changes in his or her field of work. The same is true for subject instruction-and The process of learning chemistry learners are always looking for new ways to learn the chemistry, something innovative and exciting. chemistry teachers use the benefits of various methods to achieve this goal. approaches and techniques to ensure that learners learn the chemistry in a welcoming and friendly environment. The benefits of the Eclectic Approach have been thoroughly examined and are listed below:

- This is a broad approach which includes all kinds of learning activities and gives relief to learners from monotony.
- Eclecticism involves a variety of learning activities motivated by different underlying assumptions.
- It ensures constant reflection and renewal of the teacher
- It creates a good learning environment in which the student-student relationship is improved.
- With the Eclectic Approach, it is easier and more possible for the learners to understand the subject in chemistry text with the context of culture.
- It facilitates the achievement of objectives.
- It provides the perfect opportunity for intensive role-playing and "real world" game activities.
- Eclectic method of learning is an integral part of the total learning process.
- It blends the practice of listening, speaking, reading and writing into an organic whole.
- It recommends that any method or approach can be used based on the level of the students.
- The Eclectic Approach enables teachers to choose any teaching technique which is suitable to reach the aims of the lesson.
- The teaching-learning process becomes easier and rewarding.
- The Eclectic Approach has the potential of keeping the chemistry teachers open to alternatives.
- Teaching of chemistry is made easy by creating realistic situations in the classroom through Eclectic Approach.

The Role of Teacher

Here we Adopting the role of Teacher roughly equal to CLT. we consider Teacher as a facilitator who facilitates as a slightly higher rank official who use his authority to conduct the class and make the process of teaching and learning systematic.



**Suresh and Arumugam****The Role of Learner**

The learner here is seen by us the center of teaching learning activities. His participation is very important. so the teacher will always try to involve the learners as well as the learners role in class should be cooperative and the will be allowed to communicate. Self-correct each other and ask questions about the substance provided for teaching learning activities.

Eclectic Method Paradigm.

Level -1; Identification of the hard spot of specific units.

Level-2; Teacher prepared selected unit based subject splits into certain meaning full small units

Level-3; Introduce different mixing of method (Eclectic Method)

Level-4; Power point presentation.

Microsoft software that allows users to include audio, visual, and audio/visual elements in their presentations.

It is a multimedia technology that also serves as a tool for collaboration and content sharing.

Level-5; Using dynamic aids and Static aids;

dynamic aids

This means that **the pupils are active, are involved in what they are learning.** This implies adopting a whole new approach to a lesson so that they are not just waiting, not just listening. On the contrary, they must actively participate in different ways.

examples. Movies. animation. simulations.

Static aids

Static (still) visual aids don't move or produce sound. According to our survey, the most common types are overhead transparencies, flipcharts, chalkboards and slides.

Design Techniques;

As noted in this paper, Trainer's Aid--Static Visuals covers the entire visual environment in which presentations take place. Appendix A contains the complete table of contents. Of particular interest to CAITW members are the following design considerations: color - avoid emotional colors such as orange and red, as well as multicolour visuals text - use a point system with bullets rather than sentences- use no more than 6 lines of text per visual lettering - type style - use simple style, such as san serif regular Helvetica- avoid curlicues, fancy lettering, borders, etc. as they tend to trigger distracting associations much like the Rorschach (inkblot) test- use a minimum of 18 point for transparencies and slides, and minimum of 2-inch letters for flip charts and chalkboard.

Level-6; Online learning.

Online learning is education delivered via the Internet. It is also known as "e-learning," among other things. Online learning, on the other hand, is only one type of "distance learning" the umbrella term for any learning that takes place over time and not in a traditional classroom. Because the student is responsible for engaging with the course rather than simply showing up to class on an assigned day and time, online courses teach students how to better manage their time. As a result, students not only learn from their coursework, but they also improve their time management skills.

Level-7; Blended learning.

In a report on the merits and potential of blended education, the Sloan Consortium defined hybrid courses as those that **"integrate online with traditional face-to-face class activities in a planned, pedagogically valuable manner."** Educators probably disagree on what qualifies as 'pedagogically valuable,' but the essence is clear: Hybrid education uses online technology to not just supplement, but transform and improve the learning process.

What are the characteristics of eclectic method

What are the primary characteristics or benefits of an eclectic approach?



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The main feature of this approach is the variety in adapting methodology and technique to achieve the main goal of teaching goal. Adapting such a method or theory in teaching (variety) also makes the classroom atmosphere dynamic and eliminates the monotony concept.

Chemistry

Energy released or absorbed during these processes. created, is made up known as elements. Although these atoms are made up of more elementary particles, They are the fundamental building blocks of chemical substances; there is no quantity of oxygen, mercury, or gold smaller than an atom of that substance, for example. Chemistry is thus concerned with the properties of atoms and the laws governing their combinations, as well as how knowledge of these properties can be used to achieve specific goals.

The scope of Chemistry

The days of a single person having a thorough understanding of all aspects of chemistry are long gone. Those who are interested in specific areas of chemistry communicate with others who have similar interests. Over time, a group of chemists with specialised research interests form the foundation of a field of study. Early in the history of chemistry, areas of specialisation emerged. Organic, inorganic, physical, analytical, and industrial chemistry, as well as biochemistry, continue to be of the greatest general interest. During the twentieth century, however, there was significant progress in the fields of polymer, environmental, and medicinal chemistry. Furthermore, new specialties, such as pesticide, forensic, and computer chemistry, are constantly emerging.

Inorganic Chemistry

The study of the properties and behaviour of inorganic compounds such as metals, minerals, and organometallic compounds is known as inorganic chemistry. While organic chemistry studies carbon-containing compounds, inorganic chemistry studies the remaining (non-carbon-containing) subset of compounds. However, there may be some overlap between the two fields. Organometallic compounds, for example, typically contain a metal or metalloid bonded directly to carbon.

Professional chemists need to be able to:

- make connections between topics in separate subjects
- make judgements about the most appropriate methods for investigating a problem
- clearly explain their views or conclusions to people with different backgrounds
- manage their time effectively
- think independently and flexibly
- take responsibility for continued learning.

Utilities of Eclectic Method

1. Teachers can tailor their approach to the circumstances and realistic situations in the classroom.
2. Teachers are self-sufficient enough to involve students in classroom activities such as idea exchange, group work, language workshops, and role play, among others.
3. This method reduces detachment and dullness by allowing students to multitask in order to make the classroom environment more dynamic.
4. Because different teaching strategies are used, learners develop greater understanding and memorising abilities in the Eclectic Method than in CLT and GTM.
5. Classes in this approach are frequently participatory, reciprocal, lively, and student-centered.
6. This method contributes significantly to making students extrovert and enthusiastic about classroom activities.
7. Using this method, students can develop and apply both their analytical and creative abilities.

Problems in Implementing Eclectic Method

1. Language teachers may struggle to teach the Eclectic Method due to a lack of proper training.



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2. Inadequate text books, as well as a lack of curriculum and syllabus regarding the Eclectic Approach, may impede the application of the Eclectic Method.
3. The lack of an audio-visual classroom, an uninterrupted power supply, and insufficient institutional and logistical support may be obstacles in this regard.
4. A large classroom size of 40 or more students may be an impediment to implementing this approach.
5. Students with low socioeconomic backgrounds may find it difficult to implement this method.
6. This method does not provide specific instructions.

Research Findings

- a. Equal importance must be placed on four skills: listening, speaking, reading, and writing.
- b. Monitoring from appropriate authorities regarding the implementation of the Eclectic Method in teaching must be ensured.
- c. A smart phone, computer, laptop, tablet, projector, and soundproof classroom should be available.
- d. Teachers must be motivated to change their rigid mindsets, and they must be given adequate instruction and training in the Eclectic Approach.
- e. Unusual class sizes must be reduced to a manageable 30(thirty) students.
- f. Anglophobia must be eliminated, and students must be outgoing.
- g. Standard and up-to-date text books must be written using the Eclectic Approach, and the curriculum and syllabus must be redesigned using the Eclectic Method.

CONCLUSION

At first, this study dealt in detail, the characteristics and advantages of using eclectic method to teach any subject. The study then proceeds towards incorporating Eclectic method in teaching Chemistry which needs an amalgamation of various methods to make the teaching learning experience lively and effective. This method takes care of the individual level of learning of the learners, promoting self-paced learning, and uses directive and non-directive methods of teaching that can help in framing policy on age appropriate teaching methods.

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An Interventional Study on the Efficacy of Action Observation Therapy and Mirror Box Therapy on Functional Motor Capacity of Upper Limb in Post Stroke Subjects

Thummala S Pavani^{1*} and S.Senthilkumar²

¹Research Scholar, Garden City University, Bangalore, Karnataka, Associate Professor, East Point College of Physiotherapy, Bangalore, Karnataka, India.

²Professor and Research Supervisor, School of Health Sciences, Department of Physiotherapy, Garden City University, Bangalore, Karnataka, India

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*Address for Correspondence

Thummala S Pavani

Research Scholar,
Garden City University, Bangalore, Karnataka,
Associate Professor,
East Point College of Physiotherapy,
Bangalore, Karnataka, India.
E.Mail: @gmail.com



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ABSTRACT

The intention of our pilot-study was to evaluate the effectiveness of Action observation therapy and mirror therapy with bilateral functional motor arm training which is two promising rehabilitation strategies, aimed at enhancing the motor learning and functional improvement of stroke patients through different patterns of visual feedback and observation. Action observation improves excitability of the primary motor cortex and the encoding of motor engrams as well as motor-learning. Action observation therapy (AOT) is a developing neuro rehabilitative tool, which is activated not only during the execution of an action, but also during the observation of the same action based on the existence of the mirror neuron system (MNS). Bearing in mind this "dual" activation, the AOT proposes that motor symptoms of different neurological disorders can improve with the observation and imitation of different actions. The current study focuses on elements of these therapies that have never been explored before. Recent research has shown that action observation (AO) has a clinically significant effect on stroke patients' recovery of upper limb function. We set out to test the idea that practising daily activities through AO and carrying out MBT tasks would cause the left hemisphere to become more active than the right. The idea that the same brain regions responsible for the actual execution of actions are activated during action observation and execution is now widely acknowledged in the field of neurophysiology. The mirror neuron system is a term used to describe regions that have this action observation-action execution matching mechanism. This neurophysiological mechanism is used by AOT to recover from

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motor dysfunction. In stroke patients, activation is essential for the restoration of limb function. It is unknown, though, whether there are any differences between the two approaches in terms of cortical excitability in people. The objective of this study was to determine the effectiveness of Action Observation therapy and Mirror Box therapy and also we compared the effects of Action Observation therapy and Mirror Box therapy with bilateral functional motor training in improving the functional motor capacity of upper limb in patients with sub-acute stroke and chronic stroke subjects. This study aimed to investigate the effects of Action Observation therapy and Mirror Box therapy and aimed to compare treatment effects of Action Observation therapy and Mirror Box therapy with bilateral functional motor training to improve the functional motor capacity of upper limb in patients with sub-acute stroke and chronic stroke subjects. An estimated Seventeen subjects with ischemic and haemorrhagic sub-acute stroke and chronic stroke were recruited in this study. All participants were randomly assigned to receive AOT and MBT, or bilateral functional motor training intervention for a 3-week training period (15 sessions). Outcome measures were conducted at baseline, immediately after treatment, and at 3-month follow-up. The primary outcome was the Fore arm bisection task, Fugl-Meyer Assessment, and secondary outcomes included the Box and Block Test, Motor assessment Scale. Video training was used to deliver and it was highly accepted by patients. Six weeks of motor functional training suggests improvements in the motor functioning of hand, distal shift in the pointing the middle of forearm, Video-therapy appears to be promising, as an adjunct to the bilateral functional motor training. The action observation therapy, Mirror box therapy group has showed improvements on the Fore arm bisection task, Fugl-Meyer Assessment, Motor assessment Scale, but has showed similar improvements on Box and Block Test. Moreover, the action observation therapy had a greater improvement on the Fore arm bisection task, However, the bilateral functional motor training group gained the least improvements on the Fugl-Meyer Assessment, Motor assessment Scale. The preliminary results found that the patients in the action observation therapy and MBT has shown good improvements in task completion compared with functional motor training, further large-scale study with at least 30 patients in each group to validate the study findings is needed. Video training appears to be promising and is easy to deliver as an adjunct to neuro rehabilitation and highly accepted by patients. Six weeks of intervention suggests improvement of hand function measured based on Motor assessment scale. This clinical trial will provide scientific evidence of treatment effects on motor, functional outcomes, and neural activity mechanisms after AOT and MT in patients with sub acute and chronic stroke. Further application and use of AOT and MT may include rehabilitation through web-based or video teaching.

Keywords: mirror neurons, neurological rehabilitation, rehabilitation interventions, action observation, motor recovery, Upper limb Video therapy

INTRODUCTION

The World Health Organization's (1989) definition of a stroke in an adult (16 years of age or older) was chosen, and it is defined as a quickly developing clinical symptom of a focal disruption of brain function with a likely vascular origin and lasting more than 24 hours. The primary cause of death and disability in older persons is stroke, also known as apoplexy. Although the majority of stroke survivors are able to walk independently once again, many still struggle to complete daily tasks, especially those related to self-care and housekeeping [4]. Stroke occurs due to thrombus, emboli, and haemorrhage with an incidence of 203 cases per lakh people in the age group over 20 years,



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with a male to female ratio of 1:7, and 12% of stroke cases are seen in the age group below 40 years. It is also reported that 1.2% of all deaths in India are due to stroke, and the cause of the disease depends on the severity of the lesion.

In clinical terms, a number of focused deficits are possible, including changes in loss of consciousness and impairments of sensory, motor, cognitive, perceptual, and language abilities. A stroke is the sudden loss of neurological function brought on by an interruption of blood flow to the brain. Hemiplegia (paralysis) or hemiparesis (weakness) often occur on the side of the body that is opposite the site of the lesion, and these symptoms are indicative of motor impairments. The clinical symptom of neurovascular disease of the brain is hemiplegia, or paralysis of one side of the body. It is a symptom of neurovascular illness and happens when the cerebral hemisphere or brain stem is affected by a stroke. According to the National Institute of Neurological Disorders, the word "hemiplegia" is frequently used to refer to the wide range of issues that might arise after a stroke.

CVA refers to any illnesses in which one or more brain blood vessels are primarily compromised by a pathogenic process or in which ischemia or bleeding temporarily or permanently affects a brain region. When the duration of signs and symptoms is less than 24 hours, the focal neurological deficits must be present for at least 24 hours and take longer than 3 weeks to resolve in order to be classed as a stroke or hemiplegia. The incident is categorised as a Transient Ischemic Attack Cerebral edoema, which is a symptom of severe infarcts involving the M C A, is the most prevalent cause of death in acute stroke. The acute stroke, 1985; Hachinski V, Norris J, et al. Ischemic stroke is the most frequent type of stroke, accounting for over 80% of all strokes, and is caused by two major causes. Atherosclerosis, or the slow buildup of cholesterol, is the most frequent cause of an ischemic stroke. It is brought on by a clot or other blockage in a brain artery. (<http://Stroke.Center.com>). Intra cerebral haemorrhage, caused by an aneurysm or trauma, is the abnormal bleeding into the extra vascular portion of the brain. At every stage, intra cerebral haemorrhage happens. About 12 to 24 percent of all strokes are hemorrhagic strokes [14] With 70% of all strokes being cerebral infarctions (thrombosis, embolism), it is the most prevalent type of stroke. 20% of the cases are haemorrhages, while 10% are still unknown [19]

Hypertension, diabetes, and heart disease are the three most well acknowledged risk factors for cerebral vascular disease. Hypertension is the most crucial of these elements. The fluctuation of systolic or diastolic blood pressure, pulse pressure, and various pressures are all excellent stroke predictors. Increased blood fat levels, obesity, and smoking are risk factors that have been linked to stroke. since the greatest risk factor for stroke is excessive blood pressure[20]. It is uncertain how many haemorrhages begin as ischemic strokes because some haemorrhages (hemorrhagic transition) arise inside areas of ischemia [16] The neurological abnormalities caused by a systemic feature are bilateral and have a global aspect. Impaired motor functions, balance issues, sensory deficits, cognitive limits, visual difficulties, aphasia, and depression are common disabilities following a stroke. 6 People who have survived the initial stages of a stroke typically exhibit some improvement in their capacity to move and carry out daily chores over time. According to Warlow et al.[7], a number of processes, including neuroplasticity and adaptive alterations, have been proposed to explain recovery.

Approximately 40% of stroke victims must live with a moderate to severe disability, according to statistics from the Heart and Stroke Foundation of Canada from 2003. The majority of neurological recovery occurs in the first 1 to 3 months after a stroke, while only minor changes are visible between 3 months and a year later. Early recovery is typically attributed to the resolution of regional metabolic and vascular variables. Thus, undamaged neurons that were previously blocked can restore activity due to the reduction of edoema, absorption of injured tissue, and enhanced local circulation. The continued healing is assumed to be explained by C N S plasticity. The recovery of motor function after a stroke varies significantly. Depending on the anatomical site of the lesion, the relative involvement of the arm and leg varies. Nonetheless, the arm is typically more severely injured than the leg. Furthermore, efficient hand usage necessitates a high level of fine motor control, whereas the return to walking can be accomplished with only a slight recovery of motor control in the leg.



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One of the most disabling side effects of stroke is upper limb hemiparesis, which is also the main impairment underpinning functional disability after stroke [21]. One of the most frequent conditions that physical therapists and occupational therapists treat is upper limb hemiparesis [22]. Because it reduces problems like deep vein thrombosis and pressure sores, early mobilisation is preferred [27]. Preventing complications, managing co-morbidities, and managing stroke recurrence are the top priorities in early stroke rehabilitation [26].

A decrease in the combined adverse outcome of death or worsening was linked to more extensive physiotherapy input, which may also hasten the rate of recovery [25]. However, intensive rehabilitation is costly, and many managed care organisations only pay for a set number of therapy sessions before stopping their support for rehabilitation. Additionally, the few sessions might cover a wide range of services and a lot of different talents. As a result, it's possible that repetitive exercise won't be given frequently enough for motor learning to take place. In order to improve upper extremity function after a stroke, therapy is used, which may require more repetitive practise of skills [2]. Early after a stroke, effective rehabilitation interventions can speed up recovery and reduce functional handicap. Enhanced functional results for patients also help to increase patient satisfaction and can cut down on potentially expensive long-term care costs. Strong evidence suggests that post-acute stroke cases benefit from a well-organized multidisciplinary approach.

It should be emphasised that the majority of research on the recovery of the upper limbs have looked at patients getting the frequently prescribed mainly bobath therapy, and the outcomes are likely to be favourable since they represent the effects of these therapies on the healing process. The trials that produced the favourable outcomes involved individuals whose rehabilitation involved active, task-related activities. For retraining motor skills in adult hemiplegic patients, a number of movement treatment modalities are offered in physiotherapy. Roods, PNF, Brunnstrom, and Bobath are examples of techniques that rely on reflex hierarchical theories of motor control, whereas motor relearning programme and systems theory approach derive therapeutic implications from more modern theories of motor control and motor learning as well.

Therefore, numerous studies have indicated that many of the aforementioned strategies are beneficial for stroke patients in a structured stroke unit; yet, there is still a lack of conclusive data about the way that is most effective for stroke rehabilitation (Kwakkel et al) [10]. Contrary to facilitative treatments, it is clear that increased therapy intensity utilising repetitive task-oriented procedures enhances motor and functional recovery [34].

Action Observation Therapy

- The patients in the action observation therapy group were required to observe the upper limb movements or functional actions in video clips
- (i.e., the observation phase) and to execute what they had observed to the best of their ability (i.e., the execution phase).
- Three common categories of movements and tasks were selected in the action observation therapy

protocol based on the related literature and clinical expertise:

- (a) Upper limb active range of motion (AROM) exercises
- (b) Reaching movement or object manipulation, and
- (c) Upper limb functional tasks.

The video movements were displayed from a first-person perspective to make the actions more intuitive and facilitate optimal cortico motor excitability [15]. The actors in the videos were healthy young people. Observing the actions from the first-person perspective means that the observers watch the actions as if seeing through the actor's eyes. It looks like the observers are performing the actions themselves as the same directions and space dimensions of the actors performed. During phase 1 (10–15 minutes), the patients watched AROM exercises demonstrated in the video clips on a computer screen and executed the observed exercises with both arms and hands simultaneously.



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In phase 2 (15–20 minutes), the patients were asked to observe one reaching movement or one object manipulation task, depending on the patient's motor ability, for 2 minutes in a video clip, and afterwards to execute the movements that they had observed for 3 minutes. This sequence was repeated 3 times. The reaching movements involved reaching for objects of different sizes and weights at different heights and locations. Object manipulation included in-hand manipulation, grasp and release, and transport and turning objects. During Phase 3 (30 minutes) contained one functional task in each session, starting with easy tasks and continuing with increasingly complex tasks. Each functional task was divided into 3 motor acts. For example, the action of cleaning the mouth with a tissue paper was decomposed into the following 3 motor acts: (1) moving hand toward a tissue paper, (2) taking a tissue paper, and (3) bringing the tissue paper toward the mouth and wiping. After observing a motor act in a video clip for 2 minutes, the patients were asked to execute the action they had observed for 3 minutes. For the next 15 minutes, the patients observed the functional task as a complete action for 2 minutes and then executed the entire task for 3 minutes; this sequence was repeated 3 times. Examples of the functional tasks are folding a towel, wiping a table, drinking water, opening a small drawer, and using a mobile phone.

MIRROR BOX THERAPY

Functional motor training

- The patients in this group received dose-matched bilateral arm training with mirror box provided, but no video input or for this group.
- In the active mirror box therapy intervention, the same 3 categories of movements and tasks as provided in the action observation therapy and mirror therapy groups were used.
- Treatment programs also included AROM exercises (10–15 minutes), reaching movement or object manipulation (15–20 minutes), and functional task practice (30 minutes).
- AROM exercises included bilateral shoulder, elbow, forearm, wrist, and finger movements. Object manipulation tasks were in-hand manipulation, grasp and release, and transporting and turning objects with both hands.

Examples of functional tasks were reading a magazine, folding clothes, wiping a table, and opening a small drawer with bilateral arm and hand movements. During training, the patients were required to move both of their arms and hands simultaneously as possible. Based on the patient's level of motor ability and progress, the levels of movement and task difficulty could be adjusted accordingly.

AIM

To determine the effectiveness of action observation therapy & mirror neuron therapy on functional motor capacity of upper limb in acute post stroke subjects.

OBJECTIVE OF THE STUDY

To determine the improvements in body metric representation in brain by measuring quantitatively fore arm bisection task measurement. To determine the improvements in functional motor capacity activities independently on scores of Fugl – Meyer assessment-upper limb and Motor assessment scale-upper limb as an outcome measure. To determine the improvements in dexterity of hand by applying Box and block test as an outcome measure.

MATERIALS AND METHODS

Source of data: DGS Stroke and rehabilitation centre, Hoskote,

Method of collection of data:

| | | |
|---------------|---|---------------------------------|
| Population | : | Stroke patients |
| Sample | : | Stroke subjects with hemiplegia |
| Sample design | : | Convenient sampling |





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Study design : Pre and post experimental design
Sample size : 20 subjects.
Duration of the study : 12 Weeks.

Inclusion criteria

1. Age between 40- 60 years.
2. Subjects with sub-acute and chronic stroke.
3. Subjects of both the genders.
4. Subjects diagnosed as strokes confirmed by CT or MRI scan.
5. Subjects with palpable wrist extension of grade I muscle power of MRC.
6. Subjects who will be screening under fugl-meyer score.
7. Subjects with stroke having active shoulder shrugging.
8. Subjects with scores between 10- 57 under fuglmeyer score.

Exclusion criteria

1. Subjects with MMSE score less than 20.
2. Subjects with receptive aphasia
3. Subjects with unstable cardio vascular status.
4. Subjects with psychiatric illness like severe depression, poor motivation.
5. Subjects with the presence of significant upper limb musculo skeletal and any other neurological conditions other than stroke.
6. Subjects with visual impairments.
7. Non cooperative subjects.
8. Any other deformities in hand
9. ICU dependant patients in initial stages of stroke.
10. Subjects with history or illness of tuberculosis, Meningitis and tumor.

Duration

The duration of treatment was for one hour of prescribed exercises 5 days a week and can divide the exercises in to 2 to 30 minutes session for the duration of 3 months for both the experimental groups.

Procedure

Informed consent was taken from the subject. Subjects were then screened for inclusion and exclusion criteria by doing a routine neurological examination. Subjects were also assessed for baseline data like age, gender, duration of onset of stroke, type of stroke, Fugl meyer score, MMSE, Proprioception, personal neglect and proprioception, The subjects who fulfilled the inclusion and exclusion criteria were made to understand the purpose of the study in their own language. And informed consent have been collected in the local language and subjects were explained the risks. Subjects were assigned in to the any one of the 2 groups randomly as follows-

GROUP A - Experimental group (n=10).

GROUP B - Experimental group (n=10).

The experimental group A was given Action observation therapy. The group B was given Mirror Box therapy which includes passive movements, sustained stretching, and consistent range of motion exercises, positioning, and functional motor training. The group A subjects instructed to follow the treatment protocol as demonstrated in the video sessions and tasks of functional training after being practically demonstrated. Duration- one hour of prescribed exercises 5 days a week and can divide the exercises in to 30 to 45 minutes session for the duration of 3 months.



**Thummala S Pavani and Senthilkumar****Components of AOT**

The patients in the action observation therapy group were required to observe the upper limb movements or functional actions in video clips (i.e., the observation phase) and to execute what they had observed to the best of their ability (i.e., the execution phase).

Three common categories of movements and tasks were selected in the action observation therapy protocol based on the related literature and clinical expertise:

- (a) Upper limb active range of motion (AROM) exercises
- (b) Reaching movement or object manipulation, and
- (c) Upper limb functional tasks.

The video movements were displayed from a first-person perspective to make the actions more intuitive and facilitate optimal cortico motor excitability [34]. The actors in the videos were healthy young people. Observing the actions from the first-person perspective means that the observers watch the actions as if seeing through the actor's eyes. It looks like the observers are performing the actions themselves as the same directions and space dimensions of the actors performed. They were required to first watch a video showing a specific action of the upper limb and then perform the same exercise after watching (Fig.1). A total of 30 action videos were used, which depicted the same model performing the following: the bending and extension, abduction and adduction, and pronation and supination of the shoulder joint, shrug and adduction of the scapula, bending and extension of the elbow joint, bending and extension, ulnar deviation and radial deviation of the wrist joint, warping of a thimble, empty-handed grabbing, catch and release of large and small balls, cubes and cylinders, holding and release of a coin and a key, handling of an IC card, pen, chopsticks and computer mouse, screwing of a jar lid and narrow-mouthed bottle cap, typewriting, dialling on a mobile phone, grasping and release of a spoon, feeding training, and putting on clothes (including use of a zipper and button).

Each video was approximately 50 s in duration and depicted an action as seen from straight on (20 s), right above (15 s) and right inside (15 s); the complete action was recorded 2–3 times at each angle. Each action video was numbered according to the difficulty level of the action (1 = easiest, and 30 = most difficult). Videos with similar difficulty levels were grouped into five groups of six videos each; Group 1 videos comprised the easiest actions and Group 5 videos comprised the most difficult. Patients were initially shown videos from Group 1 and instructed to try their best to simulate the action with their affected limbs. Once at least four of the actions could be performed, they moved on to the next group (higher difficulty) of videos. During phase 1 (10–15 minutes), the patients watched AROM exercises demonstrated in the video clips on a computer screen and executed the observed exercises with both arms and hands simultaneously. In phase 2 (15–20 minutes), the patients were asked to observe one reaching movement or one object manipulation task, depending on the patient's motor ability, for 2 minutes in a video clip, and afterwards to execute the movements that they had observed for 3 minutes. This sequence was repeated 3 times.

The reaching movements involved reaching for objects of different sizes and weights at different heights and locations. Object manipulation included in-hand manipulation, grasp and release, and transport and turning objects. During Phase 3 (30 minutes) contained one functional task in each session, starting with easy tasks and continuing with increasingly complex tasks. Each functional task was divided into 3 motor acts. For example, the action of cleaning the mouth with a tissue paper was decomposed into the following 3 motor acts: (1) moving hand toward a tissue paper, (2) taking a tissue paper, and (3) bringing the tissue paper toward the mouth and wiping. After observing a motor act in a video clip for 2 minutes, the patients were asked to execute the action they had observed for 3 minutes.

For the next 15 minutes, the patients observed the functional task as a complete action for 2 minutes and then executed the entire task for 3 minutes; this sequence was repeated 3 times. Examples of the functional tasks are folding a towel, wiping a table, drinking water, opening a small drawer, and using a mobile phone



**Thummala S Pavani and Senthilkumar****Group B- Mirror Box therapy-Fore arm bisection task**

Patients sat on chair, with their forearms placed radially on a table. They were asked to indicate with their index finger the midpoint of their impaired arm, considering the tip of the middle finger and the elbow (olecranon) as the distal and proximal extremes. Patients were requested to indicate the midpoint with a ballistic movement, with closed eyes. On each trial, a flexible ruler was used to measure the patient's performance, setting the 0-cm point in correspondence of the tip of the middle finger. 15 trials of bisection pointing were recorded. Before starting the task, the arm length was measured. There was no time constraint for the bisection task, but corrections were not allowed. Patients performed a total of 30 trials (15 before and 15 after each training) in every session, for a total of 60 trials per participant. A percentage score was calculated for each forearm bisection trial using the following formula: $[(p/\text{arm length}) * 100]$, where p indicates the subjective midpoint, measured with a flexible ruler on each trial. In this formula, a value of 0% corresponds to the tip of the middle finger, 100% corresponds to the elbow.

A value higher than 50% indicates a deviation of the subjective midpoint towards the elbow, i.e., proximal deviation, while a value lower than 50% indicates a deviation towards the hand, i.e., distal deviation (Sposito et al., 2012; Garbarini et al., 2015). For the analysis, we considered the difference between pre- and post-training bisections (pre-training minus post-training). The shift obtained with this formula, was positive in case of distal deviation and negative for proximal deviation. Hand movements requested during 10 min of motor training, with or without the Mirror. The patients in the active control intervention group received dose-matched bilateral arm training, but no video input or mirror box was provided for this group. In the active control intervention, the same 3 categories of movements and tasks as provided in the action observation therapy and mirror therapy groups were used. Treatment programs also included AROM exercises (10–15 minutes), reaching movement or object manipulation (15–20 minutes), and functional task practice (30 minutes). AROM exercises included bilateral shoulder, elbow, forearm, wrist, and finger movements. Object manipulation tasks were in-hand manipulation, grasp and release, and transporting and turning objects with both hands. Examples of functional tasks were reading a magazine, folding clothes, wiping a table, and opening a small drawer with bilateral arm and hand movements. During training, the patients were required to move both of their arms and hands simultaneously as possible. Based on the patient's level of motor ability and progress, the levels of movement and task difficulty could be adjusted accordingly.

Opening/closing the hands 2 min, Whole hand tapping 2 min

Single finger tapping 2 min

Whole hand lateral rotation 2 min

DATA ANALYSIS AND RESULTS

The following is the statistical analysis done in this study.

Descriptive statistical analysis has been carried out in the present study using software version SPSS 17. Results are presented on Mean + or – S D (Min- Max) and results as categorical measurements are presented in number (%) level of significance is assessed at 5 %. Wilcoxon signed rank test, Mann Whitney test, have been used to find the significance of study parameters

DESCRIPTIVES VARIABLES

Age post stroke in weeks, MMSE score, Strength grade, Visual field score, Proprioception, Personal neglect, Tactile sensation, Forearm bisection task before experiment, Forearm bisection task after experiment, FMA before experiment, FMA after experiment, MAS before experiment, MAS after experiment, BBT before experiment, BBT after experiment





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NON Parametric Tests

Mann-Whitney Test for descriptive statistics- baseline variables

NPAR TESTS

/M-W= Forearm bisection task before experiment, Forearm bisection task after experiment, FMA before experiment, FMA after experiment, MAS before experiment, MAS after experiment, BBT before experiment, BBT after experiment.

Data interpretation and results

- In the group A the pre-test score for Fore arm bisection task was 48.4 ± 0.8 which is decreased significantly to 14.6 ± 5.1 with the P value 0.05.
- In the group B the pre-test score for Fore arm bisection task was 57.0 ± 8.9 which is decreased significantly to 21.6 ± 5.18 with the P value 0.05.
- In the group A the pre-test score for FMA was 36 ± 7.68 which is increased significantly to 56.6 ± 5.96 with the P value <0.05 .
- In the group B the pre-test score for FMA was 35.6 ± 8.36 which is increased significantly to 50.70 ± 7.45 with the P value <0.05 .
- In the group A the pre-test score for MAS was 11.60 ± 2.63 which is increased significantly to 17.80 ± 3.32 with the P value <0.05 .
- In the group B the pre-test score for MAS was 11.0 ± 2.35 which is increased significantly to 18.00 ± 3.52 with the P value 0.008.
- In the group A the pre-test score for BBT was 4.26 ± 3.326 which is increased significantly to 56.6 ± 5.96 with the P value 0.009.
- In the group B the pretest score for BBT was 40.30 ± 5.375 which is increased significantly to 53.7 ± 5.29 with the P value 0.436.

NPAR TESTS /WILCOXON=Forearm bisection task before experiment, FMA before experiment, MAS before experiment, BBT before experiment with Forearm bisection task after experiment, FMA after experiment, MAS after experiment, BBT after experiment (paired) /Statistics descriptive quartiles/missing analysis.

DISCUSSION

- This study is intended to find out the effectiveness of AOT and MBT in order to improve the motor function of arm and hand in post stroke subjects following the 8 weeks protocol
- Feasible for subjects with no wrist or hand movement outcome measures are very much applicable to real life activities.
- Base line data for demographic, outcome variables were not significant from its P value with the samples matched. So homogeneity of sample was used to test the significant proportion of base line characteristics.
- In the group A the pre-test score for Fore arm bisection task was 48.4 ± 0.8 which is decreased significantly to 14.6 ± 5.1 with the P value 0.05.
- In the group B the pre-test score for fore arm bisection task was 57.0 ± 8.9 which is decreased significantly to 21.6 ± 5.18 with the P value 0.05.
- In the group A the pre-test score for FMA was 36 ± 7.68 which is increased significantly to 56.6 ± 5.96 with the P value <0.05 .
- In the group B the pre-test score for FMA was 35.6 ± 8.36 which is increased significantly to 50.70 ± 7.45 with the P value <0.05 .
- In the group A the pre-test score for MAS was 11.60 ± 2.63 which is increased significantly to 17.80 ± 3.32 with the P value <0.05 .
- In the group B the pre-test score for MAS was 11.0 ± 2.35 which is increased significantly to 18.00 ± 3.52 with the P value 0.008.



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- In the group A the pre-test score for BBT was 4.26 ± 3.326 which is increased significantly to 56.6 ± 5.96 with the P value 0.009.
- In the group B the pretest score for BBT was 40.30 ± 5.375 which is increased significantly to 53.7 ± 5.29 with the P value 0.436.
- More male subjects in experimental group, shows better functional out comes might be due to less number of females in group A and also the female subjects selected were all in the menopausal stage.
- According to Mathew J Reeves, PhD, Cheryl D Bushnell, Age-specific stroke rates are higher in men, but, because of their longer life expectancy and much higher incidence at older ages. Functional outcomes and quality of life after stroke are consistently poorer in women, despite adjustment for baseline differences in age, pre-stroke function, and co-morbidities.
- Below the age of 45 years, Stroke mortality for women and men is similar, but women aged 45–74 years have a substantially lower risk of stroke mortality than do men. The most common biological explanation for sex differences in stroke is related to sex steroid hormones, particularly estrogen.
- Timing and intensity of post stroke rehabilitation have been cited as important predictors of outcome according to (Horn et al, 2005). (Kwakkel et al., 1997, 2004).
- In this study subjects with less than one month post stroke was selected and was given immediate intervention for the duration of 2 months and which results in better out comes. Time course of upper limb recovery is well documented, According to (Parke et al., 1986; Nakayama et al., 1994). The mean time of recovery for motor impairment is three weeks post stroke but differs according to arm severity.

Limitations

- The study was not made based on the side of the hemisphere involved which might have affected the overall outcome of the study.
- Being the intervention was conducted in the hospital setting, supervision of subjects while doing the video movement therapy was challenging, which may influence the study? Due to reduce in the attention time by the patient.

Recommendations

- Further studies should be done with larger sample size.
- The hemisphere involved may be considered in the future studies.
- Further studies can be carried out with chronic stroke subjects
- And study can be done with the different sampling techniques.

SUMMARY

- Motor deficits resulting from hemiplegia is a common complication after stroke .The primary purpose of this study was to find out the effectiveness of AOT and MBT in improving the motor function of arm and hand in hospital settings. Fifteen individuals were randomly assigned into two groups for the study.
- The group B received MBT physiotherapy for stroke rehabilitation whereas the experimental group was given the AO treatment. Treatment effects were established by pre and post treatment assessment of motor function of arm and hand by using MAS score and FMA as an outcome measures. The study demonstrated significant benefit by administering the AOT and MBT in reducing the motor deficits of arm and hand following stroke.
- This study is going to benefit for the subjects for whose motor recovery is poor post stroke. Brings awareness about the modifiable and non-modifiable risk factors contributing to the stroke.
- It helps to improve their motor ability to complete their activities of daily living, self needs at their ease with in dependence maximally with assistive devices and putting back to their work with maximal functional independence.





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Frequencies of demographic variables

| GENDER | | | | | | |
|---------------|-------|---|-----------|---------|---------------|--------------------|
| Sgroup | | | Frequency | Percent | Valid Percent | Cumulative Percent |
| EXPA | Valid | M | 10 | 100.0 | 100.0 | 100.0 |
| EXPB | Valid | M | 8 | 80.0 | 80.0 | 80.0 |
| | | F | 2 | 20.0 | 20.0 | 100.0 |
| Total | | | 10 | 100.0 | 100.0 | |

| TYPE OF STROKE | | | | | | |
|-----------------------|-------|-------------|-----------|---------|---------------|--------------------|
| S group | | | Frequency | Percent | Valid Percent | Cumulative Percent |
| EXPA | Valid | ISCHEMIC | 7 | 70.0 | 70.0 | 70.0 |
| | | HAEMORRHAGE | 3 | 30.0 | 30.0 | 100.0 |
| Total | | | 10 | 100.0 | 100.0 | |
| EXPB | Valid | ISCHEMIC | 6 | 60.0 | 60.0 | 60.0 |
| | | HAEMORRHAGE | 4 | 40.0 | 40.0 | 100.0 |
| Total | | | 10 | 100.0 | 100.0 | |





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Mann-Whitney Test for descriptive statistics- baseline variables

| Ranks | | | | |
|--------------------|--------|----|-----------|--------------|
| | Sgroup | N | Mean Rank | Sum of Ranks |
| AGE | EXPA | 10 | 10.05 | 100.50 |
| | EXPB | 10 | 10.95 | 109.50 |
| | Total | 20 | | |
| MMSE SCORE | EXPA | 10 | 13.90 | 139.00 |
| | EXPB | 10 | 7.10 | 71.00 |
| | Total | 20 | | |
| STRENGTH GRADE | EXPA | 10 | 8.60 | 86.00 |
| | EXPB | 10 | 12.40 | 124.00 |
| | Total | 20 | | |
| VISUAL FIELD SCORE | EXPA | 10 | 9.70 | 97.00 |
| | EXPB | 10 | 11.30 | 113.00 |
| | Total | 20 | | |
| PROPRIOCEPTION | EXPA | 10 | 8.55 | 85.50 |
| | EXPB | 10 | 12.45 | 124.50 |
| | Total | 20 | | |
| PERSONAL NEGLECT | EXPA | 10 | 8.50 | 85.00 |
| | EXPB | 10 | 12.50 | 125.00 |
| | Total | 20 | | |
| TACTILE SENSATION | EXPA | 10 | 9.00 | 90.00 |
| | EXPB | 10 | 12.00 | 120.00 |
| | Total | 20 | | |

| Base line variables-Test Statistics ^a | | | | | | | |
|--|-------------------|-------------------|-------------------|--------------------|-------------------|-------------------|-------------------|
| | AGE | MMSE SCORE | STRENGTH GRADE | VISUAL FIELD SCORE | PROPRIOCEPTION | PERSONAL NEGLECT | TACTILE SENSATION |
| Mann-Whitney U | 45.500 | 16.000 | 31.000 | 42.000 | 30.500 | 30.000 | 35.000 |
| Wilcoxon W | 100.500 | 71.000 | 86.000 | 97.000 | 85.500 | 85.000 | 90.000 |
| Z | -.342 | -2.616 | -1.587 | -.663 | -1.643 | -1.780 | -1.371 |
| Asymp. Sig. (2-tailed) | .732 | .009 | .112 | .508 | .100 | .075 | .170 |
| Exact Sig. [2*(1-tailed Sig.)] | .739 ^b | .009 ^b | .165 ^b | .579 ^b | .143 ^b | .143 ^b | .280 ^b |





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Mann-Whitney Test for outcome variables

| Ranks | | | | |
|--|--------|----|-----------|--------------|
| | Sgroup | N | Mean Rank | Sum of Ranks |
| Fore arm bisection task before experiment percentage | EXPA | 10 | 7.65 | 76.50 |
| | EXPB | 10 | 13.35 | 133.50 |
| | Total | 20 | | |
| Fore arm bisection task after experiment percentage | EXPA | 10 | 7.20 | 72.00 |
| | EXPB | 10 | 13.80 | 138.00 |
| | Total | 20 | | |
| FMA before experiment | EXPA | 10 | 10.80 | 108.00 |
| | EXPB | 10 | 10.20 | 102.00 |
| | Total | 20 | | |
| FMA after experiment | EXPA | 10 | 12.80 | 128.00 |
| | EXPB | 10 | 8.20 | 82.00 |
| | Total | 20 | | |
| MAS before experiment | EXPA | 10 | 11.10 | 111.00 |
| | EXPB | 10 | 9.90 | 99.00 |
| | Total | 20 | | |
| MAS after experiment | EXPA | 10 | 10.35 | 103.50 |
| | EXPB | 10 | 10.65 | 106.50 |
| | Total | 20 | | |
| BBT before experiment | EXPA | 10 | 11.75 | 117.50 |
| | EXPB | 10 | 9.25 | 92.50 |
| | Total | 20 | | |
| BBT after experiment | EXPA | 10 | 11.55 | 115.50 |
| | EXPB | 10 | 9.45 | 94.50 |
| | Total | 20 | | |

For outcome variables-Test Statistics^a

| | Fore arm bisection task before experiment percentage | Fore arm bisection task after experiment percentage | FMA before experiment | FMA after experiment | MAS before experiment | MAS after experiment | BBT before experiment | BBT after experiment |
|--------------------------------|--|---|-----------------------|----------------------|-----------------------|----------------------|-----------------------|----------------------|
| Mann-Whitney U | 21.500 | 17.000 | 47.000 | 27.000 | 44.000 | 48.500 | 37.500 | 39.500 |
| Wilcoxon W | 76.500 | 72.000 | 102.000 | 82.000 | 99.000 | 103.500 | 92.500 | 94.500 |
| Z | -2.158 | -2.507 | -.228 | -1.747 | -.470 | -.116 | -.950 | -.796 |
| Asymp. Sig. (2-tailed) | .031 | .012 | .819 | .081 | .639 | .908 | .342 | .426 |
| Exact Sig. [2*(1-tailed Sig.)] | .029 ^b | .011 ^b | .853 ^b | .089 ^b | .684 ^b | .912 ^b | .353 ^b | .436 ^b |





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Wilcoxon Signed Ranks Test

| Ranks | | | | | |
|---------|--|----------------|-----------------|-----------|--------------|
| S group | | | N | Mean Rank | Sum of Ranks |
| EXPA | Fore arm bisection task after experiment percentage - Fore arm bisection task before experiment percentage | Negative Ranks | 10 ^a | 5.50 | 55.00 |
| | | Positive Ranks | 0 ^b | .00 | .00 |
| | | Ties | 0 ^c | | |
| | | Total | 10 | | |
| | FMA after experiment - FMA before experiment | Negative Ranks | 0 ^d | .00 | .00 |
| | | Positive Ranks | 10 ^e | 5.50 | 55.00 |
| | | Ties | 0 ^f | | |
| | | Total | 10 | | |
| | MAS after experiment - MAS before experiment | Negative Ranks | 0 ^g | .00 | .00 |
| | | Positive Ranks | 10 ^h | 5.50 | 55.00 |
| | | Ties | 0 ⁱ | | |
| | | Total | 10 | | |
| | BBT AFTER EXPERIMENT - BBT BEFORE EXPERIMENT | Negative Ranks | 0 ^j | .00 | .00 |
| | | Positive Ranks | 10 ^k | 5.50 | 55.00 |
| | | Ties | 0 ^l | | |
| | | Total | 10 | | |
| EXPB | Fore arm bisection task after experiment percentage - Fore arm bisection task before experiment percentage | Negative Ranks | 10 ^a | 5.50 | 55.00 |
| | | Positive Ranks | 0 ^b | .00 | .00 |
| | | Ties | 0 ^c | | |
| | | Total | 10 | | |
| | FMA after experiment - FMA before experiment | Negative Ranks | 0 ^d | .00 | .00 |
| | | Positive Ranks | 10 ^e | 5.50 | 55.00 |
| | | Ties | 0 ^f | | |
| | | Total | 10 | | |
| | MAS after experiment - MAS before experiment | Negative Ranks | 0 ^g | .00 | .00 |
| | | Positive Ranks | 10 ^h | 5.50 | 55.00 |
| | | Ties | 0 ⁱ | | |
| | | Total | 10 | | |
| | BBT after experiment - BBT before experiment | Negative Ranks | 0 ^j | .00 | .00 |





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| | | | | | |
|--|--|----------------|-----------------|------|-------|
| | | Positive Ranks | 10 ^k | 5.50 | 55.00 |
| | | Ties | 0 ^l | | |
| | | Total | 10 | | |

| Between the group analysis-Wilcoxon signed rank test-Level of significance-Test Statistics ^a | | | | | |
|---|------------------------|--|--|--|--|
| Sgroup | | Fore arm bisection task after experiment percentage - Fore arm bisection task before experiment percentage | FMA after experiment - FMA before experiment | MAS after experiment - MAS before experiment | BBT after experiment - BBT before experiment |
| EXPA | Z | -2.807 ^b | -2.814 ^c | -2.844 ^c | -2.809 ^c |
| | Asymp. Sig. (2-tailed) | .005 | .005 | .004 | .005 |
| EXPB | Z | -2.805 ^b | -2.805 ^c | -2.825 ^c | -2.807 ^c |
| | Asymp. Sig. (2-tailed) | .005 | .005 | .005 | .005 |





Some Properties of Nano K-Closed Sets in Nano Topological Spaces

K. Dass¹ and S. Subbu Lakshmi^{2*}

¹Assistant Professor, PG and Research Department of Mathematics, The M.D.T. Hindu College, (Affiliated to Manonmaniam Sundaranar University), Tirunelveli, Tamil Nadu, India.

²Research Scholar, PG and Research Department of Mathematics, The M.D.T. Hindu College, (Affiliated to Manonmaniam Sundaranar University), Tirunelveli, Tamil Nadu, India.

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*Address for Correspondence

S. Subbu Lakshmi

Research Scholar,
PG and Research Department of Mathematics,
The M.D.T. Hindu College,
(Affiliated to Manonmaniam Sundaranar University),
Tirunelveli, Tamil Nadu, India.
E. Mail: subbulakshmivallinayagam@gmail.com



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ABSTRACT

In the paper, we introduce new classes of nano closed sets in nano topological spaces called NK-closed sets, NK-open sets and some of their properties.

Keywords: NK-closed, NK-open, N_{ω} -closed, NK-int, NK-cl.

INTRODUCTION

L.Thivagar *et al.* [10] introduced the concept of nano topological spaces which was defined in terms of approximations and boundary region of a subset of a universe U using an equivalence relation on it and also defined nano closed sets, nano interior and nano closure. S.Jafari *et al.* [5], introduced the another generalization of closed sets. Topology is the branch of Mathematics, one of its purposes is to elucidate and investigate ideas of continuity within the framework of Mathematics. Bhuvaneswari. M *et al.* [1], introduced a study on nano topology.[2] Bhuvaneswari. K and Ezhilarasi. A, introduced on nano semi-generalized and nano generalized-semi closed sets in nano topological spaces. Nano generalized closed sets in Nano topological spaces is the extension of Nano closed sets.





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In the paper, we introduce new classes of nanoclosed sets in nano topological spaces called NK-closed sets, NK-open sets and some of their properties.

Preliminaries

Definition 2.1.[10]

Let U be a non-empty finite set of objects called the universe and R be an equivalence relation on U named as the indiscernibility relation. Then U is divided into disjoint equivalence classes. Elements belonging to the same equivalence class are said to be indiscernible with one another. The pair (U, R) is said to be the approximation space. Let $X \subseteq U$.

1. The lower approximation of X with respect to R is the set of all objects which can be for certain classified as X with respect to R and is denoted by $L_R(X)$. That is $L_R(X) = \cup\{R(x) : R(x) \subseteq X, x \in U\}$ where $R(x)$ denotes the equivalence class determined by $x \in U$.
2. The upper approximation of X with respect to R is the set of all objects which can be possibly classified as X with respect to R and is denoted by $U_R(X)$. That is, $U_R(X) = \cup\{R(x) : R(x) \cap X \neq \Phi, x \in U\}$.
3. The boundary region of X with respect to R is the set of all objects which can be classified neither X nor as not- X with respect to R and it is denoted by $B_R(X)$. That is, $B_R(X) = U_R(X) - L_R(X)$.

Property 2.2[10]

If (U, R) is an approximation space and $X, Y \subseteq U$ then

1. $L_R(X) \subseteq X \subseteq U_R(X)$.
2. $L_R(\Phi) = U_R(\Phi) = \Phi$.
3. $L_R(U) = U_R(U) = U$.
4. $U_R(X \cup Y) = U_R(X) \cup U_R(Y)$.
5. $U_R(X \cap Y) \subseteq U_R(X) \cap U_R(Y)$.
6. $L_R(X \cup Y) \supseteq L_R(X) \cup L_R(Y)$.
7. $L_R(X \cap Y) = L_R(X) \cap L_R(Y)$.
8. $L_R(X) \subseteq L_R(Y)$ and $U_R(X) \subseteq U_R(Y)$ whenever $X \subseteq Y$.
9. $U_R(X^c) = [L_R(X)]^c$ and $L_R(X^c) = [U_R(X)]^c$.
10. $U_R[U_R(X)] = L_R(U_R(X)) = U_R(X)$.
11. $L_R[L_R(X)] = U_R[L_R(X)] = L_R(X)$.

Definition 2.3.[4]

Let U be the universe, R be an equivalence relation on U and

$\tau_R(X) = \{\Phi, L_R(X), U_R(X), B_R(X), U\}$ where $X \subseteq U$. Then by property 2.2, $U_R(X)$ satisfies the following axioms:

1. Φ and $U \in \tau_R(X)$.
2. The union of the elements of any sub-collection of $\tau_R(X)$ is in $\tau_R(X)$.
3. The intersection of the elements of any finite subcollection of $\tau_R(X)$ is in $\tau_R(X)$.

Then, $\tau_R(X)$ is the topology on U called the nano topology on U with respect to X .

$(U, \tau_R(X))$ is called the nano topological space. Elements of the nano topology are known as nano open sets in U .

Elements of $[\tau_R(X)]^c$ are called nano closed sets with $[\tau_R(X)]^c$ being called dual nano topology of $\tau_R(X)$.

Definition 2.4. Let $(U, \tau_R(X))$ be a nano topological space and $H \subseteq U$. Then H is said to be

- (i) Nano semi-open [4] if $H \subseteq Ncl(Nint(H))$.
- (ii) Nano pre-open [11] if $H \subseteq Nint(Ncl(H))$.
- (iii) Nano semi pre-open [10] if $H \subseteq Ncl(Nint(Ncl(H)))$.
- (iv) Nano α -open [9] if $H \subseteq Nint(Ncl(Nint(H)))$.
- (v) Nano regular open [7] if $H = Nint(Ncl(H))$.

The complements of the above mentioned open sets are called their respective closed sets.

Definition 2.5. Let $(U, \tau_R(X))$ be a nano topological space and $H \subseteq U$. Then H is said to be





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1. Ng-closed [3] if $Ncl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano open.
2. Ngp-closed [6] if $Npcl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano open.
3. Ngsp-closed [8] if $Nspcl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano open.
4. $N\omega$ -closed [8] if $Ncl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano semi-open.
5. N^*g -closed [8] if $Ncl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano ω -open.
6. Nsg-closed [4] if $Nscl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano semi-open.
7. Ngs – closed [4] if $Nscl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano open.
8. $N\alpha g$ – closed [9] if $N\alpha cl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano α -open.
9. $N\alpha$ – closed [9] if $N\alpha cl(H) \subseteq V$ whenever $H \subseteq V$ and V is nano open.

The complement of Ng-closed (resp. Ngp-closed, Ngsp-closed, $N\omega$ -closed, N^*g -closed, Nsg-closed, Ngs-closed, $N\alpha g$ -closed, $N\alpha$ -closed) is said to be Ng-open (resp. Ngp-open, Ngsp-open, $N\omega$ -open, N^*g -open, Nsg-open, Ngs-open, $N\alpha g$ -open, $N\alpha$ -open).

Nano K-Closed sets

In this paper, we define and study the concept of nano K-closed (briefly, NK- closed) sets in nano topological spaces and obtain some of its properties.

Definition 3.1. A subset H of a nano topological space $(U, \tau_R(X))$ is called Nano K-closed set (briefly, NK-closed set) if $Nscl(H) \subseteq V$ whenever $H \subseteq V$ and V is $N\omega$ -open.

Theorem 3.2. Every nano closed is NK-closed.

Proof. Let H be a nano closed subset of $(U, \tau_R(X))$. Let $H \subseteq V$ and V is any $N\omega$ -open. Since H is nano closed, $Ncl(H) = H$. Also since every nano closed set is nano semi closed, $Nscl(H) \subseteq Ncl(H) = H \subseteq V$. That is $Nscl(H) \subseteq V$. Hence H is NK-closed.

Remark 3.3. The converse of the above theorem need not be true as seen from the following example.

Example 3.4. Let $U = \{s_1, s_2, s_3, s_4\}$ with $U/R = \{\{s_1\}, \{s_3\}, \{s_2, s_4\}\}$ and $X = \{s_1, s_2\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{s_1\}, \{s_2, s_4\}, \{s_1, s_2, s_4\}, U\}$. It is clear that $\{s_1\}$ is NK- closed but not nano closed.

Theorem 3.5. Every N^*g -closed is NK-closed.

Proof. Let H be a N^*g -closed subset of $(U, \tau_R(X))$. Let $H \subseteq V$ and V is $N\omega$ -open. Since H is N^*g -closed, $Ncl(H) \subseteq V$. Also since every nano closed set is nano semi-closed, $Nscl(H) \subseteq Ncl(H) \subseteq V$. That is $Nscl(H) \subseteq V$. Hence H is NK-closed.

Remark 3.6. The converse of the above theorem need not be true as seen from the following example.

Example 3.7. Let $U = \{s_1, s_2, s_3, s_4\}$ with $U/R = \{\{s_1\}, \{s_3\}, \{s_2, s_4\}\}$ and $X = \{s_1, s_2\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{s_1\}, \{s_2, s_4\}, \{s_1, s_2, s_4\}, U\}$. It is clear that $\{s_2, s_4\}$ is NK- closed but not N^*g -closed.

Theorem 3.8. Every nano regular closed is NK-closed.

Proof. Let H be a nano regular closed subset of $(U, \tau_R(X))$. Let $H \subseteq V$ and V is $N\omega$ -open. Since H is nano regular closed, we have $Nrcl(H) = H$. Since every nano regular closed set is nano closed, $Ncl(H) \subseteq Nrcl(H)$. Also since every nano closed set is nano semi closed, $Nscl(H) \subseteq Ncl(H)$. Therefore $Nscl(H) \subseteq Nrcl(H) = H \subseteq V$. Therefore $Nscl(H) \subseteq V$. Hence H is NK- closed.

Remark 3.9. The converse of the above theorem need not be true as seen from the following example.

Example 3.10. Let $U = \{s_1, s_2, s_3, s_4\}$ with $U/R = \{\{s_1\}, \{s_3\}, \{s_2, s_4\}\}$ and $X = \{s_1, s_2\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{s_1\}, \{s_2, s_4\}, \{s_1, s_2, s_4\}, U\}$. It is clear that $\{s_2, s_4\}$ is NK-closed but not nano regular closed.

Theorem 3.11. Every NK-closed is Ngsp-closed.

Proof. Let H be a NK- closed subset of $(U, \tau_R(X))$. Let $H \subseteq V$ and V is nano open. Since every nano semi-closed is nano semi pre-closed and H is NK-closed. That is $Nspcl(H) \subseteq Nscl(H) \subseteq V$. Therefore $Nspcl(H) \subseteq V$. Hence H is Ngsp-closed.

Remark 3.12. The converse of the above theorem need not be true as seen from the following example.





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Example 3.13. Let $U = \{s_1, s_2, s_3, s_4\}$ with $U/R = \{\{s_2\}, \{s_3\}, \{s_1, s_4\}\}$ and $X = \{s_1, s_2\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{s_2\}, \{s_1, s_4\}, \{s_1, s_2, s_4\}, U\}$. It is clear that $\{s_1, s_2\}$ is Ngsp closed but not NK-closed.

Theorem 3.14. Every NK- closed is Ngs-closed.

Proof. Let H be a NK-closed subset of $(U, \tau_R(X))$. Let $H \subseteq V$ and V is nano open. Since every nano open is N_ω -open and H is NK-closed, $Nscl(H) \subseteq V$. Hence H is Ngs-closed.

Remark 3.15. The converse of the above theorem need not be true as seen from the following example.

Example 3.16. Let $U = \{p_1, q_1, r_1, s_1\}$ with $U/R = \{\{q_1\}, \{r_1\}, \{p_1, s_1\}\}$ and $X = \{p_1, q_1\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{q_1\}, \{p_1, s_1\}, \{p_1, q_1, s_1\}, U\}$. It is clear that $\{s_1\}$ is Ngs-closed but not NK-closed.

Remark 3.17. NK-closed and Ng-closed are independent. It is shown by the following example.

Example 3.18. Let $U = \{s_1, s_2, s_3, s_4\}$ with $U/R = \{\{s_1\}, \{s_3\}, \{s_2, s_4\}\}$ and $X = \{s_1, s_2\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{s_1\}, \{s_2, s_4\}, \{s_1, s_2, s_4\}, U\}$. It is clear that $\{s_2, s_3\}$ is Ng-closed but not NK-closed.

Example 3.19. As in the above Example 3.18, it is clear that $\{s_2, s_4\}$ is NK-closed but not Ng-closed.

Remark 3.20. NK- closed and Ngp-closed are independent. It is shown by the following example.

Example 3.21. Let $U = \{\alpha, \beta, \gamma, \delta\}$ with $U/R = \{\{\alpha\}, \{\beta\}, \{\gamma, \delta\}\}$ and $X = \{\alpha, \gamma\}$. Then the nano topology $\tau_R(X) = \{\Phi, \{\alpha\}, \{\gamma, \delta\}, \{\alpha, \gamma, \delta\}, U\}$. It is clear that $\{\delta\}$ is Ngp-closed but not NK-closed.

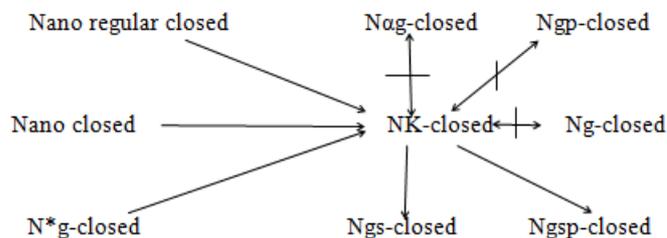
Example 3.22. In the above Example 3.21, It is clear that $\{\gamma, \delta\}$ is NK-closed but not Ngp closed.

Remark 3.23. NK-closed and N_{α} g-closed are independent. It is shown by the following example.

Example 3.24. Let $U = \{x, y, z, w\}$ with $U/R = \{\{x\}, \{w\}, \{y, z\}\}$. Let $X = \{x, z\}$. $\tau_R(X) = \{\Phi, \{x\}, \{y, z\}, \{x, y, z\}, U\}$. Let $\{x, y\}$ is N_{α} g-closed but not NK-closed and also $\{y, z\}$ is NK-closed but not N_{α} g-closed.

Remark 3.25

From the above Theorems, Examples and Remark, we obtain the following diagram, where $A \rightarrow B$ (resp. $A \leftarrow B$) represents A implies B but not conversely (resp. A and B are independent of each other).



Properties of Nano K-closed sets

Proposition 4.1. If a subset H of a nano topological space $(U, \tau_R(X))$ is NK-closed such that $H \subseteq I \subseteq Nscl(H)$. Then I is also NK-closed.

Proof. Let V be N_ω -open in U such that $I \subseteq V$. Then $H \subseteq V$. Since H is NK-closed, $Nscl(H) \subseteq V$. By hypothesis, $Nscl(I) \subseteq Nscl(Nscl(H)) = Nscl(H) \subseteq V$. Therefore $Nscl(I) \subseteq V$. Hence I is NK-closed.

Proposition 4.2. If H is N_ω -open and NK-closed then H is nano semi closed.

Proof. Let $H \subseteq H$ where H is N_ω -open and NK-closed. Then $Nscl(H) \subseteq H$. But also $H \subseteq Nscl(H)$. Therefore $H = Nscl(H)$. Hence H is nano semi closed.

Proposition 4.3. If a subset H of $(U, \tau_R(X))$ is NK-closed if and only if $Nscl(H) - H$ contains no non-empty N_ω -closed.

Proof. Let H be a NK-closed. Let F be a non-empty N_ω -closed subset of $Nscl(H) - H$. That is $F \subseteq Nscl(H) - H$. Then $F \subseteq Nscl(H) - (U - F)$. Since $U - F$ is N_ω -open and H is NK-closed, $Nscl(H) \subseteq U - F$. That is $Nscl(H) \subseteq F^c$. That is $F \subseteq [Nscl(H)]^c$. $F \subseteq Nscl(H) \cap [Nscl(H)]^c = \Phi$. Which is a contradiction. Therefore $Nscl(H) - H$ does not contain any non-empty N_ω -closed set.





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Proposition 4.4. If H and I are NK-closed sets in $(U, \tau_R(X))$, then $H \cup I$ is NK-closed in $(U, \tau_R(X))$.

Proof. Let H and I be any two NK-closed sets in $(U, \tau_R(X))$ such that $H \subseteq V$ and $I \subseteq V$ where V is $N\omega$ -open in U . Therefore $H \cup I \subseteq V$. Since H and I are NK-closed sets, $Nscl(H) \subseteq V$ and $Nscl(I) \subseteq V$. Therefore $Nscl(H) \cup Nscl(I) \subseteq V$. Therefore $Nscl(H \cup I) \subseteq V$. Hence $H \cup I$ is NK-closed.

Proposition 4.5. If H and I are NK-closed in $(U, \tau_R(X))$, then $H \cap I$ is NK-closed in $(U, \tau_R(X))$.

Proof. Let H and I be any two NK-closed in $(U, \tau_R(X))$ such that $H \subseteq V$ and $I \subseteq V$ where V is $N\omega$ -open in U . Therefore $H \cap I \subseteq V$. Since H and I are NK-closed sets, $Nscl(H) \subseteq V$ and $Nscl(I) \subseteq V$. Therefore $Nscl(H) \cap Nscl(I) \subseteq V$. Therefore $Nscl(H \cap I) \subseteq V$. Hence $H \cap I$ is NK-closed.

Proposition 4.6. For each $x \in U$, either $\{x\}$ is $N\omega$ -closed (or) $\{x\}^c$ is NK-closed in $(U, \tau_R(X))$.

Proof. Suppose that $\{x\}$ is not $N\omega$ -closed in $(U, \tau_R(X))$. Then $\{x\}^c$ is not $N\omega$ -open. The only $N\omega$ -open containing $\{x\}^c$ is the space U itself. Therefore $Nscl(\{x\}^c) \subseteq U$ and so $\{x\}^c$ is NK-closed in $(U, \tau_R(X))$.

Nano K-open sets

In this paper, we define and study the concept of nano K-open (briefly, NK-open) sets in nano topological spaces and obtain some of its properties.

Definition 5.1. A subset H of $(U, \tau_R(X))$ is called NK-open if H^c is NK-closed in $(U, \tau_R(X))$.

Proposition 5.2. For any nano topological space $(U, \tau_R(X))$, the following assertions hold:

- (i) Every nano open is NK-open.
- (ii) Every N^*g -open is NK-open.
- (iii) Every nano regular open is NK-open.
- (iv) Every NK-open is Ngs-open.
- (v) Every NK-open is Ngsp-open.

Proof. Follows from Theorem 3.2, 3.5, 3.8, 3.11, 3.14.

Theorem 5.3. A set H of U is NK-open if and only if $F \subseteq Nsint(H)$ whenever F is $N\omega$ -closed and $F \subseteq H$.

Proof. Suppose H is NK-open in U and $H \supseteq F$, where F is $N\omega$ -closed in U . Then $H^c \subseteq F^c$ where F^c is $N\omega$ -open in U . $Nscl(H^c) \subseteq F^c$. Therefore $(Nsint(H))^c \subseteq F^c$. Therefore $F \subseteq Nsint(H)$.

Conversely, suppose that $H^c \subseteq G$ and G is $N\omega$ -open in U . Then $H \supseteq G^c$ and G^c is $N\omega$ -closed. So $Nsint(H) \supseteq G^c$. That is $(Nsint(H))^c \subseteq G$. Therefore $Nscl(H^c) \subseteq G$. Therefore H^c is NK-closed. Therefore H is NK-open in $(U, \tau_R(X))$.

Proposition 5.4. Let H be a subset of a nano topological space $(U, \tau_R(X))$. For any $x \in X$, $x \in NK-cl(H)$ if and only if $V \cap H \neq \Phi$ for every NK-open set V containing x .

Proof. Suppose that $x \in NK-cl(H)$. Let V be a NK-open set V containing x such that $V \cap H = \Phi$. Therefore, $H \subseteq V^c$ and V^c is NK-closed. $NK-cl(H) \subseteq V^c$. Since $x \notin V^c$, $x \notin NK-cl(H)$. Which is a contradiction. Therefore, $V \cap H \neq \Phi$, for every NK-open set V containing x .

Conversely, Suppose that for every NK-open set V containing x such that $V \cap H \neq \Phi$. Suppose $x \notin NK-cl(H)$. Then there exists a NK-closed F of U such that $H \subseteq F$ and $x \notin F$. Therefore $x \in F^c$ and F^c is an NK-open set containing x . But $F^c \cap H = \Phi$, which is a contradiction. Therefore $x \in NK-cl(H)$.

Theorem 5.5. If $Nint(H) \subseteq I \subseteq H$ and if H is NK-open, then I is NK-open.

Proof. If $Nint(H) \subseteq I \subseteq H$ then $X - H \subseteq X - I \subseteq X - Nint(H)$. That is $X - H \subseteq X - I \subseteq Ncl(X - H)$. Since $X - H$ is NK-closed and by Theorem 4.1, $X - I$ is NK-closed and hence I is NK-open.

Definition 5.6. For any $H \subseteq X$, $NK-int(H)$ is defined as the union of all NK-open sets contained in H . That is, $NK-int(H) = \cup \{G : G \subseteq H \text{ and } G \text{ is NK-open}\}$.

Lemma 5.7. For any $H \subseteq X$, $Nint(H) \subseteq NK-int(H) \subseteq H$.

Proof. By definition of nano open and NK-open sets, it is obvious.

Proposition 5.8. For any $H \subseteq X$, the following holds.





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(i) $NK\text{-int}(H)$ is the largest NK -open set contained in H .

(ii) H is NK -open if and only if $NK\text{-int}(H) = H$.

Proof. (i) Since the union of all NK -open sets is NK -open, $NK\text{-int}(H)$ is the largest open set contained in H .

(ii) By definition of $NK\text{-int}(H)$, $NK\text{-int}(H) \subseteq H$. Since H is NK -open and $NK\text{-int}(H)$ is NK -open, $NK\text{-int}(H) = H$.

Conversely, By definition of $NK\text{-int}(H)$, $NK\text{-int}(H)$ is NK -open. Therefore H is NK -open.

Proposition 5.9. Let H be a subset of a nano topological space $(U, \tau_R(X))$. Then the following holds.

(i) $(NK\text{-int}(H))^c = NK\text{-cl}(H^c)$.

(ii) $NK\text{-int}(H) = (NK\text{-cl}(H^c))^c$.

(iii) $NK\text{-cl}(H) = (NK\text{-int}(H^c))^c$.

Proof. (i) Let $x \in (NK\text{-int}(H))^c$. Then $x \notin (NK\text{-int}(H))$. That is, every NK -open V containing x such that $V \not\subseteq H$. Thus every NK -open set V containing x such that $V \cap H^c \neq \Phi$. So $x \in NK\text{-cl}(H^c)$. By Proposition 5.4, Therefore $(NK\text{-int}(H))^c \subseteq NK\text{-cl}(H^c)$.

Conversely, Let $x \in NK\text{-cl}(H^c)$. Then by Proposition 5.4, every NK -open V containing x such that $V \cap H^c \neq \Phi$. That is, every NK -open V containing x such that $V \not\subseteq H$. This implies, by definition of $NK\text{-int}(H)$, $x \notin NK\text{-int}(H)$. That is $x \in (NK\text{-int}(H))^c$. Therefore $NK\text{-cl}(H^c) \subseteq (NK\text{-int}(H))^c$. Therefore $(NK\text{-int}(H))^c = NK\text{-cl}(H^c)$.

(ii) Follows by taking complements in (i)

(iii) Follows by replacing H by H^c in (i).

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A Study to Assess the Effect of Helper Skin Tap Technique on Level of Pain During Intramuscular Injection Among Admitted Patients in Selected Hospitals, Guwahati, Assam

Tailyang Monya¹ and Manashi Sengupta^{2*}

¹Nursing Tutor, Faculty of Nursing, North East Nursing College of Health Sciences, Ita Nagar, Arunachal Pradesh, India.

²Dean, Faculty of Nursing, Assam down town University, Guwahati, Assam, India

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*Address for Correspondence

Manashi Sengupta

Dean,

Faculty of Nursing,

Assam down town University

E. Mail: manashisengupta50gmail.com



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ABSTRACT

The word "pain" comes from the Latin word "poena" meaning a fine or penalty. An unpleasant sensation that can range from mild, localized discomfort to agony. One of the common treatment of disease is through administration of medication. Intramuscular injection of medication is a procedure commonly performed by nurse and is associated with discomfort, pain and trauma to the injected tissue. Nurses are ethically or legally responsible for managing pain and relieving suffering. Tapping over the skin is one of the various techniques to keep the muscle relaxed. Helper skin tap technique was proposed by Joanne Helfer in 1998. Helper skin tap may not only help to reduce pain due to intramuscular injection but also reduce anxiety and relax the skin and distraction of the patient. A Quasi experimental post test design and a quantitative research approach was undertaken among 60 admitted patients in Health City Hospital and down town Hospital of Guwahati, Assam. The sample consisted of adult patient (Group I- Control 30 and Group II- Experimental 30) and they were selected by using purposive sampling technique. The tools used were demographic variables and McCaffery Numerical Pain Rating Scale (NPRS). The objective of the study is to assess the effect of helper skin tap technique on the level of pain during intramuscular injection among admitted patients. The results showed that mean pain score in control group was 5.30 ± 1.149 and experimental group mean pain score was 1.07 ± 1.285 with mean difference was 4.23. The comparison was tested using unpaired t-test with obtained t value is ($t=13.45$) at $df=58$ was statistically highly significant at $p \leq 0.05$ level. Findings revealed that helper skin tap technique was effective in reducing the pain during IM injection among admitted patients in experimental group as compared to control group. The present study concluded that helper skin tap technique was effective in





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reducing the pain during IM injection among admitted patients in experimental group as compared to control group.

Keywords: Intramuscular Injection, Helfer skin tap technique, Pain, Adult

INTRODUCTION

“Pain is an uncomfortable feeling that even a tiny moment of it is enough to ruin every enjoyment” -Will Rogers

The word “pain” comes from the Latin word “poena” meaning a fine or penalty. An unpleasant sensation that can range from mild, localized discomfort to agony. Pain has both physical as well as emotional components. The physical part of pain results from nerve stimulation. Pain may be content to a discrete area as in an injury or it can be more diffuse as in disorders like fibromyalgia [1][5]. Intramuscular injection of medication is a procedure commonly performed by nurse and is associated with discomfort, pain and trauma to the injected tissue. There are several factors which influence person’s experience of pain during intramuscular injection such as anxiety, culture, age, gender and expectation of pain relief. These factors may increase or decrease the experience of pain during intramuscular injection. Even though it can cause tissue, musculoskeletal and neurological complications but are very rare and minimal as compared to the intravenous injections [2]. Helfer skin tap technique was proposed by Joanne Helfer in 1998. Helfer skin tap may not only help to reduce pain due to intramuscular injection but also reduce anxiety and relax the skin and distraction of the patient. The mechanism of helper skin tap technique is gate control theory. In helper skin tap technique while doing tapping before intramuscular injection, the nervous system will shut down the sensory gate and the pain sensation of the injection will not reach the brain. So, the injection pain goes unnoticed [5].

Objectives

The study was conducted to assess the level of pain during IM injection in control group, to assess the level of pain after providing helper skin tap technique during IM injection among admitted patients in experimental group, to compare the level of pain during intramuscular injection among admitted patients in experimental and control group and to find the association between level of pain with selected demographic variables.

MATERIALS AND METHODS

A quantitative research approach was adopted and a quasi-experimental study with post-test design was used in order to accomplish the main objectives of assessing the effect of Helfer skin tap technique.

The diagrammatic representation of the research design used is given as follows:

| Group | Intervention | Post test |
|-------|--------------|-----------------|
| E | X | OE ₁ |
| C | - | OC ₁ |

E: Experimental group consisting of 30 adults who were receiving intramuscular injection in selected hospital.

C: Control group consisting of 30 adults who were receiving standard therapy.

X: Administration of Helfer skin tap technique before and during the intramuscular injection.

OE₁: Post test assessment on the level of pain after intervention in the experimental group.

OC₁: Post test assessment on the level of pain after standard technique in the control group.

The study was conducted in Health City Hospital, Guwahati, Assam and downtown Hospital, Guwahati, Assam. The target population was 60 hospitalized patient receiving IM injection out of which 30 each were randomly assigned to the experimental group and control group. Patients who matched the inclusion criteria were selected by purposive sampling technique and assigned randomly to the experimental and control group. The tool used for the study consists of Section I: Demographic variables to assess sample characteristics. It consists of age, gender, marital



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status, education, diagnosis of patient, medication administered, site of injection, number of injections administered concurrently and previous history of IM injections. Section II: Standardized McCaffery numerical pain rating scale: The standardized McCaffery numerical pain rating scale is ranging from point 0 to point 10. '0'-No pain, '1 to 3' - Mild pain '4 to 6' – Moderate pain, '7 to 10' – Severe pain. The data collection procedure was explained to the participants. Purpose of the study was explained and informed consent was obtained from participants and assurance was given to maintain confidentiality. For adults patients with experimental group, helper skin tap technique was performed during the administration of analgesic 2ml intramuscular injection by tapping 3 times before the administration, 12 times tapping at the times of administration and lastly one tap at the time of removal of syringe. For adults patients with control group, no intervention was given. Post test level of pain was assessed for both experimental and control group within an hour using McCaffery numerical rating scale. The patient were asked to rate the level of pain from 1 to 10 in the scale.

RESULTS AND FINDINGS**Frequency and percentage distribution of patient in experimental and control group**

In the age, in case of control group majority of the patients were in the age group of 31-40 years 60% (18) and in case of experimental group majority of the patients were also in the age group of 31-40 years 70% (30). In gender, majority of the patient in case of control group were male 64% (19) and in case of experimental group majority of the patient were also male 56% (17). In educational status, majority of the patient in case of control group were graduate 66% (20) and in case of experimental group majority of the patient were also graduate 84% (25). In drug administered, all the patients in case of control group received analgesic IM injection 100% (30) and in case of experimental group, the patient received analgesic 100% (30). In reason for drug administration, all the patients in case of control group received to reduce pain i.e. 100% (30) and in case of experimental group, all the patient received drug to reduce pain i.e. 100% (30). In site of intramuscular drug administration, all the patient in control group received through ventrogluteal muscle of the hip 100% (30) whereas in case of experimental group also all received through ventrogluteal muscle of the hip 100% (30). In number of intramuscular injection administered in one day, majority of the patient in case of control group received 1 dose 53% (16) whereas in case of experimental group also majority received 1 dose 60% (18). In size of needle, all the patient in case of both control and experimental group received IM injection through 22 gauge control group 100% (30) and experimental group 100%(30). In form of drug, all the patient in case of both control and experimental group received aqueous form i.e control group 100% (30) and experimental group 100%(30). In volume of substance administered, all the patient in case of both control and experimental group received ≤ 2 ml control group 100% (30) and experimental group 100%(30). In previous exposure to intramuscular injection, all the patient in case of both control and experimental group have been exposed to intramuscular injection control group 100% (30) and experimental group 100%(30). In size of needle, all the patient in case of both control and experimental group received through 22 gauge control group 100% (30) and experimental group 100%(30). In any previous history of side effect from intramuscular injection, no patient had any side effect from IM injection in case of both control and experimental group i.e. control group 100% (30) and experimental group 100%(30).

Frequency and percentage distribution of post test level of pain during intramuscular injection among admitted patients in control and experimental group.

Figure 1 depicts the frequency and percentage distribution of post-test level of pain during intramuscular injection among admitted patients in control and experimental group. Results shows that in control group majority 23(77%) had moderate pain and 7(23%) had severe pain where as in experimental group majority 19(63%) had no pain and 11(37%) had mild pain.

Table 1: depicts the comparison of level of pain during intramuscular injection among admitted patients in experimental and control group. Findings showed that mean pain score in control group was 5.30 ± 1.149 and experimental group mean pain score was 1.07 ± 1.285 with mean difference of 4.23. The comparison was tested using unpaired 't' test with calculated 't' value is ($t=13.45$) at $df=58$ was statistically highly significant at $p \leq 0.05$ level.



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Findings revealed that helper skin tap technique was effective in reducing the pain during IM injection among admitted patients in experimental group as compared to control group.

Association between post-test level of pain with selected demographic variables among admitted patients in experimental and control group.

The selected demographic variables age, gender, educational status and number of drugs administered have no significant association on pain score of adults during intramuscular injection in the experimental and control group at $p \leq 0.05$ level of significance.

DISCUSSION

The findings shows that in control group majority 23(77%) had moderate pain and 7(23%) had severe pain where as in experimental group majority 19(63%) had no pain and 11(37%) had mild pain. The present study is supported by a quasi-experimental cross over, posttest only design study conducted by Jyoti G to compare the effect of Helper Skin Tap Technique (HST) vs Routine Technique on the pain perception among adult patients receiving intramuscular injection (Voveron). The study revealed Routine Technique, 40 (66.7%) had moderate pain, 19 (31.7%) had severe pain and 1 (1.6%) had mild pain. Among the patients who received intramuscular injection using Helper Skin Tap Technique, 43 (71.7%) had mild pain and 17 (28.3%) experienced no pain. The study findings showed that the mean pain score in control group was 5.30 ± 1.149 and experimental group mean pain score was 1.07 ± 1.285 with mean difference of 4.23. The comparison was tested using unpaired 't' test with calculated 't' value is ($t=13.45$) at $df=58$ which was highly significant at $p \leq 0.05$ level. Findings revealed that helper skin tap technique was effective in reducing the pain during IM injection among admitted patients in experimental group as compared to control group. The present study is supported by an experimental conducted by Raman N (2020) on effectiveness of Helper skin tap technique on the level of pain reduction experienced by the adult patients. The study findings depicted that the mean pain score in Experimental Group (1.16) is lesser than the mean pain score in Control Group (5.87). The calculated 't' value (unpaired 't' test) 15.67 was highly significant at $p \leq 0.05$ level. Thus, it is concluded that the Helper skin tap technique was effective in reducing level of pain among adult patients. Association between post-test level of pain with selected demographic variables was calculated using Fisher Exact test. The selected demographic variables age, gender, educational status and number of drugs administered have no significant association on pain score of adults during intramuscular injection in the experimental and control group at $p \leq 0.05$ level of significance.

CONCLUSION

The present study was conducted to assess the effect of helper skin tap technique on the level of pain during intramuscular injection among admitted patients in selected hospitals, Guwahati. The findings of the study revealed that helper skin tap technique was effective in reducing the pain during IM injection among admitted patients in experimental group as compared to control group. The study revealed that there is no significant association between pain level with selected demographic variables among admitted patients of the experimental and control group.

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Compare the level of pain during intramuscular injection among admitted patients in experimental and control group. n=60

| Comparison Level of pain | Mean | SD | Mean Difference | t test value | df | p value | Inference |
|---------------------------|------|-------|-----------------|--------------|----|----------------|-----------|
| Control group | 5.30 | 1.149 | 4.23 | 13.45 | 58 | 0.001** | S |
| Experimental group | 1.07 | 1.285 | | | | | |

*p<0.05 level of significance

**Highly significant

NS- Non significant

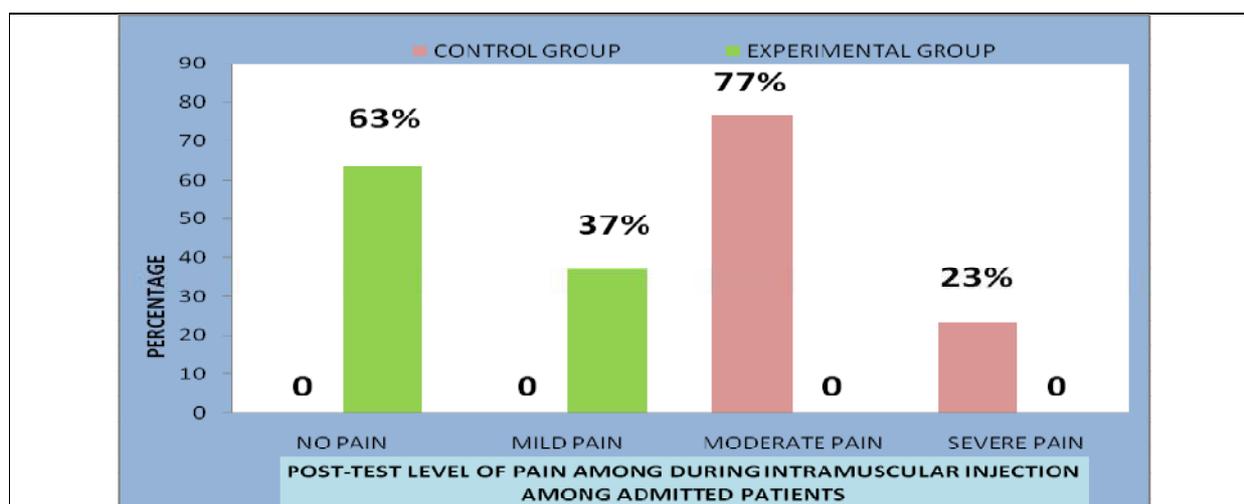


Fig 1: Percentage distribution of post-test level of pain among during intramuscular injection among admitted patients in control and experimental group.





Properties of I_ξ Continuous function in Ideal Topological Space

T.Sankili¹ and R. Asokan^{2*}

¹Research Scholar, School of Mathematics, Madurai Kamaraj University, Madurai, Tamil Nadu, India.

²Professor, School of Mathematics, Madurai Kamaraj University, Madurai, Tamil Nadu, India

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*Address for Correspondence

R. Asokan

Professor, School of Mathematics,

Madurai Kamaraj University,

Madurai, Tamil Nadu, India

E. Mail: asokan.maths@mkuniversity.org



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ABSTRACT

In this article, we introduce and study the properties of I_ξ continuous function using I_ξ open sets and also we study I_ξ closure operator and I_ξ interior operator in ideal topological spaces.

Keywords: Ideal topological space, I_ξ continuous function, I_ξ closure, I_ξ interior.

INTRODUCTION

In point set topology continuous properties are the essential part. In the year 1982 Hdeib [3] introduced the new type of open set namely ω -open set and ω -closed function and after seven years in 1989 Hdeib [3] studied ω -continuous function. A subset A belonging to an ideal topological space (X, τ, I) is said to be ω -open if for every $x \in A$, there exists an open set U_x containing x such that $U_x - A$ is countable. A function $f: (X, \tau) \rightarrow (Y, \sigma)$ where (X, τ) and (Y, σ) are topological spaces is ω -continuous if for every $x \in X$ and for every open set G in Y containing $f(x)$, there exists ω -open set U in X such that $f(U) \subseteq G$. In the year 2009 Noiri and Noorani [7] gave the pre- ω -open using ω -open. A subset A belonging to the topological space (X, τ) is said to be pre- ω -open if $A \subseteq \text{int}_\omega(\text{cl}(A))$ where $\text{Int}_\omega(A)$ is ω -interior operator of A in the space X . A function $f: (X, \tau) \rightarrow (Y, \sigma)$ is pre- ω -continuous if $f^{-1}(U)$ is pre- ω -open set in (X, τ, I) for every open set U in Y .

In the year 2009 Ekici and Noiri [2] made the notion decomposition of continuity using pre- I -open sets in ideal topological spaces. A subset A belonging to an ideal topological space (X, τ, I) is said to be pre- I -open if $A \subseteq (\text{int}(\text{cl}^*(A)))$. The opposite of pre- I -open is pre- I -closed set. A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ where (X, τ, I) an ideal topological space and (Y, σ) a topological space is pre- I -continuous if $f^{-1}(G)$ is pre- I -open set in (X, τ, I) for every open set G in Y . In [8] we produced new notion that is I_ξ open set which is finer than τ . A subset S belonging to an ideal topological space (X, τ, I) is said to be I_ξ open set if there exists closed set $F \neq \phi$, $X \in \tau^c$ such that $S \cap F \subseteq \text{int}^*(S)$. The collection of every I_ξ open set in (X, τ, I) is τ^{int^*} . The opposite of I_ξ open set in τ^{int^*} is I_ξ closed set.





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In this article, we make the properties of I_ξ continuous function using I_ξ open sets in ideal topological space (X, τ, I) . The rest of the paper is organized as follows: Section 2 gives the preliminaries, Section 3 we study I_ξ closure operator and I_ξ interior operator using the new kind of I_ξ open sets to introduce and investigate the notion I_ξ continuous function in ideal topological spaces. The relationships of the I_ξ continuous function and other familiar functions are introduced and studied.

Preliminaries

The term ideal in topological space was first coined by Kuratowski and Vaidyanathaswamy [6,9]. Jankovic and Hamlett [5] were given the concept of I open sets in ideal topological spaces. An ideal I on a non empty collection of subsets of X which satisfies the following axioms:

(i) $S_1 \in I$ and $S_2 \subseteq S_1 \Rightarrow S_2 \in I$,

(ii) $S_1 \in I$ and $S_2 \in I \Rightarrow S_1 \cup S_2 \in I$ [6,9]. Applications of ideal into various fields examined by Jankovic and Hamlett [5]. A topological space (X, τ) together with an ideal I on X and if $P(X)$ is the power set of X , a set operator $(.)^*: P(X) \rightarrow P(X)$ is said to be a local function of S in terms of τ and ideal I is defined as

For $S \subseteq X$, $S^*(I, \tau) = \{s \in X \mid U \cap S \notin I \text{ for every } U \in \tau(s), \text{ where } \tau(s) = \{U \in \tau \mid s \in U\}\}$. Furthermore $cl^*(S) = S \cup S^*(I, \tau)$ defines a Kuratowski [6] closure operator for the topology τ^* is finer than τ .

Throughout this paper (X, τ, I) (simply X) represent an ideal topological space for which there is no chance of confusion.

Definition 2.1[8] Let (X, τ, I) be an ideal topological space and $S \subseteq X$.

(i) The I_ξ closure operator of S is denoted by $I_\xi cl(S)$ and it is defined as the intersection of every I_ξ closed set containing S .

That is, $I_\xi cl(S) = \cap \{P \subseteq X \mid S \subseteq P \text{ and } S \in \tau^{int^c}\}$.

(ii) The I_ξ interior operator of S is denoted by $I_\xi(S)$ and it is defined as the union of every I_ξ open set contained in S .

That is, $I_\xi int(S) = \cup \{P \subseteq X \mid P \subseteq S \text{ and } S \in \tau^{int^*}\}$.

Properties of I_ξ Continuous function in Ideal topological spaces

Definition 3.1 Let (X, τ, I) be an ideal topological space and (Y, σ) be a topological space. Then the function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ is said to be I_ξ continuous if for every open set T in Y , $f^{-1}(T)$ is I_ξ open set in X .

Remark 3.2 Every open set is I_ξ open set, therefore every continuous function is I_ξ continuous.

The next example clearly shows that the reverse of the above remark is not true.

Example 3.3 Let (X, τ, I) be an ideal topological space, $X = \{s_1, s_2, s_3\}$, $\tau = \{\emptyset, \{s_2\}, \{s_2, s_3\}, X\}$ with ideal $I = \{\emptyset, \{s_2\}\}$. Then $\tau^{int^*} = \{\emptyset, \{s_2\}, \{s_3\}, \{s_1, s_3\}, \{s_2, s_3\}, X\}$. A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ where $Y = \{\{t_1\}, \{t_2\}\}$ and $\sigma = \{\emptyset, \{t_1\}, \{t_2\}, \{t_1, t_2\}, Y\}$ defined by $f(s_1) = f(s_3) = t_2$, $f(s_2) = t_1$.

Then f is I_ξ continuous function but it is not continuous function because open set $\{t_2\}$ in Y has $\{s_3\}$ and $\{s_1, s_3\}$ are the inverse I_ξ open images in X but these two are not open sets in X since, remark (3.4) [8].

Theorem 3.3 Let (X, τ, I) be an ideal topological space and (Y, σ) be a topological space. Then the function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ is I_ξ continuous function if and only if $f^{-1}(T)$ is I_ξ closed in (X, τ, I) for every closed set T in Y .

Proof. The proof is clear from the definition 3.1.

Theorem 3.4 A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ of an ideal topological space (X, τ, I) and a topological space (Y, σ) is I_ξ continuous if and only if for every $s \in X$ and every open set V in Y with $f(s) \in V$, there is I_ξ open set U in (X, τ, I) such that $s \in U$ and $f(U) \subseteq V$.

Proof. Assume that f is I_ξ continuous function. Let $s \in X$ and V be open set in Y containing $f(s)$. Take $U = f^{-1}(V)$. Since f is I_ξ continuous function, then U is I_ξ open in (X, τ, I) such that $s \in U$ and $f(U) \subseteq V$.





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Conversely, Suppose V is open in Y . For every $s \in f^{-1}(V)$, $f(s) \in V$. Then by hypothesis, there is I_ξ open set U_x in (X, τ, I) such that $s \in U_x$ and $f(U_x) \subseteq V$. This implies that $U_x \in f^{-1}(V)$ and so $f^{-1}(V) = \cup_{x \in f^{-1}(V)} U_x$. Hence by theorem 3.11 [1], $f^{-1}(V) = \cup_{x \in f^{-1}(V)} U_x$ is I_ξ open set in (X, τ, I) . Therefore, f is I_ξ continuous function.

Theorem 3.5 A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ of an ideal topological space (X, τ, I) and a topological space (Y, σ) is I_ξ continuous if and only if $f(I_\xi cl(S)) \subseteq \sigma cl(f(S))$ for every $S \subseteq X$.

Proof. Suppose f is I_ξ continuous and S is any subset in X . Then $\sigma cl(f(S))$ is closed in Y . Since f is I_ξ continuous function and by theorem 3.3, $f^{-1}(\sigma cl(f(S)))$ is I_ξ closed in (X, τ, I) .

Which is, $I_\xi cl(f^{-1}(\sigma cl(f(S)))) = f^{-1}(\sigma cl(f(S)))$. Since $f(S) \subseteq \sigma cl(f(S))$ then $A \subseteq f^{-1}(\sigma cl(f(S)))$.

This implies, $I_\xi cl(S) \subseteq I_\xi cl(f^{-1}(\sigma cl(f(S)))) = f^{-1}(\sigma cl(f(S)))$. Therefore $f(I_\xi cl(S)) \subseteq \sigma cl(f(S))$

Conversely, suppose that T is closed set in Y and $\sigma cl(T) = T$. Since $f^{-1}(T) \subseteq X$. Then by hypothesis, $f(I_\xi cl(f^{-1}(T))) \subseteq \sigma cl(f^{-1}(T)) \subseteq \sigma cl(T) = T$. This implies, $I_\xi cl(f^{-1}(T)) \subseteq f^{-1}(T)$, which is I_ξ closed set in (X, τ, I) . Therefore by theorem 3.3, f is I_ξ continuous function.

Theorem 3.6 A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ of an ideal topological space (X, τ, I) and a topological space (Y, σ) is I_ξ continuous if and only if $I_\xi cl(f^{-1}(T)) \subseteq f^{-1}(\sigma cl(T))$ for every $T \subseteq Y$.

Proof. Let f be I_ξ continuous and T be any subset in Y . Then $\sigma cl(T)$ is closed in Y . Since f is I_ξ continuous function and by theorem 3.3, $f^{-1}(\sigma cl(T))$ is I_ξ closed in (X, τ, I) . Which is, $I_\xi cl(f^{-1}(\sigma cl(T))) = f^{-1}(\sigma cl(T))$. Since $T \subseteq \sigma cl(T)$ then $f^{-1}(T) \subseteq f^{-1}(\sigma cl(T))$. This implies, $I_\xi cl(f^{-1}(T)) \subseteq I_\xi cl(f^{-1}(\sigma cl(T))) = f^{-1}(\sigma cl(T))$. Therefore $I_\xi cl(f^{-1}(T)) \subseteq f^{-1}(\sigma cl(T))$.

Conversely, suppose P is closed in Y and $\sigma cl(P) = P$. Since $P \subseteq Y$. Then by hypothesis, $I_\xi cl(f^{-1}(P)) \subseteq f^{-1}(\sigma cl(P)) = f^{-1}(P)$. This implies, $I_\xi cl(f^{-1}(P)) \subseteq f^{-1}(P)$. Hence $I_\xi cl(f^{-1}(P)) = f^{-1}(P)$, which is $f^{-1}(P)$ is I_ξ closed set in (X, τ, I) . Therefore by theorem 3.3, f is I_ξ continuous function.

Theorem 3.7 A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ of an ideal topological space (X, τ, I) and a topological space (Y, σ) is I_ξ continuous if and only if $f^{-1}(\sigma \text{int}(T)) \subseteq I_\xi \text{int}(f^{-1}(T))$ for every $T \subseteq Y$.

Proof. Suppose f is I_ξ continuous and T is any subset in Y . Then $\sigma \text{int}(T)$ is open in Y . Since f is I_ξ continuous function then $f^{-1}(\sigma \text{int}(T))$ is I_ξ open in (X, τ, I) . Which is, $I_\xi \text{int}(f^{-1}(\sigma \text{int}(T))) = f^{-1}(\sigma \text{int}(T))$.

Since $\sigma \text{int}(T) \subseteq T$ then $f^{-1}(\sigma \text{int}(T)) \subseteq f^{-1}(T)$.

This implies, $f^{-1}(\sigma \text{int}(T)) = I_\xi \text{int}(f^{-1}(\sigma \text{int}(T))) \subseteq I_\xi \text{int}(f^{-1}(T))$. Therefore $f^{-1}(\sigma \text{int}(T)) \subseteq I_\xi \text{int}(f^{-1}(T))$.

Conversely, suppose P is open in Y which is $\sigma \text{int}(P) = P$. Since $P \subseteq Y$. Then by hypothesis, $f^{-1}(P) = f^{-1}(\sigma \text{int}(P)) \subseteq I_\xi \text{int}(f^{-1}(P))$. This implies, $f^{-1}(P) \subseteq I_\xi \text{int}(f^{-1}(P))$. Hence $f^{-1}(P) = I_\xi \text{int}(f^{-1}(P))$, which is $f^{-1}(P)$ is I_ξ open set in (X, τ, I) . Therefore f is I_ξ continuous function.





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Definition 3.8 Let (X, τ, I) be an ideal topological space and (Y, σ) a topological space. Then the function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ is said to be I_ξ closed function if $f(S)$ is closed in (Y, σ) for every I_ξ closed S in (X, τ, I) .

Theorem 3.9 Let $f: (X, \tau, I) \rightarrow (Y, \sigma)$ and $g: (Y, \sigma) \rightarrow (Z, \rho)$ be two functions. Then the composition function gof is closed function if g is closed function and if f is I_ξ closed function.

Proof. Let S be I_ξ closed set in (X, τ, I) . Since f is I_ξ closed function then $(f(S)) = (gof)(S)$. That is, gof is I_ξ closed function.

Theorem 3.10 A function $f: (X, \tau, I) \rightarrow (Y, \sigma)$ of an ideal topological space (X, τ, I) and a topological space (Y, σ) is I_ξ closed function if and only if ${}_\sigma cl(f(S)) \subseteq f(I_\xi cl(S))$ for every $S \subseteq X$.

Proof. Assume that f is I_ξ closed function and S be any subset of X . Since $I_\xi cl(S)$ is I_ξ closed in (X, τ, I) and f is I_ξ closed function then $f(I_\xi cl(S))$ is closed in Y . This implies, ${}_\sigma cl(f(I_\xi cl(S))) = f(I_\xi cl(S))$. Since $S \subseteq I_\xi cl(S)$ then $(S) \subseteq f(I_\xi cl(S))$. This implies, ${}_\sigma cl(f(S)) \subseteq {}_\sigma cl(f(I_\xi cl(S))) = f(I_\xi cl(S))$. Therefore ${}_\sigma cl(f(S)) \subseteq f(I_\xi cl(S))$.

Conversely, let T be any closed set in Y and $I_\xi cl(S) = S$. Since $S \subseteq X$. Then by hypothesis, ${}_\sigma cl(f(S)) \subseteq f({}_\sigma cl(S)) = f(S)$. This implies, ${}_\sigma cl(f(S)) \subseteq f(S)$. Hence ${}_\sigma cl(f(S)) = f(S)$, which is $f(S)$ is a closed in Y . Therefore f is I_ξ closed function.

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A Novel Technique for Classifying Lung Lesions using Convolutional Neural Network Optimization Techniques

Ritu Nagila^{1*} and Abhishek Kumar Mishra²

¹Research Scholar, Department of Computer Science and Engineering, IFTM University, Moradabad, Uttar Pradesh, India.

²Associate Professor, Department of Computer Science and Engineering., IFTM University, Moradabad, Uttar Pradesh, India.

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*Address for Correspondence

Ritu Nagila

Research Scholar,
Department of Computer Science and Engineering,
IFTM University, Moradabad,
Uttar Pradesh, India.



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ABSTRACT

World-wide cancer is the disease with the highest recurrence rate. Lung cancer is the most fatal of all cancer kinds. Lung cancer claims millions of lives each year. Early disease detection is extremely important for cancer patients. The survival rate increases when this disease is accurately predicted. But with the current techniques, accurate lung cancer detection is time-consuming and of utmost importance. The ability to examine genes and their relationships to various diseases, such as lung cancer, has been demonstrated by the accessibility of current technologies. The hybrid approach is suggested as a solution to this problem. The identification of cancer in CT scans is addressed in this study using a hybrid strategy that combines a whale optimisation algorithm and CNN classification. With the use of this technique, doctors can accurately identify lung nodules at an early stage.

Keywords: Optimization; Classification; Lung Cancer; Nodules; Convolutional Neural Network, Whale Optimization Algorithm (WOA); Discrete Wavelet Transform (DWT); Genetic Algorithm

INTRODUCTION

Lung cancer is a frequent cancer condition that can be treated quite successfully with an early [1] diagnosis. According to ACS (American cancer Society), in the year 2018 approximately 14% are lung cancer out of all cancer types [2]. The use of image processing techniques and pattern recognition technologies to automatically recognise lung cancer from CT scans reduces human error and speeds up diagnosis [3]. In this study, CT images are examined using pipeline analysis to identify malignant tumours using image processing techniques. The first stage uses image contrast improvement and a noise drop to enhance the quality of CT pictures and the contrast of problematic spots in



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the image. In order to diagnose lung lesions accurately and effectively, a hybrid technique is suggested in this paper. The suggested method uses convolutional neural network to classify whether the detected section is cancerous or not, genetic algorithms as a segmentation method to precisely identify genes (lung nodule).

LITERATURE REVIEW

Ananya Choudhury et al. suggested a multi-objective genetic method for lung cancer segmentation in 2019 [4]. The genetic algorithm used in this method performs multi-level thresholding, and the connected component methodology is employed for classification. In this study, classification accuracy was measured in terms of true positives and false negatives, but segmentation accuracy was left out. Ammar Odeh et al. [5] suggested an early detection technique for lung cancer using genetics in 2017. In this study, the authors' accuracy was 84%. In 2017 Kamil Dimililer et.al [6] proposed a strategy for lung lesion detection using DWT. In this method DWT (Haar) is used for segmentation and extracted 4 output different images which are represented in vertical, horizontal, diagonal and approximation. Finally, all the images are processed through techniques like erosion and subtraction to extract the cancer area. By this method authors obtained 89% accuracy.

Mukesh Chandra Arya et al. [7] suggested a method in 2016 to use DWT to identify bulk tissues in chest X-ray images. By using this technique, the authors' accuracy was 86%. Manasee Kurkure et al. [8] suggested a method for identifying and categorising lung cancer in 2016. This approach uses a Nave Bayesian classifier for classification, a Genetic Candidate Group search for segmentation, and a clever edge detector. By using this technique, the authors' accuracy was 82%. This technique segments using the Canny edge detector. Due to its extensive computations and the fact that the canny detector doesn't conduct a decent operation for rotational regularity, it consumes extra computation time. All of the approaches that the authors have suggested have lower accuracy due to flaws including bad directionality, slow processing, lengthy computations, and complex computations. In order to address all of the shortcomings of the existing approaches listed above, a dynamic method for lung cancer identification in CT images is proposed in this study. CNN(Convolutional Neural Network) is utilised for feature extraction, and a genetic algorithm is used for segmentation.

METHODOLOGY

This method uses the CNN optimisation technique to identify and predict lung cancer from CT scans. The proposed method's processing methods are shown in Fig. 1. The lung cancer CT scan is initially read from the database. The captured image typically has minimal noise, and since direct noise removal runs the risk of reducing quality, processing techniques are used to eliminate the noise. A nonlinear digital filter called the median is used to cut down on noise. The Genetic method is then used to process the augmented image through segmentation. The segmented image is then processed using the feature extraction method. The CNN classifier is then given the retrieved image to determine whether the lung nodule is benign or cancerous.

Image Acquisition

The process of getting a digital image from a database is known as image acquisition. Typically, several scanners like MRIs and CTs are used to capture the images. The CT scanner produces the CT image. Through a process called computed tomography, each pixel in cross-sectional scans is given meaning [9]. This scan serves as a painless, non-invasive diagnostic technique. Additionally known as CAT, or computerised axial tomography.

Preprocessing

Pre-processing is the technique applied to images data to enhance the image. It lessens undesirable distortions and improves the characteristics of the image that are important for processing. The size of the pixel neighbourhood can be used to classify pre-processing methods. Image enhancement uses these techniques. Enhancement operations use





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sub-images of corresponding values and nearby image pixels. The noise and distortion are reduced during enhancement, which raises the quality of the image. The median filter is used in many bio-medical applications to reduce noise.

Median Filtering

Impulse and salt & pepper noise can be found in the collected CT images. The delicate details are hidden by these disturbances. By preserving the borders in an image, the median filter decreases impulsive, salt-and-pepper noise [10].

Segmentation

It extracts objects and boundary features. A challenging problem in medical images is to segment regions with boundary between object (Regions of Interest) and background [11]. To rectify this problem numerous segmentation methods have been proposed by researchers.

Classification

It is the process of removing data classes from a multiband raster image is referred as. To create topical maps, use the raster that results from image categorization after the first one. There are two types of classification: supervised and unsupervised, depending on the expert and computer's cooperation during categorization[12]. CNN, or supervised classification, is employed in this work. In order to characterise aimage, it makes use of spectral markings obtained from training examples.

Clustering

Clustering is a popular strategy to separate a significant amount of data from a small number of meetings [13]. When using k-means to cluster data, a large amount of data is split up into a large number of separate pieces and then joined into disparate groups [14]. There are two distinct phases in K-means. It leads the centroid of k in phase 1 and collects individual points from the cluster that is closer to the centroid in phase 2. One of the most widely used strategies for describing the separation of the closest centroid is Euclidean separation. The following equations are used to do the clustering.

$$D = || m(p, q) - C_n || \quad (1)$$

$$C_n = \frac{1}{n} \sum_{p \in C_n} \sum_{q \in C_n} m(p, q) \quad (2)$$

Where D is Euclidean distance and n is number of clusters

Segmentation by Genetic Algorithm

Using probabilistic techniques, GA is one form of optimisation strategy that is inspired by biological development. The concepts of "Natural Selection" and "genetic Inheritance" are used. John Holland first created this algorithm in 1975. GA is particularly suitable for challenging problems where minimal consideration is given to the fundamental research domain. A genetic algorithm maintains a population of competing solutions for the present problem and drives it forward by repeatedly applying numerous stochastic administrations. It uses stochastic operators to solve optimisation problems with workable solutions, applying these operations repeatedly to a set of feasible answers. The GA algorithm is linked with many targets. It is connected to numerous stages, such as

The analogy between chromosomes and pixels.

To carry out segmentation and feature extraction

The valid fitness function is calculated by summing all the individual objects in the group sum. For certain hurdles, it is difficult to delineate the fitness function. GA operates based on the characteristics with Selection, mutation, crossover, and recombination.





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Algorithm

- Generate initial population
- If the candidate is not best then
- Generate new population using descendant functions.
- Calculate each candidate fitness function.
- Return to the best solution (candidate).

Feature Extraction

Feature extraction is the process of breaking down and condensing a large initial set of images into smaller, easier to manage groups. Therefore, this method can make the further processing of the images simpler. The fact that these enormous datasets have a lot of parameters is their most crucial feature. These parameters require numerous calculation origins in order to be processed. The best feature from those large datasets can then be obtained by the selection and combination of variables, effectively reducing the data volume. Two well-known features, DWT, are used in this study for feature extraction, which is explained below.

Discrete Wavelet Transform (DWT)

Wavelet transform is a useful tool for a variety of image processing tasks and is used in many areas, including feature extraction, pattern identification, coding, and picture compression. A frequency domain approach is the wavelet transform. In this method, a function known as a wavelet is utilised in place of sine and cosine functions (such as Fourier transform). When a wavelet transform is used, the wavelet function keeps its shape but is moved along with the signal, compressed, and opened during the displacement, enclosing the entire signal.

This technique can produce varied resolutions for low- and high-frequency ranges, unlike the short-time Fourier transform.

Classification by CNN

A CNN typically consists of the convolutional layer, the pooling layer, and the fully connected layer. Each layer performs a different function. Each convolution neural network has two stages: a feed forward stage and a backward stage for training. The first process once the features enter the network is the point multiplication between each neuron's input and variables, followed by the application of convolution operations in each layer. From the literature, it is observed that using CNN models improves the diagnosis system performance [13]. Optimising the weights of any CNN layer is the key goal here. The recommended optimisation algorithm (GA) exhibits appropriate advancements regarding the best CNN training.

This approach generated initial weight for convolution neural network by the operation of selection, crossover, and mutation. It utilised the global optimisation and [14] survival of fittest features of genetic algorithms. Learning performance after enhancement is superior to that of a conventional convolution neural network. Simulated results show that the genetic algorithm and convolution neural network combination technique has a greater classification accuracy than conventional convolution neural network and support vector machine. Fig. 2 illustrates a straightforward lung cancer diagnosis using standard CNN. The convolution layer in Fig. 2 analyses the output of the neurons that are input-connected to the local area. The calculation is done by multiplying the weights of each neuron by the activation mass, which is the region to which they are linked. The pooling layer's primary function is to subsample the input image in order to lessen the computational burden, memory requirements, and the number of parameters (over fitting). The neural network becomes less sensitive to image displacement (regardless of position) when the size of the input image is reduced.

Proposed Whale Optimization Algorithm (WOA) based CNN

Meta-heuristics are being used more and more frequently lately in a variety of applications. For the cross-entropy loss, for example, is one of these applications[15]. Several different types of meta-heuristic algorithms have been developed in recent years. Whale optimisation algorithm (WHO) is a brand-new meta-heuristic approach that Mirjalili and Lewis introduced in 2016 [16]. The bubble net hunting technique of humpback whales served as an

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inspiration for the whale optimisation algorithm. For certain applications, although being new, it produces good results [17-21]. In order to increase the effectiveness of the procedure, this algorithm is used to minimise cross-entropy loss for images of lung cancer. The whale optimisation method is used in this work to identify cancer in cancer photos. The primary goal of this exercise is to maximise the weights of each CNN layer. The suggested optimisation algorithm demonstrates appropriate advancements regarding the best CNN training.

The WOA is a brand-new stochastic optimisation technique that was inspired by how whales hunt [16]. WOA uses a random population collection of candidate solutions, like any evolutionary approach, to search for the problem's global optimal (maximum or minimum) answer. Until the optimum value is satisfied, the algorithm keeps updating and improving the answer depending on its structure. The WOA rules' approach to developing and updating the solution is the fundamental distinction between it and other meta-heuristic techniques. The WOA is modelled after the whale's trap and attack hunting technique; the usage of bubbles in spiral movements around the prey with which the trap is built is referred to as "bubble-net feeding behaviour". Fig. 3 depicts the bubble-net feeding process' behaviour.

Fig-4 makes it obvious that the humpback whale initially blows bubbles around its meal. The whale's spiral motion is used to carry out this procedure. It then attacks the prey. The WOA's primary contributor is made up of this process. An optimised techniques of CNN with the help of WOA is shown is figure 5. According to mathematics, the explained generated bubble-net system is defined as follows:

Mathematical Model

Bubble-net feeding is a unique behaviour that can only be observed in humpback whales. In whale optimization algorithm (WOA) the spiral bubble-net feeding maneuver is mathematically modeled in order to perform optimization. WOA simulated hunting behaviour with random or the best search agent to chase the prey. WOA uses a spiral to simulate bubble-net attacking mechanism of humpback whales.

Encircling Prey

Current best candidate solution is assumed to be closes to target prey and other solutions update their position towards the best agent

$$\vec{D} = |\vec{C} \cdot \vec{X}_{best}(t) - \vec{X}(t)| \tag{1}$$

$$\vec{X}(t + 1) = \vec{X}_{best}(t) - \vec{A} \cdot \vec{D} \tag{2}$$

Where t indicates the current iteration, \vec{A} and \vec{C} are coefficient vectors, \vec{X}_{best} is the position vector of the best solution, and \vec{X} indicates the position vector of a grey wolf

$$\vec{A} = 2a\vec{r}_1 - a \tag{3}$$

$$\vec{C} = 2r_2 \tag{4}$$

Where \vec{r}_1, \vec{r}_2 are random vectors in [0, 1].

Bubble-net attacking method (exploitation phase)

In order to mathematically model the bubble-net behaviour of humpback whales, two approaches are designed as follows

1. Shrinking encircling mechanism

This behaviour is achieved by decreasing the value of a . a is decreased from 2 to 0 over the course of iterations.





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2. Spiral updating position

$$\vec{D} = |\vec{X}_{best}(t) - \vec{X}(t)|$$

$$\vec{X}(t+1) = \vec{D} \cdot e^{bl} \cdot \cos(2\pi l) + \vec{X}_{best}(t) \quad (5)$$

l is a random number in $[-1, 1]$

Search for prey

Humpback whales search randomly according to the position of each other

$$\vec{D} = |\vec{C} \cdot \vec{X}_{rand}(t) - \vec{X}(t)| \quad (6)$$

$$\vec{X}(t+1) = \vec{X}_{rand}(t) - \vec{A} \cdot \vec{D} \quad (7)$$

Algorithm

Step1: Initialize the whales population X_i ($i= 1, 2, \dots, n$)

Step2: Calculate fitness of each search agent

$X_{(best)}$ = the best search agent

Step3: while($t <$ maximum number of iterations)

for each search agent:

Update a, A, C, l and p

if($p < 0.5$):

if($|A| < 1$):

Update current agent by eq. (1)

else:

Select a random agent $X_{(rand)}$

update current agent by eq (7)

else:

update search agent by eq (5)

end-for

Check if any search agent goes beyond the search space and amend it

Calculate fitness of each search agent

Update $X_{(best)}$ if there is a better solution

$t = t+1$

end-while

Step4: return $X_{(best)}$

Dataset Description

LIDC (The Lung Image Database Consortium) database has been used to attest to and analyse the suggested method: The Lung Image Database Consortium image collection (LIDC-IDRI) consists of diagnostic and lung cancer screening thoracic computed tomography (CT) scans with marked-up annotated lesions. It is a web-accessible international resource for development, training, and evaluation of computer-assisted diagnostic (CAD) methods for lung cancer detection and diagnosis. Initiated by the National Cancer Institute (NCI), further advanced by the Foundation for the National Institutes of Health (FNIH), and accompanied by the Food and Drug Administration (FDA) through active participation, this public-private partnership demonstrates the success of a consortium founded on a consensus-based process. Seven academic centers and eight medical imaging companies collaborated to create this data set which contains 1018 cases. Some examples of the LIDC databases are shown in figure 6.

Implementation Results

On an Intel Core i10-4790K processor with 32 GB of RAM and two NVIDIA GeForce GTX Titan X GPU cards with scalable link interface (SLI), experimental simulations are done using Matlab R2017® software. To evaluate the system performance, the suggested simulations are put into practise on LIDC database for lung cancer. 10% of the data are used as the validation set, while 70% are used as the training set. As test sets, the remaining 20% are used.



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The 80/20 rule is a division known as the Pareto principle. Approximately 80% of the 20% of causes result in effects. They are chosen at random to determine which images should be used for the training, validating, or testing part. To be fair all of the dataset images underwent image processing and are scaled to 640480. The WOA approach is used to train the proposed CNN. Since the radius and number of neuron cells differ in the experiment shown (Fig. 7), the learning rate varied between 0.2 and 0.9. Additionally, practically all the training pixels will be included in the prototype neurons.

The ideal situation is to choose a neural network with the least number of neurons. According to [22], the performance ratio can be used to choose an appropriate learning rate. Fig-8 demonstrates how the performance ratio and training duration will both rise as the learning rate rises. Even though performance ratio is important, a learning rate of 0.9 is used in order to trade off performance ratio and training time. LIDC database are used as the most relevant database to vouch for the suggested strategy, as previously explained. The proposed network is trained over 30,000 iterations. to produce an accurate and independent analysis of the images. After 60 iterations of the training phase, the final results are described using the mean values. Three performance metrics that are outlined below are used to demonstrate how well the suggested system performed.

For the purpose of detecting lung cancer, various research projects have been developed. Each of these approaches has its own challenges and drawbacks. It is impossible to use every one of these techniques. Thus, three techniques have been chosen for [23] comparison with our suggested method. Automatically extracted descriptors from this method are used for a fair comparison. For this comparison, some deep learning-based systems are also used, including XG Boost and Random Forest [24], Multi-resolution patch-based CNNs [25], 3D Convolutional Neural Networks [26]. A performance comparison between the proposed system and the mentioned approaches is shown in Table 1. framework is affected by employing the WOA optimisation method. The distribution of classification performance from the previous table is displayed as a bar chart in Fig. 7 for further understanding. The excellent effectiveness of the proposed method for the diagnosis of lung cancer regions is demonstrated by experimental findings.

CONCLUSIONS

A novel method for the detection of lung cancer is put forth in this research. The suggested approach employs a meta-heuristic convolutional neural network optimisation strategy based on back propagation to train the biases and weights of the network. To do this, a lung cancer validation method that comprises a streamlined measured error between the reference and the system output is taken into consideration for the proposed optimised CNN. In this study, the whale optimisation algorithm—a recently developed algorithm—is used to reduce the error rate of the Convolutional neural network's learning stage. The proposed approach is known as CNN+WOA.

The proposed method is then put to the testing using images from two well-known database, namely LIDC (The Lung Image Database Consortium), and it is contrasted with three other well-liked categorization techniques. Final findings demonstrate the suggested system's accuracy superiority over the compared classifiers.

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Table 1: Comparison among state-of-the-art Lung Cancer detection Methods

| S.No | Method | Performance Metric | | |
|------|---------------------------------------|--------------------|-------------|----------|
| | | Sensitivity | Specificity | Accuracy |
| 1. | Proposed CNN+WOA | 0.94 | 0.91 | 0.91 |
| 2. | XG Boost and Random Forest | 0.85 | 0.61 | 0.78 |
| 3. | Multi-resolution patch-based CNN | 0.91 | 0.89 | 0.64 |
| 4. | 3D Convolutional Neural Networks CNNs | 0.86 | 0.77 | 0.89 |

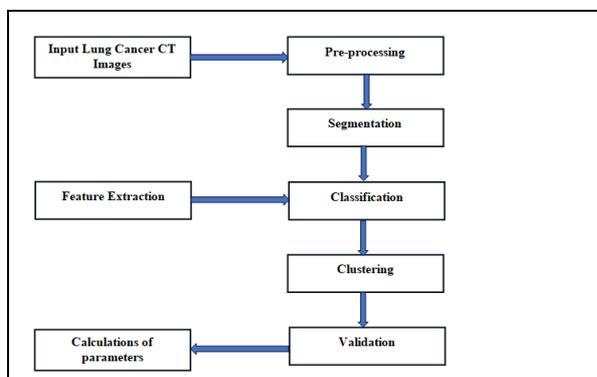


Fig. 1: Proposed Model

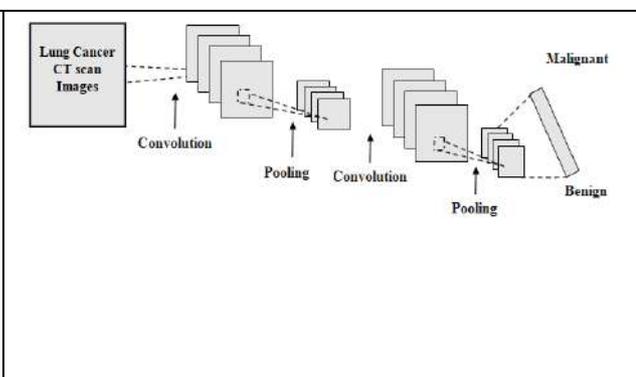


Fig. 2: A simple lung cancer detection using ordinary CNN

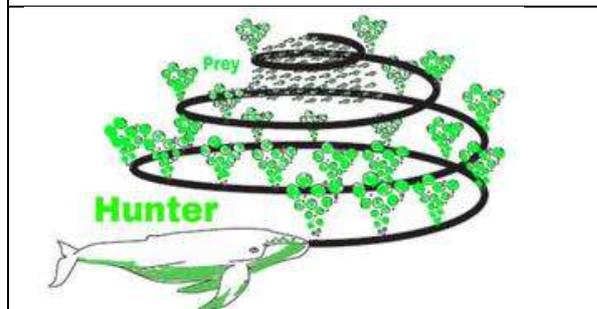


Fig. 3: Bubble-net feeding behaviour of humpback whales.

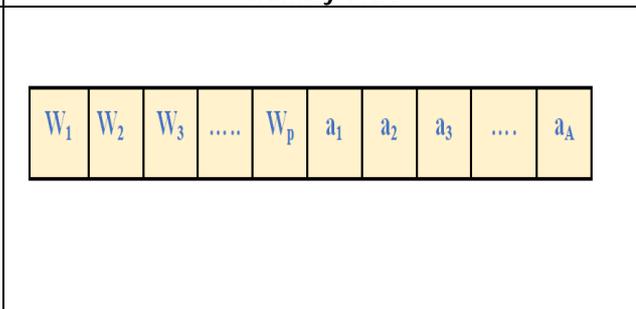


Fig. 4: The WOA search agent vector assignment on CNN

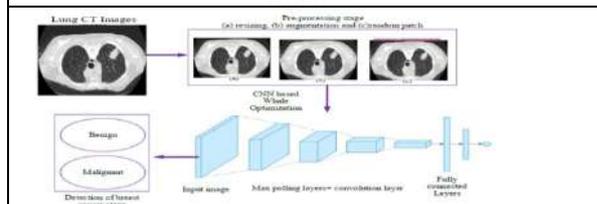


Fig 5: The WOA based CNN

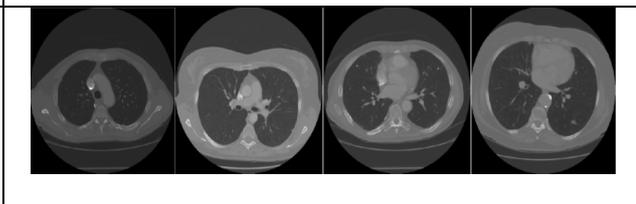


Fig 6: Some examples of the LIDC databases





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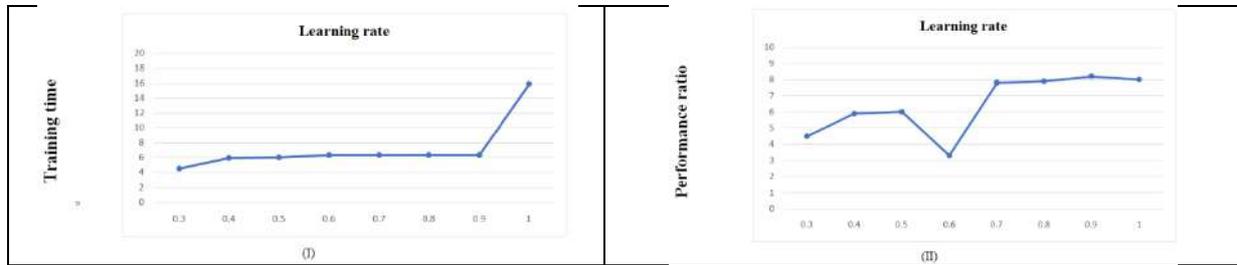


Fig-7: (I) learning rate vs. training time, (II) learning rate vs. performance ratio

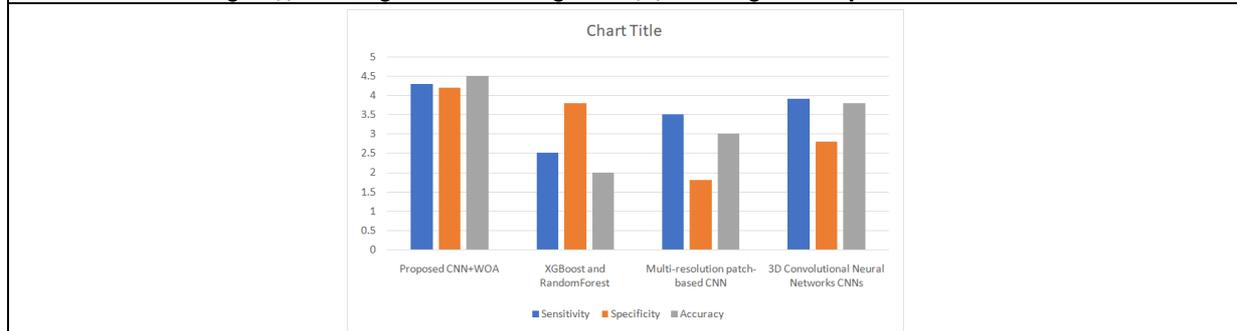


Fig. 8: Distribution of classification performance of the methods for lung cancer detection





A Philosophical Analysis of Punishment: A Comprehensive Study of Ancient and Modern Approaches

Eranew B Marak^{1*} and S.Thanigaivelan²

¹Ph.D. Research Scholar, Department of Philosophy, Annamalai University, Annamalai Nagar-608 002, Tamil Nadu, India .

²Assistant Professor, Department of Philosophy, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India.

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*Address for Correspondence

Eranew B Marak

Ph.D. Research Scholar,

Department of Philosophy,

Annamalai University, Annamalai Nagar-608 002,

Tamil Nadu, India .

E. Mail : eranewbmarak12@gmail.com



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ABSTRACT

This article examines the various philosophical perspectives on the theory of punishment, from ancient to the modern. Punishment is a philosophically hot topic, and this article aims to bring out the reasoning behind it and its relevance to modern criminal justice systems. As a starting point, the article analyses the ancient Greek and Roman perspectives on punishment, focusing on the function of punishment as a form of retributive justice. The article then proceeds on to a discussion of biblical perspectives on punishment, with an emphasis on grace and pardon. The Enlightenment-era views of punishment that sought to reform and modernize the criminal justice system are also discussed and analysed. Moving on to more contemporary perspectives, this paper talks about the retributive theory of punishment, which maintains that punishments should align with the harm committed. The article then examines the utilitarian perspective on punishment, which places a premium on punishment's deterrent function while prioritizing harm reduction. The study provides an in-depth analysis of each strategy, criticizing their merits and discussing their relevance to the state of the criminal justice system today. The paper argues that a holistic and interdisciplinary approach to punishment is required to fulfil the many demands and aims of society and that punishment should be individualized based on the nature of the crime and the offender. In the end, the article believes that a better knowledge of the philosophy of punishment can improve criminal justice. The moral, ethical, and practical difficulties surrounding the administration of justice can be better comprehended via a thorough examination and evaluation of the many methods of punishment. In this article, it argues that punishment must be approached holistically and with nuance if it is to reflect our highest ideals and values as a society.



**Eranew B Marak and Thanigaivelan****Keywords:** Deterrence, Ethics, Justice, Punishment, Retributivism, Utilitarianism

INTRODUCTION

Since the beginning of human society, somehow punishment has been used. Throughout history, several civilizations have had distinctive approaches to punishment. Punishment was seen by the ancient Greeks and Romans as a way to maintain social order and bring about change. The emphasis on retribution and rehabilitation in biblical approaches to punishment contrasts this. Theories of punishment changed during the Enlightenment period to stress utilitarianism, which promotes crime prevention through deterrence. The purpose of this essay is to examine historical punishment ideologies and their effects on current criminal justice systems. Punishment was seen by the ancient Greeks and Romans as a way to keep the peace and re-establish social order. The Greeks supported the idea of retributive justice, according to which those who commit crimes should face punishment commensurate with their conduct. With the idea of *lex talionis*, which emphasized the principle of "an eye for an eye," the Romans used a similar approach to punishment. Biblical theories of punishment have emphasized retaliation and reformation. There are countless instances of divine retribution for sin in the Old Testament of the Bible. The Old Bible has the idea of "an eye for an eye," demonstrating a belief in proportional punishment. The New Testament of the Bible, however, places a strong emphasis on love, repentance and forgiveness (Murphy, 2012). Throughout the Enlightenment era, conceptions of punishment changed towards utilitarianism, which emphasizes the prevention of future crimes through deterrence. According to the utilitarian theory of punishment, the penalty should be severe enough to prevent future offenses without being overly cruel. A well-known philosopher during the Enlightenment, Jeremy Bentham and Cessare Beccaria maintained that punishment should be used to stop future harm to society and should be planned to enhance happiness and decrease misery (Koritangsky, 2011). According to the retributive theory of punishment, the severity of the sentence should be in line with the damage the crime produced. This strategy places a strong emphasis on the notion of "just desserts," in which offenders are punished for their misdeeds in order to balance the balances of justice (Cottingham, 1979).

This method of punishment has drawn criticism for being overly severe and failing to take into account the unique circumstances of the offense and the offender. On the other side, the utilitarian perspective on punishment places a strong emphasis on deterrence as a means of preventing future offenses. According to this theory, punishment should be severe enough to prevent people from committing crimes while avoiding being overly cruel. It has been said that utilitarianism places too much emphasis on punishment and not enough on rehabilitation (Golash, 2005). The focus of the restorative and rehabilitative methods to punishment, in contrast, is on helping offenders get better and return to society. In order to stop more crimes, rehabilitation places a strong emphasis on treating the root reasons of criminal conduct, such as addiction or mental illness (Banks, 2018). On the other side, restorative justice emphasizes healing the damage brought on by the crime and mending the bonds between offenders and their victims. Some methods of punishment have drawn criticism for being excessively soft and failing to adequately punish offenders (Marshall, 1999). These varied methods of punishment have important ramifications for current criminal justice systems. The criminal justice system has historically been dominated by utilitarianism and retributive justice. But there has been a shift in recent years toward restorative justice and rehabilitation. The emphasis of the restorative justice movement is on mending the damage done by the crime and mending the bonds between criminals and their victims. Rehabilitation programs put a strong emphasis on addressing the root causes of criminal conduct and giving prisoners the skills they need to re-join society as contributing members. It has been demonstrated that these strategies lower recidivism rates and increase public safety.

METHODOLOGY

This article makes in the descriptive and analytical methods with Philosophical Analysis of Punishment: A Complete Examination of Ancient and Contemporary Approaches" would be a combination of literature study and critical



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analysis on the subject, which should include both ancient and modern philosophical ideas on punishment. Gathering and studying primary and secondary sources, such as philosophical treatises, articles, and books, can be part of this process. Following that, the researcher might conduct a critical analysis of the collected material by identifying common themes, major arguments, and divergent opinions. The examination should take into account a variety of features of punishment, including its aim, rationale, and ethical consequences with comparative ideas.

Research Objectives

- To analyze the historical approaches to theory of punishment.
- To know the ancient Greek and Roman societies, biblical approaches, and Enlightenment-era theories.
- To significantly evaluate the modern approaches to punishment, including the Retributive theory, Utilitarian approach, and Rehabilitative and Restorative approaches.
- To know the modern approaches of the theory of punishment.
- To compare and contrast the different approaches to theories of punishment and examine their merit and demerit.

DISCUSSION**Ancient theory of Punishment**

The use of punishment for criminal behaviour dates back to the dawn of human civilisation. Punishment has taken on many forms throughout history, from acts of vengeance to attempts at rehabilitating offenders. In this paper, we will examine and evaluate several historical perspectives on punishment, from those held in ancient Greek and Roman civilizations to those presented in the Bible and the beliefs developed during the Enlightenment. In order to re-establish social order and sustain stability, ancient Greeks and Romans relied on the use of punishment. To the Greeks, it was important that punishment fit the crime. The concept of *lex talionis*, or the "an eye for an eye" approach, was central to the Roman judicial system and served as a powerful deterrent to criminal behaviour (Laurence, 1960). Criminals in ancient Rome were often publicly shamed and punished in order to prevent others from engaging in criminal behaviour. Biblical perspectives on punishment, on the other hand, centre on the twin goals of restitution and redemptive change. Many examples of criminals receiving divine punishment can be found in the Old Testament of the Bible. Conversely, the New Testament stresses redemption and pardon. Indeed, Jesus Christ himself forgave sinners and taught his disciples to do the same (Brooks, 2021). By calling for repentance, forgiveness, and reconciliation, the biblical view of punishment works to return the offender to a place of righteousness.

Utilitarianism, which emphasized deterrence as a means of reducing crime, became the dominant view of punishment during the Enlightenment. Utilitarians believe that punishments should be harsh enough to deter crime but not so harsh as to be cruel. Jeremy Bentham, a major Enlightenment philosopher, advocated that punishment should be used to deter offenders from causing more harm to society by maximizing happiness and minimizing misery (Eckersley, 2015). The retributive theory, the utilitarian method, the rehabilitative and restorative approaches, and others have all arisen as viable frameworks for thinking about punishment in the modern period. Punishment, according to the retributive approach, should reflect the harm done by the offender. As opposed to this, the utilitarian view of punishment places a premium on deterring criminal behaviour. The proponents of this view argue that criminal punishment should be harsh enough to discourage offenders without being overly cruel. Some argue that Utilitarianism places too much emphasis on punishment and not enough on rehabilitation (Golash, 2005). Punishment strategies that aim to rehabilitate and reintegrate offenders into society are called rehabilitative or restorative. As a means of reducing criminal activity, one of rehabilitation's primary goals is to treat the underlying conditions that contribute to criminality, such as substance abuse or mental illness. In contrast, the goals of restorative justice are to repair the damage done by the crime and repair the relationships between offenders and victims. Punishment strategies like this have been called too soft and ineffective (Marshall, 1999). The merits and shortcomings of various forms of punishment must be carefully weighed. A major criticism levelled at the retributive



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view of punishment is that it takes neither the criminal nor the specifics of the offense into account. While the utilitarian view of punishment is helpful in reducing recidivism, it has been criticized for being too harsh and inconsiderate of offenders' rights. Although rehabilitative and restorative approaches to punishment have their place in reintegrating ex-offenders into society, they have been criticized for being overly mild and not punishing offenders sufficiently. And it's important to analyse and contrast different types of punishment to figure out which one works best for deterrence, rehabilitation, and restoration. The consequences of these methods for modern criminal justice systems should be carefully analysed.

Modern theory of Punishment

For decades, punishment has been an important part of the criminal justice system. Modern approaches to punishment are based on numerous ideologies and philosophies with distinct purposes in mind, such as deterrence, retribution, rehabilitation, and restoration. In this study, we will examine contemporary methods to punishment, such as retributive theory, utilitarian approaches, and rehabilitative and restorative techniques. The retributive philosophy of punishment stresses retribution, with punishment equal to the harm produced by the offense. This method seeks to punish the criminal for their wrongdoing while also restoring justice to the victim and society. The retributive philosophy of punishment is based on the concept of justice, which states that the penalty should be proportionate to the crime yet, some claim that this method is overly harsh and fails to consider the specific circumstances of the crime and the offender.

The utilitarian approach to punishment relies on deterrence as a means of preventing future offenses. According to this viewpoint, punishment should be severe enough to prevent people from committing crimes while avoiding excessive cruelty. The utilitarian approach to punishment has been helpful in lowering crime rates. This technique, however, has been criticized for its harshness and disdain for the offender's rights. The rehabilitative and restorative methods to punishment seek to rehabilitate and reintegrate offenders into society. As a strategy of averting future crimes, rehabilitation focuses addressing the underlying causes of criminal behaviour, such as addiction or mental illness. Restorative justice, on the other hand, focuses on mending the harm done by the crime as well as restoring relationships between offenders and their victims. Rehabilitative and restorative approaches to punishment have proved beneficial in reducing recidivism rates and fostering offender reintegration. Yet, detractors claim that these measures are too liberal and do not adequately penalize the crime committed.

It is necessary to critically analyse modern approaches to punishment and their effectiveness in accomplishing punishment's goals. The retributive philosophy of punishment has proven to be effective in punishing offenders while also restoring justice to victims and society. Yet, it has been criticized for failing to take into account the unique circumstances of the offense and the offender. By deterrence, the utilitarian approach to punishment has been helpful in averting future offenses. Nonetheless, it has been condemned for its harshness and contempt for the offender's rights. Rehabilitative and restorative techniques to punishment have been shown to be helpful in rehabilitating offenders and reintegrating them into society. They have, however, been chastised for being overly mild and failing to impose enough punishment for the crime committed. The rehabilitative approach to punishment, according to Michael Cavadino and James Dignan (2002), has been criticized for being overly optimistic about the prospects of offender rehabilitation and for ignoring the need for punishment.

It is also necessary to evaluate and contrast the various techniques of punishment in order to identify which approach is most effective in attaining the punishment's goals. According to Daniel P. Mears and Joshua C. Cochran (2013), rehabilitative and restorative approaches to punishment are more effective than retributive and utilitarian approaches in reducing recidivism and fostering offender reintegration. Yet, the retributive and utilitarian methods to deterrence and punishment are more effective. Modern ways to punishment, such as retributive theory, utilitarian approaches, and rehabilitative and restorative techniques, all have advantages and disadvantages. Although the retributive theory of punishment is successful in punishing offenders and restoring justice, it fails to take into account the unique circumstances of the offense and the perpetrator. Through deterrence, the utilitarian approach to punishment is successful in preventing future offenses.



**Eranew B Marak and Thanigaivelan****Comparison**

There have been many different methods of punishment throughout history, each with its advantages and disadvantages. The various ways to punishment, including the retributive theory, the utilitarian approach, the rehabilitative and restorative approaches, will be compared and contrasted in this essay. We will also look at their advantages and disadvantages. According to the retributive theory of punishment, the severity of the sentence should be in line with the damage the crime produced. Based on the principle of "an eye for an eye," this strategy aims to exact retribution on the wrongdoer. Retributive theory supporters contend that offenders should be held accountable for their actions and those punishments should be strong enough to discourage future offenses (Miethe, 2005). The retributive view of punishment, however, has come under fire for failing to take into account the specifics of the offense and the offender. Opponents contend that sentencing should also take into account the offender's motivation and background in addition to the harm the crime produced. The retributive theory of punishment can also result in excessive punishment and misses out on the main objective of deterring future offences (Golash, 2005).

On the other side, the utilitarian perspective on punishment places a strong emphasis on deterrence as a means of preventing future offenses. According to this theory, punishment should be severe enough to prevent people from committing crimes while avoiding being overly cruel. According to proponents of the utilitarian approach, punishment ought to be implemented in a way that maximizes happiness, minimizes pain, and serves to protect society from future harm (Skelton, 2015).

Despite its success in deterring future crimes, the utilitarian method has drawn criticism for its severity and disdain for the rights of the criminal. The utilitarian method is criticized for potentially punishing innocent people and for failing to appropriately address the underlying reasons of criminal conduct. The utilitarian approach can also result in disproportionate punishment and may not be successful in deterring some offenses (Von Hirsch et al., 2016). Rehabilitating offenders and reintegrating them into society are the main goals of the restorative and rehabilitative methods to punishment. In order to stop more crimes, rehabilitation places a strong emphasis on treating the root reasons of criminal conduct, such as addiction or mental illness (Banks, 2018). On the other side, restorative justice emphasizes healing the damage brought on by the crime and mending the bonds between offenders and their victims. These methods of punishment have received recognition for their emphasis on education and prevention (Marshall, 1999).

Yet, the rehabilitative and restorative approaches to sentencing have come under fire for being overly soft and not punishing offenders severely enough. Some critics claim that these strategies may not be successful in preventing further crimes and that they may not effectively address the damage that has been done as a result of the crime (Karim, 2020). The rehabilitative and restorative approaches could also be expensive and require a lot of resources to accomplish well. It is evident by comparing and contrasting the various methods of punishment that each method has advantages and disadvantages of its own. The retributive theory of punishment places a strong emphasis on accountability and deterrence, but it has the potential to result in disproportionate punishment and may not fully take into account unique situations. Although it can be harsh and may not address the underlying causes of criminal conduct, the utilitarian approach to punishment focuses on preventing future crimes. The emphasis on rehabilitation and restoration in the rehabilitative and restorative approaches to punishment may not be sufficient to adequately punish offenders and may not be helpful in preventing future crimes.

The specifics of the offense and the offender may ultimately determine the best course of action for punishment. To achieve the objectives of deterrent, rehabilitation, and restoration, it could be necessary to combine several methods of punishment. This philosophical analysis of punishment requires a critical examination of the evidence supporting the goals of punishment, the effectiveness of different approaches to punishment, and the ethical and moral implications of these approaches. The evidence supporting the effectiveness of deterrence, rehabilitation, and restoration is mixed and context-dependent, and each approach has its strengths and weaknesses. The ethical and



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moral implications of different approaches to punishment must also be considered, as these approaches can have differential impacts on marginalized communities.

CONCLUSION

In conclusion, the philosophy of punishment is a complicated subject that demands an in-depth comprehension of both traditional and contemporary perspectives. Several research and pieces of evidence point to punishment being effective at achieving its purposes, which include deterrence, rehabilitation, and restoration of society. However, the efficacy of various tactics varies and is dependent on a number of different elements, such as the nature of the offense, the characteristics of the offender, and the resources that are readily available for its execution. In addition, the implications that various forms of punishment have in terms of ethics and morality are not something that can be ignored. The retributive view places an emphasis on punishing the offender for the crime that was committed, but it does not take into account the unique circumstances of the criminal or the offender's capacity for rehabilitation. The utilitarian method has as its primary objective the maximization of societal welfare; yet, it may result in inequities and discrimination against groups that are already at a disadvantage. The rehabilitative and restorative approaches place a higher level of importance on the rehabilitation of the offender as well as the repair of the damage caused by the offender's actions; however, they call for a significant investment of resources and may not be appropriate for all forms of criminal behaviour. Hence, in order to have a fair and just criminal justice system, it is vital to take a balanced approach that takes into consideration the goals of punishment, the effectiveness of various measures, and the ethical implications of these ways. This calls for on-going assessment, study, and the modification of strategies in order to cater to the ever-evolving requirements of society. In the end, the objective should be to design a system that not only imposes punishments on lawbreakers but also encourages their restoration, rehabilitation, and the prevention of further criminal behaviour.

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A Cross-Sectional Study on Perceived Stress and Coping from the Situation among Women Entrepreneur

Samridhi Gupta^{1*}, Sunishtha Dhaka² and Bhawna Chahar³

¹Research Scholar, Manipal University Jaipur, Jaipur, Rajasthan, India

²Assistant Professor, Amity University, Noida, Uttar Pradesh, India

³Associate Professor, Manipal University Jaipur, Jaipur, Rajasthan, India

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*Address for Correspondence

Samridhi Gupta

Research Scholar,

Manipal University Jaipur,

Jaipur, Rajasthan, India



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ABSTRACT

India's growing economy and increased globalisation, women are benefiting from advancement in areas such as medicine, technology, access to birth control methods, contemporary household appliances, contemporary culture and traditions, and so on. These advancements help women save time and become more intelligent. However, studies have shown that women who are competitive tend to have views of women that are stereotypical. The growing responsibilities of running a business as a woman in the current period, which can include dealing with problematic workers, maintaining a healthy work-life balance, and keeping everything organised, can have a dramatic effect on the emotional and psychological well-being of women who operate their own businesses. The present study examines the relationship between perceived stress and coping through stressful situation in woman entrepreneur and compares them with housewives. 55(22 women entrepreneur and 33 housewives) participants were participated from Delhi NCR region. Ranging from 19 to 50 years of age. Purposive and snowballing sampling technique has been used to gather the data. Perceived Stress Scale and Coping Inventory for stressful situation have been used to measure the variable. Pearson's correlation and independent t-test has been used for statistical analysis. The findings revealed that there exist a significant relationship between perceived stress and coping from stressful situation and on comparison it was found that there come to be a significant difference on both the variable when compared woman entrepreneurship and housewives.

Keywords: Women entrepreneur, housewives, perceived stress, coping, cross-sectional study



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INTRODUCTION

The French term "entreprendre," meaning "one who undertakes" or "one who is a go-between," is the etymological ancestor of the English word "entrepreneur." An entrepreneur is someone who makes judgements about getting and employing resources while admitting the risk of venture, such as purchasing an item at one price with the intention of reselling it at a higher one. Entrepreneurship is the action of actively generating new wealth (Koneru, 2017). Those who take significant financial, personal, or professional risks in order to create something of value for others are the ones who get the rewards. The entrepreneur must acquire and locate the essential skills and resources, regardless of whether the product or service is novel or unique. In order for women to actively contribute to economic growth, it is necessary to create conditions that reduce the amount of time they spend on housekeeping.

The Importance of Women in the Entrepreneur

Governments and non-governmental organisations (NGOs) have played crucial roles in empowering women. Women have natural strengths that can help them succeed as business owners. The tendency for women to start businesses is both innate and inevitable. Entrepreneurship is widely seen as a major force in the advancement of the global economy. Entrepreneurs have long been credited with sparking and maintaining economic growth. There is evidence to suggest that nations with a larger share of entrepreneurs in their populations have grown and prospered more quickly than those with a smaller share (Azizah, 2023). In addition to coming up with ideas and vetting them, a woman entrepreneur must also choose a business structure, prepare a project, evaluate a product, complete all required paperwork, secure financing, buy necessary equipment, and run the company to success.

The Plight of Indian Women

Over the course of several millennia, there have been significant shifts in the position of women in India's society. Development among women in India is rich and complex, spanning from their equality with males in prehistoric times through the hardships of the mediaeval period and the pursuit of equal rights by various reformers (Komal & Sharma, 2023). While women in ancient Indian culture were given the goddess title, they were nonetheless treated as second class citizens. This prejudice has its origins in the traditional belief that women should stay at home and take care of the household. The old Hindu belief states, "Woman be a young child, be a young woman, or even be an aged one, nothing can do independently, even in her house". Nowadays, this way of perceiving is common in Indian culture. As a result, there is a significant gender gap in the level of literacy. Women are kept down and silenced in many fields because of bias based on sexuality (Mishra, Saha & Kumar, 2023).

Women Entrepreneur in India

Women are stereotyped as staying at home and having no business or economic involvement. Many cultures have the view that women should focus on domestic duties such as construction, upkeep, and child rearing. However, things are starting to look up. A growing number of contemporary Indian women are starting their own businesses, most often in the micro, and medium sectors. Considering the widespread joblessness in the country, women business owners have an increasingly important role to play. Modern mass production is too capital demanding to employ a huge workforce. Small businesses are vital, as they account for around 80% of all jobs in the economy (McAdam, 2022). It is crucial to remove the misconception that women are incapable of holding gainful employment. They can be inspired to start micro, small, and medium-sized enterprises on their own. Promoting women's business ownership is crucial to the growth of India's economy.

Cottage industries are a great way to empower women in rural areas. The government's Integrated Rural Development Programme (IRDP), Training of Rural Youth for Self-Employment (TRYSEM), and "Development of Women and Children in Rural Areas (DWCRA)" are just a few programmes designed for helping rural-based micro entrepreneurs in succeeding. The goal is to eradicate destitution via business initiatives (Kumar & Kumar, 2023). Mahajan (2013) - evaluate the initiatives taken to date to improve the economic status of women entrepreneurs in India. According to research, the Indian government has implemented several development programmes and



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policies aimed at encouraging female-dominated groups to become financially independent through the pursuit of entrepreneurial endeavours. Still, many obstacles prevent us from closing the gaps between where we are and where we need to be. Considering the disparity that exists within India between the number of male and female business owners, this is an issue of increasing importance. Women's entrepreneurship is an area that needs further investigation, and this study highlights the significance of doing so in order to strengthen existing initiatives and encourage new ones.

METHODOLOGY

Objective- To study the relationship between perceived stress and coping from stressful situation in woman entrepreneur and to compare them with housewives.

Hypothesis

H₁: There will be a significant relationship between perceived stress and coping from stressful situation among woman entrepreneur.

H₂: There will be significant more perceived stress in women entrepreneur than in Housewives.

H₃: There will be significant coping strategies during stressful situation when compare women entrepreneur and housewives.

Research Design: Non-experimental, cross-sectional research design.

Sampling: 55 (22 women entrepreneur and 33 housewives) participants were participated from Delhi NCR region. Ranging from 19 to 50 years of age. Purposive and snowballing sampling technique has been used to gather the data.

Tools

Perceived Stress Scale: It is given by Cohen et al., 1983, 10 items and five-point Likert scale ranging from never to very often. It was created to assess how stressful one's interactions with the external environment were.

Coping Inventory for stressful situation: It is given by Endler & Parker, 1999. 48-items and five-point likert scale ranging from not at all to very much. It is a more accurate predictor of preferred coping strategies because it measures coping across multiple dimensions.

Statistical analysis: To find out the relationship between the two variables, Pearson's correlation is employed and for comparison between the two groups independent t-test has been used by using SPSS version 26.0.

RESULT

The purpose of the study is to assess the correlation between perceived stress and coping from stressful situation among women entrepreneur and see the difference between housewives and women entrepreneur on these variables, the following tools has been used to measure the variables- perceived stress scale (PSS) and Coping inventory for stressful situation (CISS). The Pearson's correlation between Perceived stress and coping from stressful situation came out to be $r = .44$ which is significant at 0.01 that means if perceived stress increases coping techniques will also get build up in women entrepreneurs. Therefore, H₁ has been supported. According to Table 1, the mean difference and standard deviation for women entrepreneur and housewife participants on Perceived stress came out to be 57.95 ± 18.55 and 72.61 ± 10.55 . The standard error of difference was 3.92. The result shows that there is a significant difference between the male and female participants on perceived stress ($t = -3.73$, $p = 0.00$) that means H₂ which stated that there will be more perceived stress in women entrepreneur than in housewives has been supported.



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The mean difference and standard deviation for women entrepreneur and housewife participants on coping from stressful situation came out to be 79.50 ± 9.85 and 89.66 ± 12.88 . The standard error of difference was 3.24. The result shows that there is a significant difference between the women entrepreneur and housewives' participants on coping from stressful situations ($t = -3.13$, $p = 0.03$) that means H3 has been supported.

DISCUSSION

The present study focuses on women entrepreneur their relationship with the variable perceived stress and coping from stressful situation which is further be compared with housewives. In the following research the variables are measured by using standardized questionnaire i.e., perceived stress scale (Cohen et al., 1983) and coping inventory for stressful environment. H1 hypothesised that there will be a significant relationship between perceived stress and coping from stressful situation in woman entrepreneur. The findings revealed that there is a significant relationship between perceived stress and coping. Entrepreneurs need an internal aspect that is in themselves to assist them overcome the hard and unpredictable working environment conditions for them to reach a positive psychological condition. This is necessary for them to be successful. If they can figure out how to deal with the pressures that they are under, they will be successful despite the challenges that they are up against. A similar effect was discovered by Carnicer et al. (2019), who discovered that problem-focused coping acted as mediator between perceived stress and the psychological well-being of students in Barcelona. It is hypothesised that problem-focused coping strategies may help in management of stress and contribute to overall mental health. According to the findings of the study, an "entrepreneurial orientation" towards problem-solving in the face of a variety of stressors is beneficial to an entrepreneur's mental health. This finding is consistent with the findings of the study.

H₂ hypothesised that there will be a significant more perceived stress in women entrepreneur than in Housewives. The findings revealed that there seems to more stress in women entrepreneur the similar findings support by the research (Subashini, 2016) women entrepreneurs face additional pressure since they are often forced to choose between the needs of their families and their businesses. Women face significant challenges when attempting to balance their many responsibilities. Because women business owners are more prone to self-doubt and dissatisfaction with their current status, the stress that is associated with roles can set in motion a chain reaction that could ultimately lead to failure. This is because women entrepreneurs are more susceptible to the negative effects of stress.

H₃ hypothesised that there will be a significant difference between women entrepreneur and housewives on coping from stressful situation. The findings supported the hypothesis and some significant results has been shown by Hundera et al, 2021 which examine the connections between "role conflict, coping mechanisms, and success in the business world". It was discovered that as the degree of role conflicts shifted, so did the coping mechanisms employ by women business owners, with varying results for their ventures. They deal with the situation by putting more emphasis on their entrepreneurial responsibilities which has a favourable effect on their financial success but has an adverse impact on the nonfinancial performance when the role conflict is mild. Their financial and nonfinancial success improves when role conflict is mild, and they deal with it by integrating people or responding to all roles. When the pressure is really great, however, people tend to put their social and familial responsibilities first, which has a favourable effect on the former but has an adverse impact on the latter.

CONCLUSION

The findings of this study suggest that woman entrepreneurs can still have lives that are progressive and meaningful. The results indicated that coping mechanisms were likely helpful to woman entrepreneurs in the management of the stress that they experienced. The positive re-evaluation of situations and circumstances, the establishment of strategies, and the seeking out of assistance or alternative solutions for challenges are all examples





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of coping mechanisms that can assist business owners in managing their own resources. It is important not to undervalue the role that these strategies have in lowering stress levels. Due to the narrow nature of the research, certain inferences cannot be made, it is expected that further research will try to replicate the findings in populations that are geographically representative. In subsequent research, it may be possible to investigate the role that other factors, such as social support and the dynamics of the family, play as potential role of the connection between stress and coping. Researchers can take into consideration a variety of other factors, including personality characteristics and intangible assets. Both things have the potential to be safeguards for resources factors that are activated in response to the invasion of a stressor. They work to protect individuals from damaging effects of stress by improving potentially hazardous environmental conditions and boosting the body's capacity for healing itself.

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Table 1 .Descriptive statistics and correlation between Perceived stress and coping from stressful situation are given below:

| Variable | n | M | SD | 1 | 2 |
|------------------------------------|----|-------|-------|-------|---|
| 1. Perceived Stress | 55 | 66.75 | 15.8 | — | |
| 2. Coping from Stressful Situation | 55 | 85.60 | 12.70 | .44** | — |

****Correlation is significant at 0.01 level (2-tailed)**



**Samridhi Gupta et al.,****Table 2 Descriptive and comparison of Women entrepreneur (WE) and Housewives (HW) among given mentioned variables**

| Variables | Group | Mean | Standard deviation | Std. error of difference | T | P value |
|---------------------------------|-------|-------|--------------------|--------------------------|-------|---------|
| Perceived stress | WE | 57.95 | 18.55 | 3.92 | -3.73 | 0.000 |
| | HW | 72.61 | 10.55 | | | |
| Coping from stressful situation | WE | 79.50 | 9.85 | 3.24 | -3.13 | 0.03 |
| | HW | 89.66 | 12.88 | | | |





Plyometric and Resistance Training Impacts on Strength and Cardio-Respiratory Capacity among Male Handball Players

Ajithkumar L^{1*}, Mohanasundaram S² and Kumar P³

¹Research Scholar, Department of Physical Education and Sports, Central University of Haryana, Mahendergarh, Haryana -123031, India.

²Physical Director, Head of the Department, The Vijay Millenium, Krishnagiri- 635001, Tamil Nadu, India.

³Assistant Professor, Department of Physical Education and Sports, Central University of Haryana, Mahendergarh, Haryana-123031, India.

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*Address for Correspondence

Ajithkumar L

Research Scholar,
Department of Physical Education and Sports,
Central University of Haryana,
Mahendergarh, Haryana -123031, India.
E. Mail: ajith141363@cuh.ac.in



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ABSTRACT

The focus of the research was to examine the impact of plyometric training (PT) and resistance training (RT) on the physical and physiological (performance) parameters of male handball players. Forty-five players (aged 18 to 25) were divided into three sub-groups for an eight-week training programme that included group one (PT; n=15), group two (RT; n=15), and group three (CON; n=15). The subjects were selected from Saveetha University in Chennai. Before training several standardized measures were used to assess shoulder strength (SS), abdominal muscular strength (AMS), resting heart rate (RHR), and breath-holding time (BHT). After training, data collection was completed, and the data were analysed using computer-based SPSS software's Analysis of Covariance (ANCOVA) and the significance level at 0.05. After applying the statistical procedure, when compared to the control group, both experimental groups enhanced remarkably. Significant changes were observed in the physical parameters SS and AMS. The RT (pre \bar{X} =33.33, post \bar{X} = 38.13) group is higher than the PT group (pre \bar{X} =27.46, post \bar{X} = 28.33) on SS, and the PT (pre \bar{X} =31.00, post \bar{X} = 40.00) group is better than the RT group (pre \bar{X} =32.00, post \bar{X} = 36.00) on AMS. In physiological parameters RHR and BHT there were no significant changes between the 3 groups.

Keywords: Physical Education, Sports Training, Handball Player, Physiological, Science.





INTRODUCTION

Handball is a highly dynamic team sport that places significant physical demands on players, requiring a combination of explosive power, muscular strength, and endurance. To excel in this sport, handball players must possess well-developed upper body strength, core stability, and cardiovascular fitness. The goal of the research was to examine the impacts of PT and RT among male handball players [1]. Both PT and RT modalities offer distinct benefits and can be complementary when combined in a comprehensive training program for handball players [2,3]. PT focuses on explosive power and neuromuscular coordination, while RT goals muscular strength and hypertrophy [4,5]. Integrating both methods allows handball players to develop a well-rounded physical foundation, maximizing their performance potential in this demanding sport [6,7,8]. Understanding the influence of these training modalities on these specific parameters is crucial for designing evidence-based training programs that optimize performance and reduce the risk of injuries in this physically demanding sport [9].

SS is a fundamental attribute for handball players, as it plays a pivotal role in powerful throws, accurate passes, and robust defensive actions. Enhanced SS allows players to generate more force during throwing motions, leading to increased shot velocity and accuracy [10,11]. AMS is crucial for core stability and effective transfer of power between the upper and lower body. A strong core improves balance, stability, and overall body control, enabling handball players to perform explosive movements with precision [12]. AMS is a crucial component of overall physical fitness for handball players. A strong and stable core is essential for maintaining balance, generating power during throwing and shooting actions, and withstanding physical contact from opponents. Despite its importance, there is a dearth of research investigating the effects of PT and RT on AMS specifically handball players [13,14,15]. Understanding how these training modalities impact abdominal strength can provide valuable insights into designing comprehensive training programs that target this essential aspect of physical fitness. This study aims to contribute to the existing body of knowledge regarding the effectiveness of these training methods in enhancing core stability and overall performance in handball [16]. The findings of this study can inform coaches, trainers, and athletes about the optimal training strategies to improve AMS and support injury prevention in the context of handball [17,18].

RHR is an important indicator of cardiovascular fitness and overall health. A lower RHR suggests a more efficient cardiovascular system, enabling better oxygen delivery to work muscles during exercise [19, 4]. Improved cardiovascular fitness allows handball players to sustain high-intensity efforts for longer durations without fatigue, leading to improved performance and reduced recovery times. BHT reflects respiratory function can serve as an indicator of lung capacity and efficiency. Handball players often face situations where they need to hold their breath during physical exertion, such as during defensive actions or rapid changes in direction [20,21]. Enhancing BHT can improve the ability to maintain a sufficient oxygen supply during intense moments of the game, leading to improved performance and endurance. This study aims to provide valuable insights into designing targeted training programs that address the specific needs of handball players. Optimizing these parameters can lead to enhanced performance, reduced risk of injuries, and improved overall physical capabilities in this challenging sport [22].

MATERIALS AND METHODS

Subjects and Variables

The aim of this research was to discover the impacts of PT & RT on selected physical and physiological factors among male handball players. 45 five male handball players were chosen from Saveetha University in Chennai. 18-25 years aged players were selected as subjects In this investigation, Investigator made a conscious decision to choose the accompanying autonomous factors shown in table 1, which directly affect PT and RT.





Experimental Design

The players were selected randomly for this investigation, Forty-five players were divided into three sub-groups for an eight-week training programme that included group one (PT; n=15), group two (RT; n=15), and group three (CON; n=15). Group one as well as two was exposed to the experimental treatment of PT and RT while the control group wasn't permitted to involve any specific training.

Test Procedures

Afterwards completing the eight weeks of PT and RT, the test was conducted by below-given the test procedures for the data collection. The procedures were instructed to the subjects by the researcher.

1. Shoulder Strength - Push-Ups

Aim: The object of the test was to assess muscle strength.

Test administration: From straight-arm front leaning rest position. The exhibition brings down the body until the chest contacts the tangle and afterwards pushes upward to the straight arm uphold. The subject as indicated by their ability took the push-ups were taken in length of 1 min.

Scoring: The score was the number of right push-ups performed within 60 seconds.

2. Abdominal Muscular Strength - Bent knee Sit-ups

Aim: To find the player's core muscle strength.

Test Administration: Initiate by lying on your back, legs bent, and feet flat on the floor. Cross your arms across your chest or behind your ears. Make your abdominal muscle towards your back and use your core muscle. Raise the upper portion of your body over the ground by arcing your back and pushing your chest to your knees [22]. Maintain the movement and continue until both of your shoulders are touching the floor. Lift the upper portion of your body gently towards the initial position, keeping core tension throughout.

Scoring: Total 1 min, 1 point given for the successfully executed one sit-up.

3. Resting Pulse Rate - Radial Artery holding method (on Wrist)

Aim: To figure out the individual heartbeat during restin 60 sec.

Test Administration: Tip of 3 fingers was placed on the spread-out artery at the wrist. When the beat identities were obtained, the clock is reset to zero and the timer was started.

Scoring: The measure of beats considered is noted to beat every moment.

4. Breath-Holding Time-Holding method

Aim: To analyze the lung capacity of the players

Test administration: Take a deep breath: Inhale deeply through your nose, filling your lungs completely. Gently pinch your nostrils closed, preventing air from entering or exiting through your nose. Began timing the breath-holding duration. Maintain the breath-holding position and relax. Release your nose and exhale when you feel the urge to breathe. Remember, safety and comfort are important, so don't push yourself beyond your limits.

Scoring: How long holding their breath by utilizing the stopwatch.

Statistical Analysis

After the training, data collection was completed by the above-given test, and the Analysis Covariance (ANCOVA) was used for the data investigation, whenever it has shown significant differences Scheffe's post-hoc test was used with a 0.05 level of significance by computer-based SPSS software.

RESULTS AND DISCUSSION

The study data on the selected physical and physiological parameters have been shown in this part. The current examination was connected to decide the progressions of actual wellness and physiological factors through the PT



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and RT group of college men whose age range between 18 to 25 years. The test group was given PT and RT for a time of about two months and the data was gathered from the understudies when the preparation time frame [23]. The information identifying with factors under the examination has been measurably inspected by investigation of co-fluctuation to decide the change between the experimental group and control group for the pre-test and post-test. The acquired 'f' value was examined for significance at a 0.05 confidence level [24]. The below tables and figures show the control and experimental groups on selected strength and cardio-respiratory capacity of male handball players during the pre-test and post-test. Table 4 displays that the SS pre-test 'f' ratio was 2.72, there were no significant changes among the 3 groups. SS post-test 'f' ratio was 7.37, and significant differences were noticed between the three groups. The adjusted post-test 'f' ratio was 21.06 there significant differences were noticed between the three groups.

Table 5 denotes significant changes between the combined means among the 3 groups in Scheffe's post hoc test. The comparison between the control group and PT was 1.73. The control group and RT group were 6.013. The adjusted post-test between PT and RT groups was 4.27. All mean differences showed in the figure.1 Table 6 depicts that the AMS pre-test 'f' ratio was 0.51, there was no significant difference among the 3 groups, the AMS post-test 'f' ratio was 3.288, and significant differences were noticed between the three groups, the adjusted post-test 'f' ratio was 12.343 significant differences were obtained between the 3 groups.

Table 7 shows significant changes between the combined means among the 3 groups in Scheffe's post hoc test. The comparison between the control group and PT was 6.60. The control group and RT group were 2.60. The Adjusted post-test between PT and RT groups was 4.00. All mean differences showed in figure. 2 Table 8 shows there were no significant changes between the 3 groups because the 'f' ratio was below the table value of 3.23. So the scheffe's post-hoc test was not examined. The mean difference between PT and RT and the control group showed in the figure. 3. Table 9 shows there were no significant changes between the 3 groups because the 'f' value was below the required table value of 3.23. So the scheffe's post-hoc test was not examined. The difference in the arithmetic value of the control, PT and RT group showed in the figure.4.

CONCLUSION

When compared to the control group, the experimental groups enhanced significantly. On AMS, the PT group was better than the RT group. On SS, the RT group was higher than the PT group. On RHR, there was no significant differences between the three groups. On BHT, there was no significant differences between the three groups.

Abbreviations

Source of variance (SoV)
Degrees of freedom (df)

Sum of square (SoS)
Mean square (ms)

Conflict of Interest

There is no conflict of interest in this study.

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Table 1 - List of the parameters

| Parameters | Test Items | Measurement Unit |
|--------------------------------|-------------------|------------------|
| Physical parameter | | |
| • Shoulder Strength | Push-ups | Minute |
| • Abdominal Muscular Strength | Bent knee sit-ups | Minute |
| Physiological parameter | | |
| • Resting pulse rate | Holding Method | Minute |
| • Breath-holding time | Holding Method | Seconds |

Training Protocol

Table 2 - Plyometric Training Schedule for Experimental Group 1

| Weeks | Exercises | Sets & Rep | Intensity |
|-------|--|------------|-----------|
| | Bounds, Hurdle Hopping, Single Leg Hopping, | 2 X 8 | Low |
| 1 & 2 | Box Jumps, Depth Jumps, Tuck Jumps, | 2 X 8 | Low |
| | Chest Pass, Power Drop, Incline Chest Pass. | 3 X 6 | Low |
| | Power Drop, Vertical Toss, Single Leg Hopping, | 2 X 10 | Low |
| 3 & 4 | Depth Jumps, Box Jumps, Chest Pass, | 2 X 10 | Low |
| | Tuck Jumps, Bounds, Hurdle Hopping. | 3 X 8 | Medium |
| | Box Jumps, Depth Jumps, Tuck Jumps, | 2 X 10 | Medium |
| 5 & 6 | Hurdle Hopping, Chest Pass, Power Drop, | 4 X 10 | Medium |
| | Bounds, Vertical Toss, Single Leg Hopping. | 4 X 12 | Medium |
| | Single Leg Hopping, Bounds, Hurdle Hopping, | 5 X 10 | High |
| 7 & 8 | Power Drop, Incline Chest Pass, Tuck Jumps, | 5 X 12 | High |
| | Chest Pass, Box Jumps, Depth Jumps. | 5 X 12 | High |

Table 3 - Resistance Training Schedule for Experimental Group 2

| Weeks | Exercises | Sets & Rep | Intensity |
|-------|--|------------|-----------|
| | Barbell Overhead Shoulder Press, Seated Dumbbell Shoulder Press, Front Raise, | 2 X 8 | Low |
| 1 & 2 | Reverse Pec Deck Fly, Dumbbell Lateral Raise, Push Press, | 2 X 8 | Low |
| | Standing Barbell Shrugs, Reverse Crunches, Hanging Leg Raise, | 3 X 6 | Low |
| | Standing Barbell Shrugs, Seated Dumbbell Shoulder Press, Front Raise, Push Press | 3 X 6 | Low |
| 3 & 4 | Dumbbell Lateral Raise, Reverse Pec Deck Fly | 3 X 8 | Medium |
| | Reverse Crunches, Barbell Overhead Shoulder Press Superman Hold. | 3 X 8 | Medium |
| | Seated Dumbbell Shoulder Press, Front Raise, Barbell Overhead Shoulder Press | 3 X 10 | Medium |





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| | | | |
|-------|---|--------|--------|
| 5 & 6 | Hanging Leg Raise, Reverse Crunches, Standing Barbell Shrugs, | 4 X 10 | Medium |
| | Push Press, Reverse Pec Deck Fly, Dumbbell Lateral Raise. | 4 X 10 | Medium |
| | Front Raise, Seated Dumbbell Shoulder Press, Barbell Overhead Shoulder Press, | 4 X 12 | High |
| 7 & 8 | Push Press, Reverse Pec Deck Fly, Dumbbell Lateral Raise, | 5 X 10 | High |
| | Superman Hold, Standing Barbell Shrugs, Reverse Crunches. | 5 X 12 | High |

Table 4 - Covariant analysis for the 3 groups on SS

| | | Control group | PT Group | RT Group | SoV | SoS | df | ms | f ratio |
|---------------|------|---------------|----------|----------|-----|---------|----|--------|---------|
| Pre-test | Mean | 23.93 | 27.47 | 33.33 | B | 676.31 | 2 | 338.16 | 2.72 |
| | S.D | 9.90 | 12.16 | 11.27 | W | 5222.00 | 42 | 124.33 | |
| Post-test | Mean | 23.27 | 28.33 | 38.13 | B | 1713.64 | 2 | 856.82 | 7.37* |
| | S.D | 9.68 | 12.11 | 10.41 | W | 4880.00 | 42 | 116.19 | |
| Adjusted mean | | 27.33 | 29.07 | 33.34 | B | 254.65 | 2 | 127.32 | 21.06* |
| | | | | | W | 247.8 | 41 | 6.04 | |

*Level of significance at a 0.05.

Required table value for degrees of freedom 2&42 = 3.23.

Table 5 - Adjusted mean difference between the 3 groups on SS

| Control group | PT group | RT group | Mean diff | C.I value |
|---------------|----------|----------|-----------|-----------|
| 27.32 | 29.06 | - | 1.73 | 1.32 |
| 27.32 | - | 33.34 | 6.01 | 1.32 |
| - | 29.06 | 33.34 | 4.27 | 1.32 |

*The Scheffe's confidence circuit level at 0.05 was 1.32.

Table 6 - Covariant analysis for the 3 groups on AMS

| | | Control group | PT group | RT group | SoV | SoS | df | ms | f ratio |
|---------------|------|---------------|----------|----------|-----|---------|----|--------|---------|
| Pre test | Mean | 33.80 | 31.00 | 32.00 | B | 60.40 | 2 | 30.20 | 0.51 |
| | S.D | 6.16 | 8.30 | 8.27 | W | 2456.40 | 42 | 58.48 | |
| Post test | Mean | 33.4 | 40.00 | 36.00 | B | 331.60 | 2 | 165.80 | 3.28* |
| | S.D | 5.82 | 6.01 | 9.00 | W | 2117.60 | 42 | 50.41 | |
| Adjusted mean | | 32.31 | 40.89 | 36.18 | B | 540.37 | 2 | 270.18 | 12.30* |
| | | | | | W | 897.50 | 41 | 21.89 | |

*Level of significance at a 0.05.

Required table value for degrees of freedom 2&42 = 3.23.

Table 7 - Adjusted mean difference between the 3 groups on AMS

| Control group | PT group | RT group | Mean diff | C.I value |
|---------------|----------|----------|-----------|-----------|
| 33.40 | 40.00 | - | 6.60 | 2.48 |
| 33.40 | - | 36.00 | 2.60 | 2.48 |
| - | 40.00 | 36.00 | 4.00 | 2.48 |

*The Scheffe's confidence circuit level at 0.05 was 1.32.





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Table 8 Covariant analysis for the 3 groups on RHR

| | | Control group | PT group | RT group | SoV | SoS | df | ms | f ratio |
|---------------|------|---------------|----------|----------|----------|---------|----|-------|---------|
| Pre test | Mean | 78.33 | 73.53 | 75.80 | B | 172.98 | 2 | 86.49 | 2.32 |
| | S.D | 4.19 | 6.09 | 7.56 | W | 1565.47 | 42 | 37.27 | |
| Post test | Mean | 78.27 | 73.33 | 76.13 | B | 183.64 | 2 | 91.82 | 2.59 |
| | S.D | 4.57 | 6.10 | 6.93 | W | 1486.00 | 42 | 35.38 | |
| Adjusted mean | | 75.96 | 75.55 | 76.22 | B | 3.27 | 2 | 1.63 | 0.88 |
| | | | | | W | 97.37 | 41 | 2.38 | |

*Level of significance at a 0.05.

Required table value for degrees of freedom 2&42 = 3.23.

Table 9 Covariant analysis for the 3 groups on BHT

| | | Control group | PT group | RT group | SoV | SoS | df | ms | f ratio |
|---------------|------|---------------|----------|----------|----------|---------|-------|--------|---------|
| Pre test | Mean | 40.33 | 31.93 | 34.13 | B | 569.20 | 2.00 | 284.60 | 1.27 |
| | S.D | 11.79 | 16.05 | 16.64 | W | 9432.00 | 42.00 | 224.57 | |
| Post test | Mean | 40.80 | 33.40 | 34.87 | B | 460.58 | 2.00 | 230.29 | 1.02 |
| | S.D | 12.62 | 15.99 | 16.21 | W | 9489.73 | 42.00 | 225.95 | |
| Adjusted mean | | 35.95 | 36.92 | 36.20 | B | 7.30 | 2.00 | 3.65 | 1.17 |
| | | | | | W | 128.27 | 41.00 | 3.13 | |

*Level of significance at a 0.05.

Required table value for degrees of freedom 2&42 = 3.23.

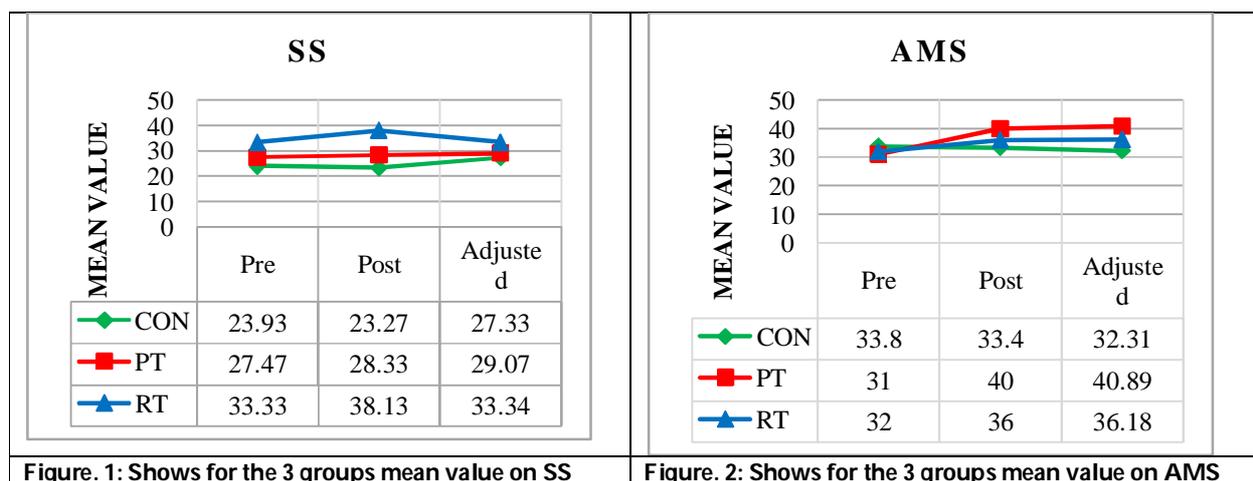


Figure. 1: Shows for the 3 groups mean value on SS

Figure. 2: Shows for the 3 groups mean value on AMS





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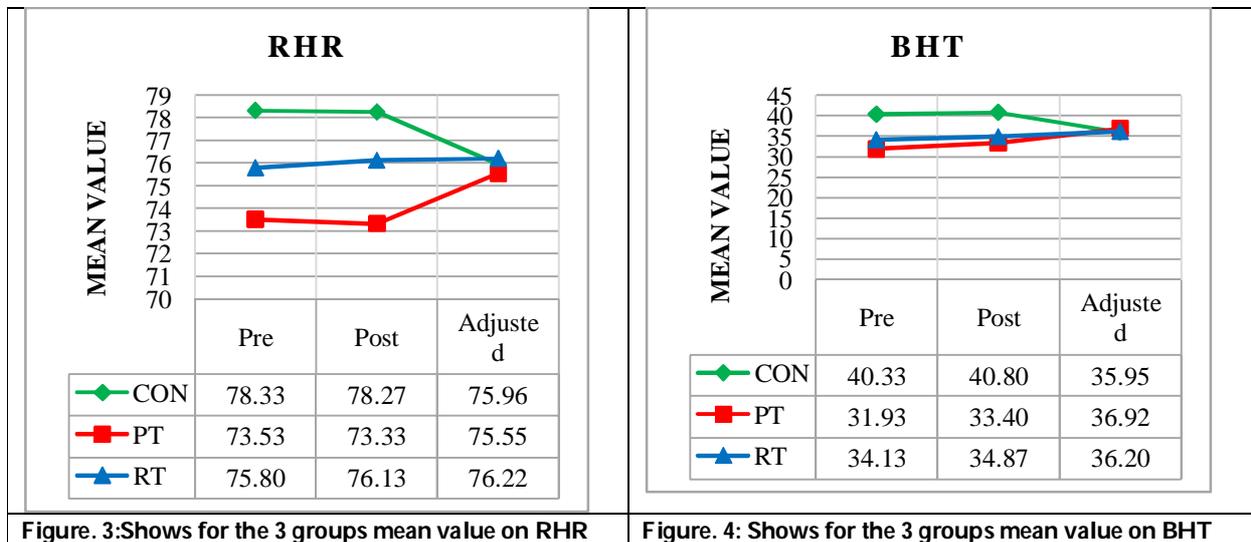


Figure. 3: Shows for the 3 groups mean value on RHR

Figure. 4: Shows for the 3 groups mean value on BHT





Prediction of COVID-19 Infections using Classification Algorithms in Machine Learning

C. Dharmadevi^{1*} and S. Thaddeus²

¹Assistant Professor, Department of Computer Science, Sacred Heart College (Autonomous), Tirupattur, Tamil Nadu, India.

²Assistant Professor, PG and Research Department of Computer Science, Don Bosco College (Co-Ed), Yelagiri, Tamil Nadu, India.

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*Address for Correspondence

C. Dharmadevi

Assistant Professor,
Department of Computer Science,
Sacred Heart College (Autonomous),
Tirupattur, Tamil Nadu, India.
E.Mail: dharmadevi@shcpt.edu



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ABSTRACT

The pandemic disease COVID-19 creates a global urgency initiates a necessity for a novel technology to support the healthcare experts, government to tackle the disease. The proofs of exiting studies using Artificial Intelligence (AI) and Machine Learning (ML) algorithms on the earlier epidemic motivate the researchers to propose a new approach for rapid investigation, prediction and medication with less human intervention. In this research work, various machine learning classification algorithms such as Logistic regression, Decision Tree, K-Nearest Neighbor, Naive Bayesian classifier (NBC), Random Forest and Support Vector Machine (SVM) are used to predict the COVID-19 for the standard data set and their efficiencies are compared. The promising results demonstrate that SVM is more efficient and gives better accuracy than other classification algorithms. Further, the analysis detects the symptoms and co-morbidities which have high impact in the prediction.

Keywords: COVID-19 Pandemic Disease, Machine Learning Algorithms, Classification Algorithms, Support Vector Machine, Naive Bayesian Classifier.

INTRODUCTION

Several epidemics disease invaded people in World history [1]. The pandemic disease SARS-CoV-2, a coronavirus, quickly infected people in more than 185 countries and causes death of millions of people from June,2020 [2, 3

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Jonwards. In order to combat the widespread COVID disease, WHO, scientists, and medical professionals are looking for novel technologies to screen infected patients at different stages, locate the best clinical trials, control the virus' spread, develop a vaccine to treat infected patients, and track contacts of the infected patient [4]. The review of previous research works identifies Machine Learning algorithms and Artificial Intelligence algorithms are well suited for creating such efficient predictive models to assist diagnosis and treatment of COVID-19 with less human intervention [1][5]. Furthermore, in this research work, the popular ML classification techniques Naive Bayesian, Decision Trees, Support Vector Machine and Random Forest are applied to COVID-19 data set with symptoms and co-morbidity for prediction and analysis. The performances of the ML algorithms are evaluated and compared using precision, recall, and area under the ROC, as well as classification accuracy [3] and symptoms with high impact are identified.

The remaining sections of this research work are organized as follows: Section 2 listed out the relevant research studies identified and reviewed. Section 3 shows the materials and methods used in COVID-19 prediction model. In Section 4, the comparison of efficiency of models, results and interpretations are specified. Finally, the results of this study are presented in Section 5.

Related Work

This section present a comprehensive review of research studies on the technologies and models applied to predict the unusual pandemic of Covid-19. The studies furthest compares the efficiency and accuracy of different AI and ML classification models employed in recent times for the integration and dataset kinds, the definitive performance of each proposed model, and an indication of the benefits and drawbacks of contemporary methods. Samuel Lalmuanawma [1], addressed the application of Machine Learning (ML) as well as Artificial Intelligence (AI) application on the previous pandemic to encourage healthcare experts and researchers to propose new models for screening and predicting like MYCIN [7], forecasting like XG Boost, contact tracing with digital contact tracing [8, 9] and drug development for the novel Coronavirus outbreak. This research work also identifies the errors and challenges addressed in the proposed algorithms. [2] Peipei Wang proposed the Logistic model into a time series prediction machine learning model Fb Prophet model to predict the development of the epidemic curve. This research work predicts the epidemic curve, summarized the epidemic size of five different countries Brazil, India, Peru, Russia and Indonesia from which pointed out that the peak infection turning point of COVID-19 differs from county to country. [3] Zohair Malik carried out this research work to identify the change of weather parameters including temperature and humidity has impact in the spreading rate of COVID-19. The confirmed case details with weather conditions of few different regions were taken and the correlation of temperature and humidity to COVID were calculated using different linear regression machine learning models [24, 25, 26]. The outcome of the research depicts that change in temperature and humidity affects the mortality rate.

Milind Yadav [4], in the research work instead of Linear Regression Models used Support Vector Regression Models to foresee the spread of coronavirus across regions, to analyze country wise growth rate and the reformation type, to predict the imprecise end of the epidemic, to analyze the spreading rate of the COVID-19 virus and to identify the impact of weather condition on coronavirus effectively and efficiently. Furthermore, Sun L et al [6] reviewed and found four important medical features. Support Vector Machine is used as the main feature classification model to classify the clinical, laboratory, and demo-graphic information based on GHS, CD3 %, total protein, and patient age. The study found that the model was reliable and useful for identifying severe/critical COVID cases.

Dangi et al. [10] suggested a technique for short term weather forecasting using wavelet denoising with cat boost algorithm by relating the temperature and population density of infected cities around the world to anticipate the novel virus COVID-19 infection in 35 large urban areas in India from March to April 2020. Sajadi et al. [11] suggested a simplified ML based weather model knowing and analyzing the growth and transmission rate of COVID-19 in a particular region able to forecast the regions that are in high risk in the upcoming weeks. This prediction helps the public health care sectors to concentrate more on that region reducing the community spread of COVID-19.



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Demongeot et al. [12] in their proposed research work proved that less humidity and hot weather climatic conditions are associated with a reduction in epidemic viral illnesses like SARS-Cove and MERS-CoV. The assumed temperature dependence of infectivity of COVID-19, the new coronavirus has created a high research interest in the medical domain. Similarly, this research work objective is to find temperature-dependent key factors, such as the infection coefficient rising with cold or dry conditions, from the COVID-19 spread dynamics. The proposed method by Vamsidhar Enireddy [11] uses ResNet50 deep learning feature extraction technique to extract the features from X-Rays and the prediction is done using SVM classifier. Using only ResNet50 gives 94% accuracy whereas combining SVM with ResNet50 feature selection gives 100% accuracy.

The Analysis of performance or accuracy of different Machine Learning Classification Algorithms used for the COVID-19 prediction is shown in Table .1.

MATERIALS AND METHODS

The systematic review of earlier COVID-19 prediction models as given in Table 2.1 shows that Machine Learning Algorithms are widely employed in the prediction with less human intervention which produces better results with good accuracy. However, choosing the correct model is depends on the type, size of the dataset and factors used for prediction. Hence, this research work proposed a methodology given in Fig.1 to identify a model that fit for an effective and efficient prediction of COVID-19 and analysis of impact of symptoms and co-morbidities.

Data Preparation

This investigation is based on the COVID Data set that is obtained from Kaggle website, an online open source repository [10]. Here, we used the COVID-19 data set of INDIA with symptoms and co-morbidities. The dataset has 127 instances with 21 attributes. The details of the attributes are given in Table 2. The Age and Body Temperature are numerical type whereas all the other attributes are of categorical type having either 1 or 0.

Implementation of ML models

The COVID-19 data set with symptoms and co-morbidities taken from the public domain are cleaned and applied with various ML classification algorithms such as Naïve Bayesian classifier, Logistic regression, Decision Tree, K-Nearest Neighbor, Random Forest and Support Vector Machine and the results are recorded. The implementation was carried out using Python jupyter to find the accuracy of the algorithms and to identify the factors which have significant impact in the prediction of covid. In the Table 3, the accuracy of the classification algorithm, f1-score and execution time for prediction are mentioned whereas Fig.2 and Fig 3 shows the graphical representation of accuracy and f1-score, execution time of classification algorithms. The precision- recall curve obtained from the various classification algorithms and precision score of SVM model are shown in Fig.4 and Fig. 5.

DISCUSSION

This section depicts the parameters and presents the outcomes of the classification models that help to compare and identify the right predictive model for the prediction of COVID-19. From Table 2, Fig.2 and Fig.3 the classification algorithm SVM produces results with more accuracy in less execution time. Though, the accuracy of Naïve Bayesian, SVM and Random Forest are nearer, SVM takes less execution time than the rest of the two algorithms. Also, compare to other classification algorithms SVM has high f1-score which reveals that SVM is better than other algorithms. The ROC curve displayed in Fig.4 and Fig.5 used to analyze the efficiency of the classification algorithm also portrays that SVM has high Precision score than rest of the algorithms. The results of correlation co-efficient represents the factors breathing problem, loss of smell, drowsiness are highly significant features in the prediction whereas change in appetite and dry cough symptoms have less importance in finding the covid.





CONCLUSION

Medical professionals can utilise ML techniques as a helpful diagnostic tool to help them analyse the data at hand and create expert systems. ML techniques have been widely adopted in the field of healthcare. The Pearson-correlation co-efficient is used to find the most relevant factors from the symptoms, co-morbidities and demographic factors for the prediction of infection in the COVID-19 data set. Fig.4 shows the correlation of symptoms for a patient to be identified covid positive, Fig.5 displays the correlation of co-morbidities which has high chance for the infection and correlation of demographics and Fig.6 indicates whether the demographics of the patient has impact in the infection or not. This research paper presented two of the most popular ML classification techniques used to foresee the COVID – 19, namely Support Vector Machine (SVM) and Naïve Bayesian (NB). From the experiments carried out, it was showed that prediction of COVID- 19 with symptoms and co-morbidities is more accurate when using Naive Bayesian Classification Model for the given data set. Moreover, this model can be applied to predict for any kind of disease like diabetes, heart disease with proper symptoms.

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Table 2.1. Analysis of Performance measure or accuracy of different ML Classification Algorithms

| Author | ML Model | Type of Data | Data Set | Performance Measure / Accuracy |
|------------------------------------|--|---|--|--|
| Peipei Wang,. | FbProphet – a Time series Logistic Prediction Model | Global Epidemic Time Series Data | ArcGIS Platform | Significant improvement in prediction |
| Zohair Malki [3] | Regression Model (20 different regression and classification models) | Demographic data with temperature humidity and wind speed | Country level aggregate data from 21 states, 3144 countries and 5 territories | KNN: MSE – 1.49381e+07 RMSE - 3782.07, Decision Tree:MSE- 35927 RMSE – 184.686 |
| Milind Yadav [4] | Linear Regression, Polynomial Regression and SVR | Clinical Data | Number of Positive cases, recoveries, deaths from 22/1/2020 to 24/4/2020 – 93 days | Linear Regression – 65.01% Polynomial – 98.82% SVR – 99.47% |
| Dana Bazazeh [5] | SVM, Random Forest, Bayesian Networks | Clinical Data | Wisconsin original breast cancer 669 instances | SVM – 97.0% Random Forest – 96.6% Bayesian Networks – 97.1% |
| Sun L et al [6] | SVM | Clinical, Laboratory Features, Demographics | 336 – COVID-19 with PCR kit test; 310 non-critical and 26 critical cases | Accuracy: 77.5% Specificity: 78.4% AUROC reaches 0.9996 training and 0.9757 testing dataset |
| Wu, J., Zhang,[7] | Random Forest Algorithm | Clinical, | 253 samples : 169 patients (suspected), 49 commercial clinic samples, 24 infected patients | Accuracy : 97.95%, MCC – 95.94%, AUC – 1.00 Sensitivity – 97.5, Specificity – 99.09 |
| Ribeiro, M. H. D. M., et al ., [8] | Support Vector Regression and stacking-ensemble | Clinical | 40, 581 | Accuracy: Error in range of 0.87%-3.51% one, 1.02%-5.63% three and 0.95% -6.90% six day ahead |





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| | | | | |
|-----------------------------|---|--------------|----------------------------------|---|
| Yan, L. <i>et al.</i> , [9] | XGBoost classifier | Clinical | Blood samples of 75 features 485 | Accuracy – 90% |
| A. Farid, G. [10] | Stack Hybrid Classification method with SVM, NB, JRIP and Random Forest | CT images | 51 SARS and COVID CT images | SVM- 86.27%, NB – 96.07%, JRIP – 94.13%, Random Forest – 92.95% |
| Vamsidhar Enireddy [11] | ResNet50 and SVM, | X-Ray images | 50 X-Ray images | ResNet50 - 94% ResNet50+SVM – 100% |

Table 2. Attributes of COVID-19 dataset taken from Kaggle website

| Name of the Attribute | Type of the Attribute | Name of the Attribute | Type of the Attribute |
|-----------------------|-----------------------|--------------------------------------|-----------------------|
| Age | Numerical | Gender | Categorical |
| Body Temperature | Numerical | Dry Cough | |
| Sour Throat | Categorical | Weakness | |
| Breathing Problem | | Drowsiness | |
| Pain In Chest | | Travel History To Infected Countries | |
| Diabetes | | Heart Disease | |
| Lung Disease | | Stroke Or Reduced Immunity | |
| Symptoms Progressed | | High Blood Pressure | |
| Kidney Disease | | Loss Of Sense Of Smell | |

Table 3. Performance measure of various classification Algorithms

| Algorithm | Accuracy | f1-score | Time in seconds |
|---------------------|----------|----------|-----------------|
| Logistic Regression | 74.90 | 0.61 | 0.064 |
| KNN | 76.47 | 0.35 | 0.065 |
| SVM | 85.04 | 0.86 | 0.055 |
| Naïve Bayesian | 84.36 | 0.33 | 0.056 |
| Decision Tree | 75.64 | 0.80 | 0.058 |
| Random Forest | 83.72 | 0.68 | 1.799 |

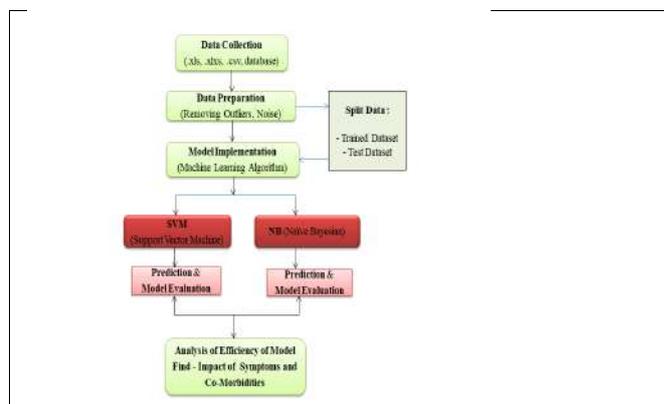


Fig.1. Methodology for prediction of COVID-19 using Machine Learning (ML) – Classifier Models

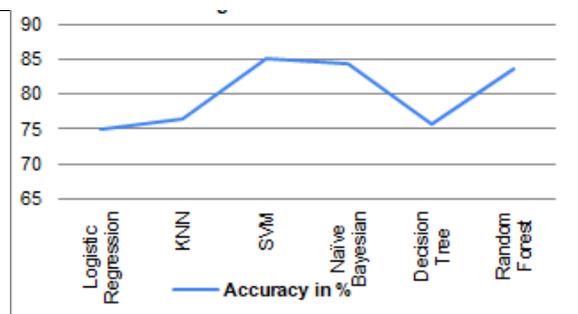


Fig.2 Accuracy of Classification Algorithms





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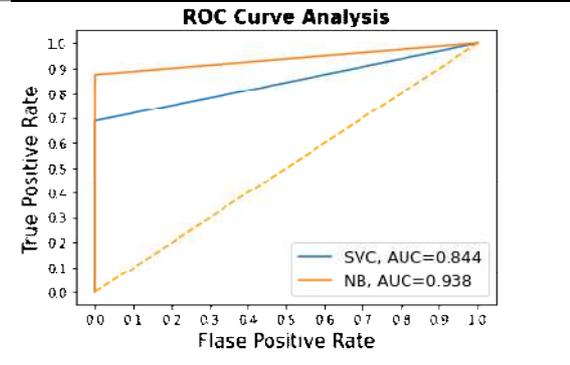


Fig.3. f1-score and execution time of ML Classification Algorithms

Fig.4. ROC Curve Analysis

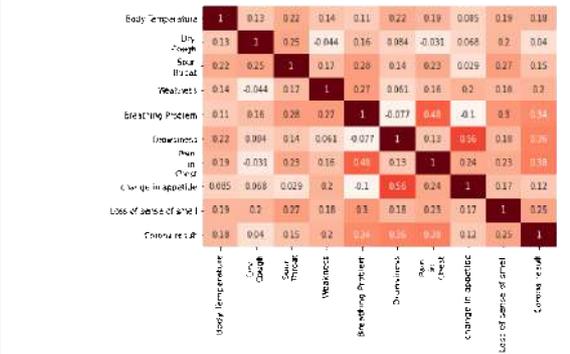
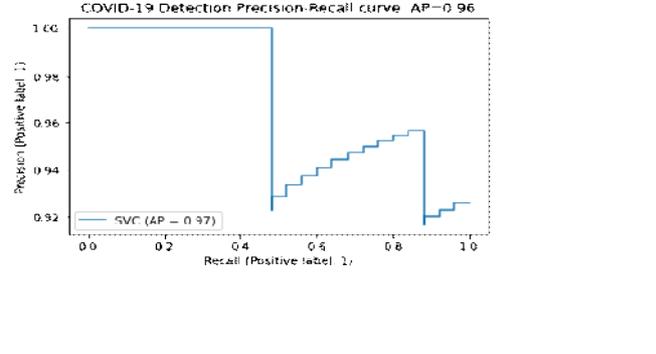


Fig.5. AP Score of SVM

Fig. 6 Correlation of Symptoms

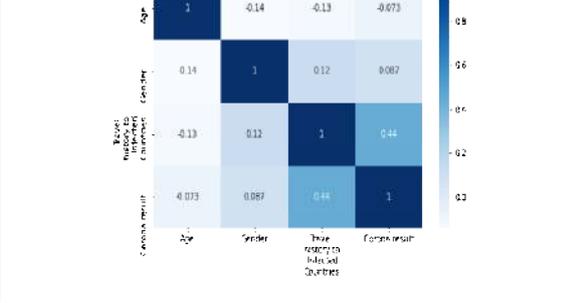
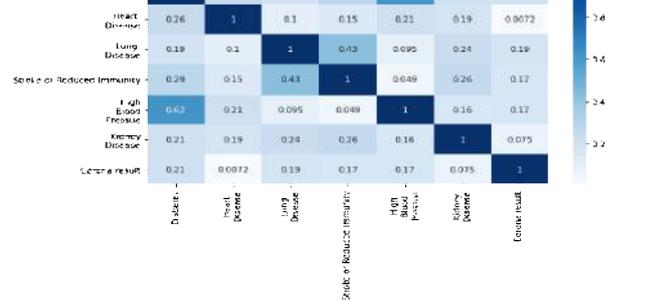


Fig.7 Correlation of Co-morbidities

Fig.8 Correlation of Demographics





Bio-Synthesis Characterization of Cobalt Oxide / Silicon Oxide Core Shell using *Aristolochia indica* Extract and their CV Analysis

Thangeswari.S^{1*}, Amuthavalli K², Vinoline Golda.T¹ and Infant Francita Fonseka.C¹

¹Research Scholar, V.O.Chidambaram College, Thoothukudi- 628 008, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli- 627 012, Tamil Nadu, India.

²Associate Professor, V.O.Chidambaram College, Thoothukudi- 628 008, Affiliated to Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli- 627 012, Tamil Nadu, India.

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*Address for Correspondence

Thangeswari.S

Research Scholar,
V.O.Chidambaram College,
Thoothukudi- 628 008,
Affiliated to Manonmaniam Sundaranar University,
Abishekapatti, Tirunelveli- 627 012,
Tamil Nadu, India.
E.Mail: thngam8991@gmail.com



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ABSTRACT

In this study, a natural extract from the *Aristolochia indica* plant is used as a powerful chelating agent in the environmentally friendly manufacturing of the $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shells. The crystalline structure, surface, morphology and optical properties of the $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell were investigated using a number of characterization techniques. X-ray diffraction (XRD) analysis proved that cubic $\text{Co}_3\text{O}_4/\text{SiO}_2$ crystals were produced. Using Fourier Transform Infrared Spectroscopy, the stretching vibration of the Co-O and Si-O core shells was studied (FTIR). The average grain size of the $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shells, as observed by scanning electron microscopy (SEM), is 21 nm. The optical characterization clearly revealed the information on the direct band gap for the core and for the core-shell. Through cyclic voltammetry investigation, the electrochemical performances of the Co_3O_4 core and the $\text{Co}_3\text{O}_4/\text{SiO}_2$ core-shell nanoparticle supercapacitor were explored.

Keywords: Bio synthesis, *Aristolochia indica*, $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shells, pseudocapacitor and bandgap.





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INTRODUCTION

Supercapacitors have received a lot of attention in recent years because of their high energy density, quick recharge capabilities, and long cycling life. They are utilized in hybrid electric vehicles, digital communication equipment, and even as a backup power source. It is well known that the performance of the supercapacitor is significantly influenced by the crystallinity, shape, specific surface area, chemical compositions, and structural stability of nanomaterials. Any transition metal oxides that fall into the nanosized zone have been proven to have even more alluring applications, including heterogeneous catalysts, gas sensors, lithium ion batteries, electrochromic devices, solar energy absorbers, ceramic pigments, optical devices, etc [1]. Recently, a number of physical and chemical-based methods have been used to synthesis the different Co_3O_4 nanostructures and improve their ability to have a high theoretical specific potential and good electrochemical characteristics. The restricted potential or energy density and low cycling stability of new cobalt oxide composite materials for the supercapacitor have been thoroughly examined [2]. Precipitation, sol-gel combustion, sonochemical techniques, thermal breakdown, chemical vapor deposition, wet polymerization, laser ablation, solvothermal techniques, micro-emulsions, and hydrothermal techniques are some examples of these. In addition to these conventional synthesis procedures, the green synthesis process has a number of advantages, including cost effectiveness, a lack of need for extra chemicals, and environmentally friendly practices with little waste production [3].

These Co_3O_4 nanoparticles are employed as magnetic resonance contrast agents, medication delivery mechanisms, and adjuvant in human immunization procedures. The metal salt, a reducing agent, and a stabilizing agent for regulating nanoparticle size and avoiding their aggregation are three crucial requirements for the metallic nanoparticles that are biosynthesized using plant extract. The bioreduction, synthesis, and stability of metal nanoparticles may involve biomolecules such proteins, enzymes, amino acids, carbohydrates, alkaloids, terpenoids, tannins, saponins, phenolic compounds, reducing sugars, and vitamins. The amount of nanoparticle synthesis is significantly influenced by the availability of polyphenols, enzymes, and other chelating agents in plants [4]. In the current study, a $\text{Co}_3\text{O}_4/\text{SiO}_2$ core-shell was made utilizing a natural extract of *Aristolochia indica*. Analysis was done on the Co_3O_4 core and $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell nanoparticles' physical, optical, and capacitance properties.

Experimental Technique

Extraction preparation

20 grams of the dried *Aristolochia indica* stem were added to a 250 ml Erlenmeyer flask. The mixture was then heated to 55 °C after 100 cc of distilled water was added. After 10 minutes, the color of the solution changed from colorless to bright yellow. The solution was filtered using Whatman No. 1 filter paper to get removal of the residue once it had cooled to room temperature.

The creation of a Co_3O_4 core

20 mL of dried stem extract and 1 mL of cobalt salt solution were mixed in a glass beaker and stirred at 700 rpm for two hours to create the Co_3O_4 core. A color change indicates the creation of Co_3O_4 core (from light purple to dark purple). The resultant solution was centrifuged for 3 minutes at 1000 rpm, the supernatant solution was collected, and the leftover material was dried by baking it for 8 hours at 100 °C. Following that, Co_3O_4 core particles were created and calcined for three hours at 360 °C. Finally, a black Co_3O_4 core was gathered and used for further investigation.

Formation of silica shells

0.1 M of the Co_3O_4 core was covered with SiO_2 by adding 3.5 ml of ammonia and 5 drops of TEOS (Tetraethylorthosilicate) while agitating the Co_3O_4 suspension, with a 5-minute pause between each TEOS drop. This was crucial since it made it possible to alter the SiO_2 thickness by changing the TEOS concentration. The mixture was then stirred continuously for a further hour, during which the ammonia concentration acted as a catalyst to quicken the reaction. After being washed with DD (Double distilled) water and ethanol, the suspension was centrifuged to



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separate the particles from the liquid in 50 ml Eppendorf tubes. The black $\text{Co}_3\text{O}_4/\text{SiO}_2$ core-shell of the collected particles was recovered and used after five hours of drying at 300°C .

RESULT AND DISCUSSION

X-ray diffraction

Co_3O_4 core particle XRD patterns are shown in Fig. 1(a), and the strong peaks they exhibit show the product to have good crystalline purity. The Co_3O_4 planes (220), (311), (400), (422), (511), and 440, as well as the Co_3O_4 core peaks at 2θ of 31.4° , 36.98° , 59.4° , and 65.4° , are attributed to the diffraction peaks (JCPDS NO: 653103). The lattice parameter "a," which equals 8\AA , is inferred from the XRD data. The value for Co_3O_4 is constant. Muhammad Hafeez *et al.* discussed reports of a similar nature [5]. The strength of the Co_3O_4 core's diffraction peaks decreased after the SiO_2 shells were coated, and the peaks for the core-shell were relocated from 36.98° to 37.08° and from 65.4° to 65.52° . The XRD peak intensity and position may change, according to Abdur Rahman *et al.*, if the metal core's surface is oxidized or if any metal compounds are produced.

After the formation of a SiO_2 shell on a Co_3O_4 core, the strongest peak for the core is moved to a point from the lattice spacing of 2.43062-2.42466, roughly displaced by 0.306 percent. The Debye-Scherrer equation is used to determine the average particle size.

$$D = k\lambda / \beta \cos\theta$$

Where λ is the X-ray wavelength is 1.54 nanometers, D is the crystallite size (in nanometers), k is the crystallite form factor, and β is the whole width at half maximum of the diffraction peak at the diffraction angle. The average diameters of the Co_3O_4 core and $\text{Co}_3\text{O}_4 / \text{SiO}_2$ core-shell were calculated to be 14.99 nm and 21.5 nm, respectively, by resolving the peak.

Structural analysis

A scanning electron micrograph of the Co_3O_4 core and $\text{Co}_3\text{O}_4 / \text{SiO}_2$ core shell is shown in Figure 2. The morphology reveals spherical particles with typical grain sizes ranging from 10 nm to 31 nm for core particles. Similar findings were reported by Wang F *et al.* (2015), Xu H *et al.* (2014), Raman V *et al.* (2015), and Montazeezohori M *et al.* (2017).

Analysis with Energy Dispersive X-rays (EDX)

The outcomes of the EDX analysis of the Core and Core Shell are shown in Figure 3. The strong signals Co, O, C, and Si are seen in the spectrum. Cobalt and oxygen were present in the synthesized samples shown in Figure 3(a), and cobalt oxide and silicon oxide were created in the samples shown in Figure 3(b). Additionally, no new maxima for any other elements were observed. The synthesised sample was found to contain only Co, Si, and O and was free of any impurities, according to the elemental analysis.

Functional group analysis

Figures 4(a) and 4(b) display the FT-IR spectra of Co_3O_4 core and $\text{Co}_3\text{O}_4 / \text{SiO}_2$ core shell particles with wavelengths ranging from 4000 to 400 cm^{-1} (b). Figure 4(a) shows that the stretching vibration of C-H alkanes causes the broadband at 3424 cm^{-1} and 2924 cm^{-1} , and that the stretching and bending modes of water are responsible for the peaks at 1628 cm^{-1} and 1627 cm^{-1} , respectively [6]. A high band appears at 664 cm^{-1} due to the stretching vibration of the metallic oxygen link of the Co-O band. The same kind of result, according to C. Indra Priyadarshini *et al.* (2020), was attained [1]. Figure 4(b) showed Si-O-Si bands that were 1101 cm^{-1} in intensity, matching earlier reports by Zhigang Yi *et al.* (2019) [7]. The conspicuous band at 564 cm^{-1} is connected to the stretching and bending vibrations of the silicon-oxygen link. Halim Z *et al.* (2019)'s investigation of strong bands in the 500 cm^{-1} and 1080 cm^{-1} range, corresponding to the O-Si-O and Si-O-Si bending and stretching vibrations resulted in same findings [8]. The sharp





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band at 661 cm^{-1} results from the Co-O stretching of Si-O-Co bonds. The Co_3O_4 core is encased in a SiO_2 shell, in accordance with reports by Subrata Mandal et al. (2018) [9].

Optical characteristics

The optical absorption properties of the produced Co_3O_4 and $\text{Co}_3\text{O}_4/\text{SiO}_2$ Cores and core shells were investigated using UV-vis spectroscopy at room temperature. The conclusion, which displays absorption bands in the 333 nm core and 280 and 242 nm core shell wavelength ranges, is shown in Figure 5. The charge transfer to $\text{O}^{2-}-\text{Co}^{2+}$ and $\text{O}^{2-}-\text{Co}^{3+}$, respectively, resulted in the formation of these bands. Similar results were obtained by Vijayanandan A et al. (2018), and Sakthisabarimoorthi A (2018) [10, 11]. Figure 5 displays the optical transmittance spectra of Co_3O_4 core and $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell nanoparticles (b). As the amount of coating material is increased, it is seen that the lattice faults lead to a reduction in transmittance and a little movement of the absorption edge towards the visible light region. The optical band gap spectra of the core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ are shown in Figure 6. The band gap of the p-type semiconductor Co_3O_4 can be calculated using the following equation:

$$(\alpha h\nu)^2 = K (h\nu - E_g)$$

$h\nu$ is the photon energy, expressed in electron volts (eV), and α is the absorption coefficient. E_g is the energy of the absorption band gap, and K is a constant in relation to the substance. The two E_g values for the core and core shell are 2.8, 2.4, 2.6, and 2.2 eV as a result of the absorption bands in Figure 6. While the charge transfer process between O^{2-} and Co^{3+} might cause the second band gap, the charge transfer from $\text{O}^{2-}-\text{Co}^{2+}$ may be the reason for the first direct band. Cobalt exists in both the +2 and +3 levels, as is clear. Similar findings were made by A.K. Sarfraz and S.K. Hasanain (2013), Qiao, L. et al. (2013), and Kandula S. et al. (2015).

Because of the higher value of the extinction coefficient k , Figure 7 demonstrates that the core Co_3O_4 sample exhibits a stronger absorption than the core $\text{Co}_3\text{O}_4/\text{SiO}_2$ sample. The amazing transparency of Co_3O_4 and SiO_2 is demonstrated by the frequent changes in the value of k as coating materials are added. It was observed that the extinction coefficient was high. The figures show how much energy is lost as a result of electromagnetic radiation moving through the substance's medium. It is clear that at high frequencies, the significance of the k values rises substantially, and at lower wavelengths, it falls slightly. The coated SiO_2 components are very transparent when compared to the core Co_3O_4 sample. The variation in the k values might be brought on by light scattering at the point where Co_3O_4 and SiO_2 nanoparticles converge.

The measurements of the refractive indices and the electrical properties of the obtained samples may be connected. You can use the following relationship to determine the refractive index (n) from the reflectance (R):

$$n = \left(\frac{1 + R}{1 - R} \right) + \sqrt{\frac{4R}{(1 - R)^2} - k^2}$$

The refractive index, or n , is a crucial physical variable relevant to small-scale atomic inter actions. Figure 8 depicts the variation of the refractive index n with wavelength for core Co_3O_4 and core $\text{Co}_3\text{O}_4/\text{SiO}_2$. Figure 8 shows that the increase in coated SiO_2 -containing materials causes a general rise in the refractive index because the transmission of core Co_3O_4 is greatly reduced. It is used to produce electronic polarization and an applied electric local field inside optical materials. The additional coating components almost led to a shift in the refractive index values. Research from 2020 by Mohamed R.M. and colleagues indicates that the maximum refractive index value is appropriate for optical apparatus. Electron mobility inside the lattice optical conductivity was the primary cause of the optical properties' initial appearance. These composites' electrical conductivity suffers as a result of the Co_3O_4 component. The transmission varies with optical conductivity as a function of photon energy for all synthetic composite materials. The increase in optical conductivity of the high photon energy region is due to the high absorbance of the composite samples in comparison to the core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ and possibly the electron driven by photon energy, as well as the addition of additional coated material with SiO_2 content. The creation of new levels in the band gap,



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which make it simpler for electrons to transition from the valence band to these local levels and into the conduction band, is what is responsible for this increase. As a result, according to Shahat Adel El et al., (2017) the band gap closes and conductivity increases.

Photoluminescence spectroscopy

Three photoluminescence peaks, which were recorded between 300 and 900 nm, could be seen in the PL spectra of the Co_3O_4 core and $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell in Fig. 9 (a) and (b). Green emission happens between 500 and 600 nanometers, UV emission happens between 300 and 400 nanometers, and blue emission happens between 400 and 500 nm. The oxygen vacancy in the Co_3O_4 core and core shell is what is responsible for this peak. The photon energies of the peaks at 341 nm, 467 nm, and 552 nm in the core are respectively 3.6, 2.7, and 2.3 eV, while the photon energies of the peaks at 316 nm, 456 nm, and 532 nm in the core shell are respectively 3.9, 2.7, and 2.3 eV.

Cyclic Voltammetry

Core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ served as the working electrodes for the cyclic voltammetry, while CGE served as the reference electrode in the electrolyte of 0.1 M KOH. Figures 10(a) and (b) show the CV curves of the core (Co_3O_4) and the core shell ($\text{Co}_3\text{O}_4/\text{SiO}_2$) with the scan rate varied from 50 mV/s to 200 mV/s, and, for the core and core shell, respectively, 50 mV/s, 100 mV/s, 150 mV/s, and 200 mV/s in the potential window range from -0.9 V to 0.9 V. As the scan rate rose, the CV curves in figures 10(a) and (b) expanded. Due to the fact that it demonstrates how closely the voltammetry current correlates with the scan rate, this is a great example of how a super capacitor will behave. The oxidation and reduction peaks were seen on the CV curves. This illustrates that the pseudo capacitance of the electrode is the main contributor to its electrochemical capacitance. Compared to the core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$, the core Co_3O_4 showed a small current potential window and weak reversible redox activity. The capacitance values were calculated for various scan rates. The voltage curve in Figure 11 ranges from 50 mV/s to 200 mV/s. All of the electrodes showed a steady increase in scan rate, in contrast to the values that declined for the supercapacitor. The capacitance of the core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ was examined at various current densities ranging from 4 mA/cm² to 9 mA/cm² and 4 mA/cm² to 7 mA/cm², respectively.

CONCLUSION

A green synthesis method was employed to successfully produce the core shell of Co_3O_4 and SiO_2 utilizing *Aristolocia indica*. Physical properties were examined using XRD and FESEM. In order to identify the functional group, FTIR was utilized. The optical properties were investigated using UV and PL characterization. The direct band gap demonstrates that the generated core shell particles are semiconducting by nature. Future applications of this information include supercapacitors, batteries, and solar cells. Because a semiconducting substance will function better in energy storage devices. The experimental data on transmittance and absorbance were evaluated using an ultraviolet (UV-Vis) spectrophotometer. The core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ become powerful UV-light blockers (up to 370 nm), whereas they only block UV-light in the range of 240-333 nm, with the addition of Co_3O_4 coating materials like SiO_2 . On the other hand, the transmittance of Co_3O_4 and SiO_2 drops to less than 20% when the core and core shell contents increase. For highly loaded composites, this transmittance decrease is particularly pronounced in the NIR band. The band gap is narrowed with the use of coated SiO_2 components. This lessens as a result of the particle size reduction. This phenomenon is known as red shift in ready composites. Composite samples' optical characteristics are identified. Up to 300 nm, the VELF values saw a dramatic change. The SELF values decreased when more coating materials were introduced into the Co_3O_4 . Analyses of all the optical characteristics of the Co_3O_4 and SiO_2 samples under study show that these composite materials are better suited for their great potential as optoelectronic devices and UV-shielding coatings. The pseudo-capacitive behavior of the core and core shell was investigated using cyclic voltammetry, and the current densities of the core and core shell were calculated over the ranges of 4 mA/cm² to 9 mA/cm² and 4 mA/cm² to 7 mA/cm², respectively.





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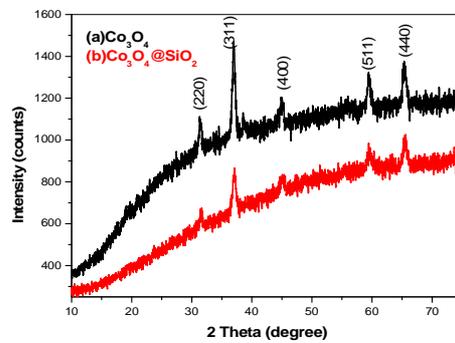


Figure 1 The XRD patterns of Co_3O_4 core and $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell

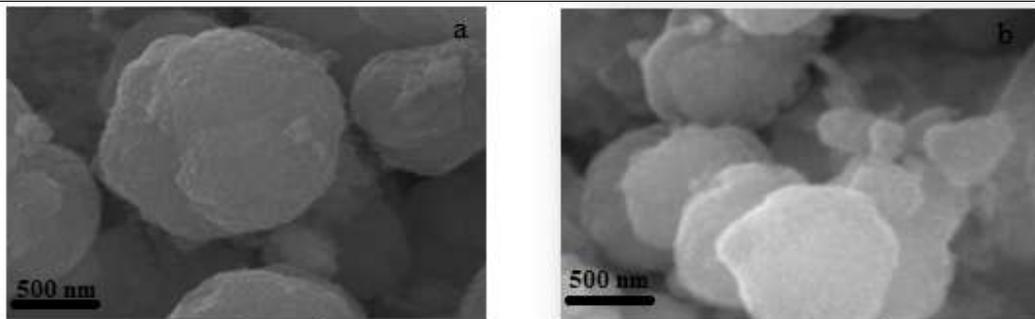


Figure 2 Morphology and Distribution of (a) Co_3O_4 core Nanoparticle, (b) $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell Nanoparticles

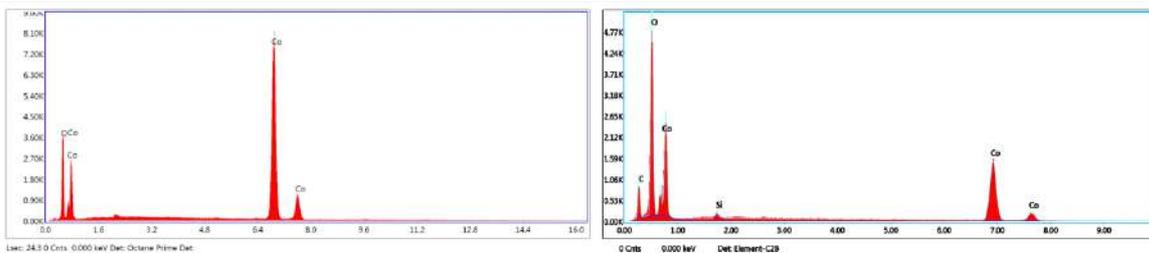


Figure 3 EDX spectra of (a) Co_3O_4 core Nanoparticle, (b) $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell Nanoparticles

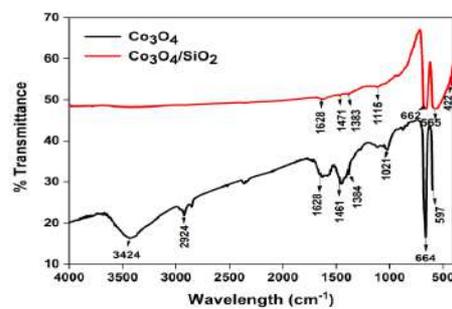


Figure 4 FTIR spectra of (a) Co_3O_4 core Nanoparticle, (b) $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell Nanoparticles





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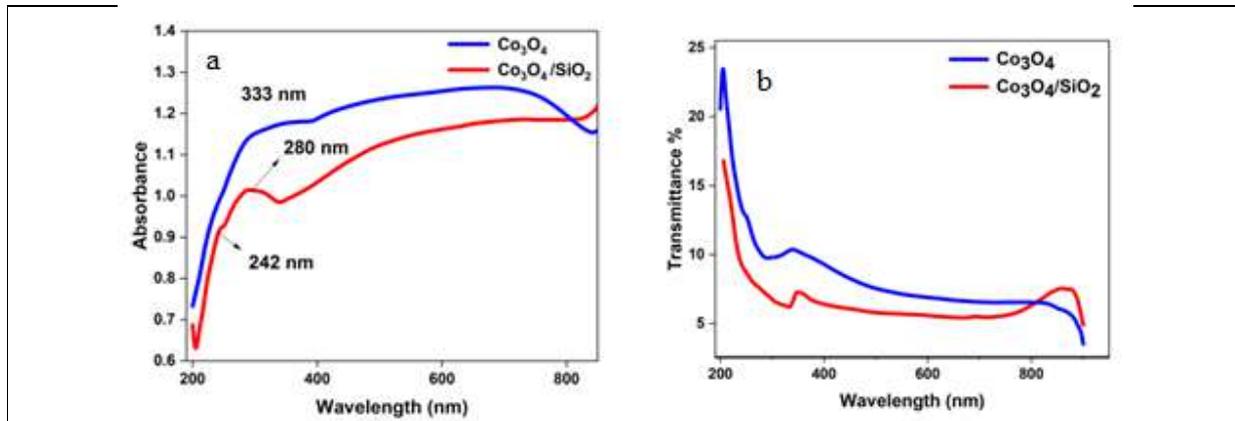


Figure 5 (a) UV-vis Absorbance spectra and 5 (b) Transmittance % spectra of core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$

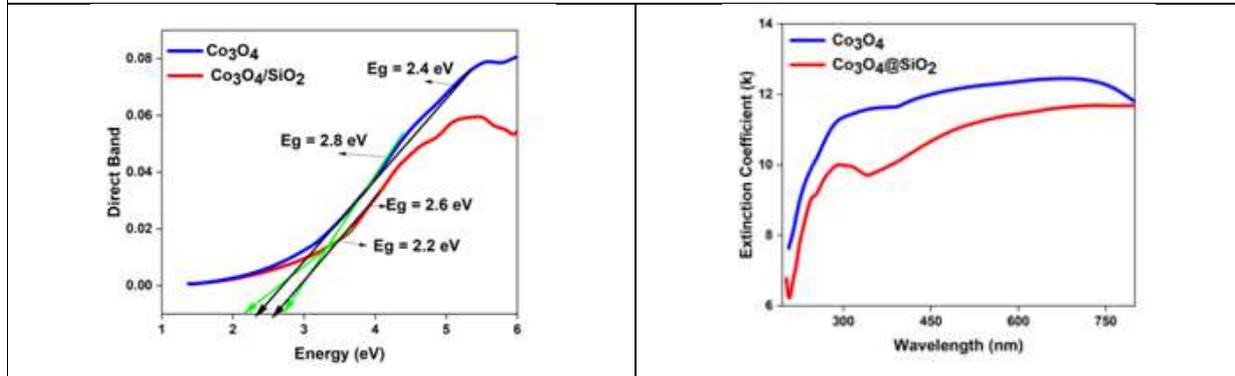


Figure 6 Tauc's plots of variation of optical band gap for core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$

Figure 7 Variation of Extinction coefficient k for core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$

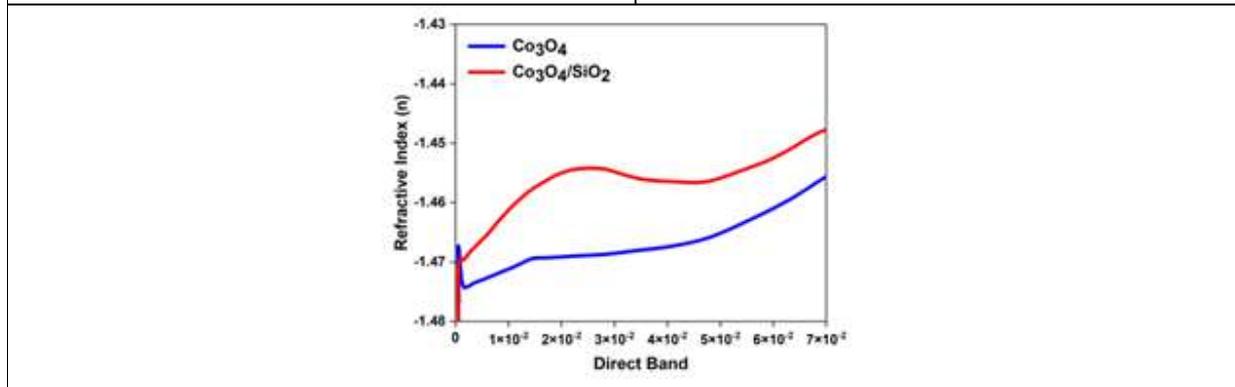


Figure 8 Dependence of Refractive Index for core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$





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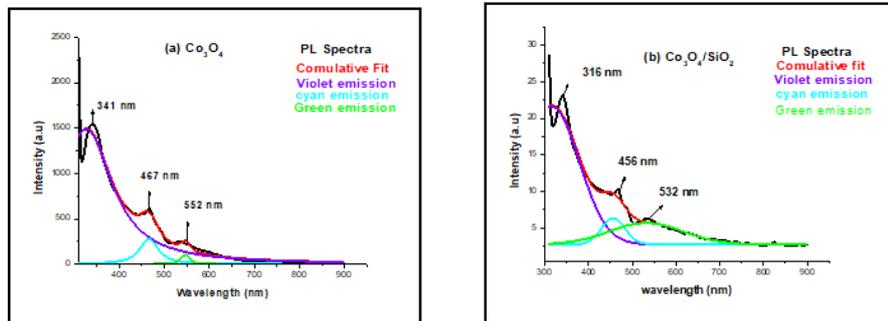


Figure 9 Photoluminescence spectroscopy of (a) Co_3O_4 core (b) $\text{Co}_3\text{O}_4/\text{SiO}_2$ core shell

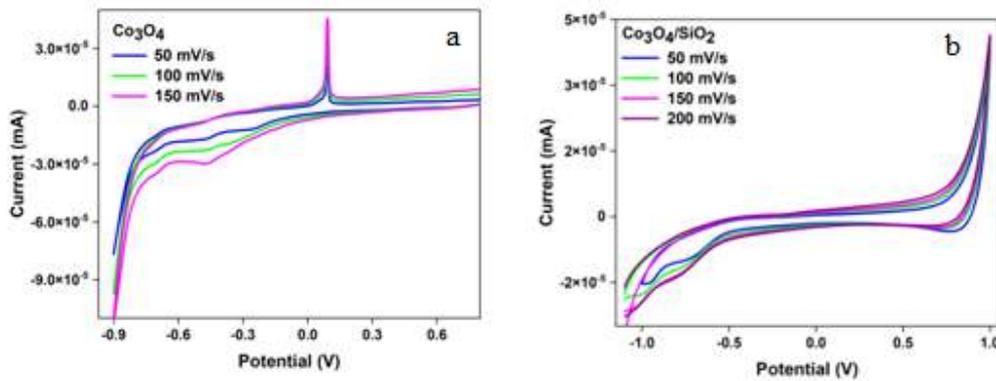


Figure 10 (a) and (b) CV curves of the core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$ electrode at different scan rates

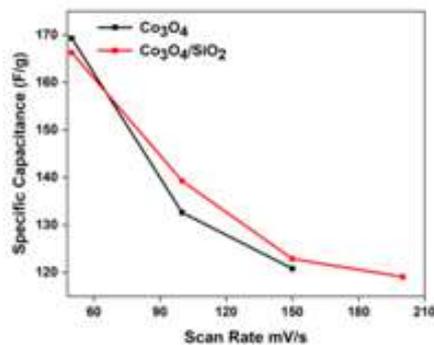


Figure 11 Comparison of specific capacitance values of core Co_3O_4 and core shell $\text{Co}_3\text{O}_4/\text{SiO}_2$





Improved Soft Thresholding Technique for Denoising Ultrasound Images”

Sima Sahu^{1*} and P. Sowjanya²

Associate Professor, Department of ECE, Malla Reddy Engineering College, Maisammaguda, Hyderabad, Telangana, India

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*Address for Correspondence

Sima Sahu

Associate Professor,
Department of ECE,
Malla Reddy Engineering College,
Maisammaguda, Hyderabad,
Telangana, India
E.Mail: simahal@mrec.ac.in



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ABSTRACT

An improved algorithm for denoising ultrasound image is proposed in this paper. Generally ultrasound image is affected by multiplicative noise called speckle noise. Removal or reduction of speckle noise is highly essential in the disease diagnosis process. This paper added the features of statistical modeling in soft thresholding to develop an improved denoising method. Thresholding the wavelet coefficients require threshold value and in this paper the threshold value is calculated by modeling the wavelet coefficients. The heavy-tailed wavelet coefficients are modeled using a suitable Cauchy probability density function (PDF) to estimate the signal and noise variance information of the wavelet coefficients. Performance parameters such as Peak Signal-to-Noise Ratio (PSNR), Structural Similarity (SSIM) and Edge Preservation Index (EPI) are used in this paper to evaluate the efficacy of the proposed method. From the simulation results it is found that the proposed denoising method has achieved improved results than the state-of-the-art methods.

Keywords: Soft thresholding, Cauchy PDF, denoising, Speckle noise





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INTRODUCTION

Ultrasound images are contaminated by speckle noise which results the degradation of image quality and it affects the disease diagnosis processes. So in medical image processing, an image denoising process is highly required. In literature several filtering approaches have been implemented by authors for reducing speckle noise from ultrasound images. These filtering approaches can be broadly categorized in to three sections. They are algorithmic, transform, and statistical modeling approaches. Algorithmic approaches include Adaptive median [1], Bilateral filtering [2], Rotating kernel transformation [3], Non-local mean filter [4] and Anisotropic diffusion filter [5]. Transform approach involves filtering using wavelet transform [6], Contourlet transform [7], and Curvelet transform [8]. Statistical modeling approached filters are designed by utilizing the statistical behavior of the noise and signal. Filters based on algorithmic approach suffer from limitations like loss of structural details and high computational complexity. Filters based on transform approach suffer from determination of accurate threshold value. Statistical modeled filters are generally depending on the choice of prior and estimator [9, 17].

Wavelet thresholding filter is a common type of wavelet-based filter. The basic steps of this type of filters involve generation of wavelet coefficients, modification of wavelet coefficients and recovering of image from the modified wavelet coefficients. For modifying the wavelet coefficients a threshold value is required and the denoising efficiency depends on the threshold value. Soft and Hard thresholding methods are widely used by the researchers and have been proved more promising in removing the multiplicative noise. This paper applies soft thresholding to the logarithmic transformed wavelet coefficients and the thresholding value is found out by modeling the wavelet coefficients statistically. The organization of the paper is as follows. Section 2 discusses about homomorphic approach, Cauchy PDF, statistical modeling of wavelet coefficients and Soft thresholding. Section 3 gives the denoising methodology. Simulation results are elaborated in section 4 and conclusion is given in section 5.

BACKGROUND

This section discusses the preliminaries of the proposed denoising method. Homomorphic approach which is used as a process for converting the speckle noise into white Gaussian noise is discussed in this section. Cauchy PDF which is used for modeling the wavelet coefficients is discussed. Soft threshold methodology is also discussed in this section.

Homomorphic Approach

The basic steps of homomorphic approach are logarithmic (LOG) transformation and exponential (EXP) transformation. This approach decomposes the multiplication response as given below [10]:

$$P(m(t)n(t)) = P(m(t)) + P(n(t)) \quad (1)$$

P defines homomorphic system and is logarithmic in nature. A homomorphic system is shown in Fig. 1:

Cauchy PDF

A highly suitable probability density function is required for estimating the noise-free wavelet coefficients. This paper utilizes Cauchy PDF for modeling the wavelet coefficients due to its long-tail structure. Assuming X as a Cauchy random variable, Cauchy PDF and cumulative distribution function (CDF) are defined by the following expressions [11]:

$$f(x) = \frac{1}{\pi} \frac{b}{(x-m)^2 + b^2} \quad (2)$$

$$F(x) = \frac{1}{2} + \frac{1}{\pi} \tan^{-1} \left(\frac{x-m}{b} \right) \quad (3)$$

Where m is the location and b is the scaling parameters. The PDF and CDF graphs are shown in figures 2 and 3 respectively.





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Statistical Modeling of Wavelet Coefficients of Ultrasound Image

Wavelet coefficients are generated from the logarithmic transformed ultrasound image are modeled using Cauchy PDF to find out the signal and noise information. The distribution of wavelet coefficients in the approximation sub-band at level-1 is shown in figure 4. The noise-free signal density is calculated from the detailed sub-bands. The noise density is calculated from the diagonal detail sub-band of at level-1 [12].

Soft Thresholding

Soft thresholding procedure narrows the wavelet coefficients C whose values lies above the threshold level. The soft thresholding function given by Zaki et al. [13] is:

$$Soft_{t_h} = \begin{cases} 0; & |C| \leq t_h \\ sgn(C) [|C| - t_h]; & |C| > t_h \end{cases} \quad (4)$$

t_h is the threshold value which is defines as:

$$t_h = \frac{\sigma_N^2}{\sigma_S} \quad (5)$$

σ_N^2 is the variance of the noise and calculated from the diagonal sub-band coefficients at level-1 ($D1$). It is given by [14]:

$$\sigma_N^2 = \left(\frac{\text{median}(D1)}{0.6745} \right)^2 \quad (6)$$

σ_S is the standard deviation of the noise-free signal defined by the fundamental theorem of probability as:

$$\sigma_S = \sqrt{\overline{C^2}} \quad (7)$$

$\overline{(\)}$ is the expectation operation.

METHODOLOGY

The complete denoising procedure is explained through the following procedural steps and also the explained through the block diagram given in the figure 5.

- Step 1. Input ultrasound image.
- Step 2. Application of homomorphic approach to the input ultrasound image.
- Step 3. Application of discrete wavelet transform (DWT) to the image obtained from step2.
- Step 4. Calculation of threshold value.
- Step 5. Application of soft thresholding to recover the wavelet coefficients.
- Step 6. Application of Inverse discrete wavelet waveform (IDWT) to recover the image.
- Step 7. Application of exponential operation (EXP) to the image obtained from step 6 to get the noise free ultrasound image.

Comparisons are given in tables 1, 2, and 3. From tables 1, 2 and 3 it can be viewed that the PSNR(dB), SSIM and EPI values are better for the proposed method than the state-of-the-art methods irrespective of the noise variance value. The PSNR improvement of the proposed method is 10.42% and 8.22% than the Donoho's Soft threshold [16] and Zaki et al. [13] methods respectively for noise variance of 0.1. The SSIM improvement of the proposed method is 2.27% and 0.72% than the Donoho's Soft threshold [16] and Zaki et al. [13] methods respectively for noise variance of 0.1. The EPI improvement of the proposed method is 3.21% and 1.99% than the Donoho's Soft threshold [16] and Zaki et al. [13] methods respectively for noise variance of 0.1.





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CONCLUSION

A speckle denoising technique using the improved soft thresholding was presented in this paper. At first the speckle noise which is a multiplicative noise was converted in to additive noise by using the logarithmic procedure. Further, multiresolution was applied to the image and resulted wavelet coefficients were modeled using Cauchy PDF and Gaussian PDF to get the signal and noise parameters. The threshold value was calculated from these information and further, the wavelet coefficients were soft thresholded to obtain the updated wavelet coefficients. From the comparison results it is seen that the proposed improved soft thresholding technique obtained better result than the state-of-the-art methods. In future the proposed method may be applied for other imaging modalities to enhance the diagnosis results.

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Table 1.PSNR (dB) Parameter comparison for different methods

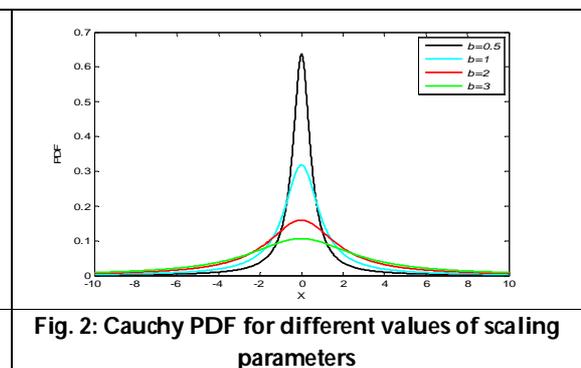
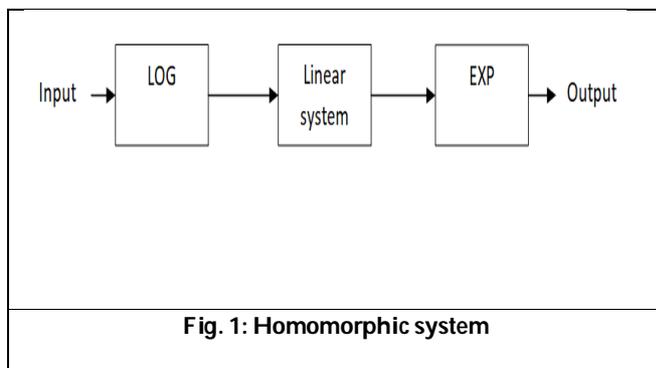
| Noise Standard deviation | Donoho's Soft threshold [16] | Zaki et al. [13] | Proposed Method |
|--------------------------|------------------------------|------------------|-----------------|
| 0.1 | 27.66 | 28.34 | 30.88 |
| 0.2 | 26.39 | 27.05 | 29.32 |
| 0.3 | 25.82 | 26.98 | 28.66 |
| 0.4 | 24.55 | 25.77 | 27.52 |

Table 2. SSIM Parameter comparison for different methods

| Noise Standard deviation | Donoho's Soft threshold [16] | Zaki et al. [13] | Proposed Method |
|--------------------------|------------------------------|------------------|-----------------|
| 0.1 | 0.943 | 0.958 | 0.965 |
| 0.2 | 0.863 | 0.903 | 0.954 |
| 0.3 | 0.825 | 0.862 | 0.930 |
| 0.4 | 0.739 | 0.840 | 0.906 |

Table 3. EPI Parameter comparison for different methods

| Noise Standard deviation | Donoho's Soft threshold [16] | Zaki et al. [13] | Proposed Method |
|--------------------------|------------------------------|------------------|-----------------|
| 0.1 | 0.872 | 0.883 | 0.901 |
| 0.2 | 0.856 | 0.871 | 0.892 |
| 0.3 | 0.821 | 0.834 | 0.886 |
| 0.4 | 0.808 | 0.825 | 0.875 |





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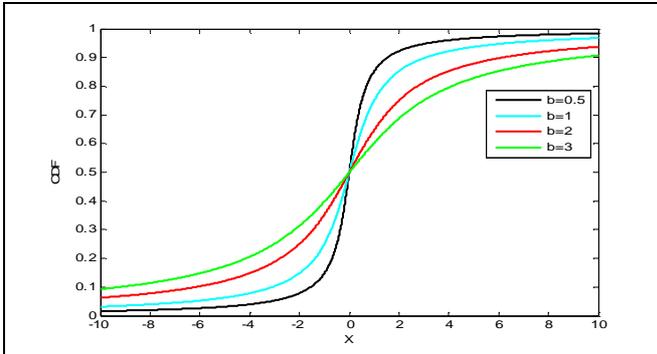


Fig. 3: Cauchy CDF for different values of scaling parameters

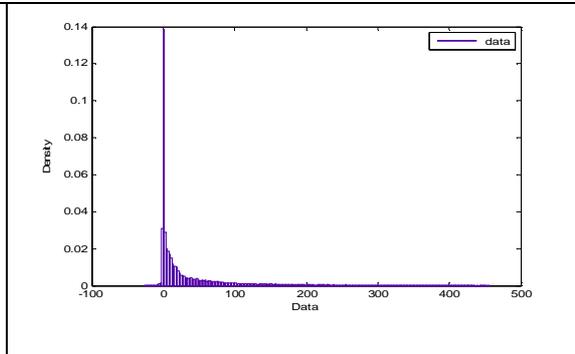


Fig. 4: Wavelet coefficient distribution in approximation sub-band at level-1

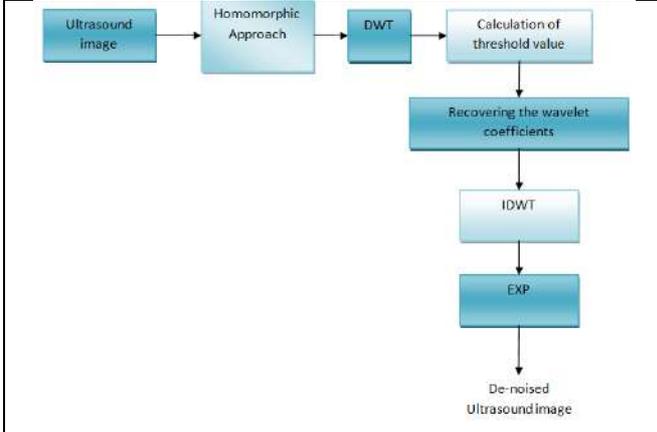


Fig. 5: Block diagram of the proposed method

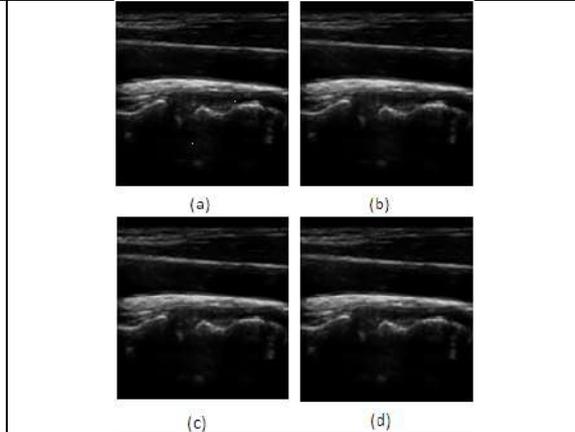


Fig. 6: De-noising performance (a) Original image (b) Donoho's Soft threshold method [19] (c) denoising result with Zaki et al. [10] (d) proposed method.





A Comprehensive Review on Nanoemulsion Drug Delivery System

Rachana G Y^{1*} and Prakash s Goudanavar²

¹M Pharma, Department of Pharmaceutics , Adichunchanagiri College of Pharmacy , B G Nagar, Karnataka, India.

²Department of Pharmaceutics , Adichunchanagiri College of Pharmacy , B G Nagar, Karnataka, India.

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*Address for Correspondence

Rachana G Y

M Pharma,
Department of Pharmaceutics ,
Adichunchanagiri College of Pharmacy ,
B G Nagar, Karnataka, India.
E.Mail: Rachanagy7773@gmail.com



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ABSTRACT

The design of effective formulations for drugs is a long-standing difficulty. Instability or low solubility in the vehicle can significantly limit drug efficacy. The nanoemulsion drug delivery technology, which is being used to boost the bioavailability and solubility of lipophilic medicines, is one of the most promising technological advancements. Recent interest in lipid-based drug delivery (LBDD) is linked to advancements from the past 10 to 15 years. Such a medication delivery method boosts solubility and lymphatic transport, which increases bioavailability. Nanoemulsions are excellent pharmaceutical drug delivery devices because of their great stability, clarity, and simplicity of production. The nanoemulsion is the carrier that has attracted the greatest attention. The advantages and disadvantages of nanoemulsion are discussed in this article, along with its preparation techniques and therapeutic uses. The literature study makes it obvious that studies on nanoemulsion systems containing different medications for different therapeutic applications has accelerated quickly. As a result, it could be used as a novel, less affordable carrier in a therapy to boost bioavailability while lowering dose-related systemic toxicities.

Keywords: Nanoemulsion, Aqueous phase, Oil phase



**Rachana et al.,**

INTRODUCTION

Nanoemulsion are defined as the thermodynamically stable, isotonic translucent dispersions of water and oil with droplet sizes between 20 and 500 nm.[1] Researchers have become interested in these formulations because of their simplicity in production and scaling, stability, and enhanced bioavailability.[2]

Nanoemulsion benefits are follows:

- It is possible to increase the bioavailability and water solubility of lipophilic drugs by using a nanoemulsion.[3]
- Using nanoemulsions can improve the consistency of data on plasma concentration and bioavailability of medications.[4]
- Fine oil droplets assist the drug to be distributed widely throughout the digestive tract and hasten stomach emptying, which lessens the discomfort that results from the drug's prolonged contact with the gut wall.[5]
- Nanoemulsions can be created with the least amount of energy (heat or mixing), can last longer than standard micellar solutions, and have a better solubilization capacity.[6]
- Moreover, they provide extensive open interfacial zones and exceptionally low interfacial tension. Additionally, they offer benefits in terms of speed of action over current self-emulsifying devices. Excellent kinematic stability and optical transmittance may be features of nanoemulsions.[7]
- Because the features in most nanoemulsions are substantially smaller than the visible wavelength, they seem optically clear even under high loading.[8,9]
- To get high shear, one typically uses a microfluidic or ultrasonic method, which shrinks the particle size to the nanoscale [10].

Classification

There are three main forms of nanoemulsions, depending on the proportion of the water and oil components.

- a) Oil in water (O/W) nanoemulsions.
- b) Water in oil (W/O) nanoemulsions.
- c) Bi-continuous nanoemulsions.[11]

Major Components of Nanoemulsion

Oil

The selection of a suitable oily phase, particularly in O/W nanoemulsions, has a major influence on the choice of other nanoemulsion ingredients. The oils with the finest solubilizing qualities for the selected new medicine is often utilised while creating nanoemulsions. This makes it simpler to pack the most particles into the nanoemulsions. medicament possible. Triglycerides, which naturally occur in Oils and fats are blends of fatty acids with various unsaturation degrees and chain lengths. As having a group shown, triglycerides are categorised (12 carbons), and to lower the quantity of unsaturation and increase their resistance to oxidative deterioration, they can be synthetic hydrogenated. The capacity of the drugs to dissolve is typically balanced while using an oily phase.[12]

Surfactants

The surfactant should assist in the micro emulsification of the viscous phase and should be effective in solubilizing the hydrophobic drug molecules. The surfactant of choice affects how the nanoemulsion is created. The hydrophilic surfactant polysorbate 80, with an O/W nanoemulsion is created with an HLB value of 10. A mix of hydrophilic (high HLB) and lipophilic (low HLB) surfactants may be required to produce a nanoemulsion.[13] Surfactants in fluids above their minimum micellar concentration (CMC) boost the solubility of pharmaceuticals by generating spaces in solutions for hydrophobic drug interactions. It becomes more liquid because the hydrophobic centre improves drug trapping. The surfactant assembles at the water/ oil interface in the presence of abundant oil to generate emulsions that allow the medication to breakdown in the inside layer of oil. As opposed to that, small surfactants particles with trapped oil occur when the liquid level is very low; they are referred to as nanoemulsions.[14]





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Co-surfactant

The improved drug trapping by the hydrophobic core increases its solubility. If there is an abundance of oil present, the medication may breakdown in the inner oil phase due to surfactant concentrations that induce emulsions to form at contact between oil and water. Yet, when the oil concentration is very low, the creation of nanoemulsionstiny surfactant droplets containing trapped oil occurs. To change the system's overall HLB, it is common to combine a cosurfactant with a very low HLB and a surfactant with a high HLB. The cosurfactant might not have the same independent ability to form micelles or even other self-associated structures as the surfactant does. It is known that hydrophilic cosurfactants, especially those containing types of alcohol with intermediary long - chain such hexanol, pentanol might lessen the contact between the oil and the water.[15]

Co-solvents

For the best SMEDDS, surfactants must normally be present in quantities more than 30% weight-per-weight. Organic solvents including ethanol, glycerol, and propylene glycol (PG) are appropriate for oral delivery since they co-solvently dissolve significant amounts of the hydrophilic surfactants increasing the environment's hydrophobicity by lowering water's dielectric constant. traditional SMEDDS may have alcohols and other combustible co-solvents that vaporise, precipitating the drug into the flexible or rigid gelatine capsules. Because of this, alcohol-free preparations have been made.[16]

Aqueous phase

The aqueous phase's Characteristics influence the stability and dispersion of the nanoemulsion. Hence, when creating a nanoemulsion, the pH and ionic composition of the aqueous phase should be considered. The physiological environment has a pH between 1.2 and 7.4, which is equivalent to the stomach's pH. The existence of different ions in the biological conditions can also have a big impact on how nanoemulsions behave. The stability and water content of nanoemulsions can both be influenced by electrolytes, as is well known. To assess the attributes of the resulting nanoemulsion in aqueous phases, it is therefore best to use changes in pH and/or ionic strength.[17]

Methods of Preparation of Nanoemulsion

The FDA has classified the common food ingredients that are used to make nanoemulsions as "Generally Considered to be safe These emulsions are made in large amounts by mixing an aqueous phase with a liquid oil phase utilising a mechanical extrusion technique under high stress that is readily available. High-pressure equipment is ideal for producing nanoemulsions since they have very small particle sizes. The carefully consider the best preparation technique. selected according on the desired formulation since the stability is significantly impacted by the distribution of droplet sizes characteristic of the nanoemulsion. techniques for nanoemulsion preparation that are high and low energy are given below [18].

High energy emulsification method:

Oil in water nanoemulsions with a 20% oil content can be made using this method. With the significant increase in interfacial area brought on by the homogenisation process and the correlation between droplet diameter diameters and oil content, surface-active chemicals are only present in emulsions with high oil concentrations. Furthermore, the most the shear-behaviours of oil-concentrated emulsions when they thin is caused by the creation of droplet clusters, which ultimately leads to an increase in droplet size [19].

High pressurized homogenization method.

In this condition, an aqueous solution, an oil phase, and surfactants are involved and under great pressure. The valve lets the microparticles through first slowly. Thus, the positive-displacement pump generates the pressure while also generating a flow rate that is essentially constant. In between valve and seat, a fast-moving liquid with small particles flows. As speed rises, pressure falls. A homogenised nano emulsion is the fluid's final discharge.[20]



**Rachana et al.,****Micro fluidization**

A microfluidizer is a piece of apparatus used in the unusual mixing procedure known as micro fluidization. The substance is forced into the air compartment. of a positively displacement (PD) pump, which is made of small channels known as "microchannels." (500 to 20000 psi). The product passes through the micro - channels as well as the area of impingement, creating very small particles that are smaller than a micron in size.

Method

To create a thick emulsion the two solutions' hydrophilic and oily phases are combined and passed through a line-based homogenizer. The rougher emulsion is further transformed into a steady nanoemulsion using a microfluidizer. The fine emulsified is constantly driven into the detection until the necessary particle size is attained, the chamber of the microfluidizer. [21,22]

Ultrasonication:

Methods of ultrasonication rely on ultrasound waves (20 kHz and up). you can use them to shrink a pre-formed emulsion or create a nanoemulsion on-site. A piezoelectric probe used in benchtop sonicators produces a strong disruptive force at its tip [23] Cavitation bubbles are created when ultrasonic vibrations are applied to a sample, and they expand until they explode. This implosion generates shock waves, which then cause a jet stream to form in the surrounding liquid, pressurising the dispersed droplets and causing them to condense in size.[24] The results of an analysis of the operational parameters show that as the sonication time and input power are increased, the droplet size decreases. The functionality of the probes in an ultrasonicator depends on their accessible dimensions. It should not touch any solid surfaces since the relative positioning of the probe in the sample, changes the pattern of wave reflections and pressure distribution. A coarse emulsion is made procedurally by adding a while physical stirring is being applied, a homogenous oil phase to aqueous solution will transition. Afterwards, in order to obtain the necessary qualities for nanoemulsion, the emulsion is exposed to ultrasonication at converter for brief time cycles. Comparing ultrasonication to certain other high energy procedures, it utilises the least energy. One significant drawback of this method is contamination brought on by the probe. Professional homogenizers based on sonication have been created for scale-up application, using nanoemulsion to flow through a specific column capable of creating ultrasonic waves.[25]

*Low Energy Emulsification Methods***Phase Inversion Temperature**

By using chemical energy from phase changes that occur along the emulsification path, this approach produces fine dispersion. The proper phase transitions are achieved using the phase inversion temperature (PIT) approach, which is based on variations in the solubility of polyoxymethylene-type surfactant with temperature[26] As the temperature rises, the dehydration of the polymer chain causes this surfactant to become lipophilic. However, at low temperatures, the surfactant monolayer exhibits a significant positive spontaneous curvature that gives rise to an oil-swollen micellar solution phase [27].

Spontaneous Emulsification

Three steps are involved in the production of emulsions during spontaneous emulsification. combining oil and a lipophilic surfactant to produce an even organic solution in a fluid that is water soluble. In order to establish the homogenous aqueous phase, moisture combined with a hydrophilic surfactant The o/w emulsion was quickly produced by penetrating of external aqueous layer of the organic solvent, which resulted in the development of nanodroplets, after the organic phase had been injected into the water while being stirred magnetically. To allow the system time to settle, the magnetic stirring continued for an additional 30 minutes. The liquid solvent vaporized completely under reduced pressure in 45 minutes. Oil nanodroplets were mixed with a hydrophilic surfactant in a hydrophilic water solution.



**Rachana et al.,****Method for Solvent Displacement**

The oil phase is combined with the surfactant- and co-surfactant-containing water phase. After the oil phase has been dispersed in a solvent such as acetone or ethanol. The reason being that it occurs naturally at room temperature, this procedure requires no energy. The viscosity, Surfactant type, and increased concentration of the emulsion are the defined criteria that must be accomplished in order to obtain the required nanoemulsion. [28,29]

Characterization of nanoemulsion**Droplet Size Analysis**

The droplet size distribution of a nano-emulsion, one of the key physicochemical characteristics, can be measured by diffusing to use a Coulter LS-2 light-scattering analyser for particle sizes. [30,31] The scattering of laser light by the particles is used to determine the size and distribution of the particles.

Polydispersity Index

The samples' The average length and polydispersity index were determined by photon correlation spectroscopy. Testing with a He-Ne laser was carried out at 25 °C.

Viscosity

The thickness of the nanoemulsion was measured using 1 ml of the formulation. At 37.0°C for 10 minutes, a flow stress of 100 s1 can be applied while the spindle speed was set to 100 rpm. The viscosity of the modified nanoemulsion was measured.

Refractive Index

The index of refraction (n) of the medium indicates the connection between the wave's phase speed in the media and its speed (c) in a standard medium.

Zeta potential

It is a technique used to assess the physical stability over time of nanoemulsions as well as the surface charge properties. The surface charge is measured using a device called the Zeta PALS. The studies used dilute nanoemulsion compositions 16, and the values were computed utilising the electrophoretic movement of the oil droplets. Zeta potentials of at least 20 mv are desirable.[32]

Drug Content

Using the C18 column 15 and a reverse phase HPLC technique, drug content was assessed.[33]

Percent Transmittance

The UV-VIS Absorption spectrum 17 was utilized to assess the % transmittance spectrophotometrically. of the created nanoemulsion formulations [34].

Application of nanoemulsion in various medication delivery system

Nanoemulsion, which is a proven effective medication delivery method, circumvents the drawbacks of conventional methods. Using oral, topical, and other medication administration methods can present several difficulties that the nanoemulsification procedures can exactly remove. Medicines now function more effectively in nanoemulsions due to extended retention periods. Nanoemulsion antimicrobial medications can quickly eradicate microorganisms by breaking through their cell walls [35].

Oral delivery

Nanoemulsion has the potential to significantly alter oral medication delivery systems. The conventional systems' numerous shortcomings have been resolved. When creating oral dosage forms, consideration was given to the drug's solubility, absorption rate, and targeted administration of the drug. The development of nano emulsified drug delivery techniques has made it possible to solve each of these issues in a single step. Drugs with the transmittance

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was measured spectrophotometrically using the UV-VIS absorbance spectrum ensuring better solubility. This is possible because the particles in these nanoemulsions are extremely small and contain both hydrophilic and lipophilic units. A high rate of medication absorption is caused by these tiny particles' ease with which they can bypass the epithelial layer [36].

Percutaneous Route

on drug entrapment in submicron sized droplets of o/w emulsion is more effective and may have no unfavourable side effects. The common chemical skin penetration boosters known as organic solvents, on the other hand, are usually connected to skin toxicity, hypersensitivity, and irritation. In addition, the unique huge domestic hydrophobic core of o/w sub micronized droplet size enables great aspects of sustainability for topically active water-insoluble drugs and helps in having to deliver water, an outstanding softener, to the epidermis, such as NSAIDs, diazepam, drugs [37].

Nasal Route

Due to its several benefits over intravenous and oral delivery, particularly liver bypass, the nasal approach has drawn a lot of interest. To boost absorption, for instances, a lipid-soluble rennin-inhibitor was given to an o/w emulsion. This solubilized the medicine in the emulsion's inner phase and prolonged the duration that the emulsion droplets were in contact with the nasal mucosa, hence improving absorption. In vivo testing has shown that nasal absorption by emulsion is more increased and extended than nasal absorption by water solution. other medications that can be delivered by the nose include insulin and testosterone. [38,39]

Use of Nanoemulsions in Cosmetics

Nanoemulsions have recently garnered more significance for the optimal distribution of active substances in specific skin layers and as potential aesthetic delivery systems. Considering that nanoemulsions are naturally lipophilic, they for the transportation of lipophilic substances, are preferred to liposomes. The enveloped viruses E. coli, benefit from them in a manner like liposomes.[40]

Antimicrobial Nanoemulsions

The size range of antimicrobial oil-in-water emulsions is 200-600 nm. They are made of water and oil, and substances that have antibacterial properties like alcohol and surfactants keep them stable. The sprouting spores are susceptible to the nanoemulsion's antibacterial characteristics because of its broad spectrum. The toxicity of the nanoemulsions to bacteria at doses that do not irritate skin or mucous membranes makes them unusual. There is a safety buffer since each even while a nanoemulsion droplet contains very little detergent, when combined, they have enough power and surfactant to select the pathogens that need to be killed, while protecting healthy cells. presently, the nanoemulsion can perform topical antibacterial activities at a rate.

Optical delivery

The pharmacological effects and bioavailability of Lacrimal secretion limits the effectiveness of popular eye drops used to give ophthalmic drugs. and mucous membrane drainage. Most of the medication travels to the digestive system through the tear-draining nasolacrimal duct. After that, it may be absorbed, with sometimes unfavourable results. This problem must be resolved by leaving the drug on the eye for a longer period.^[41]

CONCLUSION

The topic of modern pharmaceutical sciences research has been nanoemulsion. because they can overcome Since they are greater stable than other emulsifiers and because they have fewer restrictions than traditional pharmaceutical therapy, nanoemulsions are currently being studied for compatibility with a few drug formulations. Nanoemulsions can be produced by scientists utilising a variety of techniques. Nanoemulsification-based nanoparticles, nanocarriers, and nanocapsules are also currently being developed. By using nanoemulsions,



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physiological membrane piercing becomes easier. By using nanoemulsification, a wide spectrum of drugs with considerably lower dosages, targeted distribution, higher local concentrations, and less side effects could be produced. In fact, the hurdles to creating more specialised and targeted drug delivery are being removed by nanoemulsions. As a result, it is quickly becoming a popular formulation option and has started a new era in drug therapy.

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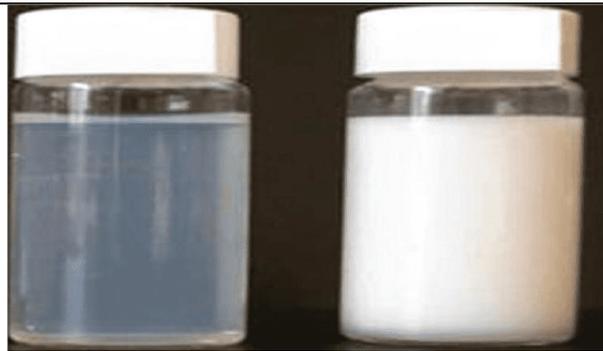


Figure 1. Nanoemulsion size 35nm and microemulsion size 1 µm.[10]

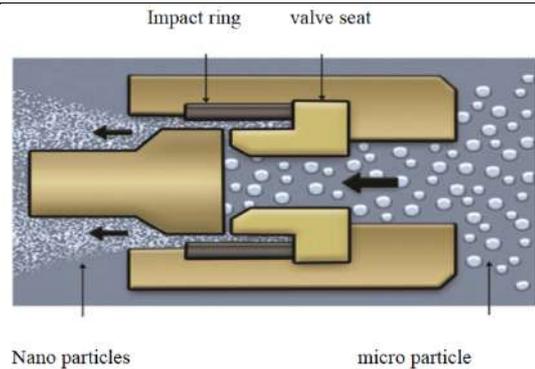


Figure 2 High pressure homogenizer. [20]

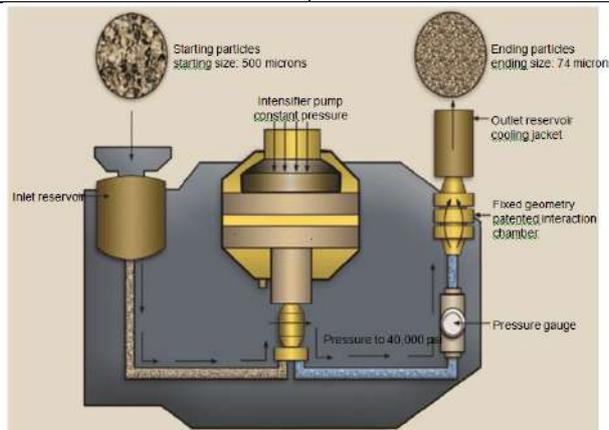


Figure 3 Microfluidizer [22]





Adoption of Electric Vehicles: A Review of Technology, Transformation and Policies

Mudasir Ahmad Tass^{1*}, Nisar Ahmad Kakroo² and Iqbal Ahmad Hakim³

¹Research Scholar, Department of Management Studies, University of Kashmir, Sri Nagar, Jammu and Kashmir-190006, India

²ICSSR- Doctoral fellow, Department of THLS, University of Kashmir, Sri Nagar, Jammu and Kashmir-190006, India.

³Professor, Department of Management Studies, University of Kashmir, Sri Nagar, Jammu and Kashmir - 190006, India.

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*Address for Correspondence

Mudasir Ahmad Tass

Research Scholar,
Department of Management Studies,
University of Kashmir, Sri Nagar,
Jammu and Kashmir-190006, India
E.Mail: sahil.tass@gmail.com



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ABSTRACT

The adoption of electric vehicles (EVs) is considered a promising solution to address the negative impacts of conventional vehicles on the environment and human health. This paper provides a comprehensive review of the current state of EVs, including their types, technology, adoption, government policies, environmental impact, and future prospects. The review reveals that EVs have the potential to significantly reduce air pollution, greenhouse gas emissions, and noise pollution. However, their adoption has been hindered by various factors such as perceived usefulness, ease of use, and risk, which can be addressed through policy interventions and infrastructure development. The paper highlights the significance of the Faster Adoption and Manufacturing of Electric Vehicles (FAME) scheme in India and other international policies and initiatives to support the adoption of EVs. The review also identifies the technological advances and battery development as promising opportunities for the future of EVs. The paper concludes by providing implications for policy and practice, including the need for incentives and infrastructure development to promote EV adoption and recommends further research on the consumer trends and challenges in the adoption of EVs.

Keywords: Electric vehicles, technology acceptance model, FAME, environmental impact, battery development, sustainability.





INTRODUCTION

India has one of the world's fastest-growing economies, a rapidly expanding population, and rising transportation demand. However, the road transport industry contributes significantly to the nation's greenhouse gas emissions, with over 23% of the total emissions coming from this sector (Agency, 2021). India has been actively encouraging the use of electric vehicles (EVs) as a way to combat this problem and reduce both the nation's reliance on fossil fuels and air pollution (MOEF&CC, 2019). The Faster Adoption and Manufacturing of Electric Vehicles (FAME) scheme, which provides financial incentives to electric vehicle manufacturers and consumers, is one of the policy efforts the Indian government has undertaken in order to accelerate the adoption of EVs (Industries, 2020). As a result, the number of EVs on Indian roads has increased significantly in recent years, with the total number of EVs sold in India in 2021 reaching approximately 6.8 lakh units, a 140% increase from the previous year (Singh & Singh, AditiSpecialist, 2023). Despite this growth, the adoption of EVs in India remains low compared to traditional gasoline-powered vehicles. The Technology Acceptance Model (TAM) states that perceived usefulness, perceived ease of use, and perceived risk are key determinants of the intention of accepting new technologies (Davis, 1989). Therefore, it is crucial to fully understand the factors influencing EV adoption in India and to find strategies for boosting awareness among consumers.

This paper aims to provide a comprehensive review of the technology, transformation, and policies related to the adoption of EVs in India. The paper draws on a range of literature, including academic articles, government reports, and industry publications. The literature review covers both national and international research to provide a comprehensive understanding of the adoption of EVs in India. The paper is structured as follows: Section II provides a summary of different types of EVs and the technology behind them. Section III discusses the adoption of EVs and the factors influencing their adoption, including knowledge about EVs, perceived usefulness, perceived ease of use, and perceived risk. Section IV examines the government policies and incentives in India that support the adoption of EVs, including FAME scheme, rebates, and infrastructure development. Section V discusses the environmental benefits of EVs, including air pollution reduction, greenhouse gas emissions reduction, and noise pollution reduction. Section VI explores the future of EVs in India, including technological advances, battery development, consumer trends, and challenges and opportunities. Finally, Section VII provides a summary of the key findings and implications for policy and practice.

The primary objective of this paper is to offer a comprehensive analysis of the present status of electric vehicle (EV) adoption in India. Through an in-depth review the study aims to add to the existing body of literature by identifying important research gaps and providing viable areas for further investigation in this field.

Literature Review

The adoption of EVs is influenced by various factors, including technology, consumer behavior, government policies, and infrastructure. In this literature review, we will examine the existing research on these factors and identify the gaps that need to be addressed.

Technology

The Technology Acceptance Model (TAM) is a widely accepted framework for understanding consumer adoption intention towards new technologies (Davis, 1989), including EVs. Several studies have used TAM to investigate EV adoption intention in various contexts. For instance, a study conducted by (Adu-Gyamfi *et al.*, 2022) found that perceived usefulness, perceived ease of use, and social influence significantly influenced consumer adoption intention towards EVs.

Consumer Behavior

The inclination towards EVs is also significantly influenced by consumer behaviour. Some studies have investigated the factors that influence consumer behavior towards EVs. For instance, a study conducted by (Karagoz *et al.*, 2020)





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in Iran found that environmental concern, perceived benefits, and social influence significantly influenced consumer behavior towards EVs. Another study conducted by (Yang & Lee, 2022) in China found that perceived value, perceived quality, and brand image significantly influenced consumer behavior towards EVs.

Government Policies

Regulations by the government are essential in promoting the use of EVs. In order to promote the use of EVs, the number of countries have enacted policies including tax incentives, subsidies, and infrastructure development. For instance, to encourage the use of EVs, the Indian government introduced the Faster Adoption and Manufacturing of Electric Vehicles (FAME) scheme in 2015 (Ministry of Heavy Industries and Public Enterprises, 2019). The scheme provides financial incentives to manufacturers and buyers of EVs. Similarly, several other countries have implemented similar policies to encourage the adoption of EVs.

Infrastructure

Another significant aspect that affects EV adoption is infrastructure. For EV adoption, the availability of charging stations is essential. One of the biggest obstacles to the adoption of EVs is the absence of charging infrastructure. Several research have looked into the connection between EV adoption and the accessibility of charging infrastructure. For instance, a study conducted in the Netherlands by (Sierchula et al., 2014) discovered that the accessibility of charging infrastructure has a considerable impact on EV adoption.

Gaps in the Literature

While several studies have investigated the factors that influence EV adoption intention, the literature still has multiple gaps that need to be addressed. One such gap is the absence of research in the Indian context. India is a unique context due to reasons such as lack of charging infrastructure, low awareness among consumers, and costly upfront expenditures. Thus, it is important to conduct research in the Indian context to understand the factors that influence EV adoption intention and develop strategies to overcome the barriers to adoption.

Significance & Relevance

The adoption of EVs has the potential to address several environmental and economic challenges facing the world. The transition to EVs can dramatically cut greenhouse gas emissions, resulting in a major contribution to the transportation sector. Adoption of EVs can also lessen reliance on fossil fuels and improve energy security. Economic opportunities in the industrial and service sectors may arise as a result of the adoption of EVs. The adoption of EVs is not without obstacles, either. Some of the obstacles to acceptance are the high upfront prices, shorter driving distances, and a lack of charging infrastructure. It takes a thorough grasp of the variables influencing EV adoption intention to get over these obstacles. The existing literature has identified several factors, including technology, consumer behavior, government policies, and infrastructure, that influence EV adoption intention. Nevertheless, there are still gaps in the literature that must be filled, especially in the context of India.

The government of Indian has implemented several policies, including the FAME scheme, to encourage the adoption of EVs. However, the impact of these policies on EV adoption intention is yet to be fully understood. Consequently, conducting research within the Indian context holds significant importance in comprehending the factors impacting EV adoption intention and formulating strategies to address adoption barriers. The adoption of EVs has the potential to address several environmental and economic challenges facing the world. However, the adoption of EVs is not without its challenges, and overcoming these challenges requires a comprehensive understanding of the aspects that impact EV adoption intention. Conducting research in the Indian context is crucial to understand the issues that affect EV adoption intention and develop policies to encourage the adoption of EVs in India.

Objective

This paper's main goal is to provide a thorough evaluation of the research on the factors that affect EV adoption, with particular focus on the Indian context. The paper aims to identify the factors that influence EV adoption intention and highlight the gaps in the literature that need to be addressed.





METHODOLOGY

In this paper, we present a literature review on our topic of interest. A comprehensive review of relevant studies was conducted using multiple academic databases, including Scopus, Web of Science, and Google Scholar. We aim to provide an up-to-date analysis and synthesis of current knowledge and findings in the field in this review paper. The search have included articles published between 2010 and 2023. The search terms include a combination of the following keywords: "electric vehicle," "plug-in electric vehicle," "battery electric vehicle," "EV adoption," "technology acceptance model," "consumer behavior," "government policies," "infrastructure," "FAME scheme," "charging station," "range anxiety," and "Indian context." The inclusion criteria for the papers include publication in journals and relevance to the factors influencing EV adoption intention in the Indian context. Papers that are non-English, as well as conference proceedings and presentations are excluded.

RESULTS AND DISCUSSIONS

Electric vehicles (EVs) have been in advance fame as an alternative to conventional gasoline-powered automobiles due to their potential to decrease greenhouse gas emissions, improve air quality, and promote energy security. In this section, we will provide an outline of the different types of EVs and the technology behind them.

Overview of Electric Vehicles

Types of Electric Vehicles

Plug-in electric vehicles (PEVs), battery electric vehicles (BEVs), and hybrid electric vehicles (HEVs) are the three primary categories of EVs.

Plug-in Electric Vehicles (PEVs)

PEVs are vehicles that primarily run on electricity and include an internal rechargeable battery that can be charged by connecting the car to an external power source. Plug-in hybrid electric vehicles (PHEVs) and extended-range electric vehicles (EREVs) are the two categories of PEVs. PHEVs have equally an electric motor and a gasoline-powered engine. They can function in electric-only mode for a limited range, after which the gasoline engine will start to power the vehicle. EREVs, on the other hand, have a larger battery than PHEVs and can operate solely on electric power for a longer range. Once the battery is depleted, a gasoline-powered generator will kick in to power the vehicle (Chakraborty et al., 2022).

Battery Electric Vehicles (BEVs)

BEVs are vehicles that rely solely on electricity as their source of power. A rechargeable battery fuels an electric motor that powers them. BEVs don't have internal combustion engines and don't emit any pollution at all. They have a limited range, typically between 100-300 miles, before requiring a recharge (Wicki et al., 2023).

Hybrid Electric Vehicles (HEVs)

HEVs combine an internal combustion engine with an electric motor. The engine receives additional power from the electric motor, which lowers pollution and consumption of fuel. HEVs rely on regenerative braking for recharging their batteries because they cannot be plugged in (Karoń, 2022).

Technology behind Electric Vehicles

Charging Stations and Charging Time

Charging stations are essential for EVs, as they provide a place to recharge the battery. There are three levels of charging: Level 1 (120-volt AC), Level 2 (240-volt AC), and Level 3 (DC fast charging). Level 1 and 2 charging can be done at home or at public charging stations and typically take several hours to fully recharge the battery. Level 3 charging can charge the battery up to 80% in 30 minutes, making it ideal for longer trips (Muratori et al., 2021).





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Range and Battery Life

Range anxiety is a common concern among potential EV buyers, as the range of EVs is typically lower than that of gasoline-powered vehicles. However, advances in battery technology have increased the range of BEVs, with some models having a range of over 300 miles. Battery life is also a concern, as the cost of replacing the battery can be significant. However, the lifespan of EV batteries has been increasing, with many manufacturers offering warranties of up to 8 years (Miao *et al.*, 2019).

Environmental Benefits

EVs have the capability to decrease greenhouse gas emissions and enhance air quality. Studies have shown that the total emissions from EVs are lower than those from gasoline-powered vehicles, even when considering the emissions from power plants that generate the electricity used to charge the EVs. Additionally, EVs produce no tailpipe emissions, which can significantly improve air quality in urban areas (Z. Xia *et al.*, 2022). The different types of EVs and the technology behind them provide consumers with a range of options to choose from. Advances in battery technology and charging infrastructure are making EVs a more viable option for consumers. Moreover, the battery life of EVs has also improved significantly with technological advancements. Lithium-ion batteries are the most common type of batteries used in EVs, and they have a lifespan of about 10-15 years, which is equivalent to the lifespan of a typical gasoline-powered car (Miao *et al.*, 2019). Additionally, the cost of lithium-ion batteries has decreased by 85% since 2010, making EVs more affordable for consumers (Colin McKerracher, 2020).

One of the main advantages of EVs is their environmental benefits. EVs emit less greenhouse gases compared to gasoline-powered cars, which makes them a more sustainable alternative to traditional vehicles (Colin McKerracher, 2020). Furthermore, EVs have no tailpipe emissions, which helps to reduce air pollution in urban areas (Gandoman *et al.*, 2019). The widespread adoption of EVs could have significant impacts on reducing air pollution, particularly in densely populated areas.

EVs have derive a long way since their inception, and progressions in technology have made them a more viable alternative to traditional gasoline-powered cars. The different types of EVs available, such as PEVs, BEVs, and HEVs, offer consumers a range of options depending on their needs. The technology behind EVs, including charging stations, battery life, and environmental benefits, has improved significantly, making EVs more attractive to consumers. As a result, the adoption of EVs has the potential to decrease air pollution and greenhouse gas emissions, making them an important part of the transition towards a more sustainable transportation system.

Adoption of Electric Vehicles

An essential step in creating a sustainable transportation system is the adoption of electric vehicles (EVs). However, there are a number of reasons why the adoption of EVs has been so delayed. The Technology Acceptance Model (TAM) is a widely recognized framework employed to comprehend the adoption of new technologies, including EVs. The factors influencing EV adoption within the TAM framework were proposed by (Davis, 1989). Perceived usefulness states the degree to which a technology is believed to be useful in attaining a particular goal or task (Davis, 1989). In the case of EVs, perceived usefulness can be related to factors such as cost savings on fuel and maintenance, reduced environmental impact, and improved performance (Cui *et al.*, 2023). Studies have found that perceived usefulness is positively related to the intention to adopt EVs (Bryła *et al.*, 2023). Therefore, it is important for manufacturers and policymakers to promote the usefulness of EVs to increase their adoption.

Perceived ease of use states the degree to which a technology is supposed to be easy to use (Davis, 1989). In the case of EVs, perceived ease of use can be related to factors such as the availability of charging infrastructure and the ease of charging (Cui *et al.*, 2023). Therefore, it is important for manufacturers and policymakers to make EVs and their charging infrastructure more accessible and user-friendly. Perceived risk refers to the potential negative consequences associated with the adoption of a technology (Davis, 1989). In the case of EVs, perceived risk can be related to aspects such as the range concern, battery life, and the accessibility of charging infrastructure (Cui *et al.*, 2023). Studies have found that perceived risk is negatively associated to the intent to adopt EVs (Bryła *et al.*, 2023). Therefore, it is important for manufacturers and policymakers to address these perceived risks and concerns to



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increase the adoption of EVs. Various factors can affect the adoption of EVs. Studies have identified factors such as demographic characteristics, financial incentives, and government policies as important factors that influence the adoption of EVs (Bryła et al., 2023; Cui et al., 2023; Gandoman et al., 2019). Additionally, the accessibility of charging infrastructure, the choice of EVs, and the cost of EVs also play an important role in the adoption of EVs (Colin McKerracher, 2020). In order to enhance the adoption of EVs, it is crucial for manufacturers and policymakers to take these considerations into account.

Government Policies and Incentives

Government regulations and incentives play a big role in determining whether electric vehicles are adopted. The Indian government has launched a number of programmes to encourage the usage of electric vehicles nationwide. The Faster Adoption and Manufacturing of Electric Vehicles (FAME) scheme, one of the key initiatives, was introduced in 2015 with the goal of encouraging the production and uptake of electric vehicles in India (GOI, 2020). The government offers financial incentives to both consumers and manufacturers of electric vehicles under this scheme.

Governments employ incentives and rebates as essential policy tools to support the adoption of electric vehicles. Incentives offered by the Indian government to consumers of electric vehicles include reduced taxes, lower registration costs, and subsidies for the purchase of electric vehicles (GOI, 2020). Additionally, a number of Indian state governments offer further incentives for the purchase and use of electric vehicles (Aayog, 2023). Building up the infrastructure is essential for the widespread use of electric vehicles. The Indian government has taken a number of actions to encourage the growth of the nation's infrastructure for electric vehicle charging. The government offers financial support to states under the FAME programme for the construction of a charging infrastructure. The government has also set a goal of putting in 2.7 million charging stations nationwide by 2030 (GOI, 2020).

International policies and initiatives also have a significant role in promoting the adoption of electric vehicles. India signed the Paris Agreement in 2016, which aims to limit the increase in global temperature to well below 2 degrees Celsius within pre-industrial levels (United Nations, 2021). To achieve this, India has set a target of achieving 30% electric vehicle adoption by 2030 (GOI, 2020). India has also joined the International Solar Alliance, which intends to encourage the use of solar energy globally (International Solar Alliance (ISA), 2022). The government incentives and policies play an important part in promoting the adoption of electric vehicles. The FAME programme, rebates and government subsidies, as well as infrastructure development, are just a few of the measures that the Indian government has done to promote the production and usage of electric vehicles. International policies and initiatives, such as the Paris Agreement and the International Solar Alliance, also play a significant role in promoting the adoption of electric vehicles.

Impact of Electric Vehicles on Pollution and Environment

Electric vehicles have become an attractive alternative to traditional vehicles due to their potential environmental benefits. The growing concern about air pollution and climate change has led to an increase in research on the impact of electric vehicles on pollution and the environment.

- **Air Pollution Reduction:** Electric vehicles have the potential to reduce air pollution in urban areas. Studies have revealed that as compared to gasoline and diesel cars, electric vehicles produce significantly less nitrogen oxides (NOx), particulate matter (PM), and volatile organic compounds (VOCs) into the atmosphere (Palit et al., 2022). Electric vehicles also do not emit greenhouse gases (GHGs) during operation, which further contributes to air quality improvement.
- **Greenhouse Gas Emissions Reduction:** One important technology for reducing GHG emissions in the transportation industry is electric vehicles. According to a study by (Aijaz & Ahmad, 2022), electric vehicles have the capabilities to reduce GHG emissions by up to 90% compared to vehicles using gasoline. However, the extent of the GHG emissions reduction depends on the source of electricity used for charging the vehicles. In regions where electricity generation relies heavily on fossil fuels, the GHG emissions reduction may not be as significant.





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- **Noise Pollution Reduction:** Another potential advantage of electric vehicles is the decrease of noise pollution. Electric vehicles are significantly quieter than old vehicles, which could lead to a reduction in noise pollution in urban areas (Khreis et al., 2023).

It is important to note that the environmental benefits of electric vehicles depend on the life cycle assessment (LCA) of the vehicles, including the manufacturing, operation, and disposal phases (X. Xia & Li, 2022). A comprehensive LCA is necessary to fully understand the environmental impact of electric vehicles. Overall, the potential environmental benefits of electric vehicles make them an attractive option for addressing air pollution and climate change concerns.

Future of Electric Vehicles

Electric vehicles (EVs) have a bright future ahead of them, with the potential for considerable technology breakthroughs, battery developments, and consumer trends.

Technological Advances

Technological advancements are expected to bring major improvements in EV performance, efficiency, and safety. Advances in power electronics, motors, and control systems have improved the efficiency of EVs (Trinko et al., 2023). Moreover, the integration of artificial intelligence and machine learning technologies is anticipated to enable the development of more efficient EVs, resulting in improved battery performance and increased driving range (Liu et al., 2022).

Battery Development

Battery technology plays a vital role in the development and progress of EVs. Current advancements in battery technologies, such as solid-state batteries, are expected to increase the energy density, reduce the cost, and enhance the safety of EVs (Fan et al., 2021). Furthermore, progresses in battery recycling technologies are expected to reduce the environmental impact of battery production and disposal (Liu et al., 2022).

Consumer Trends

Consumer trends are critical for the growth of EVs. The rising demand for sustainable transportation, coupled with decreasing battery costs and increasing driving range, is expected to boost the adoption of EVs (Palit et al., 2022). In addition, the growing popularity of shared mobility services and electric autonomous vehicles is expected to drive the development of the EV market (Zhu et al., 2022).

Challenges and Opportunities

Despite the numerous benefits, the widespread adoption of EVs faces several challenges. These challenges contain high upfront costs, absence of charging infrastructure, limited driving range, and consumer anxiety about battery life and maintenance (Ke et al., 2022). However, these challenges also present opportunities for further innovation, investment, and policy intervention to address them and accelerate the growth of the EV market. The future of EVs is promising, with significant opportunities for technological advancements, battery developments, and consumer trends. However, the widespread adoption of EVs will require continued investment, innovation, and policy intervention to address the challenges and capitalize on the opportunities.

CONCLUSION

The adoption of electric vehicles is gaining momentum due to advancements in technology and increased awareness of the benefits of electric mobility. Plug-in electric vehicles (PEVs), battery electric vehicles (BEVs), and hybrid electric vehicles (HEVs) are among the several types of electric automobiles; with BEVs are the most environmentally beneficial. Electric vehicle technology, including charging stations, battery capacity, and range, has advanced dramatically over time. The FAME plan in India and other government subsidies and tax credits are only a few



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examples of the policies and incentives that have been instrumental in fostering the adoption of electric vehicles. Additionally, electric cars significantly reduce noise pollution, air pollution, and greenhouse gas emissions. The future of electric vehicles is promising due to technological advances, battery development, and changing consumer trends. However, challenges such as infrastructure development, battery cost, and range anxiety need to be addressed. Implications for policy and practice suggest that governments should continue to promote the adoption of electric vehicles by providing incentives, such as rebates and tax credits, and investing in infrastructure development. Companies should also invest in the research and development of electric vehicle technology to improve battery life and range, and provide affordable electric vehicles to the consumers. Recommendations for future research include investigating the impact of electric vehicle adoption on the electricity grid and the potential of vehicle-to-grid (V2G) technology. Additionally, research could be conducted on the impact of electric vehicle adoption on the automotive industry and its supply chain. The adoption of electric vehicles has significant implications for the environment, public health, and the automotive industry. Continued research, policy support, and investment in technology are essential to promote the adoption of electric vehicles and create a sustainable future.

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Assessing the Bioactive Constituents of *Dodonaea viscosa* (Viraali) through GC-MS

P. Parameswari^{1*}, T. Vinciya², Sanjukta Sainath Singh¹ and A. Dhanalakshmi³

¹Associate Professor, Department of Pharmaceutics, Mohamed Sathak AJ College of Pharmacy, Chennai, Tamil Nadu, India.

²Assistant Professor, Department of Pharmacology, Mohamed Sathak AJ College of Pharmacy, Chennai, Tamil Nadu, India.

³Lecturer, Department of Pharmacology, Mohamed Sathak AJ College of Pharmacy, Chennai, Tamil Nadu, India.

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*Address for Correspondence

P. Parameswari,

Associate Professor,

Department of Pharmaceutics,

Mohamed Sathak AJ College of Pharmacy,

Chennai, Tamil Nadu, India.

E. Mail: paramupharma10@gmail.com



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ABSTRACT

The present effort was intended to examine *Dodonaea viscosa* for phytochemical compounds and differentiate the chemical constituents of plant using GC-MS. The shade dried leaf of plant powder of *Dodonaea viscosa* was extracted with Methanol overnight, filtered and concentrated. The GC Clarus 500 (Perkin Elmer) used in the investigation employed a column packed with Elite- 5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30mm x 0.25mm x 0.25 μ mdf) and the components were separated using Helium (1mL/min) as the carrier gas. The 2 μ l sample extract injected into the instrument was detected by the Turbo mass gold detector (Perkin Elmer) with the aid of the Turbomass 5.2 software. The GC-MS analysis provide different peaks decisive the presence of seven phytochemical for Viraali Leaf dry powder The phytochemical and GC-MS profiling methanolic extract of *Dodonaea viscosa* revealed the presence of bioactive compounds with important medicinal properties. Hence, the occurrence of these phytochemicals might be responsible for the curative effects of the plant.

Keywords: *Dodonaea viscosa*, Methanolic extract, GC-MS analysis, Phytoconstituents.





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INTRODUCTION

Plants are used as medicines in a variety of cultures and serve as a source of many effective drugs due to the presence of convinced bioactive compounds for pharmaceutical industries. Plants contain different phytochemicals, also known as secondary metabolites. Phytochemicals are helpful in the treatment of certain disorders by their individual, additive, or synergic actions to improve health. Phytochemicals are vital in pharmaceutical industry for growth of new drugs and training of therapeutic agents. The development of new drugs starts with identification of active principles from the natural sources. The selection of plant extracts is a new approach to find therapeutically active compounds in various plant species.

Phytochemicals such as flavonoids, tannins, saponins, alkaloids, and terpenoids have several biological properties which include antioxidant, anti-inflammatory, anti-diarrhea, anti-ulcer, and anticancer activities, among others. During the last decade, use of traditional medicine has long-lasting globally and has gained attractiveness. With the unbelievable expansion in the use of traditional medicine worldwide, safety and efficiency as well as quality control of herbal medicines and traditional therapies have become important concerns for both health authorities and the public. There is still a considerable lack of research data in this field. In the absence of pharmacopoeia data on the various plant extracts, it is not possible to isolate or standardize the active contents having the desired effects. Screening of active components from plants has direct to the development of new medicinal drugs which have efficient protection and treatment role against various diseases.

Dodonea visosa is a shrub by means of various ethnomedical applications. The roots are used to care for wounds, leaves for gastrointestinal tract, skin and rheumatism, bone fracture, diarrhea, as antibacterial, antifungal, and as antidote for snakebite. There is a report of its anti-HIV role. This plant is reported to have antiplasmodial, to cure sore throat and cold, as antidiabetic and anti-inflammatory. The plant is reported to have antioxidant and anticholinesterase activities. The leaf extracts of this plant are reported to contain flavonoids, terpenoids, saponins, reducing sugars, and steroids. Huge number of medicinal plants and their purified constituent has shown advantageous therapeutic potentials. With this situation, this study was aimed to identify the phytoconstituents present in methanolic extract of *Dodonea visosa* using GC-MS analysis. Gas chromatography-mass spectroscopy (GC-MS) is a combined analytical technique used to determine and recognize compounds present in a plant sample. GC-MS plays an essential role in the phytochemical analysis and chemotaxonomic studies of medicinal plants contain biologically active components.

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used for the research were of analytical grade.

Collection and Preparation of Plant

The leaf of plant were collected from the natural habitats of Panruti, Cuddalore District of Tamilnadu, India. The samples were washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaf of plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Plant Sample Extraction

Fifty grams of powdered sample was extracted with methanol overnight and filtered through ash less filter paper with sodium sulphate and the extract was concentrated. The extract was analyzed using the Clarus 500 GC-MS (Perkin Elmer). 2 μ L of the methanolic extract of *Dodonea visosa* was employed for GC-MS analysis.





GC-MS Analysis

The Clarus 500 GC (Perkin Elmer) used in this analysis. It employed a fused silica column packed with Elite -5MS (5%Diphenyl / 95% Dimethyl poly siloxane, 30mm x 0.25mm x 0.25µm df) and the components were separated using helium as carrier gas at a constant flow of 1 mL/ min. The 2 µL sample extract injected into the instrument. It was detected by the Turbo gold mass detector (Perkin Elmer) with the aid of Turbo mass 5.2 software. During the GC process the oven was maintained at a temperature of 110°C with 2 min holding. The injector temperature was set at 250°C. The different parameters involved in the operation of the Clarus 500 MS were also standardized. The Inlet line temperature was 200°C and source temperature was 200°C. Mass spectra were taken at 70 eV; a scan interval of 0.5s and fragments from 45-450 Da. The MS detection was completed in 36 min. The detection employed the NIST ver. 2.0 year 2005 library.

RESULTS

The results concerning to GC-MS analysis led to the identification of number of compounds from the GC fractions of the methanolic extract of *Dodonea visosa*. These compounds were identified through mass spectrum attached with GC. The active principles with their retention time (RT), molecular formula (MF), molecular weight (MW) and concentration (%) were tabulated in the given tables. The results revealed that the presence of seven phytochemical for Viraali Leaf dry powder such as cis-5,8,11,14,17-Eicosapenta enoic acid ,(S,Z)-Heptadeca-1,9-dien-4,6 -diyn-3-ol,5,8,11,14-Eicosatetraenoic acid methyl ester, (all-Z)-,4,7,10-Hexadecatrienoic acid methyl ester ,8,11-Octadecadiynoic acid methyl ester , 10-Heptadecen-8-ynoic acid, methyl ester, 6,9,12-Octadecatrienoic acid, methyl ester.

DISCUSSION

Gas Chromatography- Mass Spectrometry (GC-MS) is a expensive tool for consistent detection of bioactive constituents. This study results were interpreted. By interpreting these compounds, it is originate that *Dodonea Visosa* possesses various therapeutically applications. The present study characterized the chemical profile of *Dodonea Visosa* using GC-MS. The GC chromatogram shows the relative concentration of various compounds accomplishment eluted as a function of retention time. The heights of the peak point out the relative concentration of the presented components. The mass spectrometer analyzes the compounds eluted at different times to identify the nature and structure of the compounds. The examination done that the stronger extraction capacity of methanol have produced number of active constituents responsible for many biological activities. So these might be utilized for the development of traditional medicines and further investigation needs to elute novel active compounds from the medicinal plants which may be created a new way to treat many incurable diseases including cancer. To separate volatile substances in a mixture, gas chromatography is normally utilized. The biological components of the extracted solvent were examined using a gas chromatography-mass spectrometer (GCMS).

Ryu et al., 2011 reported that the Phytol have antioxidant, antiallergic antinociceptive and anti-inflammatory activities. latest studies have exposed that phytol is an excellent immunostimulant. It is better-quality to a number of commercial adjuvants in terms of longterm memory induction and activation of both innate and acquired immunity. According to Saikia et al., 2010 Phytol exposed antimicrobial activity against *Mycobacterium tuberculosis* and *Staphylococcus aureus*. Phytol was observed to have antibacterial activities against *Staphylococcus aureus* by cause damage to cell membranes as a result there is a leakage of potassium ions from bacterial cells. Phytol is a key acyclic diterpene alcohol that is a precursor for vitamins E and K1. It is used along with simple sugar or corn syrup as a hardener in candies Cyclohexene (5.09%): antioxidant and anti-diabetic. Methyl palmitate (18.56%) - used in textiles, spin, finishers, and food as an emulsifier or oiling agent. Detergents, soaps, shampoos, shaving creams, and other cosmetic goods are made with methyl stearate (12.57%). Dibutyl phthalate (18.64%) was once used in nail polish, however, it is now prohibited. Elemicin (1.99 %) is a flavoring ingredient that can also be used medicinally in high dosages. Ketorolac (1.07 %) is an NSAID that is used to relieve acute pain for a brief period of time (5 to 7 days)





following an injury, dental issue, surgery, or childbirth. These are the biochemical compounds that **Sathya *et al.*** reported in 2017 and which are used as an ingredient in sunscreens and cosmetics. Octocrylene (1.42%) is also used as a component in hair products.

According to a 2013 study by Murugesan *et al.*, the GC-MS-MS analysis identified 42 phytochemicals as components of *M. dubia* leaf extract. In the GC-MS-MS chromatogram, the peaks of the compounds were identified along with their retention time, molecular formula, molecular weight, and concentration (peak area percent). The compounds discovered in the previous study were octadecanoic acid (15.71%), hexadecanoic acid (11.10%), humelene (3.24%), caryophyllene (6.07%), aromadendrene (3.53%), and germacrene-D (2.89%), which has been shown to have pesticidal activity by Shailesh (2012). The compounds discovered in the current study were octadecanoic acid (15.71%), hexadecanoic acid (11.10%), humelene (3.24%), caryophyllene (6.07%), aromadendrene (3.53%), and germacrene-D (2.89%), which has been shown to have pesticidal activity by Shailesh (2012). According to a report by Hassan *et al.* from 2014, the main components of the essential oil extracted from *Vitex pseudo-negundo* leaves were 2,6,6-Trimethylbicyclo [3.1.1]hept-2-ene (18.2 %), 1,8-Cineole (16.7 %), Phenol, bis(1,1-dimethylethyl), Viridiflorol (8.32 %), dl-Limonene (4.4%) (1.31 %). The compounds 9,12,15- Octadecatrienoic Acid, Methyl Ester reported that Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepato protective, Nematicide, Insectifuge, Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor, Antiandrogenic. Phytol Isomer is reported that it can be used for Anticancer, Anti-inflammatory, Hypocholesterolemic, Nematicide, Anticoronary.

CONCLUSION

Several phytochemical evaluation have been carried out in leaf part of *Dodonea visosa* using GC-MS. This analysis showed the existence of various compounds with different chemical structures. The occurrence of various bioactive compounds proves the purpose of *Dodonea visosa* for various disorders. However, selection of individual phytochemical constituents may proceed to find an innovative drug. Hence, this type of effort will be supportive for in intensity study.

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Table: 1 Compounds identified in the methanolic extract of Viraali leaf dry powder:

| No | RT | Name of the compound | Molecular Formulae | Molecular Weight | Peak Area % |
|----|---------|---|--|------------------|-------------|
| 1 | 16.66 | cis-5,8,11,14,17-Eicosapenta enoic acid | C ₂₀ H ₃₀ O ₂ | 302 | 0.47 |
| 2 | 23.54 | (S,Z)-Heptadeca-1,9-dien-4,6 -diyn-3-ol | C ₁₇ H ₂₄ O | 244 | 15.14 |
| 3 | 23.75 | 5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)- | C ₂₁ H ₃₄ O ₂ | 318 | 3.57 |
| 4 | 23.99 4 | 4,7,10-Hexadecatrienoic acid, methyl ester | C ₁₇ H ₂₈ O ₂ | 264 | 3.75 |
| 5 | 24.82 | 8,11-Octadecadiynoic acid, methyl ester | C ₁₉ H ₃₀ O ₂ | 290 | 2.91 |
| 6 | 25.69 | 10-Heptadecen-8-yenoic acid, methyl ester, (E) | C ₁₈ H ₃₀ O ₂ | 278 | 71.93 |
| 7 | 25.82 | 6,9,12-Octadecatrienoic acid, methyl ester | C ₁₉ H ₃₂ O ₂ | 292 | 2.23 |

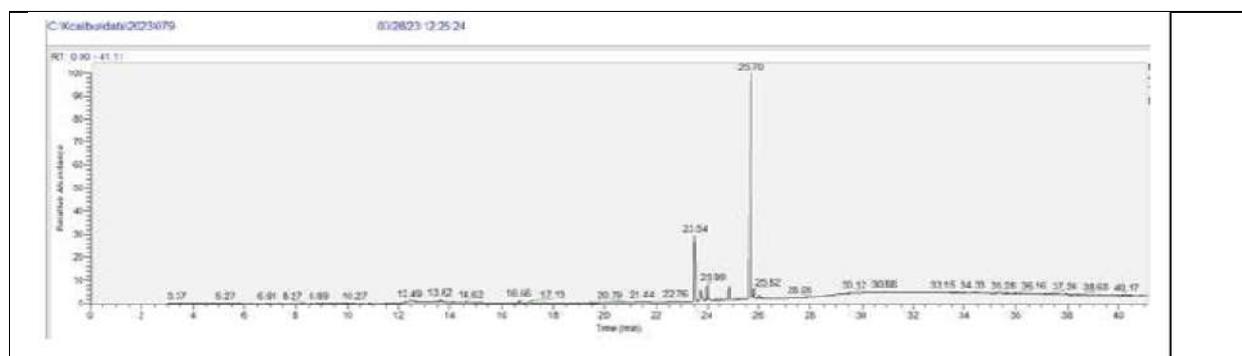


Fig 1: GC MS chromatogram of the methanolic extract of Viraali leaf dry powder





Impact of Chemical and Environmental Hazards on Pharmaceutical Occupational Health Safety

B.Hima Bindu¹ and Lakshmi Prasanthi Nori^{2*}

¹Student, Department of Quality Assurance, Shri Vishnu College of Pharmacy, Bhimavaram, West Godavari (Dt), Andhra Pradesh, India.

²Department of Regulatory affairs, Shri Vishnu College of Pharmacy, Bhimavaram, West Godavari (Dt), Andhra Pradesh, India.

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*Address for Correspondence

Lakshmi Prasanthi Nori

Department of Regulatory Affairs,
Shri Vishnu College of Pharmacy,
Bhimavaram, West Godavari (Dt),
Andhra Pradesh, India.

E.Mail: prasanthi_pharm@yahoo.com



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ABSTRACT

Hazardous situation is one that poses a threat to one's health, safety, property, or environment. Any actual or potential situations brought on by some sectors that may result in property damage, injury to workers, or even death. Industrial risks or hazards are significant problem today and are major occupational health and safety (OHS) issue in pharmaceutical manufacturing. Globally, there have been significant advancements in chemical safety and responsible chemical management in recent years. Natural and synthetic chemical production and distribution have rapidly increased, raising concerns about their effects on the environment and public health. Some Industrial facilities pose risks due to the nature of their operations and the substances they utilise which are even more advantageous when they are situated adjacent to residential areas. Residents are particularly vulnerable to mishaps during the festivities and the risks can be identified. As the pharmaceutical industry's pollution may cause poisoning and rashes, and product deterioration, which is likely detrimental to pharmaceutical product quality manufacturing.

Keywords: Hazard, Threshold limit, Flammable materials, OSHA Act, Styrene



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INTRODUCTION

The Indian Pharmaceutical Industry is making great steps to match the global standards for producing APIs and quality medications as well as entering the expanding fields of research, goods, and clinical trials because it is a highly regulated and R&D-driven business. Like other chemical sectors, the pharmaceutical industry also faces significant environmental issues, particularly in the production of API [1]. The health consequences of working in the pharmaceutical sector are not well understood. The industry appears orderly on the outside but the manufacturing of pharmaceuticals necessitates a meticulously sanitary working environment, and the white lab coats worn by employees heighten the sense of security [2]. Although it may appear deceptive, exposure to hazardous industrial chemicals can occur during the production of pharmaceuticals and other medicines. More than 2000 people have died in a series of terrible catastrophes over the past few decades, including the Deepwater Horizon oil spill, the Buncefield fire in the UK in 2005, and the devastating toxic gas release in Bhopal, India, in 1984 as shown in Figure 1 which has resulted in fatalities [3].

Risks can be classed as ecotoxic, carcinogenic, persistent, or bio-accumulative, which refers to waste that builds up as it moves up the food chain and causes harm to the environment or causes or contributes to the development of cancer. There are various types of hazards and regardless of the form, any materials that has the potential to endanger people's physical and mental health is considered a chemical hazard. According to Safe Work Australia and the Hazard Communication Standard (HCS) of the Occupational Safety and Health Administration (OSHA), it is important that the employees are aware of the risks and hazards associated with their daily work and the manufacturers and importers are required to evaluate the hazards of the chemicals they produce or import, and prepare labels and safety data sheets as shown in Figure 2 to convey the hazard information. The chemicals or substances which has the potential to threaten the surrounding natural environment or adversely affect people's health including pollution and natural disasters. These environmental hazards are classified into three interrelated categories (biological, chemical and physical) based on the properties of their causes. There are various environmental contaminants or toxins like carcinogens, teratogens, mutagens, neurotoxins and endocrine disruptors which interferes with the body and leads to various diseases. Little is known about the health risks of working in the pharmaceutical industry where exposure to hazardous industrial chemicals may occur during the production of pharmaceuticals and other medicines. General health risks in the production of pharmaceuticals include formaldehyde exposure, repetitive motion diseases, dust and noise exposure, and UV radiation exposure. The health impairment which is caused due to pharma products has been observed mainly in hormone and antibiotic production leading to various problems. The negative impact of the production of pharmaceutical products on the natural environment is well known which seriously affects the living near production plants and residents with waste pharma products.

The Safer Pharma campaign is run by Health Care Without Harm (HCWH) Europe to draw attention to the unfavourable relationship between pharmaceutical production and the environment. It also challenges the healthcare sector to clean up its production, which promotes the emergence of drug resistance. This campaign has sparked a number of initiatives to try to better understand the effects of pharmaceutical production on the environment and how it can be mitigated. The chemical hazards are noxious, erosive, exasperated, combustible and mutagenic Hence, chemical dangers are classified according to the Workplace Hazardous Materials Information (WHMI) system are exhibited in Table 1.

Risks associated with organic synthesis

Industrial risks associated with organic chemical synthesis fall into three categories:

- To begin with, the active substances employed to assault and alter the structure, by their very nature, organic molecules are especially good at the chemical substances in the human body to attack and change them, therefore creating results that are quite toxic.



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- Second, the majority of organic synthesis intermediate components are frequently distinguished by the eagerness with which they engage in.[4] They are active and can combine chemically with other organic substances and receives a wide range of harmful properties from this.
- Third, even though the finished goods are medications that are absorbed into the body, however, could result in severe poisoning when exposed to industrial pollutants.[5]

Hazardous waste

Hazardous waste is defined as any waste that is potentially harmful or dangerous and can include solids, liquids, contained gases, or sludge. [6] The two categories of hazardous wastes are listed wastes and characteristic wastes. The listed wastes are included on one of four hazardous waste lists (F, K, P and U). On two of these lists—the P and the U, which both include industrial chemical goods—are pharmaceuticals. Solid wastes are those that are neither listed nor display a characteristic. [7] According to state and/or local rules, particularly those governing medical waste, these solid wastes should be disposed of. Generally, the waste form codes describe the general physical and chemical characteristics of a hazardous waste and there are various management method codes as shown in Table 2 and Table 3 which describes the type of hazardous waste management system used to treat or dispose of hazardous waste. For this, the Environmental Protection Act,1986 was established to provide sufficient protection system from the waste to the environment, which gives the power to the central government to regulate all forms of waste and tackle the problems present in different regions of India.

TLV concept

American Council of Government Industrial Hygienists has established Threshold Limit Values (TLV).For around 800 chemicals, a list of the threshold limits has been created as shown in Figure 3. Worker exposure to chemicals other than these should be avoided.[8] The three distinct TLV categories listed were

- **Time weighted average (TVW)** - The TWA of concentration for an 8- and 40-hour workday/ week, to which nearly every employee may be exposed multiple times daily with no negative consequences.
- **TLV-STEL** -This cannot go over the weighted average of 15 minutes, even if the weighted average of eight hours is used, at any point throughout a working day is inside of the TLV. This is the maximum employee concentration possible.[9] Short period of exposure without displaying symptoms of chronic or irreparable annoyance and significant tissue injury or narcosis to fundamentally lower labour efficiency or raise the chance of an accident.
- **TLV-CEILING** -The highest level of focus that may not be surpassed at any time throughout the time at work. The potential toxicity is not taken into account by numerical TLV values. The fact that TLVs are not meant to constitute strict distinctions between concentrations that are secure and risky should be highlighted.[10] Each and every professional in occupational health must possess a copy of the biological exposure indices for most recent and TLVS.

Management of hazardous gases

Cylinders are filled with compressed gases and brought to the location of use. The following are significant safety measures must be observed:

- Neither the cylinders nor the other may be released or permitted to collide.
- It is forbidden to tamper with the safety devices fitted on the cylinders.
- Make use of common tools and special valves. Typically, which are provided by the producers.
- The cylinders need to be protected from extreme weather conditions, especially a rapid temperature rise.
- A standard label stating must be attached to the cylinders. Depending on the label's colour, you can tell if the gas is either inert, corrosive, or flammable.
- Cylinders must be divided between full and empty ones.[11]

Management of over-exposure to chemicals

- Removal from exposure -The first step is the rapid eviction of the individual from the area. The use of respirators and lifelines is required as aid.



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- Resuscitation - Resuscitation refers to bringing back to life of someone who appears to be dead (collapsed or shocked). The same level of supportive care should be provided in a medical emergency.
- Decontamination - It is necessary to take off clothing and shoes right away for a person whose skin or belongings have been affected. After that, it is advisable to take a brisk soap-water shower, paying particular consciousness to the fingernails and scalp.[12]
- Symptomatic Treatment - Regardless of the particular substance, subtle overexposure can result in number of symptoms that need basic supportive medical therapy. Examples include controlling bronchospasm and convulsive convulsions.[13]

Risk assessment

The process of system design begins here. Determine the hazardous situation's location and timing, as well as potential exposure, with the aid of field investigations.[14] In terms of ignition, the hazard features can be identified as corrosivity, toxicity, and reactivity which reflects the danger to human health according to the guidelines established by OSHA (EPA), the Environmental Protection Agency, and the Resource Conservation and Recovery Act.[15] The knowledge and the information gleaned from the precaution measures serves as a basis for more effectively addressing the gas's potential risk.

Pharmaceuticals in environment and assessment of environment hazard

Pharmaceuticals are exposed to the environment through a variety of channels, including hospital and industry effluents, land-based applications such as biosolids and recycled water etc. However, the active compounds in waste water are not always successfully removed by sewage treatment services.[16] As a result, pharmaceuticals end up in the aquatic environment, where they have an immediate impact on aquatic creatures and can enter food chains. In a recent investigation, the effluents from a local waste water treatment plant near Visakhapatnam, India were discovered to contain exceptionally high amounts (mg/L) of numerous medications.[17] Globally, the discovery of waste medicines in the environment poses concerns related to their introduction into aquatic and terrestrial life as well as animals, and is developing into a severe issue for both regulators and the pharmaceutical industry. With the current levels of knowledge being so limited regarding pharmaceutical effects, environmental mobility, and fate, significant progress on this topic is just not possible. Risk assessment of pharma chemicals include the detection of the inherent dangers at each stage and an estimation of the risks resulting from these hazards.[18] This is necessary to account for the potential rising effects of many medications hitting the same receptors. Nanotechnology holds enormous promise for the future of medicine and health care. But liposomes and polymer-based systems are two examples of the nanoscale vehicles that have been developed for "housing" and delivering pharmaceuticals. However, there is a paucity of knowledge regarding the fate of these vehicles in the environment and their ecotoxic impacts.[19]

Case study- Vizag gas leak

The towns of Visakhapatnam and Assam had unexpectedly become involved in an environmental conflict as the whole nation was beneath attack from the greatest century's epidemic due to corona virus. Lack of proper supervision caused accidents in industries.[20] On the night of May 7, 2020, Visakhapatnam witnessed a significant gas leak from one of its plants, LG Polymers, which produces chemical products like polystyrene and its copolymers and it spread over five kilometres covering several villages as shown in Figure 4. Styrene gas if inhaled causes headache, fatigue, irritation of eyes, head reeling, fainting and led to the death of 12 people, left around 585 affected and seriously harmed the local vegetation.[21] A 5 judge bench was assembled by the National Green Tribunal to investigate the leak and determine its full scope of causes.[22] When the ongoing examination revealed information indicating that the company had engaged in an ignorant style of operations and may have disregarded Standard Operating Procedures (SOP), which caused the leak and also imposed a penalty on the corporation. The company also enquired for environmental clearance from the Ministry of Environment, Forestry, and Climate Change (MoEFCC), under the heading of projects operating in breach of the aforementioned EIA notification, and documents were submitted that indicated that the company had not complied with environmental standards were in use but did not have a current environmental clearance in compliance with the provisions of the Environment Impact



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Assessment Notification of 2006(EIA).[23]The Andhra Pradesh Police filed a complaint against LG Polymers using this information as a basis and accusing them of violating many sections of the Indian Criminal Code for their negligence in inflicting harm and risking the lives of people.[24] The massive environment loss and potential long-term effects it may have on the local biodiversity, even though officials allegedly responsible for the leak have been detained and questioned, and seem to have received little attention.[25]

CONCLUSION

Currently, pharmaceutical compounds are being merged into the environment in extremely large quantities regularly and the present system of regulations of their release is not able to control the untreated or partially treated pharma effluents. The current system of regulations for their discharge is unable to manage the untreated or partially treated pharmaceutical effluents, which are currently being released into the environment in extraordinarily large quantities on a regular basis. The most crucial components of an ideal pharmaceutical safety management are perceptiveness and comprehending the risk of hazardous facilities and hazard release. An efficient risk and hazard assessment enables the creation of a plan for handling incidents and the application of techniques. They are very important substances used in the production of a variety of polymers, copolymers, and glass- reinforced plastics. Plastics and on exposure to these have a negative impact on human health which can even be lethal in some cases. The hazardous gas leak that occurred in Bhopal and Visakhapatnam caused a lot of illness and some fatalities polluting the air and people as it spread throughout the world. Therefore, we should take care of our future and defend against the risks of tragedies. So, it is vital to rethink the production process while considering how it would affect the environment, workers, and inhabitants' health.

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Table 1: WHMI classification of chemical hazards

| CLASS | EXAMPLES |
|---------|---|
| CLASS A | Compressed gas Dissolved gas or liquefied gas |
| CLASS B | Flammable gases Flammable and combustible liquids Flammable solids Flammable aerosols Reactive flammable material |
| CLASS C | Oxidizing materials-oxidizer and organic peroxide Oxidizer: chlorates, nitric oxide, peroxides, permanganates, |





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| | |
|---------|--|
| | Per chlorates, nitrites, nitrates easily oxidize metal powder Organic peroxide: Tetrahydrofuran, diethyl ether, dioxane, methyl isobutyl ether |
| CLASS D | Poisonous and infectious materials: cyanide, tea salts, asbestos |
| CLASS E | Corrosive materials: Inorganic acids and bases, hydrogen fluoride |
| CLASS F | Dangerous reactive materials: Ethylene dioxide, organic azides, Na, Li, Ca Pyrophosphoric materials: White phosphorous, diethyl aluminium chloride, lithium |

Table 2: P- listed Hazardous Waste Pharmaceuticals

| P-Listed pharmaceutical | Waste code |
|--------------------------|------------|
| Arsenic trioxide | P012 |
| Epinephrine | P042 |
| Nicotine | P075 |
| Nitroglycerin | P081 |
| Physostigmine | P204 |
| Physostigmine salicylate | P188 |
| Warfarin | >0.3% P001 |

Table 3: U- listed Hazardous Waste Pharmaceuticals

| U-Listed pharmaceutical | Waste code |
|-------------------------|------------|
| Chloral hydrate | U034 |
| Paraldehyde | U182 |
| Chlorambucil | U035 |
| Phenol | U188 |
| Cyclophosphamide | U058 |
| Reserpine | U200 |
| Daunomycin | U059 |
| Resorcinol | U201 |
| Dichlorodifluoromethane | U075 |
| Saccharin | U202 |
| Diethylstilbesterol | U089 |
| Selenium sulfide | U205 |
| Lindane | U129 |
| Hexachlorophene | U132 |

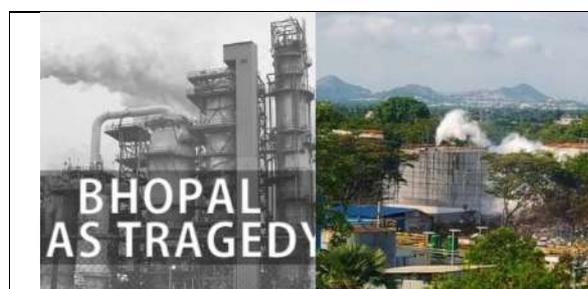


Figure 1: Bhopal tragedy and Visakhapatnam gas leak

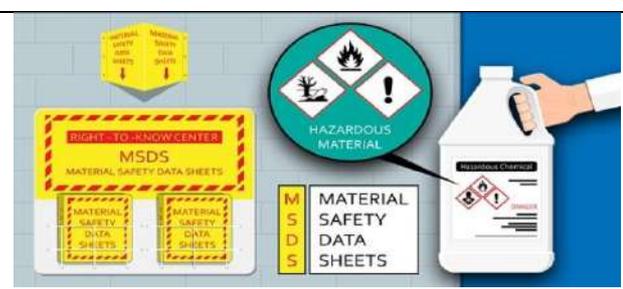
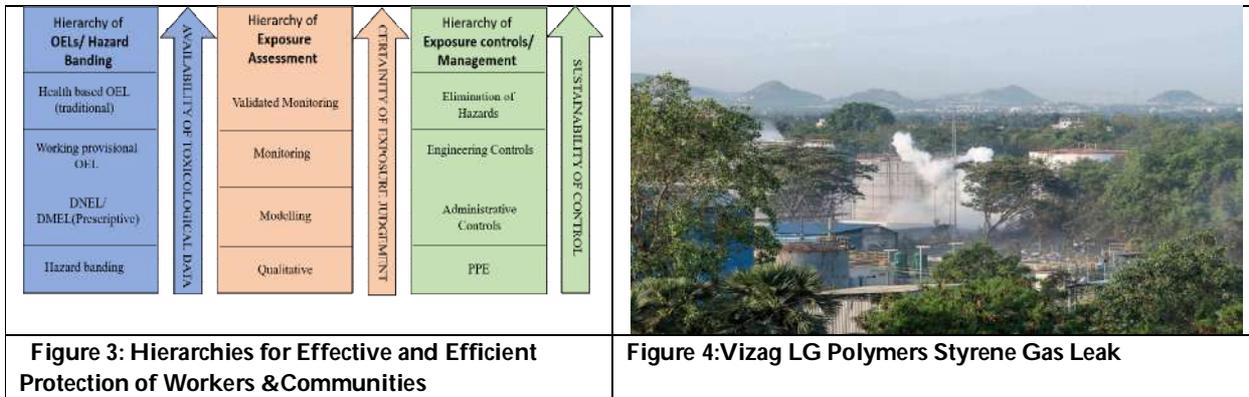


Figure 2: MSDS for Hazard Identification Assessment





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Synthesis and Characterization of Europium doped Barium Hexaferrite for the Application of Microwave Absorbing Materials

M.Easwari^{1*}, Sr.S.Jesurani², Kanagesan Samikannu³, Maria Therese⁴ and K. Chellemmal¹

¹Research Scholar, Department of Physics, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Tamil Nadu, India. Affiliated to Mother Teresa Women's University, Kodaikanal, and Assistant Professor of Physics, Nadar Saraswathi College of Arts and Science, Theni, Tamil Nadu, India.

²Principal, Associate Professor of Physics, Department of Physics, Jayaraj Annapackiam College for Women (Autonomous), Periyakulam, Tamil Nadu, India. Affiliated to Mother Teresa Women's University, Kodaikanal, Tamil Nadu, India.

³Low Dimensional Materials Research Center, Department of Physics, University of Malaya, Kuala Lumpur, Malaysia.

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Accepted: 04 Sep 2023

*Address for Correspondence

M.Easwari

Research Scholar, Department of Physics,
Jayaraj Annapackiam College for Women (Autonomous),
Affiliated to Mother Teresa Women's University, Kodaikanal,
Periyakulam, Tamil Nadu, India.



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ABSTRACT

In this paper europium doped Barium hexaferrite ($\text{BaFe}_{12-x}\text{Eu}_x\text{O}_{19}$) with concentration ($x=0.01, 0.02, 0.03, 0.04$ and 0.05) is prepared via sol-gel method based on the reaction between $\text{Ba}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ and Europium nitrate in distilled water. The X-ray pattern confirms that synthesized nanomaterials were in pure phase and they possess hexagonal crystal structure having $p6_3/mmc$ space group. Crystallite size decreased with increment in concentration of Europium in Barium Hexaferrite lattice. Constituent elements and chemical composition were analyzed using EDAX spectrum. The hysteresis study is carried out for saturation magnetization using VSM. The resulted product has the property of the hard magnetic material.

Keywords: Europium, Barium Hexaferrite, XRD, SEM, EDAX, VSM.





INTRODUCTION

Now a day's hexagonal ferrites (hexaferrites) grabbing greater attention due to their high Curie temperature, high saturation magnetization, large uniaxial magnetic anisotropy, high coercivity, low dielectric loss and good chemical stability. Barium hexaferrites ($\text{BaFe}_{12}\text{O}_{19}$ /BaM) are widely used in various devices such as multimeter devices, sound devices, perpendicular magnetic recording devices etc [2-9]. The crystal structure of BaM is described by the space group $p6_3/mmc$. The unit cell of BaM consists of two structural blocks 'R' and 'S' with stacking sequence RSR*S*, ordered along the direction of hexagonal caxis. The S and R blocks have spinal and hexagonal structure respectively. The S* and R* are the rotated blocks obtained by 180° rotation of S and R block along the c-axis respectively [10]. The synthesis technique of $\text{BaFe}_{12}\text{O}_{19}$ plays a vital role in determining the structural, electrical and optical properties. Various synthesis methods were reported for the synthesis of high purity $\text{BaFe}_{12}\text{O}_{19}$ powders such as sol-gel method [11], hydrothermal synthesis [12], combustion method [13] etc.

In this work europium doped barium hexaferrite powders were prepared using combustion synthesis technique due to its low cost and less time consumption.

MATERIALS

All the reagents are the analytical grade and used as received, without further purification. Barium Nitrate [$\text{Ba}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$], Ferric nitrate [$\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$], Europium nitrate [$\text{Eu}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$], Maltose, Ethylene glycol [$\text{C}_6\text{H}_6\text{O}_2$], Oxalic acid.

EXPERIMENTAL

Europium doped Barium hexaferrite were synthesized by sol-gel method with five concentrations ($x=0.01$ to 0.05). Chemicals used were of high purity grade namely $\text{Ba}(\text{NO}_3)_2$, $\text{Fe}(\text{NO}_3)_3$ and $\text{Eu}(\text{NO}_3)_3$. For chelation in the process oxalic acid were used. The solution was slowly evaporated at 80°C until becoming a highly viscous gel. The obtained gel dried at hot air oven. Finally, the powder was sintered in muffle furnace at 700°C for 3 hours.

RESULT AND DISCUSSION

XRD

X ray diffraction peaks confirmed that synthesized nanoparticles were in pure phase and all possess hexagonal crystal structure and belongs to space group $P6_3/m$.

FTIR measurements

The FTIR absorption spectra for $\text{BaFe}_{12-x}\text{Eu}_x\text{O}_{19}$ is shown in Fig. for $x=0.01$ to 0.05 wave number 400 cm^{-1} to 1500 cm^{-1} .

Magnetic Property

Magnetic Hysteresis loop for Europium doped Barium Hexaferrite for $x=0.01$ to 0.05 is depicted in Fig. Magnetization curves showing ferromagnetic behavior, high coercive field values are found.

FESEM

FESEM representative micrographs of the samples with $x = 0.01$ to 0.05 . The prepared ferrites are single domain. It seems that the small amount of Eu substitution will not affect the crystal structure and morphology of the samples significantly.





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CONCLUSION

Europium ions doped barium ferrite nano particles were prepared by combustion synthesis technique and the structure of the prepared nano particles is hexagonal as confirmed by PXRD pattern analysis. FESEM micrographs confirm the formation of plate like structure. Vibration between the crystallographic sites (4f1 and 4f2) of ferric ions of the prepared samples was observed in FTIR analysis.

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Table 1. X ray diffraction

| BaFe _{12-x} Eu _x O ₁₉ | 2 theta (deg) | Particle Size (nm) | Average Particle Size (nm) |
|--|---------------|--------------------|----------------------------|
| x=0.01 | 33.77 | 44.49 | 47 |
| | 31.71 | 47.15 | |
| | 36.80 | 47.60 | |
| | 62.81 | 50.24 | |
| x=0.02 | 33.81 | 44.49 | 47 |
| | 32.67 | 47.27 | |
| | 36.27 | 47.52 | |
| | 62.83 | 50.25 | |
| x=0.03 | 33.8 | 44.49 | 47 |
| | 32.8 | 47.28 | |
| | 37.29 | 47.66 | |
| | 54.33 | 48.19 | |
| x=0.04 | 33.77 | 44.49 | 48 |
| | 32.59 | 47.26 | |
| | 54.54 | 50.81 | |
| | 62.51 | 50.16 | |
| x=0.05 | 33.84 | 44.49 | 47 |
| | 32.83 | 47.29 | |
| | 36.28 | 47.52 | |
| | 54.71 | 48.28 | |

Table 2. Magnetic Hysteresis

| Molar ratio | Magnetic Property | | |
|--|---|---------------------------------------|--------------------------------|
| BaFe _{12-x} Eu _x O ₁₉ | Magnetization (M _s) (emu/g) | Retentivity (M _r) (emu/g) | Coercivity H _c (Oe) |
| X=0.01 | 39.73 | 21.36 | 4060.8 |
| X=0.02 | 34.70 | 16.45 | 2166.4 |
| X=0.03 | 26.80 | 14.82 | 5033.7 |
| X=0.04 | 25.99 | 14.12 | 5098.8 |
| X=0.05 | 24.15 | 12.04 | 3939.5 |

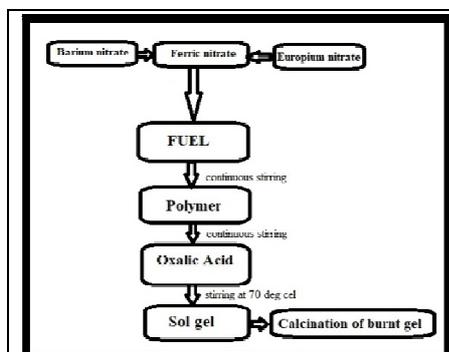


Fig 1Block diagram

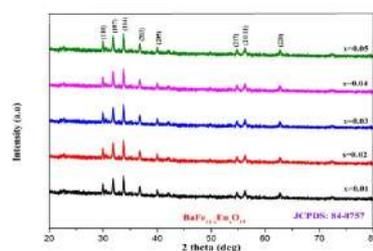


Fig 2 X ray diffraction





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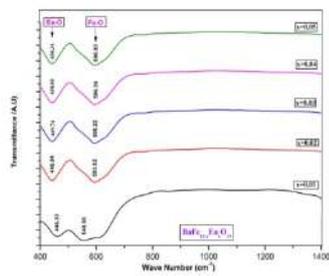


Fig 3 FTIR

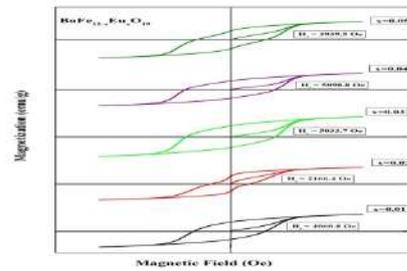


Fig 4 Magnetic Hysteresis

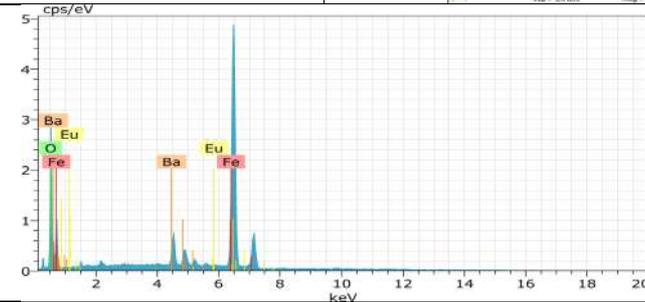
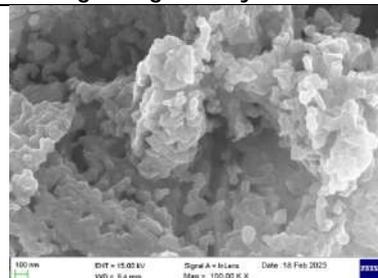
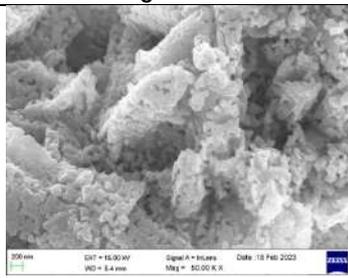


Fig 5 BaFe_{12-x}Eu_xO₁₉ (x=0.02)

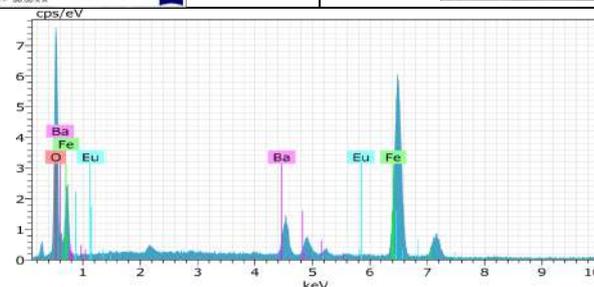
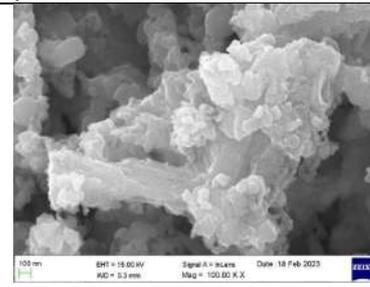
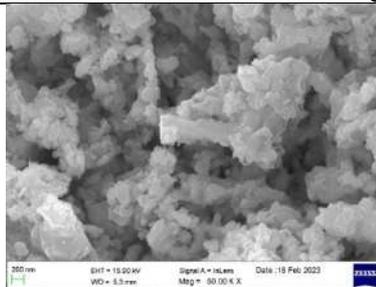
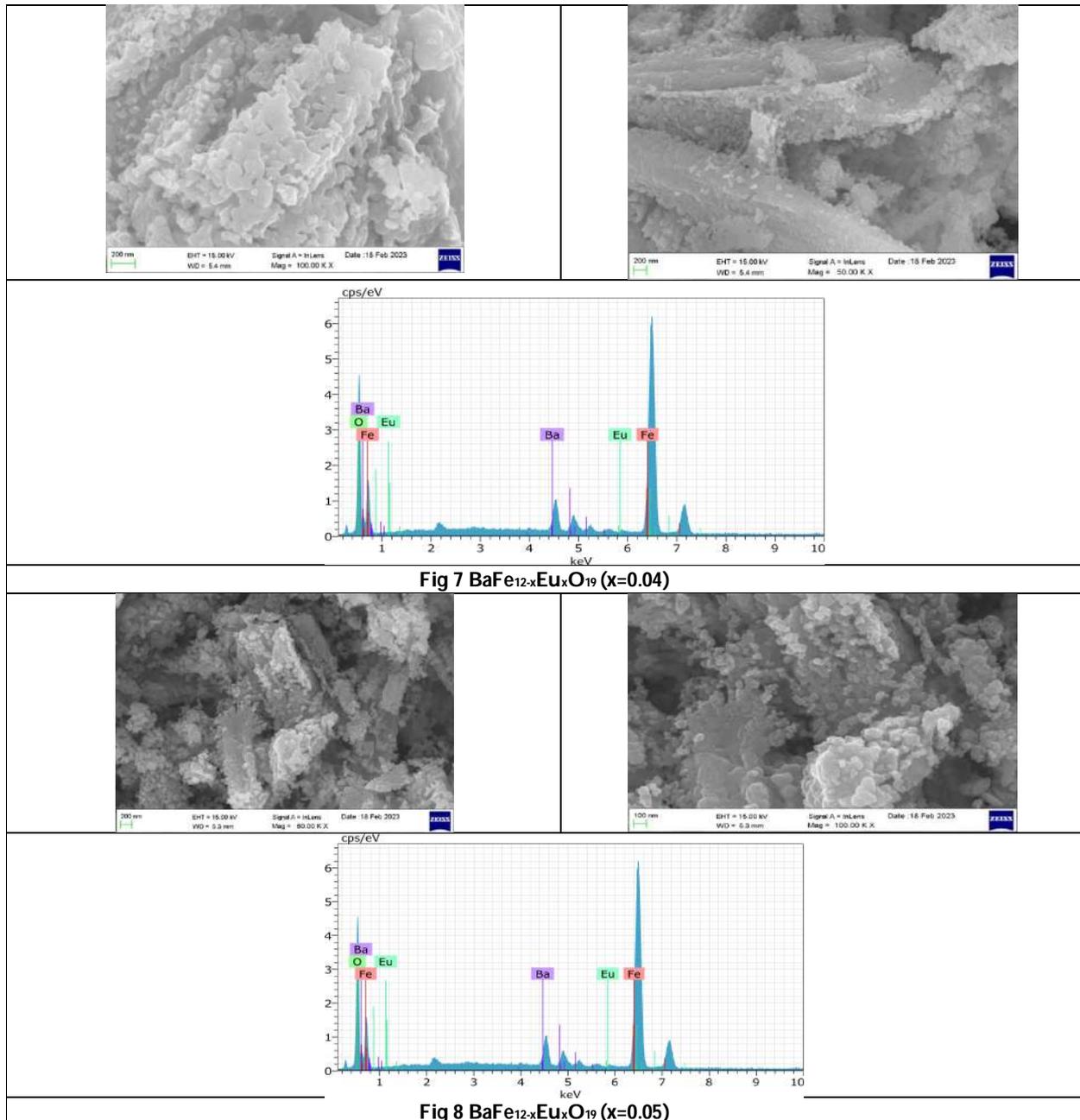


Fig 6 BaFe_{12-x}Eu_xO₁₉ (x=0.03)



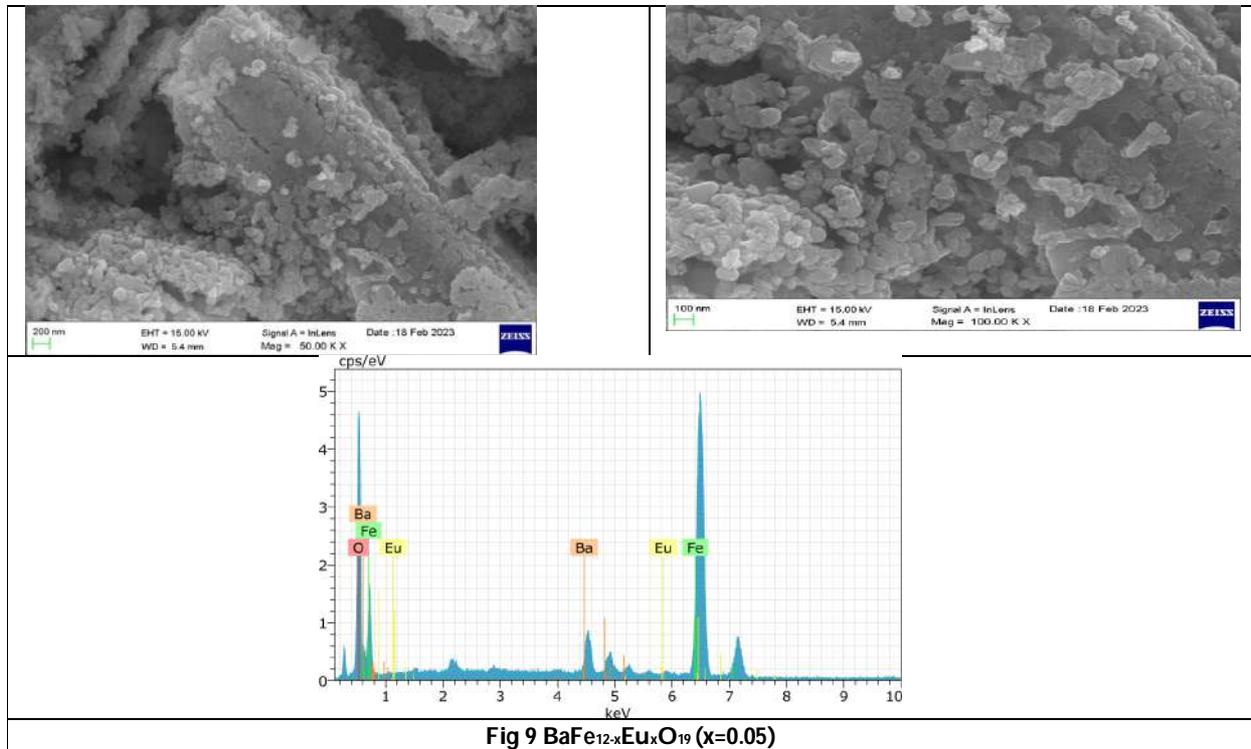


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***In silico* Molecular Docking Studies on the Phytoconstituents of *Vitex negundo* for Its Fertility Activity**

Sanjukta Sainath Singh^{1*}, P Parameswari², T. Vinciya³, A. Dhanalakshmi⁴

¹Associate Professor, Department of Pharmacology, Mohamed Sathak AJ College of Pharmacy, Chennai-600119, Tamil Nadu, India.

²Associate Professor, Department of Pharmaceutics, Mohamed Sathak AJ College of Pharmacy, Chennai-600119, Tamil Nadu, India.

³Assistant Professor, Department of Pharmacology, Mohamed Sathak AJ College of Pharmacy, Chennai-600119, Tamil Nadu, India.

⁴Lecturer, Department of Pharmacology, Mohamed Sathak AJ College of Pharmacy, Chennai -600119, Tamil Nadu, India.

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***Address for Correspondence**

Sanjukta Sainath Singh,

Associate Professor,

Department of Pharmacology,

Mohamed Sathak AJ College of Pharmacy,

Chennai-600119, Tamil Nadu, India.

E.Mail: sanjuktagharami@gmail.com



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ABSTRACT

To study the effectiveness of phytocomponents from *Vitex negundo* against CYP 17 using computational molecular docking studies. The characterization of polycystic ovarian syndrome is hyper insulinemia, menstrual irregularities, long-term metabolic disturbances, and hyper androgenism. Adrenal and ovarian androgen synthesis are induced by an enzyme, CYP 17 (P450c 17 α). Due to increase in the activity of this enzyme, hyperandrogenism mainly occurs. By inhibiting the enzymatic activity, excess androgen synthesis can be prevented in ovarian theca cells. Literature study has reported that the gas chromatography mass spectrometry analysis on the ethanolic extract of the leaves of *Vitex negundo* possesses 11 compounds. Molecular docking analysis was performed for the reported 11 compounds against CYP 17 using Schrodinger Glide software. These results were compared with the docked score of fertility inducing drug clomiphene citrate. All 11 compounds have exhibited moderate to potent inhibition with a range of -5.5 to -9.5. gamma. - Sitosterol have showed potent inhibition with the docking score of -9.5 respectively. The results revealed out that the compounds present in *Vitex negundo* can reduce the activity of CYP 17. This study throws a light on establishing the novel drug for infertility through experimental procedures.

Keywords: *Vitex negundo* , Glide-score, Infertility, *In silico*, CYP 17, Polycystic ovarian syndrome.





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INTRODUCTION

In worldwide, up to 15% of reproductive-aged couples were affected by infertility[1]. Anovulation and fallopian tube obstruction are the two major factors of female infertility[2]. The most common endocrine disorder is polycystic ovarian syndrome (PCOS) which is characterized by hyperinsulinemia, hyperandrogenism, menstrual irregularities, and long-term metabolic disturbances in female[3]. Women with PCOS are frequently affected by diabetes, metabolic syndrome and obesity which further subjected to increased risk of infertility due to the ovulatory dysfunction that is associated with adverse reproductive outcomes[4]. Mainly 5-10% of women in the reproductive age are affected by PCOS. PCOS is well acknowledged to be a state of genetic disorder. The most common symptom of PCOS is hypersecretion of androgen. Most of the women with PCO have an elevated level of androgen. Specific gene and its loci for PCOS as follows:

- Gene CYP 11A1 contains coding and promoter regions for the translation of protein, P450 SCC
- Promoter region of CYP 17 encodes a specific androgen regulating protein, P450 17 α
- Gene which encoding the enzyme, leptin plays a vital role in reproductive function and obesity.

The steroidogenic enzyme, CYP 17 functions as hydroxylation and lyase. It is present in the zona reticularis and zona fasciculata of the respective gonad tissues and adrenal cortex. In the first step of enzymatic activity, hydroxylation of pregnenolone and progesterone at the C17 position occurs resulting in the formation of 17-hydroxypregnenolone and 17-hydroxyprogesterone. During the second step of enzymatic action, C17-C20 bond of 17-hydroxypregnenolone and 17-hydroxyprogesterone are cleaved to generate dehydroepiandrosterone and androstenedione respectively[5]. Increase in CYP 17 activity in adrenal and ovarian sites induces hyperandrogenism in PCOS. In PCOS patients, gene that encodes CYP 17 has been overexpressed and androgen has been converted more efficiently to testosterone than normal theca cells[6]. The main side effects of the commercial synthetic drug, clomiphene citrate used for infertility are congenital heart disease and congenital malformation[7]. Thus, naturally extracted compounds from the medicinal plants have been preferred and developed as a potent therapeutic agent to treat infertility disorders. Easily accessible, scanty availability and lesser side effects must be the targeted role in identifying medicines from plants for all disease related problems.

Vitex negundo Linn., locally known as 'Nirgundi/ Sindhvar' is an important medicinal plant and is used for treatment of a wide spectrum of health disorders in traditional and folk medicine; some of which have been experimentally validated. It is widely planted as a hedge plant along the roads. *Vitex negundo* Linn. is a woody, aromatic shrub growing to a small tree. It commonly bears tri or penta foliate leaves on quadrangular branches, which gives rise to bluish-purple coloured flowers in branched tomentose cymes. *Vitex negundo* is used as folk medicine in most of the states of India, Bangladesh, and Southeast Asia. The leaves are most potent for medicinal use. *Vitex negundo* has been used to several ailment such as inflammation, eye disease, toothache, ulcers, fever, asthma, headache, digestion problems, sinuses, bronchitis, antibacterial, antipyretic, antihistaminic, analgesic, insecticidal, antidote for snake bite, etc. The leaves of these plants have been shown to have mosquito repellent effects as well[8][9]. The plant is also found to have anticancer, rheumatoid arthritis healing and hepato protective potentials. The leaves are used for treatment of inflammation, skin-ulcers, gonorrhoea, and bronchitis. They are also used as tonics, vermifuge to treat catarrhal fever. Oral administration of the leaves claims to have antihyperglycemic, antibacterial, antipyretic, antihistaminic agents, and implantation activity.[10][11]

Literature studies have reported that the gas chromatography mass spectrometry (GC-MS) analysis of ethanolic extract from the leaves of *Vitex negundo* showed the presence of 11 compounds. In modern drug design process, molecular docking studies are generally applied to identify the protein ligand interactions. Exploitation of natural active compounds which has potent therapeutic properties with least side effects using *in silico* docking analysis is necessary to manage female reproductive disorders. The framework of this study is mainly on finding out the binding effectiveness of phytoconstituents present in the ethanolic



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extract from the leaves of *Vitex negundo* against CYP 17 using molecular docking studies. Furthermore, the results obtained were compared with the docking score of known fertility inducing drug clomiphene citrate against CYP 17. This study provides a suitable platform for novel drug development for PCOS.

Docking Study

Preparation of the Protein

In the present study, the X-ray crystal structure of CYP 17 (PDB: 3RUK) was downloaded from the RCSB Protein Data Bank [PDBID: 5L3J] [12]., but the raw structure could not be applied directly for molecular docking studies. Since the raw structure describes only heavy atoms, cofactors, waters, metal ions and multimeric and it fails to provide information on topologies, bond orders, or formal atomic charges. The protein was further processed by removing the water molecules, ligands and other heteroatoms. The resulted clean structure of the protein was further optimized for docking by the addition of hydrogen bonds and Amberff14SB standard residues.

Preparation of the Ligand

The structures of the synthesized compounds were drawn using ChemSketch software where the output is saved as .mol2 files. Then these compounds were saved in 3D form using UCSF chimera Auto dock software. The ligand molecules were processed by the removal of water molecules and the addition of hydrogen bonds and charges. The charges were included as Amberff14SB standard residues and in Gastregier form in non-standard residues [13].

Docking using Auto Dock Vina

The docking study was performed on a crystal structure of CYP 17 (PDB: 3RUK) using Auto docking Vina in UCSF chimera software, in which there is an online version of AutoDock provided by opal web service. This method of docking allows only a single compound to be docked at a time. The pre-optimized compounds were open and pre-processed similarly and converted to pdqbt format. All the torsion angles in the small molecules were set free so as to perform flexible docking. Grid box of size 75x 75x75 with a centre spacing of zero was defined along x, y and z-axis. The grid box was adjusted to cover the active site of the protein. The analysis of binding and interaction of ligands with residues on active site was carried out by using Discovery studio 4.1

RESULTS AND DISCUSSION

The size of the active site was determined by generating the receptor grid. The most feasible orientation of the ligands in the binding pocket is predicted, and the strength of the interaction in the particular orientation was quantified from a scoring function. precision was preferred over standard mode mainly to determine the better correlation between good poses and good scores. Docking analysis was performed between ligands and the target protein CYP 17. The more negative value on the scoring function indicates better docking, and the docking of ligand and protein were ranked according to the corresponding scoring function. The scoring function of docking program was exhibited in the form of G-score. G-score indicates the binding ability of a ligand to the specific conformation of the protein receptor. The accuracy of a docking procedure is determined from the object scoring function which was predicted even in lowest energy pose (binding conformation). In this study, the validation of docking procedure was done by eliminating the inhibitor compound with the protein, CYP 17 that was evaluated from the binding energy, H-bonds, and G-score. 11 molecules were identified through ethanolic extract from the leaves of *Vitex negundo* using GC-MS analysis. Molecular interaction and binding affinity of ligand analogs with protein, CYP 17 were studied by docking every ligand into the active site of the protein, and the docking results of the ligands. For each minimized complex, the different interaction energies such as van der Waals energy, intermolecular hydrogen bonding and electrostatic energy were reckoned. All 11 compounds have exhibited moderate to potent inhibition with a range of -5.5 to -9.5. gamma.-Sitosterol have showed potent inhibition with the docking score of -9.5 respectively. The results revealed out that the compounds present in *Vitex negundo* can reduce the activity of CYP 17. This study throws a light on establishing the novel drug for infertility through experimental procedures. Docking studies using Glide software have proved that the above





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inhibitors fit into the binding pocket of the protein, CYP 17. Although the results, it was observed that successful docking has been correlated with good intermolecular hydrogen bonding and lipophilic interactions between the ligand and the receptor. From this study, it was inferred that the phytoconstituents exist in the *C. fruticosa* could be promising alternatives for the development of fertility drugs.

CONCLUSION

During drug discovery, effective screening procedures could be applied to reduce cost and time. In this study one of the effective methods, molecular docking has been used to analyze the binding ability between 11 compounds with CYP 17. Compounds such as gamma-Sitosterol have good docked pose with the protein by analyzing its G-score. This showed the importance of molecules from different herbals as docking agents. From this investigation, initial screening on identifying the potential fertility drugs from *Vitex negundo* has been developed for PCOS. This study throws a light on establishing the novel drug for infertility through experimental procedures.

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Table 1: Glide scores for the phytoconstituents from ethanolic extract of leaves of *Vitex negundo*

| S no | Name of the compound | G- score |
|------|---|----------|
| 1 | Viridiflorol | -7.8 |
| 2 | Ethyl .alpha.-d-glucopyranoside | -5.7 |
| 3 | BETA. ISO METHYL IONONE | -6.8 |
| 4 | BETA.-CHAMIGRENE | -7.5 |
| 5 | Phytol | -6.1 |
| 6 | ETHYL LINOLATE | -6.0 |
| 7 | 1,1,6-trimethyl-3-methylene-2-(3,6,9,13-tetramethyl-6-ethenye-10,14-dimethylene-pentadec-4-enyl)cyclohexane | -7.5 |
| 8 | Dodecenyl succinic anhydride | -6.3 |
| 9 | Squalene | -5.5 |
| 10 | Vitamin E | -7 |
| 11 | gamma.-Sitosterol | -9.5 |
| 12 | Clomiphene citrate | -5.6 |

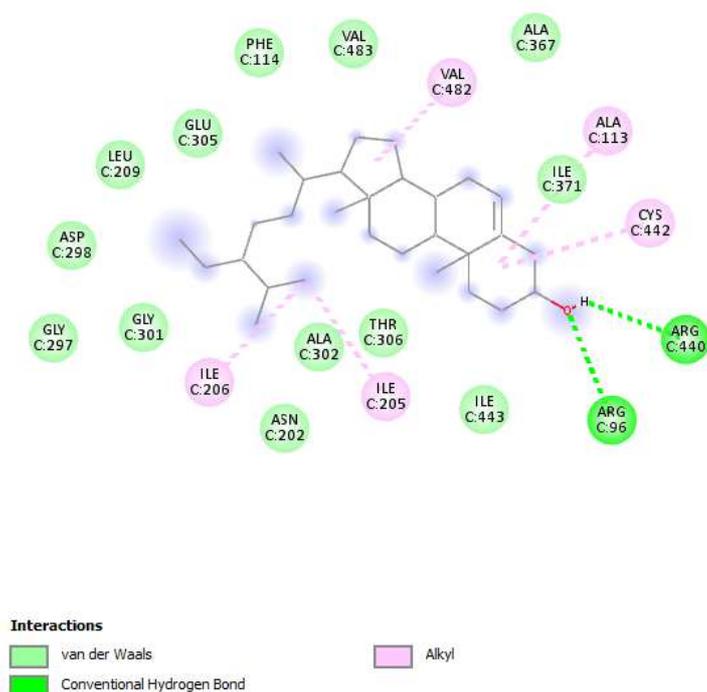


Fig : 1 Ligand interaction of gamma.-Sitosterol with CYP 1





CRLinkage : Privacy-Preserving Certificate Linkage / Revocation in Vanets Without Linkage Authorities

C.Rajkumar^{1*}, T.A.Sangeetha² and C.Rajkumar³

¹Research Scholar, Department of Computer Science, Kongu Arts and Science College, Erode, Tamil Nadu, India

²Associate Professor, Head and Department of Computer Science, Kongu Arts and Science College, Erode, Tamil Nadu, India

³Assistant Professor, Department of Computer Applications, Dr.SNS Rajalakshmi College of Arts and Science, Coimbatore, Tamil Nadu, India.

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*Address for Correspondence

C.Rajkumar

Research Scholar,
Department of Computer Science,
Kongu Arts and Science College,
Erode, Tamil Nadu, India.



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ABSTRACT

V2V (vehicle-to-vehicle communication) technology is predicted to become increasingly prevalent in the future, boosting transportation efficiency and safety. This predicted large-scale adoption, however, is contingent on overcoming certain hurdles. To reduce driver misuse, communications sent between authorized vehicles must be verified. This entails the construction of a Vehicle Public Key Infrastructure (VPKI). Unlike standard PKIs, VPKIs are intended to protect honest drivers' privacy by preventing their vehicles from being readily identified or monitored. We presented privacy-preserving certificate linkage/revocation in VANET without linking authority using Shuffled Binary Tree Encoding and Shuffled Binary Tree Decoding techniques. The Security Credential Management System is a potential VPKI solution that meets these standards and is one of the key standardization candidates in the United States (SCMS). In this paper, we make two substantial advances in our endeavor to improve SCMS and overcome its flaws. To begin, we discuss and resolve two birthday attacks on the SCMS certificate revocation procedure, thereby strengthening the system's long-term privacy. Second, we recommend that Linkage Authorities be removed from the SCMS architecture (LAs). This strategy reduces deployment costs and the attack surface of the system, notably against specific forms of replay assaults.

Keywords: CRLinkage, VANET, VPKI, Security, Routing, Multipath





INTRODUCTION

Transportation efficiency and road safety might be improved with the help of ITS [1]. Vehicle-to-everything (V2X) communications are essential for ITS systems to achieve this goal [2, including not only Vehicle-to-Vehicle (V2V) communications but also Vehicle-to-Infrastructure (V2I) and Vehicle-to-Pedestrian (V2P) communications]. These innovations allow for the consistent dissemination of critical data, including a vehicle's current position, velocity, acceleration, and course [3]. V2X has several promising applications; however there are security and privacy concerns with its extensive use [4]. For example, a rogue or faulty vehicle may send out misleading information that would actually increase the risk of collisions rather than reduce them. In addition, since vehicles are programmed to continually broadcast their whereabouts, this technology might be used to follow individuals or even to vehicle out widespread surveillance. In order to (1) issue digital certificates to vehicles for signing their messages, (2) revoke certificates from misbehaving vehicles, and (3) prevent reliable vehicles from being identified or tracked through message analysis, numerous proposals have been made to set up a secure and private Vehicular Public-Key Infrastructure (VPKI) [5].

Because they conceal the certificate owner's identity, VPKI that are based on pseudonym certificates are highly valued [6, 7]. The Security Credential Management System is a front-runner for standardization in the United States (SCMS). [8] SCMS provides vehicles with a number of interchangeable temporary pseudonym certificates, increasing the likelihood that they will be able to elude detection. Pseudonym certificates may be issued and revoked quickly and easily thanks to the system's streamlined processes. Certificates are issued by a Pseudonym Certificate Authority (PCA) when a Registration Authority (RA) creates a large number of public keys in response to a single vehicle request. Certificate revocation, on the other hand, just only entering a minimal amount of information given by Linkage Authorities (LA) (CRL). The final system protects the confidentiality of compliant automobiles, even from PCAs, LAs, and RAs that aren't cooperating. Because of its appealing features, recent studies [9-11] have aimed to improve SCMS's layout.

In this article, we improve the SCMS certificate linking and revocation processes in two separate (but mutually compatible) ways. In this paper, we show that the original SCMS's revocation privacy degrades with time, and then we fix this problem for next to nothing [12–15]. Then, we suggest a way to streamline SCMS's design by doing away with the requirement for LAs (LANs). To this end, SCMS is redesigned such that PCA and RA may effectively fill the roles of the LAs without risk to themselves or others. As a consequence, we are able to simplify the SCMS's architecture [16–20] by removing the potential failure/collusion point posed by LAs in the first iteration of the system.

BACKGROUND STUDY

Alfadhli, S. et al. [1] these authors proved an innovative multi-factor authentication method for VANETs, which shields V2V and V2I communications from being tracked. The security and privacy of the system were greatly improved by the introduction of dynamic multi-factor authentication-related materials and the removal of the need that such items be kept in a perfect TPD at all times. The GROOV protocol was shown to be secure against tampering by the author, who conducted both physical and cloning tests. Kumar, H., & Singh, D. [3] The purpose of these authors was to look at the adoption of an efficient approach for transferring CRL across all VANET nodes in a timely and effective way. Using both V2I and I2I communication, this system successfully distributes CRL segments among VANET nodes. The quantity of CRL components in a vehicle affects its ranking in this method. A vehicle of lesser rank may seek components from a vehicle of equal or higher status. More CRL components may be reused across automobiles if each automobile was assisted in finding a better fit. The results reveal that this system requires fewer piece list messages than the C2C Epidemic model to move more CRL pieces among vehicles in a given time period.

Lu, Z. et al. [5] The CMT and MPT were used by the author to enhance the standard blockchain structure and create a unique authentication technique for VANETs that protects user privacy. The author's suggested BPPA system has transparent certificate issuance and revocation as well as secure authentication, conditional privacy, high efficiency,



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and scalability. First, the transactions were logged in the blockchain, making the semi-TAs' behaviour visible and traceable to all organisations. Second, the blockchain's global consensus process and standard cryptographic primitives enable a safe authentication technique in the face of attack scenarios. Third, the authority's secret key was used to encrypt the relationship between a vehicle's certifications and its true identity. As a consequence, the true identification of a target vehicle may only be exposed if disputes arise.

Qi, J., & Gao, T. [7] For cross-domain vehicle authentication, these authors investigate a safe and efficient anonymous identity-based authentication technique. The usage of pseudonyms protects the anonymity of automobile users. By mandating a λ -interval pseudonym acquisition policy, the author can make sure that all domain-level pseudonyms expire at the same time as the TA and RSUs, hence prohibiting any possibility of pseudonym linking. When the revocation event happens, all pseudonyms were unlinked, which improves privacy protection. The Bloom filter was also used to optimise CRL. Rajput, U. et al. [9] A practical method for implementing conditional privacy on a VANET was explored in this article. The weaknesses of both pseudonymous and group signature methods were remedied by the hybrid method. Several fresh concepts, such as a lightweight pseudonym with a trapdoor method to avoid CRL, were introduced in these authors. The trapdoor's efficient operation makes monitoring possible. The CA in this system was designed in a modular fashion with scalability and cloud computing deployment in mind. Modules like this effectively handle a broad variety of network needs, such as vehicle registration, the generation and distribution of pseudonyms and regional cryptographic credentials, and vehicle revocation. It was noteworthy that this study also presented the concept of geographical zones in a VANET scenario, whereby a group of vehicles may interact with one another without exposing their identities.

Roshini, T. et al. [11] The author research an ID-based method for Vanet in this study. As part of the simulation, the author subjected the system to a variety of realistic scenarios beyond just the one "heavy load" scenario. In this analysis, the author focuses on two limitations for authentication in vanets that aim to protect users' anonymity. The clustering structure greatly decreases the certificate burden during the massive deployment of vanets, and the keys were scattered among Upper and Lower level TAs to protect the master secret. The hierarchical pseudonym-based strategy was unsecure and did not provide enough privacy during collision. Master secrets were not always retained in single TAs in an open-access scenario, and certificate management was a challenge for large-scale vanet deployment. Simplicio Junior, M. et al. [13] The widespread implementation of ITS depends on two factors: (i) data authentication, which allows vehicles to screen out spoofed communications, and (ii) user privacy, which protects trustworthy motorists from being watched by their peers or the system. The Security Credential Management System (SCMS) was an effective answer to these issues since it allows for the fast, scalable, and secure issuance and revocation of pseudonym certificates.

Tuladhar, K. M., & Lim, K. [15] An international system for CRL distribution and management in hierarchical VANETs was presented in these authors. The authors suggest broadcasting numerous regional CRLs effectively across hierarchical VANETs by dividing the global CRL into smaller chunks. The size and amount of false positives in the CRL were reduced with the application of the dual bloom filter in this approach. Based on the findings reported here, it seems that a compressed CRL size much less than the global CRL size may be feasible. In addition, a domain's size may be dynamically modified to suit a variety of circumstances, such as the number of vehicles in the domain or the target size of the regional CRL. Wei, L. et al. [18] these authors research were considering a technique that combines identity-based conditional privacy preservation with message recovery and SSK updating. The suggested CPPA method used elliptic curve encryption and provided message recovery to shorten the pseudo identity, traffic emergency message, and matching signature. Accordingly, the CPPA system would be favoured for protecting transmissions of time-critical traffic emergency alerts. The intended SSK updating method allows only unrevoked autos to get the most current SSK through an unsecured public channel, hence increasing the security of the SSK, which was the CPPA system's cornerstone. The CPPA approach and SSK updating algorithm developed have the ability to successfully protect traffic emergency message transmissions.

MATERIALS AND METHODS





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Network Model

An intelligent vehicle as conceived in effectively contains a series of sensors (face radar, reverse radar, etc.) that receive valuable information about the environment, which is not usually perceived by the driver alone.

Road Side Unit:

The roadside machine is a computer that is attached next to or at a certain spot, for example, parking or intersection.

CRLinkage model

The Proposed model is generated using Shuffled Binary Tree Encoding and Shuffled Binary Tree Decoding

Shuffled Binary Tree Encoding

Our tree encoding strategy was motivated by a well-liked binary search method for one-dimensional sorted arrays. Figure 1 depicts a dataset with $N = 8$ training images that we'll use to illustrate the idea behind our encoding approach (note that the data instances are indexed from 0 to $N-1$). The binary-to-decimal conversion procedure, $h: R-d_b$, where b is the number of bits in the binary form, is appended to the labels of each training set vector ($N \times b$). Contrarily, Figure 1 a, $b = 3$ demonstrates that $h(x_0) = 000$, $h(x_1) = 001, \dots$, and $h(x_7) = 111$. It is also well known that the arrangement of the binary representations' 0 and 1 bits creates a binary tree if the values of $h(x_i)$ are written vertically, as in Figure 1 All data is kept on the first level of the $b+1$ -layered tree (the root). Recursive partitioning of the vectors, similar to conventional binary search, begins at the root node, which in this case is the whole training dataset. Specifically, when there are l levels in a tree, the left child (zero-node) stores all of the information from the parent node whose l th bit in binary is 0. The right child (one-node) does the same (remember that bit indices start at 1), but only for those elements in the binary representation of the parent node where the l th bit is 1. x_0, \dots, x_3 are offspring of the left child node in the first level of Fig. 1 because their first bit is 0, whereas x_4, \dots, x_7 are offspring of the right child node because their first bit is 1. Each successive node in the tree might be partitioned in a similar fashion.

Shuffled Binary Tree Decoding Algorithm

Receive beam forming generates both the user signal and the background noise signal, which are combined and sent into the decoder as input. A CS decoder, a tree decoder, or both are used in the decoding process. Common sub-blocks supplied by all users may be recovered by conventional CS decoders using CS algorithms. By tracing the connections between the different sub-blocks in the coding trees produced by the tree decoder, the original messages may be recreated.

CS Decoder For each sub-slot s , the received signal can be expressed as

$$Y_s = A\Delta H_s + Z_s = AX_s + Z_s \text{ ----- (1)}$$

Row sparsity in the matrix X_s allows for its possible recovery through computational science methods like Approximate Message Passing (AMP). If there is a lot of scattering, the Gaussian model may be used as a good statistical approximation to the actual channel coefficients. In mmWave communications, scatterers aren't a factor, hence the entrance H_s can't be roughly modelled by a Gaussian distribution. As a solution, we provide a novel method of detecting physical motion. Here, we use the Gaussian mixture (GM) model for activity detection and the GM-AMP model for channel estimation to estimate the unknown prior distribution. Given these values for the coefficients, the equation $X_s = [x_{s,1}, x_{s,2}]$ may be written. One such example is the formula $X_s = [x_{s,1}, x_{s,2}]$.

$$px(x; p, w, \mu, v) = (1 - \rho)\delta(x) + \rho \sum_{i=1}^I w_i N(x; \mu_i, v_i), \text{ ----- (2)}$$

in where (px) is the Dirac delta, $(\delta(x))$ is the sparsity rate, and $(\rho \sum_{i=1}^I w_i N(x; \mu_i, v_i))$ is the number of GM components, w_k , μ_k , and v_k are the weight, mean, and variance of the k th GM component, respectively. The sparsity rate quantifies how sparse the vector really is. You may train the EM technique to adjust the weights, averages, and





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dispersion numerous times. Estimates of X_s , denoted by X_s , are provided by the CS algorithm for each individual sub-slot s . The activity detector $a_k(s)$ is defined using maximum-ratio-combining (MRC) as

$$a_k(s) = \begin{cases} 1, & \sum_{i=1}^{N_{RF}} n_i |X_{k,i}^{(s)}| \geq \epsilon \\ 0, & \sum_{i=1}^{N_{RF}} n_i |x_{k,i}^{(s)}| < \epsilon \end{cases} \dots\dots (3)$$

where ϵ is a threshold, and n_i is expressed as

$$n_i = \frac{|x_{k,i}^{(s)}|}{\sqrt{\sum_{j=1}^{N_{RF}} |x_{k,j}^{(s)}|^2}} \dots\dots (4)$$

$K_s = k$ and $a_k(s) = 1$ for every k between 1 and N . The shared codebook's indices of transmitted code words are compiled by the activity detector. For each set of s , let $A = k \mid a_k(s) = 1$, where k may be any positive integer from 1 to N . Since there is only one sub-block linked with each codeword, retrieving the sub-block after the discovery of a codeword is fast.

RESULTS AND DISCUSSION

We simulate our proposed protocol in NS2 Simulator. We compare our CRLinkage Model with the A Geographic Routing Based on Road Traffic and Multi-hop Intersections in VANETs (GRBRT) and A Geographic Routing Over VANETs (GROOV) and SDN-Enabled Spectral Clustering-Based Optimized Routing (SeScR). The Network size is 550 x 480 m. and the network parameters as communication overhead, signature generation, verification and delay. The network model Delay comparison chart is compared with GRBRT, GROOV, SeScR and CRLinkage method represented in figure 1. X-axis denotes the number of vehicles and Y-axis denotes the shortest route. Throughput with respect to the number of vehicles is compared with GRBRT, GROOV, SeScR and CRLinkage method represented in figure 2. X-axis denotes the number of vehicles and Y-axis denotes the shortest route. Network Overhead with respect to the number of vehicles is compared with GRBRT, GROOV, SeScR and CRLinkage method represented in figure 3. X-axis denotes the number of vehicles and Y-axis denotes the shortest route. Packet Loss Ratio is compared with GRBRT, GROOV, SeScR and CRLinkage method represented in figure 4. X-axis denotes the number of vehicles and Y-axis denotes the shortest route.

CONCLUSION

Vehicle-to-everything (V2X) communication technologies are becoming more ubiquitous, and many standardization initiatives are concentrating on them. Many approaches for developing a vehicle public key infrastructure have been offered due to the necessity for security and privacy (VPKI). SCMS is well-known for its scalable, efficient, and privacy-protecting pseudonym certificate issuance and revocation mechanism. We study with the SCMS linkage and revocation process in this work and suggest two design modifications. The first concerns attacks that may degrade the system's (forward) privacy as more certificates are issued and revoked over time by exploiting the birthday paradox. The suggested method, which is based on security strings, has the potential to either (1) prevent this reduction in security at a minimal cost, or (2) boost system security without incurring any overhead. Furthermore, we demonstrate how the job of LAs may be securely reallocated to the PCA and RA, therefore removing the necessity for LAs to exist as separate organizations. Shuffled binary tree certificates result in better security and less implementation costs with minimal delay. For further to introduce in ML algorithm for analyzing the delay with various performance measures.

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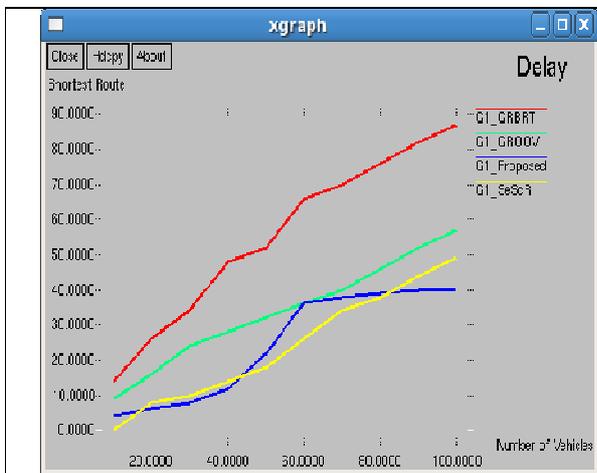


Figure 1: Delay comparison chart

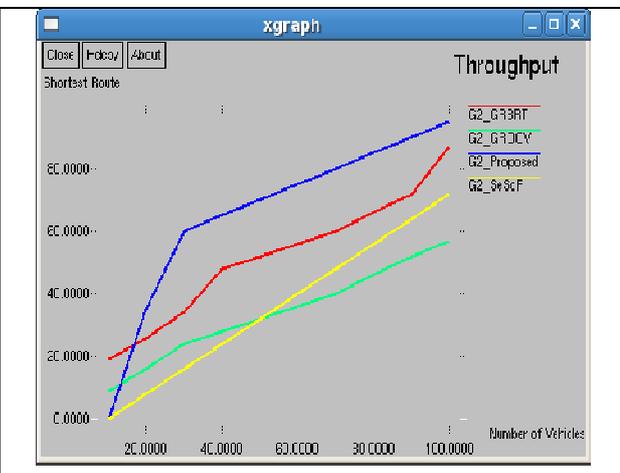


Figure 2: Comparison chart for throughput with respect to the number of vehicles

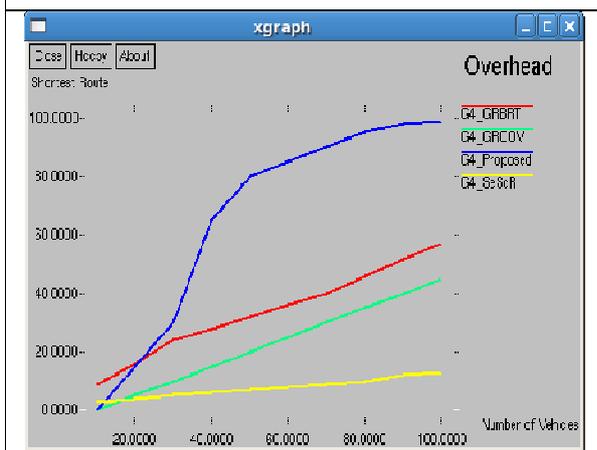


Figure 3: Comparison chart for overhead with respect to the number of vehicles

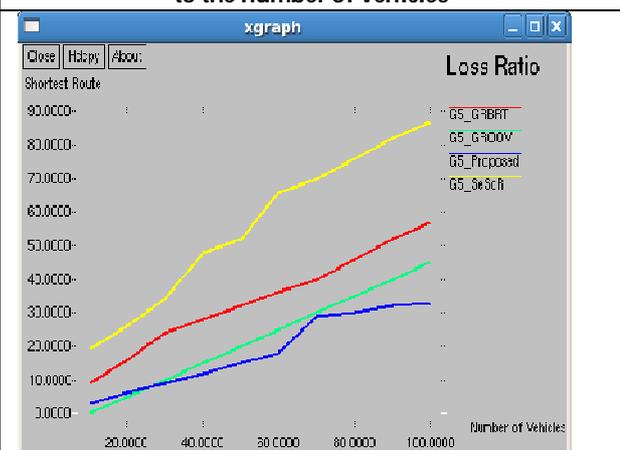


Figure 4: Comparison chart for Loss Ratio





***In silico* Design, Characterization and Biological Evaluation of Novel 3-Substituted Coumarin Derivatives**

Bijula .K¹, Cyril Jacob², Shadiya.C.K³, AnilKumar², Kavitha.S² and Akash Marathakam^{4*}

¹M.Pharm Student, Department of Pharmaceutical Chemistry, National College of Pharmacy Kozhikode, Kerala-673601, India.

²Assistant Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy Kozhikode, Kerala-673601, India.

³Associate Professor, Department of Pharmaceutical Chemistry, National College of Pharmacy Kozhikode, Kerala-673601, India.

⁴Professor and Head, Department of Pharmaceutical Chemistry, National College of Pharmacy Kozhikode, Kerala-673601, India.

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***Address for Correspondence**

Akash Marathakam

Professor and Head,
Department of Pharmaceutical Chemistry,
National College of Pharmacy Kozhikode,
Kerala-673601, India.
E.Mail: amarathakam@gmail.com



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ABSTRACT

Coumarins are the heterocyclic aromatic organic compounds in which benzene and α -pyron groups are fused. Coumarin derivatives play important role in medical field with Pharmacological activities such as antimicrobial, antiviral, antidiabetic and anticancer activity etc. Ortho-hydroxy benzaldehydes and 1-ethylacetoacetate or malonic acid after a 2 h reflux in ethanol in the presence of a catalytic amount of different heteropolyacids (HPAs) generate 3-acetyl coumarin. These compounds were characterized by IR spectroscopy, ¹H NMR spectroscopy. Docking studies were done to know about their docking scores and by using software's were Chems sketch 2017, ChemBiodraw, Aurgus lab 4.0.1, Discovery studio 2017 R2 and SCF Bio. Their biological activities were evaluated by different in-vitro methods such as anticancer, anti-inflammatory and antioxidant activities.

Key words: Ethyl coumarin-3-carboxylate; Perkin reaction; Novel 3- substituted coumarin derivatives; Anti-cancer activity ; Molecular docking





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INTRODUCTION

Drug discovery and development is a process by which new drugs are discovered or designed with remedial benefit. Discovery may involve screening of chemical libraries, identification of the active ingredient after natural remedy or and ultimately regulatory approval [1]. Drug design resultant from an understanding of the target. This includes study on microorganisms and animal, clinical trials used for drug discovery process are made by synthesizing compounds in a multi-step process, followed by in vivo and in-vitro biological screens and further investigating the talented candidates for them.[2,3] Pharmacokinetic properties, metabolism and potential toxicity, and these drug development processes has shown in various failures ascribed to low pharmacokinetics (39%), lack of efficacy (30%), animal toxicity (11%), adverse reactions in humans (10%) and various commercial and various other factors. At present, due to failure of traditional drug discovery, the process has been transformed with the introduction of genomics, proteomics, bioinformatics and advanced technologies like, combinatorial chemistry, high throughput screening (HTS), virtual screening, de-novo design, *In-silico* ADME/T screening and structure-based drug design [2,3].

New technologies for synthesizing a novel drug against a selected target for a specific disease usually involves combinatorial chemistry (combiChem), microwave assisted organic synthesis (MAOS) and high-throughput (HTS) biological screening methods. *In silico* methods have been developed and contain quantitative structure activity relationships, databases, pharmacophores, similarity searching, homology models and other molecular modelling, which uses a computer. These methods have been frequently used in the drug discovery process for identifying drug targets via bioinformatics tools and for the illumination of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterisation.[4,5]. Intervention of computers at certain steps reduces the cost and time required in the drug discovery process. *In silico* designing is a computer-based method whose technologies are useful in drug target identification, optimization etc [6]. Nowadays the use of computers and computational methods pervades all phases of drug discovery and forms the core of structure-based drug design [7]. Important roles of computation in drug discovery are virtual screening and de novo design. *In silico* ADMET estimation and highly developed methods for determining protein-ligand binding and structure-based drug design. Computers are used to modify chemical structures that might work against it. Drug will bind to the target cell, which causes the disease [8]. The steps involved in drug discovery process are target identification, target validation, lead identification, lead optimization, testing of the Active Compound (Pre-Clinical Phase) and clinical trials [9-10].

Docking is often used to predict the binding alignment of drug molecules against protein targets in order to assess the affinity and action of the drug. Therefore, molecular docking plays a vital role in the rational drug discovery process. The principal of docking is to computationally simulate the molecular identification process and to attain an optimized conformation so that the free energy is minimized. Modern drug discovery is mainly based on the application of computer techniques and plays a significant role in rational drug design [6,11]. Coumarin is a phytochemical (benzopyrone); a toxin found in many plants, notably in high concentration in the vanilla grass, strawberries, cherries, cinnamon, sweet clover and having vanilla like flavour and is oxygen heterocycle.[25] Structure of Coumarin can occur either free or combined with the sugar glucose(coumarin glycoside). It has a sweet scent, readily recognized and has been used in perfumes since 1882. Coumarins are naturally occurring polyphenolics distributed widely in plants, fungi, and bacteria and have found applications for centuries in traditional medicine.[26] The biosynthesis of coumarin in plants is via hydroxylation, glycolysis and cyclization of cinnamic acid.

They are widely used as additives in food, perfumes, cosmetics, pharmaceuticals, dispersed fluorescent and laser dyes, insecticides and in optical brighteners. Coumarins constitute an important class of compounds with several types of pharmacological agents possessing anticancer, anti-HIV, anticoagulant, spasmolytic and antibacterial activity among others. of the many actions of coumarins, antioxidant and antiproliferative effects stand out. A large number of structurally novel coumarin derivatives had shown substantial cytotoxic activity in vitro and *in vivo*.





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MATERIALS AND METHODS

All experiments were carried out under room temperature unless stated otherwise. Chemicals and reagents such as Salicylaldehyde, Ethyl acetoacetate, Piperidine, Butanol, Isopropanol and Ethanol. Melting points were measured with a laboratory melting point apparatus and were uncorrected. Proton nuclear magnetic resonance (¹HNMR) spectra were recorded on a ppm (400MHz) spectrometer using CD₃OD as solvent and tetramethyl silane (TMS) as an internal standard. All chemical shifts (δ) were reported in parts per million (ppm) and spin-spin coupling constants, J, were expressed in Hz. IR spectra was recorded on a Shimadzu FTIR-8400S spectrometer (Shimadzu Corporation, Japan). Molecular docking done by using softwares such as Chesketch 2017, ChemBiodraw, Aurgus lab 4.0.1, Discovery studio 2017 R2, SCF Bio.

In-silico docking

Molecular docking studies were performed in maestro 11.2 using glide (Schrödinger, LLC, New York, NY, 2017). This is an interactive molecular graphics program from docking calculations, for identification of the probable binding site of the bio molecules, and for visualizing ligand receptor interactions. All compounds were build using maestro build pane and optimized to lower energy conformation using Ligprep which uses an OPLS_2005. The coordinate for enzyme for anticancer activity (PDB ID-2Y0B and for anti-inflammatory activity (PDB ID: 1HNE). Were taken from RCSB Protein Data Bank and prepared for docking using 'protein preparation wizard' in maestro11.2. Water molecules in the structure were removed and termini were capped by adding ACE and NMA residue. The bond orders and formal charges were added for hetero groups and hydrogen were added to all atoms in the structure side chain that are not close to binding cavity and do not participate in salt bridges were neutralized. After preparation, the structure was refined to optimize the hydrogen bond network using opls_2005 force field. The minimization was terminated when the energy converged or the RMSD reached a maximum cut off of 0.30Å. Grids were then defined around the refined structure by centering on ligand using default box size. The extra precision XP docking mode of all the compounds was performed on a generated grid of protein structure. The final evaluation of ligand protein binding was done with glide score (docking score).

General procedure

Synthesis of Ethyl coumarine-3-carboxylate by Perkin method: In a one litre of round bottom flask, add 25ml salicylaldehyde and 27.5ml ethyl acetoacetate. Mix well. Then add 0.5ml of piperidine as base. The mixture was refluxed for 2 hours. After completion of the reaction, the mixture was transferred into a glass beaker and it gently cooled in running water. The precipitated product was filtered with suction. Then product was dried. Crude product yield was found to be 82% it was recrystallized by using ethanol and melting point was found to be 119 – 122° C.^[54]

Synthesis of 3- substituted coumarin derivative A

Step 1: Synthesis of ethyl coumarin -3-carboxylate

In a one litre of round bottom flask, add 25ml salicylaldehyde and 27.5ml ethyl acetoacetate. Mix well. Then add 0.5ml of piperidine as base. The mixture was refluxed for 2 hours. After completion of the reaction, the mixture was transferred into a glass beaker and it gently cooled in running water. The precipitated product was filtered with suction. Then product was dried. Crude product yield was found to be 82% it was recrystallized by using ethanol and melting point was found to be 119 – 122° C. [54]

Step 2: Synthesis of 3- substituted coumarin derivative A

About 0.01 mole ethyl coumarin-3-carboxylate, 0.01 mole butanol and 0.01 mole of piperidine mix well and reflux it for about 1-2 hrs on water bath. The reaction mixture was then poured into cold water, pale yellow coloured product will be separated in hot, filter it dry and recrystallize it from ethanol.



**Synthesis of 3- substituted coumarin derivative B:****Step 1: Synthesis of ethyl coumarin -3-carboxylate**

In a one litre of round bottom flask, add 25ml salicylaldehyde and 27.5ml ethyl acetoacetate. Mix well. Then add 0.5ml of piperidine as base. The mixture was refluxed for 2 hours. After completion of the reaction, the mixture was transferred into a glass beaker and it gently cooled in running water. The precipitated product was filtered with suction. Then product was dried. Crude product yield was found to be 82% it was recrystallized by using ethanol and melting point was found to be 119 – 122° C.

Step 2: Synthesis of 3- substituted coumarin derivative B

About 0.01 mole ethyl coumarin-3-carboxylate, 0.01 mole isopropanol and 0.01 mole of piperidine mix well and reflux it for about 1-2 hrs on water bath. The reaction mixture was then poured into cold water, pale yellow coloured product will be separated in hot, filter it dry and recrystallize it from ethanol.

Synthesis of 3- substituted coumarin derivative C

Step 1: Synthesis of ethyl coumarin -3-carboxylate: In a one litre of round bottom flask, add 25ml salicylaldehyde and 27.5ml ethyl acetoacetate. Mix well. Then add 0.5ml of piperidine as base. The mixture was refluxed for 2 hours. After completion of the reaction, the mixture was transferred into a glass beaker and it gently cooled in running water. The precipitated product was filtered with suction. Then product was dried. Crude product yield was found to be 82% it was recrystallized by using ethanol and melting point was found to be 119 – 122° C.

Step 2: Synthesis of 3- substituted coumarin derivative C

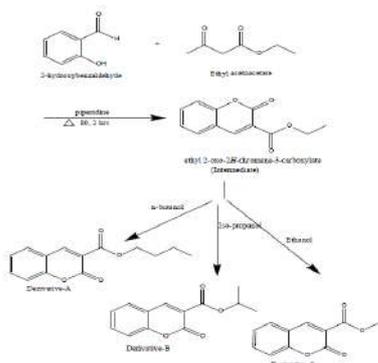
About 0.01 mole ethyl coumarin-3-carboxylate, 0.01 mole ethanol and 0.01 mole of piperidine mix well and reflux it for about 1-2 hrs on water bath. The reaction mixture was then poured into cold water, pale yellow coloured product will be separated in hot, filter it dry and recrystallize it from ethanol.

CHARACTERIZATION

Butyl 2-oxo-2H-chromene-3 -carboxylate (3SCD-A): Dark yellow crystal, yield -76%; Melting point 136.47°C, Molecular formula C₁₄H₁₄O₄; Molecular weight (gm/mol)-246.09 IR (KBr, cm⁻¹) 3067.25 (C-H stretching on Aromatic ring), 1556.56 (Aromatic C=C), 2934.32 (C-H stretching on alkane), 1731.03 (C=O stretching on ester). ¹HNMR ppm (400MHz in CD₃OD): 0.90-1.19 (CH₃-methyl 1 beta C-C) doublet; 1.47-3.97 (CH₂- methylene 1 beta C=O) doublet; 7.43-7.86 (CH- H on coumarin) doublet; 8.55 (H- 1-C=O cis from ethylene in coumarin) singlet. Isopropyl-2-oxo-2H-chromene-3 carboxylate(3SCD-B): Yellow crystal, (yield 80%); Melting point 110.2°C, Molecular formula C₁₃H₁₂O₄; Molecular weight (gm/mol)-232.07. IR (KBr, cm⁻¹) 3067.02(CH stretching on alkene), 3028.75(CH on stretching), 2934.56 (CH stretching on alkane), 1605.20 (C=C stretching on aromatic), 1743.23 (C=O stretching on ester). ¹HNMR ppm (400MHz in CD₃OD): 1.11 (CH₃-methyl 1 beta C-C) singlet; 4.87 (CH₃- methylene 1alpha C-C) singlet; 7.38-7.86 (CH- H on coumarin) multiplet;

Reaction Employed

8.55 (H- 1-C=O cis from ethylene in coumarin) singlet. Methyl 12-oxo-2H-chromene-3-carboxylate (3SCD-C): Yellow





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crystal, (yield 80%); Melting point 102.03°C, Molecular formula C₁₁H₈O₄; Molecular weight (gm/mol)-204.04 IR (KBr, cm⁻¹) 3180.38(CH stretching on aromatic), 3030.53 (CH stretching on alkene), 2980.28 (CH stretching on alkane), 1743.14 (C=O stretching on ester), 1571.12 (C-C stretching on aromatic), 1483.37(C=C stretching on aromatic) ¹HNMR ppm (400MHz in CD₃OD): 3.71 (CH₃-methyl 1 alpha -OC) singlet; 7.38-7.86 (CH- H on coumarin) multiplet; 8.55 (H- 1-C=O cis from ethylene in coumarin) singlet.

BIOLOGICAL EVALUATION

Anticancer activity Trypan blue exclusion method: the test compounds were studied for short term invitro cytotoxicity using Dalton's lymphoma ascites cells (DLA). The tumour cells aspirated from the peritoneal cavity of tumour bearing mice were washed thrice with PBS or normal saline. Viable cell suspension (1x 10⁶ cells in 0.1 ml) was added to tubes containing various concentration of test compounds and the volume was made upto 1ml using phosphate buffered saline (PBS). Control tube contains only cell suspension. These assay mixture was incubated for 3 hrs at 37°C. Further cell suspension was mixed with 0.1ml of 1% trypan blue and kept for 2-3 min and loaded on haemocytometer. Dead cells taken up the blue colour of trypan blue but live cells not take. The number of stained and unstained cells was counted separately [30,31,36,39].

$\% \text{ cytotoxicity} = \frac{\text{No. of dead cell}}{\text{No. of dead cell} + \text{No. of live cell}} \times 100$

Anti-inflammatory activity (Protease inhibition assay):

100 ml of bovine albumin was added to 0.1 ml of sample. This was incubated at room temperature for 5 minutes. Reaction was inhibited by the addition of 0.25ml of trypsin followed by centrifugation. The supernatant was collected, and absorbance was observed at 210 nm. Naproxen was used as a control. The experiment was carried out and percent inhibition of protease inhibition was estimated. [34,57,71]

$\% \text{ Inhibition} = 100 - \left(\frac{A1 - A2}{A0} \right) \times 100$

Where A1 = absorbance of the sample,

A2 = absorbance of the product control

A0 = absorbance of the positive control.

Anti-oxidant activity Hydrogen peroxide scavenging activity: The H₂O₂ scavenging potential of the test compounds were detected according to the method of Ruch et al. Solution of H₂O₂ (40 mM) was assembled in phosphate buffer (pH 7.4). 20, 40, 60, 80 and 100 mg/ml concentrations of the test compounds in 3.4 ml phosphate buffer were added to H₂O₂ solution (0.6 ml, 40 mM). The absorbance value was recorded at 230 nm [32,33,58]. The percentage scavenging of H₂O₂ was calculated as: $\% \text{ of scavenging} = \left[\frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \right] \times 100$

RESULTS AND DISCUSSION

In silico molecular analysis of diverse 3 substituted coumarin derivatives have been evaluated. And all the compounds were checked by 'Lipinski rule of five'. None of the compound violates the rule. Lipinski rule analysis of proposed 3 substituted coumarin derivatives by SCFBio online software obtained Molecular mass (MM), Hydrogen donor (HD), Hydrogen acceptor (HA), LogP and Molecular refractivity (MR). The derivatives such as 3SCD-A (246.09, 0.4, 0.16, 45.64), 3SCD-B (232.07, 0.4, 0.50, 42.38), 3SCD-C (204.04, 0.4, 0.49, 36.56). The proposed derivatives were docked with various cancer targets and inflammatory targets and the docking scores obtained are as follows. Docking scores for synthesized compounds against 2Y0B is SCD-A (-7.245), SCD-B (-8.545), SCD-C (-7.799). Then the Docking scores for synthesized compounds against 1HNE3SCD-A (-7.969), 3SCD-B (-6.990), 3SCD-C (-8.893). The percentage inhibition of synthesized compounds was found out by hydrogen peroxide free radical scavenging activity technique. The concentrations of synthesized compounds were compared with the standard ascorbic acid at 440nm.





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CONCLUSION

The present study reports the successful synthesis of various 3-substituted coumarin derivatives and assessment of their anticancer, anti-inflammatory, antioxidant activity. First the proposed derivatives were screened on the basis of lipinski rule of five using SCF Bio online software. After the screening, three derivatives were selected namely, 3SCD-A, 3SCD-B and 3SCD-C. Then it was made to dock with receptor selected from PDB. Here for anticancer activity docking was done on caspase (PDB ID: 2Y0B), and for anti-inflammatory activity, on Leukocyte elastase (PDB ID: 1HNE). It showed good docking score compared to the standard drugs. The ethyl coumarine-3-carboxylate was synthesized by perkins Condensation reaction from this using different alcohols the 3 derivatives were synthesized. After that, characterization of the derivatives was performed by IR, NMR and MASS spectroscopy. From the synthesized derivatives, 3SCD-B and 3SCD-C showed good anticancer activity, were as 3SCD-C and 3SCD-A showed good anti-inflammatory activity, and 3SCD-A and 3SCD-C showed good antioxidant activity. These results make these synthesized 3 substituted coumarin derivatives an interesting lead molecule for more synthetic and biological evaluation. These compounds certainly hold great promise towards pursuit to discover novel class of anticancer, anti-inflammatory, antioxidant agents in order to further improve these activities in future.

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Table . 1 List of Perkins condensation reaction products

| Compound Code | Structure | IUPAC name of the compound |
|--|-----------|---|
| ethyl coumarin-3-carboxylate | | ethyl 2-oxo-2H-chromene-3-carboxylate |
| 3- substituted coumarinderivative A (3SCD-A) | | butyl 2-oxo-2H-chromene-3-carboxylate |
| 3- substituted coumarinderivative B (3SCD-B) | | isopropyl 2-oxo-2H-chromene-3-carboxylate |
| 3- substituted coumarinderivative C (3SCD-C) | | methyl 2-oxo-2H-chromene-3-carboxylate |

Table 2 Antioxidant Value with Standard Deviation

| Compound code | Percentage inhibition | | | | | |
|---------------|-----------------------|------------|------------|------------|------------|-------|
| | 10(mcg/ml) | 20(mcg/ml) | 30(mcg/ml) | 40(mcg/ml) | 50(mcg/ml) | IC50 |
| 3SCD-A | 28.3 | 41.8 | 51.2 | 64.8 | 74.4 | 28.7 |
| 3SCD-B | 19.3 | 21.4 | 35.8 | 40.2 | 48.9 | 45.4 |
| 3SCD-C | 26.7 | 35.8 | 45.6 | 52.8 | 61.3 | 33.7 |
| Ascorbic acid | 32.6 | 48.4 | 59.5 | 74.2 | 81.5 | 25.32 |

Each value expressed as percentage of activity mean \pm deviationTable .3 *In-vitro* Cytotoxicity of Synthesised Compounds Against Dia

| Derivatives | Percentage growth inhibition (%) (concentration in $\mu\text{g}/\text{ml}$) | | | | | |
|---------------|--|----------------|----------------|-----------------|-----------------|--------|
| | 10 | 20 | 50 | 100 | 200 | IC 50 |
| 3SCD-A | 6.78 \pm 0.8 | 7.86 \pm 0.1 | 8.57 \pm 1.3 | 10.7 \pm 2.4 | 16.7 \pm 1.9 | 375.41 |
| 3SCD-B | 7.33 \pm 1.03 | 9.5 \pm 1.73 | 14.3 \pm 2.0 | 21.41 \pm 1.2 | 32.9 \pm 0.93 | 222.37 |
| 2SCD-C | 6.62 \pm 0.9 | 7.54 \pm 0.4 | 8.58 \pm 0.9 | 10.4 \pm 1.4 | 17.2 \pm 1.8 | 377.43 |
| 5-Flurouracil | 20 | 32 | 50 | 58 | 70 | 82.60 |





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Table 4. *In-vitro* anti-inflammatory activity of synthesized compounds

| Compound code | Percentage inhibition± standard deviation | | | |
|---------------|---|----------------|----------------|------------------|
| | 25mcg/ml | 50mcg/ml | 100mcg/ml | IC ₅₀ |
| 3SCD-A | 31.4312±0.1672 | 37.5781±0.1267 | 49.5241±0.1521 | 73.818 |
| 3SCD-B | 21.5832±0.1217 | 29.8234±0.1167 | 34.2346±0.0974 | 102.170 |
| 3SCD-C | 42.1456±0.1201 | 48.2356±0.1034 | 59.4765±0.0863 | 58.388 |
| Diclofenac | 78.4924±0.0973 | 88.4861±0.0521 | 93.2154±0.0307 | 34.590 |

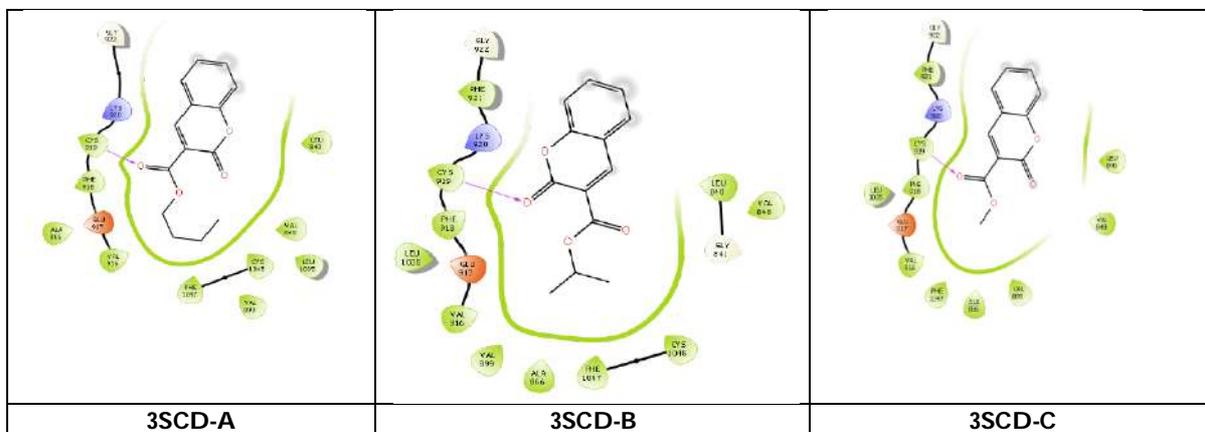


Fig. 1 Docking Images of Synthesized Compounds In 2y0b Targets

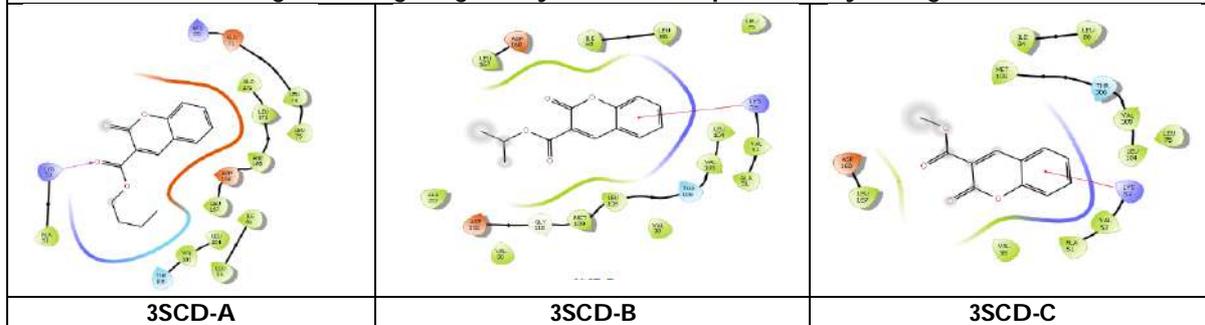


Fig. 2. Docking scores for synthesized compounds against 1HNE

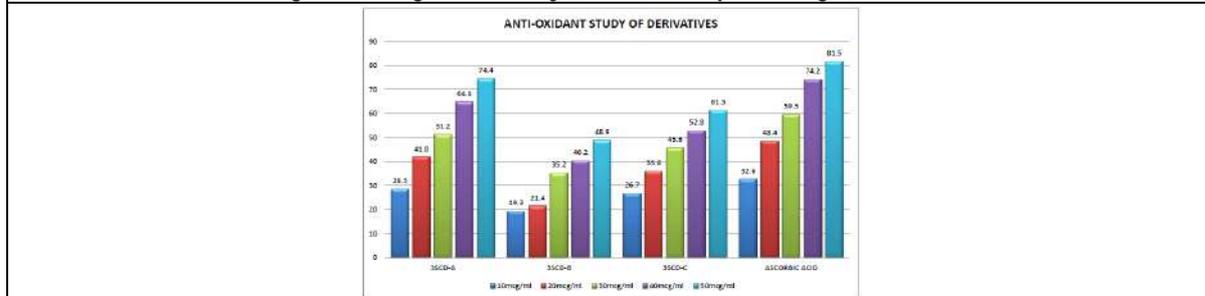


Fig. 3 Anti-Oxidant Activity Study of Derivatives





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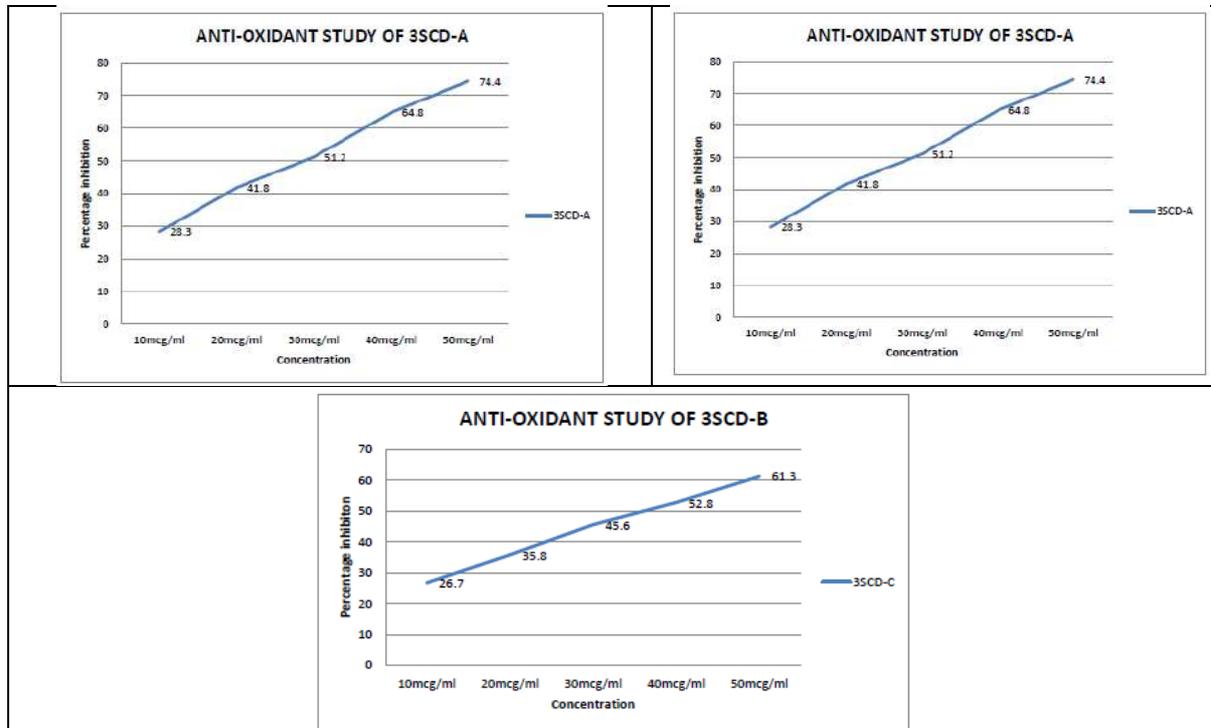


Fig. 4. Hydrogen peroxide scavenging assay of 3SCD-A

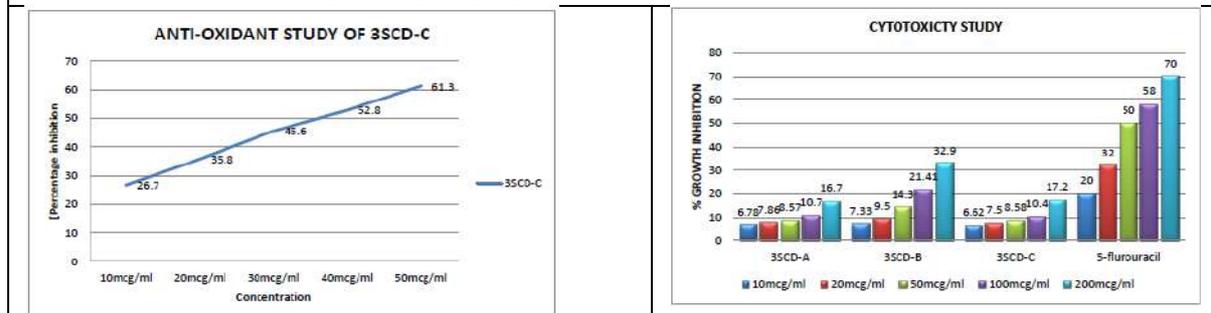


Fig. 5. Hydrogen peroxide scavenging assay of 3SCD-B

Fig 6. In-vitro cytotoxicity of synthesized compound against DLA cell lines

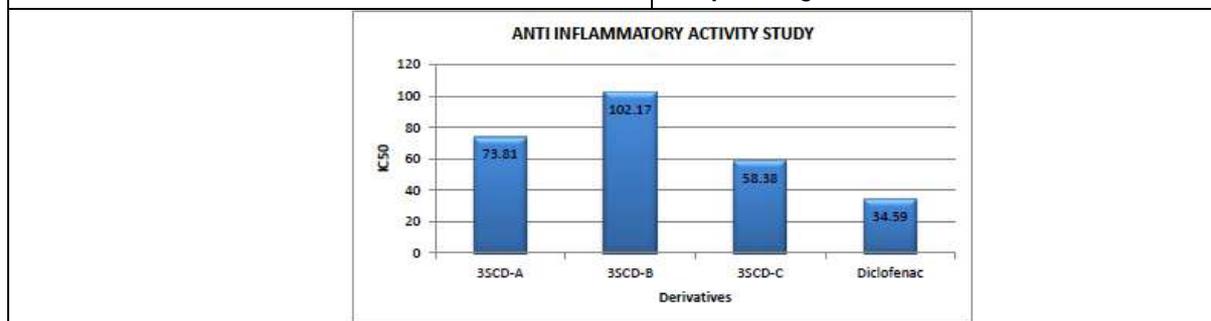


Fig 7. 11Comparison of IC₅₀ of synthesized compounds on protein denaturation





Human Development throughout The Tea Garden Community - An Analysis with Special Reference to Dibrugarh District In Assam.

Bhabajyoti Saikia*

Assistant Professor, Department of Management, Faculty of Commerce and Management, Assam down town University, Guwahati, Assam, India.

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*Address for Correspondence

Bhabajyoti Saikia,

Assistant Professor,

Department of Management,

Faculty of Commerce & Management,

Assam down town University.

E. Mail: saikia.bhabajyoti@gmail.com



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ABSTRACT

The tea industry is significant to Assam and has a unique impact on both the state's economy and the overall economy of the country. Assam has retained its reputation abroad and has a sizable portion of the global tea market. Assam's tea tribes are primarily the labor of the tea industry and are regarded as one of the state's exploited and backward communities. Tea tribes typically live in dangerous environments. Tea companies typically exploit tea tribes in every way possible, resulting in frequent conflict with management. Poverty, backwardness, illiteracy, alcoholism, low standard of living, and lack of health care are all problems that Assamese tea tribes face. Moreover, the tea business in Assam employs more than 9.86 lakhs people in the state on a daily basis, which is around half of the average number of workers employed nationwide. As a scientific method of measuring a country's development, the Human Development Index (HDI) has several advantages over traditional income-based measures of development. It reflects the actual scenario of human well-being development. Despite the fact that the Human Development Index is primarily a spatial dimension, it can be used to construct the Human Development Index by tribes, communities, ethnicity, and religion. This paper attempts to examine the state of human development in the tea garden community in Dibrugarh district in Assam. HDI for the community is calculated using both old and new UNDP methodology. Both HDI values are extremely low when compared to the state and national averages.

Keywords: Assam, Dibrugarh, Economy, Healthcare, Human Development Index (HDI), Labors, Tea.



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INTRODUCTION

Conventionally, a country's development was measured solely by its income level. Per capita income and national income were the most widely used concepts for measuring a country's development. With an increase in these two indicators, the country is said to be moving closer to development. However, only the level of income or income-based measures of development cannot accurately reflect a nation's development [3]. Many fast-growing developing countries' development experiences have revealed that their high GNP growth rates have failed to alleviate socioeconomic deprivation among significant segments of their populations. Development in the broad sense of a nation means improving both social and economic indicators rather than just income level [1]. In this regard, the United Nations defined economic development as an improvement in both economic and social conditions, as well as cultural and institutional change. As a result, the concept of a nation's development cannot be limited solely to income level. The increase in physical output or national income is merely a tool for economic development. Thus, it can be concluded that human well-being is the ultimate goal of development of any country or any region [2].

Additionally, there are numerous flaws in the income-based measurements of development such as GDP, GNP, GNP per capita, etc. One of the main criticisms of these metrics is that they neglect to take into account welfare and income distribution issues as well as non-marketed or non-priced subsistence production, which includes much of housewives' labor. Due to these drawbacks, several initiatives have been made to fix these issues as well as develop other composite indicators that could supplement or replace these conventional metrics and the Unitary Index (Drewnovsky and Scott, 1966), the UNRISD approach (United Nations Research Institute on Social Development, 1970), the Adelman and Morris Approach (Irma Adelman and Cynthia Taft Morris, 1967), the Physical Quality of Life Index (D. Morris, 1979), and finally the Human Development Index are some of the prominent examples (UNDP, 1990).

Human Development – A Concept

The process of expanding or increasing people's options and raising their level of wellbeing is known as human development. The true freedom that everyday individuals have to choose who they are, what they do, and how to live is what human growth is all about [10]. People's options are limitless or subject to change as time goes on. However, there are three choices that are necessary at all developmental phases. These include living a long and healthy life, learning and receiving an education, and having access to the numerous resources that are necessary for living a decent level of living. Numerous additional opportunities in human existence would not be attainable without these options [11, 13, 15]. There are some extra possible choices that are highly valued by many people. These are: economic freedom, social freedom, political freedom, self-respect, and human rights. Keeping this in mind, the Human Development Report, 1991 elaborated on the concept of human development as follows: "People must be at the centre of human development. People must be woven into development, not development into people. It must be development of the people, for the people, by the people."

Human Development Index – An Introduction

The Human Development Report was published for the first time in 1990. United Nations Development Programme commissioned and introduced the idea of the Human Development Index and the architect behind this was Dr. Mahbub-UI-Haq who designed this concept. In the year 1970, at the World Bank, and later, Dr. Haq served as Pakistan's Minister of Finance and claimed that existing measures of human progress were in adequate account for the true goal of development—improvement in individuals' lives. The Human Development Index quantifies a country's average performance in three fundamental dimensions: i) a long and healthy life, also known as life expectancy ii) human educational attainment and iii) standard of living being. Thus, the human development index (HDI) can be considered as a composite indicator of life expectancy at birth, educational attainment (which is calculated as the weighted average of the adult literacy rate and the combined gross enrolment ratio), and income index (measured in terms of GDP per capita, US dollars).



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Despite the fact that the Human Development Index has mainly space dimension, it can still be used to create Human Development Indexes by tribes, communities, religion and ethnicity. In a country with a higher HDI value, there may be cast- and religion-specific variations, state- or region-specific variations, and variance by religion. This study is an effort to examine the human development of the tea garden community in the Dibrugarh district in Assam, India, and to create a community-specific Human Development Index using the UNDP methodology.

Tea ranks first among the various factors that significantly contribute to Assam's economy. Assam's economy would be incomplete without the tea industry. Assam's tea industry is said to be the state's economic backbone. It makes a significant contribution to the state's NSDP. The tea industry is an important part of Assam's identity, and it is recognized both in India and around the world. Tea industry and tea garden laborers, on the other hand, are two sides of the same coin.

Both of these are complementary to one another. The tea industry would be incomplete without the tea garden laborers. These tea garden laborers in Assam not only contribute a sizable portion of the state's population but also playing an important role in the state's tea production, which, in turn, contribute to the state's economy. Thus, the growth of Assam's tea industry depends either directly or indirectly on the workers in the tea gardens. However, in comparison to the other communities in the state, this community's social and economic situation is still marginalized. They are among the most backward. Assam's exploited community as a result of decades of tea estate management's continuous exploitation and the government's indifferences (Saikia B., 2008).

Health and education levels, as well as hygiene, housing quality, diet, and per capita income etc. of this community are gravely impoverished. So, in order to help this community develop, a root and in-depth analysis of their social and economic situation is absolutely necessary. Additionally, there are some elements which are needs to be studied in order to provide a precise image of level of this community's growth. This study will further assist in having a good understanding of the challenges they face towards their development and also the community as a whole.

Objectives of the study

The following objectives have been established in order to make the present research systematic and precise:

- To analyze the level of human development among the tea garden community people in Assam's Dibrugarh District.
- To establish a human development index for the community of the tea garden.
- To propose some policy suggestions based on the study's findings.

Hypothesis Of The Study

Based on a review of available literature and personal experience and following field observations, the hypothesis established is as follows:

- The level of human development among the community of labors of the tea garden is very much lower when compared with the average of state and national level.

METHODOLOGY

The primary and secondary sources of data have been used in this study. The primary data are gathered using the assistance of formulated inquiries from a sample of respondents. Additionally, experts in the field of study and concerned authorities are consulted, and data has been collected. The vital information has been gathered with the use of the multistage sampling collection method. In terms of tea production and cultivation is considered, it has been found that Assam is leading the position within India. On the other hand, Dibrugarh district in Assam is the leader in both tea cultivation and production. Assam's Dibrugarh district is also known as the "Tea City of Assam". Furthermore, the Dibrugarh district has the highest concentration of tea garden community members in the state.





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Thus, the current study's field investigation is limited to the Dibrugarh district of Assam. The entire Dibrugarh district is divided into four branches based on the distribution of tea gardens and the population of tea garden communities: Moran, Dibrugarh, Naharkatia and Tingrai Branch.

Two branches namely Moran and Dibrugarh branch are purposefully chosen in the early phase, taking the size of the branches into consideration. In the second phase, one commercially significant tea garden is chosen from each branch, and then 30 percent of the households in each tea garden are chosen at random. As a result, the sample for primary data is chosen in the manner described below:

The study's secondary data sources, on the other hand, from where the data and information have been gathered are as follows:

- Tea garden records – this include records from offices of tea garden offices, hospitals of tea garden, and schools of tea garden.
- Brochures collected from various tea-related organizations, Publications and pamphlets of Tea Board of India, Assam Chah Mazdoor Sangha (ACMS), Assam Branch Indian Tea Association (ABITA) and Bharatiya Chah Parishad (BCP).
- Government booklets and publications- Assam Statistical Handbooks published by the Directorate of Economics and Statistics, Assam, Human Development Report 2019 released by OKD Institute for Social Change and Development, Guwahati and Institute for Human Development, New Delhi, Assam Economic Survey, issued by the Directorate of Economics and Statistics, Assam, Booklet of Directorate for Welfare of Tea and Ex-Tea Garden Tribes, Assam; various publications of Tea Tribes Welfare Department, Assam.

In addition to these, government and institution reports, extensive research papers and books about the Human Development Index, the Assam tea industry, interaction with the residents of the tea garden community were reviewed, alongside numerous websites have been browsed, in order to provide the necessary secondary data such that a complete picture of the human development in the tea garden community in that particular Dibrugarh district in Assam can be produced.

Limitations Of The Study

With a few overarching objectives, this study attempts to examine the state of human development in the tea garden community. The researcher is making every effort and using a particular plan to achieve these objectives. However, despite meticulous design, the study still has certain drawbacks. The study's primary limitations include:

- Only the Dibrugarh district in Assam is included in the study's scope. Despite the fact that members of the tea garden community are dispersed throughout the state, only the Dibrugarh district was chosen for this study.
- While gathering data, the researcher frequently encountered several challenges. It was discovered that people are extremely introverted, and they frequently refuse to provide all of the information required for the study. As a result, some information is derived solely from observation.

STUDY FINDINGS AND CONCLUSION

In the study area, the percentages of young and old dependents are 41.36% and 4.16%, respectively. The total percentage of dependents is 45.52, indicating an extremely high dependency ratio of 83.49. The gender ratio sampled is less than the average for the state (958, according to the 2011 census) and the country (943, according to the 2011 census). Males and females make up 482 and 452 of the sample's total 934 participants, respectively. Consequently, the sample population's total gender ratio is 937. The sample population's primary sources of income are wages, revenue from livestock and poultry, and income from trade and commerce. Total annual income from all sources among the 161 households in the research region is estimated to be Rs. 3,88,50,366; this translates to per-household and per-capita incomes of Rs. 2,42,814 and Rs. 41,595 respectively. This level of per capita income is significantly lower than the national and state averages. Annual per capita income in Assam and India in 2016-17 was Rs. 67,303/- and Rs. 1,26,349/-, respectively (at current prices).



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The sample population has very low asset ownership status. The majority of the sample population's assets are represented by land, animals and poultry, consumer durables, cash in banks, insurance policies, and so forth. Land ownership among the sample population is not particularly prevalent. Only 4% of the sample households in the research area possessed 2.5 bigha (0.826 acres) of land each household possess. In terms of bank accounts, 149 out of the sample's households (93%), have bank accounts. Only 34 out of 149 households, or 23%, have bank deposits which are more than Rs. 10,000. 115 households still have bank deposits that are less than Rs. 10,000. However, just 11% of respondents in the study area reported having life insurance.

In the study region, 51 people died overall in the previous five years. Consequently, the average yearly growth rate is 10 (10.2). Therefore, the overall mortality rate for the tea garden community in the research locations is 10.7 per thousand. According to the 2011 census, the crude death rate of the tea garden community in the studied areas is significantly higher than the state and national average. According to the 2011 census, Assam and India's crude death rates were 7.2 and 7.1, respectively. There were 117 live births in the study area over the last five years. As a result, the crude birth rate in the sample population is estimated to be 24.6. According to the 2011 census, this crude death rate is higher than the state (22.8) and national (21.8) averages. The natural growth rate of the sample population is estimated to be 13.9 based on the crude birth rate and crude death rate, which is lower than the national and state averages. According to the 2011 census, the natural population growth rates in Assam and India were 15.6 and 14.7 percent, respectively.

Major diseases and minor diseases are divided into two groups for the research of disease prevalence in the sample population. In terms of serious illnesses, 8.45% of the respondent's family members had TB, 1.4% had cancer, 4.22% had high blood pressure, 5.63% had respiratory issues, 7.04% had heart disease, 5.63% had malaria, and 4.22% had encephalitis. However, with regard to mild illnesses, practically every family member in the sample household has a small illness such a cough, fever, etc. The family members of the sample homes, on the other hand, also reported joint pain (36.1% of the sample households), diarrhea (26.3% of the sample households), stomach ache (38.8% of the sample households), and jaundice (37.5% of the sample households).

It is evident from the study that ten infants' deaths were recorded in the study region in the last five years. Consequently, the sample population's rate of infant mortality is predicted to be 87, which is significantly more than the state and national average incidence of infant mortality in India which was 34 and Assam was 44, respectively, based on the data gathered from SRS – Maternal Mortality Bulletin. The sample population's life expectancy at birth is calculated using the abridged life table method. Using this method, the sample population's life expectancy is estimated to be 60.1 years, which is lower than the national and state averages. Infant mortality rates in Assam and India were 63.9 and 67.9, respectively, according to the "Abridged Life Table- 2015-19," published by Office of the Registrar General & Census Commissioner, India.

It is discovered that there are insufficient health care facilities accessible in the research area. The main health clinics and tea garden hospitals are the closest hospitals to the sample population. However, they fall short in terms of health care services. The local primary health facility and tea garden hospital offer a very limited number of medical services. Moreover, awareness of various healthcare facilities amongst the sample respondents is very poor and also awareness on various health related schemes launched by government are also not very much fruitful. A decent image of vaccination status is noticed in the sample population where it was found that 84% of total sample households have vaccinations for their children. Although it does not include all of the vaccines that are recommended. It contains only those vaccines that are provided for free from the government. ASHA employees are taking an active role in this regard. Only 36% of the households have access to safe drinking water, while the remaining 64% rely on unclean drinking water.

The respondent's literacy rate is projected at 61.03, with 71.09% male and 51.09% female respectively. The rate of literacy is determined to be lower when compared with the state and national average. Overall literacy rates for India and Assam according to census 2011 is 72.19 and 74.04 respectively. Adult literacy rate for the sample



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population is estimated to be 47.64%, which is lower than the national average according to Census 2011. The estimated enrollment ratio for the primary level is 81%. In terms of educational facilities in the study area, only primary and upper primary educational opportunities exist. Estimated average years of education are 4.03 years. The HDI value was calculated using an old UNDP methodology for the sample population has been measured to be 0.429. The HDI value for the sample population, on the other hand, is calculated at 0.253 using UNDP's new methodology. This HDI score is less and according to the UNDP Human Development Report of 2018, the nationwide average is 0.640 and state average is 0.557. Thus, the tea garden community's HDI value falls under the low human development category.

Recommendations

According to the study's findings, the following measures are proposed to improve the level of Human growth in the tea garden community. The average daily wage for laborers in tea gardens is remarkably low compared to the hard work they put in. The management of the tea garden should look into this and it should be raised in order to maintain a minimal quality of living of those labors. Likewise, the casual or temporary workers get very less amount of salary. Additionally, they find work only during the busiest times of the year for production. Thus, they are classified as seasonal unemployment. Thus, facilities such as additional sources of income or alternative income source should be provided for improvement in the nature of their livelihood.

Hospitals in tea gardens should be modernized. Maximum health-care facilities should be provided in the tea garden hospitals and it must ensure easy access to health-care facilities for the tea garden workers. School dropout rates are extremely high among children of the tea garden community. Particular attention and activities to combat school dropouts must be undertaken and it should be arranged by the tea garden area administration and garden management. In addition to general education, specialized technical education, or some kind of vocational education programme should be implemented by the by the state or national government, specifically for the unemployed youth belongs to that particular community which will in turn give them the confidence and opportunity in creating jobs for themselves.

A programme to promote adult literacy should be implemented in locations of the tea gardens with certain times and dates along with suitable curriculum for the adult tea garden workers. Alcoholism is a significant issue among the tea garden community. From the research area, it was observed that the majority of adults (that includes both (male and female) are alcoholic addicts. It poses serious health and social risks to their lives. Consequently, both the production and consumption of alcoholic drinks and beverages should not be permitted to sell near tea garden locations.

The majority of the study's sample households consume toxic water, which could lead to occurrence of different diseases. The garden management should provide safe drinking water facilities for the tea garden community. The female tea garden employee needs to receive the health-related facilities, as well as government works. The availability of maternity leave or child care is not provided to female garden laborers. Consequently, they do not receive the essential opportunities to care for their child during the prenatal and postnatal phases. It might lead to vulnerable to illnesses and child malnutrition. Therefore, in order to have a safe pregnancy and birth, leaves should be given to them in addition to all the facilities. In addition, for improved female and children health, a female-specific doctor (gynecologist) should also be made available in that area for their benefit.

It's important to develop the money saving habit, and should be made popular among the workers in tea gardens. For this purpose, particular sub-branch or branches of nationalized and commercial banks should be opened around the tea gardens. Although, even after the introduction of Pradhan Mantri Jan DhanYojana (PMJDY), many households have opened bank accounts, but it was noticed that deposits in these accounts are significantly low. Thus, extra effort should be made by the garden administration and management who can implement and encourage the community for money savings. Moreover, various NGOs can also come forward and take action in





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this matter. People should be educated on the variety of government initiatives and programmes relating to social and economic advancement which are available for them. It is very much important to spread awareness of those initiatives and programmes such that they can effortlessly obtain the data and can reap the benefits.

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Enhancing Access Time Recovery using ARM through Intersection on CSS: A Matrix-based Approach for Redefining FIS in Textual Data

Sandeep Kumar^{1*} and Rahul Kumar Mishra²

¹Research Scholar, School of Computer Science and Applications, IFTM University, Moradabad, India.

²Professor, School of Computer Science and Applications, IFTM University, Moradabad, India

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*Address for Correspondence

Sandeep Kumar

Research Scholar,

School of Computer Science and Applications,

IFTM University, Moradabad, India.

E.Mail: shyamsandeep28@gmail.com



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ABSTRACT

In data mining association rule mining is an important technique which is used to discover interesting relationships and correlations between variables in large datasets. Frequent itemsets are a fundamental concept in association rule mining, and Apriori is a well-known algorithm which is used to discover frequent item-sets. However, the performance of an Apriori decreases as the size of the dataset increases, as it generates a huge number of candidate itemsets. In this research paper, we propose an improved Apriori algorithm that uses the intersection method to enhance the efficiency of frequent itemset generation. The proposed algorithm reduces the number of candidate itemsets, which improves the efficiency of the algorithm. We also evaluated the proposed algorithm on a real-world dataset, and the results show that it outperforms the traditional Apriori algorithm in terms of execution time complexity.

Keywords: Frequent item-set, Association Rule Mining (ARM), Apriori Algorithm, KDD (Knowledge Discovery in Databases), Market Basket Analysis

INTRODUCTION

Data mining remains a highly sought-after practice in today's landscape[1], as organizations from diverse sectors confront mounting data volumes and a need for actionable insights to drive their operations. The exponential growth in data generated by businesses, governments, and individuals necessitates effective data mining techniques to derive meaningful and valuable insights. In the current competitive business environment, organizations strive to gain a competitive edge by leveraging data-driven insights to enhance decision-making [2]. Data mining plays a pivotal role in this regard, offering valuable insights that enable organizations to optimize their operations, reduce costs, and boost revenues. With the advent of big data, organizations increasingly seek to personalize their products

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and services to cater to specific customer needs. Data mining facilitates the analysis of customer data, revealing patterns that can be utilized to customize offerings accordingly. Moreover, data mining empowers organizations to develop predictive models capable of forecasting future trends and outcomes. These models prove instrumental in making informed decisions and anticipating market changes or shifts in customer behavior. By leveraging data mining, organizations can proactively adapt their strategies and stay ahead of the curve.

Knowledge Discovery in Databases

KDD (Knowledge Discovery in Databases) represents a comprehensive approach to data mining that encompasses multiple essential steps in extracting knowledge from data [3], [4]. The KDD process consists of distinct stages, including data cleaning, data integration, data transformation, data mining, and interpretation of the results. This systematic framework ensures accuracy and meaningfulness throughout the data mining endeavor. The initial step in the KDD process involves [5] data cleaning, which aims to eliminate errors, inconsistencies, and missing values present in the dataset. This stage holds significant importance as it ensures the data's quality and suitability for subsequent analysis. Following data cleaning, data integration comes into play, where data from various sources are combined to create a unified dataset. Through data transformation, the format of the data is modified to facilitate analysis, including operations such as normalization or scaling.

Data mining serves as the foundation of the KDD process [2]. It encompasses a variety of statistical and computational techniques aimed at analyzing data and unveiling patterns and connections. Within this step, various methods are employed, including clustering, classification, regression, and association rule mining. Clustering allows for the grouping of similar data points, while classification enables the prediction of the class or category of new data points based on past observations. Regression comes into play when predicting continuous variables using other variables, while association rule mining identifies patterns and relationships between variables. The final phase of the KDD process involves interpreting the obtained results. This entails scrutinizing the identified patterns and relationships and comprehending them within the context of the relevant domain. Such interpretation holds significant importance as it ensures that the outcomes carry meaning and can be effectively utilized for decision-making purposes.

Association Rule Mining

ARM widely used data mining technique that is used to discover interesting relationships between different items in a dataset [6]. ARM has numerous applications in different domains, such as marketing, healthcare, and finance. The frequent itemset mining algorithm (FIS) is one of the most popular algorithms used for ARM. [7] FIS is used to identify sets of items that frequently co-occur in a dataset. Textual data is a type of data that is often challenging to analyze using traditional FIS algorithms. This is because textual data requires preprocessing steps to convert it into a numerical representation that can be used by FIS algorithms. [8] Preprocessing steps include tokenization, stemming, stop word removal, and other normalization techniques. These preprocessing steps can be time-consuming and can lead to the loss of important information. To address this problem, several modifications have been proposed for the Apriori algorithm, including partition-based algorithms, parallel algorithms, and algorithms that use sampling techniques.

Related Work

Several modifications have been proposed for the Apriori algorithm to improve its efficiency. One approach is to use partition-based algorithms, such as PFP and FP Growth. [9] These algorithms partition the dataset into smaller subsets and mine frequent item-set in each subset. Another approach is to use parallel algorithms, such as Dist-Eclat and Dist-Apriori. [7] These algorithms parallelize the frequent itemset generation process to improve the efficiency of the algorithm. One more approach is to use algorithms that use sampling techniques, such as Random Sampling and Stream Sampling. These algorithms sample a subset of the dataset and mine frequent itemsets in the sample. The frequent itemsets generated in the sample are then used to estimate the frequent itemsets in the entire dataset. Similarly, other researchers have proposed techniques for preprocessing textual data, such as stemming, stop word removal, and tokenization. However, these preprocessing techniques can be time-consuming and can lead to the loss





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of important information. Other researchers have proposed techniques for mining textual data directly without preprocessing. [10] proposed a technique for mining association rules from text documents using a graph-based algorithm. However, this technique was computationally expensive and was limited to small datasets. Agrawal et al. (1993)[9], [11] proposed a technique for extracting frequent itemsets from transactional databases using an apriori-based algorithm. However, this technique required the conversion of textual data into a binary matrix representation.

Apriori Algorithm

Apriori algorithm[12] is a popular algorithm used in data mining and machine learning for mining frequent itemsets and association rules. The algorithm works by identifying the most frequent items in a dataset and then using them to generate association rules. The Apriori algorithm[13], [14] uses the concept of support, which is the number of times an item or itemset appears in a dataset. It also uses the concept of confidence, which is the probability of the occurrence of a consequent given the occurrence of an antecedent in the dataset. The Apriori algorithm works in two phases:

1. Generating frequent item sets
2. Generating association rules

Here is the pseudocode for the Apriori algorithm

Apriori ()

Input: A database of transactions D , a minimum support threshold min_sup

Output: A set of frequent itemsets F

1. $C_1 = \{ \text{all items in } D \}$
2. for $k = 1$ to ∞ do
3. $C_{k+1} = \text{Apriori-gen}(C_k)$
4. for each transaction t in D do
5. for each candidate itemset c in C_{k+1} do
6. if c is a subset of t then
7. increment the count of c
8. $F_{k+1} = \{ c \text{ in } C_{k+1} \mid \text{count}(c) \geq min_sup \}$
9. if F_{k+1} is empty then
10. break
11. return $\cup_k F_k$

Example

A transaction database containing a collection of transactions serves as the input data for the Apriori algorithm. An item set constitutes each transaction. To identify the frequent things, the method begins by counting the frequency of each item in the transaction database. To create candidate itemsets of size $k + 1$, the frequent items are used. The transaction database is then used to tally the candidate itemsets in order to gauge their support. A candidate itemset is eliminated if its support falls below the required minimum. Up until no new frequent item sets can be discovered, this procedure is repeated. Association rules are generated using the final frequent item sets. Let see an illustration of the Apriori Algorithm. In this example we take 6 transactions with 5 item shown in table 1

Let us consider the $min_support_threshold$ value ($s = 33.33\%$) and $max_confident_threshold$ ($c = 60\%$) then

Minimum Support Count = $(33.33/100) * 6$

Minimum Support Count = 2

$[P1 \wedge P4] \Rightarrow [P5]$ //confidence = $\frac{\text{sup}(P1 \wedge P4 \wedge P5)}{\text{sup}(P1 \wedge P4)} = \frac{2}{2} * 100 = 100\%$ //Selected

$[P1 \wedge P5] \Rightarrow [P4]$ //confidence = $\frac{\text{sup}(P1 \wedge P4 \wedge P5)}{\text{sup}(P1 \wedge P5)} = \frac{2}{2} * 100 = 100\%$ //Selected

$[P4 \wedge P5] \Rightarrow [P1]$ //confidence = $\frac{\text{sup}(P1 \wedge P4 \wedge P5)}{\text{sup}(P4 \wedge P5)} = \frac{2}{3} * 100 = 66.67\%$ //Selected





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[P1] \Rightarrow [P4 \wedge P5] //confidence = $\frac{\sup(P1 \wedge P4 \wedge P5)}{\sup(P1)} = \frac{2}{4} \times 100 = 50\%$ //Rejected
 [P4] \Rightarrow [P1 \wedge P5] //confidence = $\frac{\sup(P1 \wedge P4 \wedge P5)}{\sup(P4)} = \frac{2}{3} \times 100 = 66.67\%$ //Selected
 [P5] \Rightarrow [P1 \wedge P4] //confidence = $\frac{\sup(P1 \wedge P4 \wedge P5)}{\sup(P5)} = \frac{2}{4} \times 100 = 50\%$ //Rejected

So here for the minimum confidence >60 there are four strong result.

The Apriori algorithm has been widely used for association rule mining and has been applied in many areas, including market basket analysis, recommendation systems, and fraud detection. However, the algorithm has a high computational complexity, making it unsuitable for very large datasets. To overcome this limitation, variations of the Apriori algorithm, [12] such as Partitioning Apriori and Sampling Apriori, have been developed to improve the efficiency of the algorithm

Proposed Algorithm

The New Hybrid Apriori Algorithm operates in two different steps. In the first step, a large transaction set is converted into a sparse matrix. Each data item's presence is represented by a "1," while its absence is represented by a "0" in the corresponding transactions. Additionally, a new column called SUM is appended to the dataset, which calculates the total sum of each transaction.

Algorithm: Hybrid-Apriori()

Define:

$\alpha \rightarrow \{ \text{Min_Sup}() \}$
 CSSk \rightarrow Candidate Subset
 DSSk \rightarrow Discarded Subset
 FIS \rightarrow Set of frequent item set

Input:={Tj \in Ii}

Output:={FIS}

Initialize: Let $\alpha = x$;

Ii = {I1, I2, ..., In} // $\forall I_i \geq 1 \ \& \ \forall n \leq K$ //Set of n-Item set, where $K = \text{Max}(\text{Sum})$
 Tj = {T1, T2, ..., Tm} // $\forall T_j \geq 1$ // Data Set contains m-Transactions
 Ek = {E1, E2, ..., Ek} // $\forall E_k \geq 1$ //Set of nth -Element item set
Txni = {T1, T2, ..., Tm} // $\forall T_j \geq 1$ // No. of Transactions involve in ith element item set of CCSi
 CSSn = { } & DSSn = { } // Candidate & Discarded Subset of nth -Element item Set
Ln = { } // Labeled CSS with no. of transactions involve for nth -Element item Set

Start

Step-1: While;

if $\text{Min_Sup}(E_k) \geq \alpha$,
 Then $\forall I_i \parallel E_k \in \text{CSS}_i \ \text{CSS}_i \leftarrow E_k$
 else
 $\forall I_i \parallel E_n - k \in \text{DSS}_k \ \& \ \text{DSS}_i \leftarrow E_n - k$
 $i = i + 1$;
 if $i > n$
 Go to Step-3

Step-2:

Do

For $i = 1$;
 LO \leftarrow (Ii // Support // Ek) // Find Lo along with Transaction involved.
 For $i = 2 \dots n$; $L_{i-1} \ \text{CSS}_i \parallel \text{Txnm}$ // find L1 to Ln-1
 Repeat for each i: $\text{Txnm} \leftarrow \text{Intersection} [L_o, L_{n-1}]$ then $E_n \leftarrow \text{Txnm}$;

Step-3:

FIS = [CSS₁, CSS₂, ..., CSS_n] // Set of all frequent items

End;



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To determine the maximum number of items, denoted as K , the SUM column is sorted. Currently, K is significantly smaller than the total number of items involved in the transactions (denoted as " n "). However, in the worst-case scenario, K can be equal to n . The reduced item set value, K , is then passed to the second stage of the algorithm known as the 'New-Apriori' algorithm (referencing the Neeraj Paper). This algorithm treats the item set with K as having the most items among all the transaction sets. To locate all possible viable Frequent Item Sets (FIS), the intersection approach in Association Rule Mining (ARM) is applied. This approach, as shown in Figure 2 and detailed in the suggested algorithm section, enables the identification of FIS.

RESULT ANALYSIS

In our implementation of Hybrid Apriori, we utilized a dataset consisting of 39,039 transactions obtained from a US-based department store, which is publicly available on Kaggle. The dataset underwent initial cleaning and preprocessing stages. The statistics presented in Table 3 showcase the results achieved by applying Apriori and the proposed Hybrid Apriori on variable length support. The calculations were performed using Python on an Intel i7 processor with 16 GB of RAM, utilizing data ranging from low to high volume transaction sets. Based on the experimental data, Hybrid Apriori outperformed the traditional Apriori algorithm for DS-1, which represents the dataset with the highest number of transactions (22,039). This outcome is illustrated in Figure 3. and DS-3, which has the lowest set of transactions (5000) and efficiency with a 27.48% rate of improvement over the Apriori Algorithm at a minimum support of 30%, as illustrated in figure 5.

CONCLUSION

In our experiments, we compared the performance of Hybrid Apriori (our proposed algorithm) with Apriori using different minimum support values (Min_Supp) in three different datasets (DS-1, DS-2, and DS-3). The results were quite remarkable. In DS-1, New Apriori achieved speeds that were 11.71%, 6.62%, and 5.47% faster than Apriori for Min_Supp(30), Min_Supp(60), and Min_Supp(80), respectively (see Figure 4). Similarly, in DS-2, it performed 4.29%, 3.34%, and 3.34% faster for the same support values (see Figure 5). In DS-3, the speed improvements were even more significant, with Hybrid Apriori being 237.48%, 12.58%, and 3.71% faster than Apriori for the respective minimum support values (see Figure 6). These findings unequivocally demonstrate that our proposed New Apriori algorithm represents a significant improvement over the original Apriori algorithm. It consistently achieves better performance across all the parameters we considered, resulting in more favorable outcomes. We are excited about the potential impact of our algorithm in various applications and industries. Its enhanced performance can lead to improved efficiency and faster processing times in association rule mining tasks

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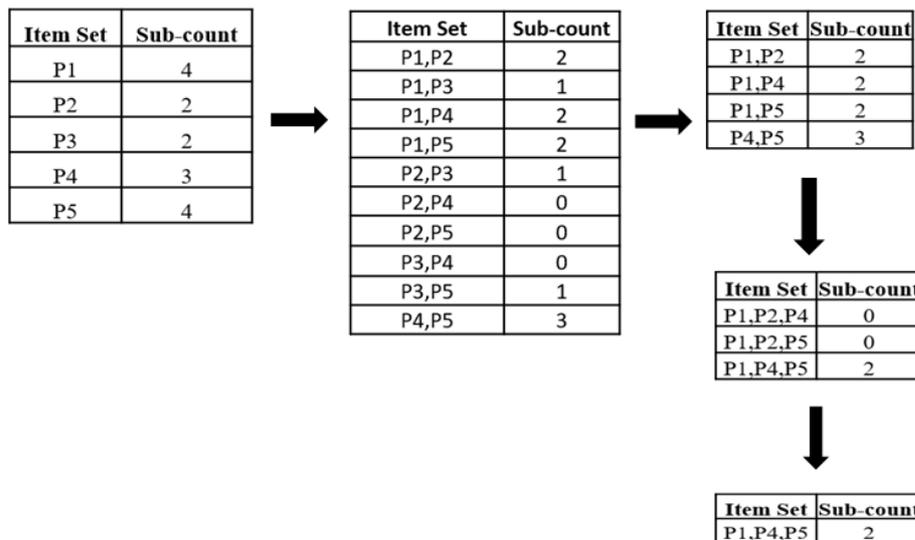
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Table 1: Transaction Table with Item-Set

| Transaction ID | Item |
|----------------|----------|
| T1 | P1,P2,P3 |
| T2 | P1,P2 |
| T3 | P1,P4,P5 |
| T4 | P4,P5 |
| T5 | P5,P3 |
| T6 | P1,P4,P5 |

Table 2: Apriori Algorithm Solutions





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Table 3: Comparison table

| Sr | Data Set(DS) | Number of Transactions | Min _ Support in % | Total no. of Item appear in Txn | Max No. of Items appear in Txn | Execution Time of Apriori in MiliSec (T_{AP}) | Execution Time of Hybrid Apriori in MiliSec (T_{HA}) | Execution Time Difference ($T_{AP}-T_{HP}$) | Execution Rate(%) |
|----|--------------|------------------------|--------------------|---------------------------------|--------------------------------|---|--|---|-------------------|
| 1 | DS-1 | 22039 | 30 | 4215 | 1117 | 1090.493 | 962.777 | 127.716 | 11.71 |
| 2 | | | 60 | 4215 | 1117 | 918.453 | 857.63 | 60.823 | 6.62 |
| 3 | | | 80 | 4215 | 1117 | 871.656 | 823.919 | 47.737 | 5.47 |
| 1 | DS-2 | 12000 | 30 | 3996 | 304 | 684.298 | 478.396 | 205.902 | 30.08 |
| 2 | | | 60 | 3996 | 304 | 479.682 | 459.078 | 20.604 | 4.29 |
| 3 | | | 80 | 3996 | 304 | 486.986 | 470.676 | 16.31 | 3.34 |
| 1 | DS-3 | 5000 | 30 | 3900 | 604 | 339.494 | 246.191 | 93.303 | 27.48 |
| 2 | | | 60 | 3900 | 604 | 294.796 | 257.691 | 37.105 | 12.58 |
| 3 | | | 80 | 3900 | 604 | 272.548 | 262.413 | 10.135 | 3.71 |

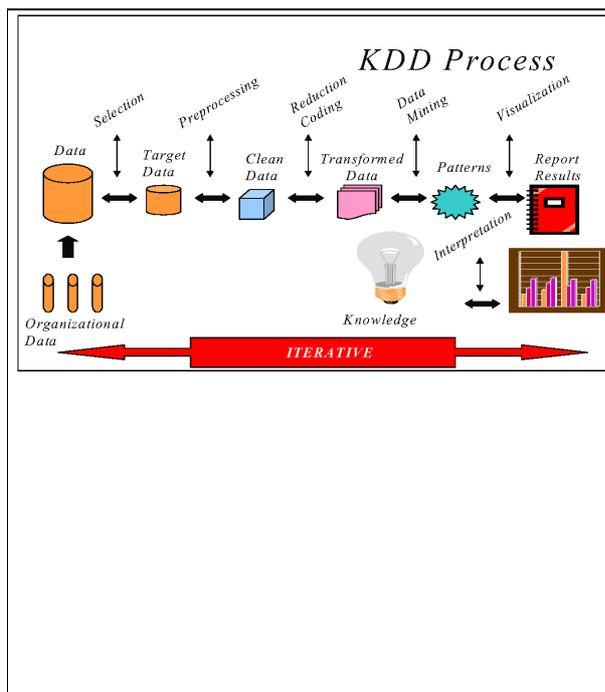


Figure 1: KDD Process (Source: Susan P. Imberman)

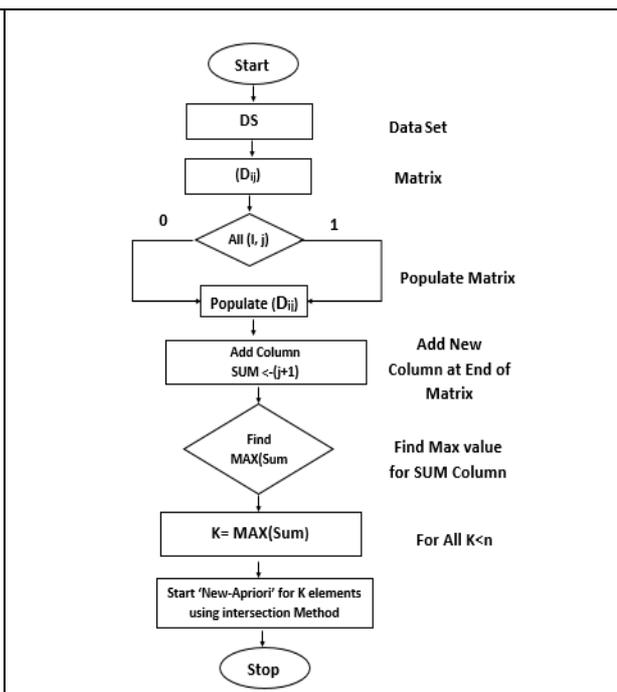


Figure 2: Flow Chart of New- Apriori Algorithm





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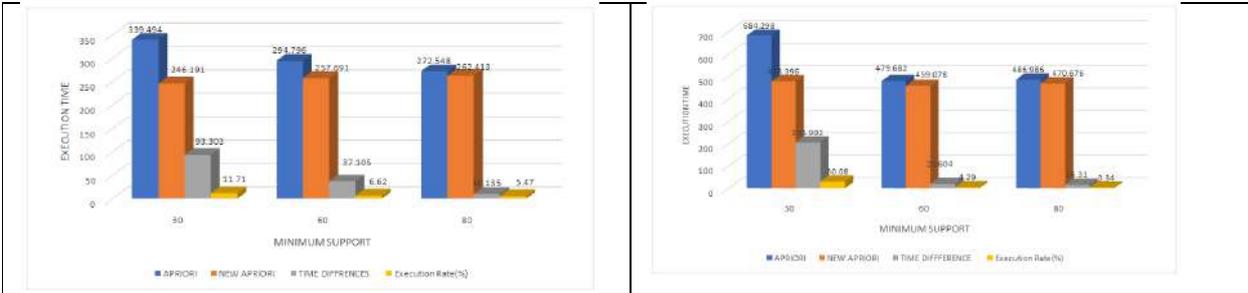


Figure 3: DS-1 Analysis

Figure 4: DS-2 Analysis

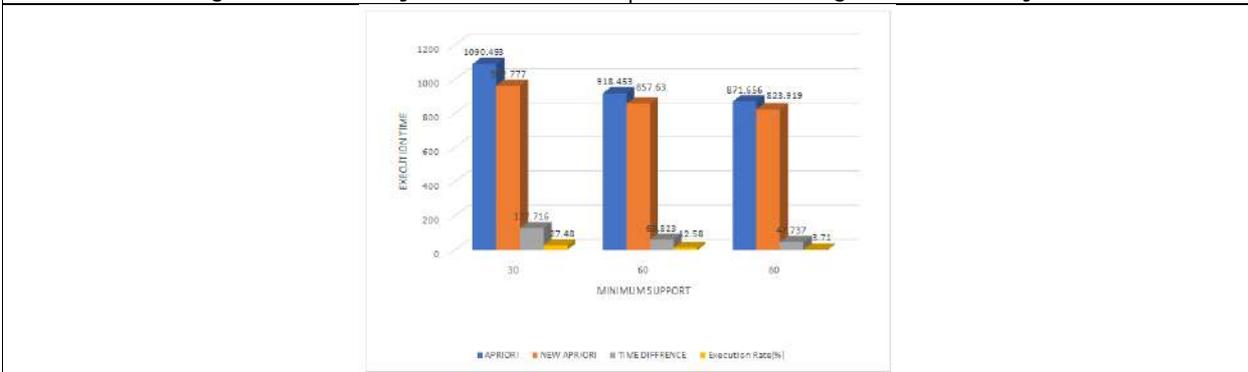


Figure 5: DS-3 Analysis





Indian Music as a Source of Spiritual Salvation

Mani Sachdev¹ and Radhika Mohan Gupta^{2*}

¹Professor, Department of Arts, Manipal University Jaipur, Jaipur, Rajasthan, India

²Assistant Professor, Department of Arts, Manipal University Jaipur, Rajasthan, India.

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*Address for Correspondence

Radhika Mohan Gupta

Assistant Professor,

Department of Arts,

Manipal University Jaipur,

Rajasthan, India.

E.Mail: radhikamohan.gupta@jaipur.manipal.edu



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ABSTRACT

Our minds continue to battle our daily problems and concerns, and it is difficult to remember life's higher purpose. Music, Bhajan, Kirtan, and chanting help move the mind away from its everyday concerns by giving it something else to focus on—something spiritually engaging, repetitive, and filled with divine vibration. The following paper sheds light on how Indians view music and connect it to spirituality, which is believed to lead them on a path of salvation.

Keywords: Music, Salvation, sound, Rhythm, Art.

INTRODUCTION

One of the noblest purposes of art in India is that it is a means of explaining existence through life and nature. Classical music does this exceptionally well when the artist, feeling the soul through his inward eye, incorporates both the artistic and the material world into it. Music is that fine art in which we express our feelings through tone and rhythm. Music primarily gives us a sense of beauty. It is a beautiful form of art, pleasing to the heart. Who isn't enchanted by music? According to the Indian concept, music, which is an ocean of sweet sound waves, is a divine gift given to all. This ocean is a source of intense pleasure that encompasses not only gods, demons, humans, animals, and birds but also the vegetation.

In India, it is not only the emotional aspect of music and aesthetics that is considered important, but it is the spiritual aspect as well, which is considered equally important. In Indian thought, the principle of art is based on the principle of *unity in diversity*. Indian music searches for this principle in the practice of religion also.





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The main goal of music in India is to make the listener feel the soul, which in itself is divine. Therefore, we find Indian classical music immersed in spirituality, the knowledge and practice of which leads us to divine life. In some of the religious discourses of Bhaktiyoga, Swami Vivekananda said, 'The greatest means of constant meditation of God is music.' [1].

नाहं वसामिवैकुण्ठे योगिनां हृदये न वै।

महदक्ताय न्नगार्थं तत्र त्रिष्टाभि नारदा। [2]

Krishana says to Narad, a great guru of Bhakti, "I do not live in heaven, not in the heart of the yogi, but where my devotees sing songs of glory in my praise."

गीतं वाद्यं तथानृतं त्रयसं गीतमुच्यते Shlok 21 of Sangeet Ratnakar [3]

Songs, musical instruments and dance are all three called music.

According to Sharangdev, Brahma has collected music in Samaveda.

"गीतेन प्रीयते देवः सर्वज्ञः पार्वतीपतिः।

गोपिपतिरन्तोऽपीव शध्वनिशंगतः।

सामगीतिरतो ब्रह्मा वीणा आसक्तः सस्वती।

किमन्येयक्षगन्धर्वदेवतदानवमानवाः" Shlok 26-27 of Sangeet Ratnakar [3]

(Shankar, the husband of omniscient Parvati, is pleased with the song. The sound of Vanshi subdues Gopipati Krishna. Brahma is engaged in Saamageet. Saraswati is attached to the Veena. What about other Gods, Yakshas, Gandharvas, Demons, and human beings?)

Sharangdev says that music has so much power that it takes a person to the transcendental world. This is the reason that when the mother recites a lullaby, the child falls asleep.

"अज्ञातविषयास्वादो बालः पर्यङ्किक्रमातः।

रुदनगीतामृतं पीत्वा हर्षोत्कर्षप्रपद्यते" Shlok 26-27 of Sangeet Ratnakar [3]

(The child who has not yet received the knowledge of subjects and knows only to sleep in the cradle also ends his crying after receiving the nectar of the song and becomes happy.)

"वने चरस्तुणाहारश्चिन्मृगशिशुः पशुः।

लुब्धो लुब्धकसंगीते गीते यच्छति जीवितं" Shlok 28 of Sangeet Ratnakar [3].

(It is astonishing that the forester who roams in the forest and whose food is grass, such as an antelope cub, also dedicates his life to being fascinated by the music of the huntsman.)

"तस्य गीतस्य माहात्म्यं के प्रशंसितुमिशते।

धर्मार्थकाममोक्षानमिदं वैकसाधनमा ॥" Shlok 29 of Sangeet Ratnakar [3].

(Who can describe the greatness of that song, which is the only means of Dharma, Artha, Kama, and Moksha.)

The beauty of music is said to be divine (Shlok 30 of Sangeet Ratnakar [3]). "God dwells where there is music." We have references to the Veda of music from the Bhagavad Gita, where Lord Krishna says, "Vedanam Samvedo hasme in," (वेदानां सामवेदोऽस्मि देवानामस्मिन्वासवः।) {22nd verse of the 10th chapter of Shrimad Bhagavad Gita [4]}, i.e., I am Samaveda amongst the Veda. Out of the four Vedas, Samaveda is predominant in music. Its compositions are rhythmic, and it can be sung. Sama means 'song' or 'music.' The hymns of Samaveda are sung. Music is believed to have originated from Samaveda itself.

According to Indian belief, music is the other form of the divine. Music, musical instruments, and vocal music are all synonymous. The meaning of "I am Samaveda amongst the Veda" means that it is nature's musical zone, a form of divine vibration through which nature is created every moment. The medium of this is a mere sound. In Indian tradition, it is called the sound of Om.





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ओमित्येतदक्षरमिदं सर्वतस्योपव्याख्यानं भूतं भवद् भविष्यदिति सर्वमोङ्कार एवा
यच्चान्यत्रिकालातीततदयोङ्कार एवा।

(OM is this imperishable word, OM is the universe, and this is the exposition of OM. The past, the present, and the future; all that was, all that is, all that will be, is OM. Likewise, all else that may exist beyond the bounds of time is also OM)[5]. The three letters अ, उ, and म, pronounced together as Om, are the representative symbols of all possible sounds. The sound अ is the least differentiated of all sounds and therefore is called the fundamental sound, "aksaranamakaro," says Krishna in the Gita (X, 33)[4], that is, "I am "अ" among letters."

All human speech is produced in the space within the mouth, beginning with the root of the tongue and ending in the lips, with 'अ' representing the throat sound, 'उ' the rolling forward of the impulse from the throat, and ending with 'म,' the closing lip sound. Correctly pronounced, Om will represent the entire phenomenon of sound production. Just as Om represents the 'Undifferentiated', the other sounds represent the differentiated aspects of the one, i.e., Nada-brahman.

The Katha-Upanishad mentions Om as the supreme syllable as well as an aid to salvation:

सर्वे वेदायत्पदमामनन्ति तपासिसर्वाणि च यद्ब्रुवन्ति
यदिच्छन्तो ब्रह्म चर्यं च रन्ति तत्ते पदं संप्रहेण ब्रवीम्योमित्येतत्।
एते एव हि अक्षरं ब्रह्म एतत् एव हि अक्षरं परम्। एतत् एव हि अक्षरं ज्ञात्वा यः यत्तुच्छति तस्य तत्सिद्ध्यति।
— Katha Upanishad, Chapter 2 valli-15th & 16th verse [6]

I will tell you briefly about the place and the end which all the Vedas proclaim and which all the austerities proclaim, for the attainment of which human beings practice to lead a holy life. OM is that goal, "O Nachiketas. that is, this Brahman is imperishable, this is the ultimate element; the person who knows this imperishable, gets whatever he wants."

In other words, because of its unchangeable nature or power of the soul, the supreme element is perishing or attaining physical expression. On the other hand, after merging with its perishing form, the same element is getting merged with the non-perishable. Nature is coming to an end from where it started. Rabindranath Tagore believed there was more significant potential in Indian music for religious feelings than daily experiences. He said, "It disengages the spiritual from the happenings of life; it sings of the relationship of the human soul with the soul of things beyond." 'Our music pulls the listener beyond the limits of everyday human pleasures and sorrows. It takes us to the lovely realm of renunciation that lies at the universe's core.' [7] Amidst it, all is a powerful song. Wherever we look around us in the universe, whether it is the flowing rivers, the seasons, or spirituality, there is a grand verse recital taking place. This is why Indian music attains its natural form. In India, practitioners consider music the greatest source of knowledge and truth. On the one hand, we have the unexpressed, unstruck sound, and on the other, we have the evident song. The imagination of sound in nature, which has been done in Indian philosophy for ages, is an easy effort to understand through music.

The Concept Of Nada

Music is considered to be Brahm, the supreme self. The natural and scientific reason for the origin of the song is Kampan (vibration). Those vibrations and agitations that are regular are musical. Regularity is a universal principle of beauty. Beauty thinkers of music have termed irregular music as noise, detached it from the world of beauty and only given recognition to regular music, considering it as a song. Vibrations produce sound. When we talk about sound, Naad is very important in Indian tradition in terms of sound. Naad is actually the science of sound. Its principle is that the whole world is based on sound. That is, the entire universe is the creative form of the principles of sound. These sounds are sound waves not only in the external environment but also in our bodies at different levels of consciousness. The body is formed by its mixing and change. The whole world is believed to be under the sound of Naad. Wherever there is life in the world, there is Naad; that is, there is sound. Naad is the essential element of music. Moreover, the ancient texts are filled with the worship of Naad, wherein ,Naad is also referred to as Naad Brahma. The origin of the fundamental basis of music is the sound of the Naad. The sound is also said to be





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the attribute of the ether According to Naad Yoga, the only sacred sound was Om in the beginning. After this, the universe was created by sound waves.

Since time immemorial, all systems of Indian spiritual practice have unanimously considered Om as a unique sound symbol of God. Whether any holy name designates it in various sadhana paths, it is considered to be the original source of all kinds of special sounds. This is the basis of the Vedas. In this lies the soul of the Vedas and its real meaning. The Brahma that emanates from the sound of Omkar is so powerful and omnipotent that it does not allow any particle of the universe to stagnate. India has always had a rich tradition of science, from churning the ocean to fierce storms and volcanoes to capturing the subtle vibrations of planets and constellations to arranging for the mutual exchange of ideas. As a result of the worship of Naad-Brahm, the seekers here have been called the lords of the transcendental abilities and the lords of the heavenly wealth. In the process of the production of sound, vibration or movement is produced due to air pressure or airflow; this is where the Naad originates. When sound is produced, this movement/vibration is sometimes steady and sometimes irregularly, erratic and unsteady. In Sangeet Ratnakar, talking about the origin of the sound, it says that 'Naad' (sound) is composed of two words, (a) 'Nakaar,' i.e., 'Praan' (the life breath), and (b) 'Dakaar', i.e., 'Agni' (fire). It is with the help of 'praan' and 'Agni' that a song is composed.

Two beauty features are essential: 'sanjeevta', i.e., 'Life Breath', and 'Prakash,' i.e., 'Inherent power'. The symptom of the life element is speed or movement, where there is vibration and movement in the emergence of a song. In this manner, the song has both a life element and an element of beauty. The symptom of 'Dakaar' (Agni or fire) is light, and its inherent feature is power. The sun is a combination of both light and power. So, the second quality of the song, 'Dakaar,' i.e., Agni, is full of light and power. Light is a symbol of knowledge. Beauty brightens up because of light. In this manner, the song is a fusion of animation, movement, power, and light.

नकारं प्राणनामानं दकारामनली

जातः प्राणान्मिसंयोगात्तेननादो अभिधीयते IIShlok 6 of Sangeet Ratnakar [3].

('Nakaar' means 'life,' and 'Dakar' means 'fire.' Due to the union of Prana and Agni, it is called 'Naad.')

This AnhadNaad (sound) is believed to have emanated from the voice of God, which was heard and accepted by the sages who reached the peak of our spiritual progress. A fundamental feature of Indian thought has been that the ultimate goal of all sciences, arts, scriptures, etc., is self-realization. In the Indian tradition, the art of music has also been accepted as a form of worship and austerity rather than merely an art. The Yogis and Maharishis regarded sound as a form of Brahman and referred to it as the Sagun form of Nirguna Brahman. The concept of Brahman in the Upanishads is a philosophical concept, not a religious concept. The concept of Brahman is not only a unified form of the natural forces mentioned in the Vedas but an integrated form of everything from the vast universe to fine particle-like substances, from abstract physical forces to emotions. There is much similarity between this and the energy-related facts of modern science. In the end, the Upanishads called him 'neti-neti' even after so many descriptions of Brahman. Whatever is said concerning him can still not be fully described. In other words, Brahman is indescribable. Similarly, science has also not been able to give a specific definition of energy; energy is undefined. There is a beautiful light within the universe, which is the reason for attaining the supreme blissful, eternal ecstasy. Yogis always think of Him from the point of view of yoga and knowledge. That light is blissful, formless, and transcendent, or the 'Paratpar Brahman'. The realization of 'Paratpar Brahman' is proved only by the worship of 'Pranava' or the 'Naad'. Divine qualities have been accepted in the power of music.

The search for Naad is the exploration of Brahman in the heart. A sadhana practitioner must focus on this Naad steadily, the concept of which is unclear in the initial state. The heart is the centre of desires, effects of past actions, and various types of feelings and tendencies, from which many kinds of movements arise. As a result, this sound can neither be heard nor felt in the normal course of life. To overcome these difficulties, one has to practice with caution and restraint. For this, peace has to be created in the heart, and efforts must be made to focus the attention on the Naad continuously.





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Purity of heart is necessary because Indian music, after affecting the material level, also affects the spiritual level, and the artist worships the divine. The listeners can also take complete pleasure in it when they rise above the material level and reach the spiritual level. This is because, in Indian philosophy, the internal senses have been given the same importance as the external sensory organs. It is a matter of misfortune that nowadays, people have started considering alphabets and linguistics as true knowledge, whereas this type of knowledge is the outer cover of man. True knowledge is full of originality, like our inner qualities of love, compassion, and kindness, and not like colourful clothes, which only help to increase outward pomp. When all these inner fundamental senses start developing in a person, we should assume that he has got true knowledge. In this context, it is also worth noting that we develop ethics, public dignity, and positive thinking methods through true knowledge. It should be seen in practice that such a person respects the elderly and understands family boundaries. If he takes care of his responsibilities and duties towards the society or nation, it shows the transformation of one's knowledge into education, i.e., the person has attained true knowledge. It is clear that internal senses are directly related to inner change. True knowledge can create ability, bring the soul closer to nature and make it melodious. We need knowledge that is not dry and is beautiful, less like a stubble tree, but fruitful and dense—onethat can create love, kindness, compassion, and attraction. We should acquire the knowledge that changes our fundamental qualities and cultivate them. This is possible only when your education is linked not only with bookish knowledge but also with life. Knowledge does not mean merely acquiring alphabet knowledge. Instead, an effort should be made to eradicate the indiscretion, ignorance, stupidity, or inertia within us. Our educationists, religious leaders, and saint-mahatmas should come forward and suggest ways to the people that can help overcome inner darkness. The darkness here refers to the darkness that spreads within our inner being, such as the customs, desires, and vices inside us; they are the darkness within us. The question worth considering here is how we can awaken them, how we can manifest them and how we can move them forward. This should be the primary aim of true knowledge.

Music As A Method For Moksha

Music has been given as much importance in India as the six systems of Indian philosophy (Sankhya, Yoga, Nyaya, Vaisheshika, Mimamsa and Vedanta). Similar to Indian philosophies, music also remains a matter of contemplation. In Indian Philosophy, the main goal of humans has been to interface with God. At the same time, it has also been recognized that music can be a powerful medium for this connection. Music, the highest medium of attaining this, has been linked with spirituality. Apart from Samaveda, we find that *sacred* texts like Brahman Sanhita, Gandharva Veda, Pratishakhya, Naradiya Shiksha, and Chandogya Upanishad are proof of this. Natyashastra, Vishnudharmottar Puran, Sangeet Ratnagar, etc., are epics with the same theme.

Indian singers, musicians, and dancers identified the soul of music because their goal was to attain salvation and establish a relationship with God. So for this reason, they utilized music as a platform to achieve their goal. Sangeet Makarand says, "(music) increases affection amongst all dependents (Bhaktas), castes, and kings." [8]

'The Shruti caste, which is skilled at playing the Veena, attains salvation because of their skill and knowledge of rhythm,' says Yagyavalkya Smriti [9].

वीणावादनतत्त्वज्ञःश्रुतिजातिविशारदः

तालज्ञश्चाप्रयासेनमोक्षमार्गंनियच्छति | | Shlok 15 of Yagyavalkya Smriti [9].

(The person who knows the principle of playing the Veena, is proficient in Shruti and caste, and easily knows the way to liberation.)

Highlighting the significance of music, Sangeet Chudamani mentions that song is the only saviour and offers unlimited rewards that neither yoga nor meditation can provide.

योगध्यानदिकंयस्मात्सर्वलोकानुरंजनं

तस्मादनं तफलमगीतं स्यादभुक्तिमुक्तिदम् | Verse 11 of Sangeet Chudamani [10].

In Sangeet Ratnakar, music has been defined as 'Song, instrument, and dance, all three constitute music.' [3].

These three are the different forms of sound. It is for this reason that sound is universal. It is the first medium of behaviour.





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गीतं नादात्मकं वाद्यं नादव्यक्त्या प्रशस्यते
तद्ध्यानुगतं नृत्तनादाधीनमतस्त्रयम्।
नादेन व्यज्यते वर्णः पदं वर्णात्पदाद्ब्रह्म।

वचसोव्यवहारोऽयं नादाधीनमतोजगत् २। Shlok 1-2 of Sangeet Ratnakar [3].

Songs and instruments are forms of Nada because it is through the expression of Nada that they attain beauty and elegance. The dance which imitates both (song and instrument) is also Naad. Therefore, songs, instruments, and dance are three forms of sound or Naad. Not only these three, but the whole world is also full of sound or Naadatmak. Because sound is expressed by alphabet, alphabets and sentences are described by words. Hence, the entire behaviour of speech is under the sound of Naad.

Sangeet Ratnakar's book mentions, "Songs and instruments are resonating because only through the expression of sound can they attain beauty." Following these two dances also resonate.

So, songs, instruments, and dances are all resonating. Not only these three, but the whole world is also full of resonance because sound leads to syllables, syllables leads to verse, and verse finds expression in sentences. Even speech behaviour is based on resonance.

The word that all the Vedas recite and all austerities proclaim, desiring that men live a life of religious study, is Om! The syllable, indeed, is Brahma! That is the highest syllable! You can have whatever you want! That is the best support. That is the supreme support. With that support, one becomes happy in the Brahma world. "

Rasanubhuti

Rasa: The Artistic Experience of Raga

Here we should understand the meaning of "rasa" as a pure feeling of beauty. Rasa is deeply sacred because, from a spiritual point of view, art is seen as pure and extreme joy and not merely as the creation of feelings like beauty, anger, and comedy. Usually, it is said that the soul of art is rasa, and the goal of art is to make one get the feeling of rasa. Rasa is produced with the sprouting of these feelings; the rasa is expressed as art-oriented beauty. It is called the "aesthetic value of art" in English. (Hhiriyanna or Natyashastra definition of rasa). It is said that these expressions are present in a person's heart (hriday).

The most important aspect of Indian music, closely related to the concept of "raga," is the concept of "rasa". Rasa is the vital force of raga; nay, it is the transcendental experience of the *ananda* of (Nada)

रसोवैसः। रसं ह्येवायं लब्धवानन्दी भवति। को ह्येवान्यात्कः प्राण्यत्पदेषु आकाशानन्दो न स्यात् [11].

He is the form of rasa. One who gets this rasa becomes blissful. When the creature has attained this bliss, this rasa, he himself becomes blissful.

Rasa is more fundamental than even the Ananda, or bliss of the Absolute, i.e., *Saccidananda*. If raga is considered the melodic core of Indian music, rasa is its artistic experience. For example, when we take a morsel of food and move it in the mouth with our tongue, trying to extract its vital juice or sap, we feel a peculiar relish and delight characteristic of that particular type of food. Similarly, every raga inherently carries its rasa, experienced by both the performer and the discernible listener. Thus, raga and rasa go hand in hand. There can be no raga without an accompanying rasa.

The word "rasa" has been understood in the following three aspects:

- (i) In the physical sense, it denotes juice or essence, for example, the juice of a fruit.
- (ii) In the psychological sense, it tells you about the quality of the juice, how delectable or otherwise it is.
- (iii) In the metaphysical or spiritual sense, it is known as rasanubhuti, that is, an experience, which is beyond the self-centred ego.

In order to achieve artistic excellence, one must merge their identity with the state of rasa-experience.

It is challenging to find a word in any other language that can convey the meaning of the word 'rasa' in its fullness. It is a sort of "feeling par excellence," or the eternal value that is felt like an end in itself. Such a feeling is sublime and



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transempirical and is directed inward. Rasa is not comparable to any common experience we encounter in our everyday life. It is therefore designated as *Alaukika*, *Lokottara*, or *Vilaksana* because it is super sensuous.

Rasa is lokottara, i.e., we don't have such an experience in normal life. It is a transcendent, supermundane experience. It can be enjoyed only as part of one's being, as an intrinsic, inseparable aspect of one's self, by those who are deeply sensitive to beauty. Not all are capable of this experience which takes you to the path of salvation. The primary source of traditional Indian classical music is found in the Vedas, Upanishads, and Aagams, and the fundamental nature of Indian music has always been religion. The relationship between the sound of music and 'Naad Brahm' is intense. Indian classical music has been considered an expression of 'NaadBrahm' (OM is representative of the same). In fact, 'NaadBrahm' and om are synonymous. According to Indian tradition, Om is the sound through which other sounds find expression. If all those features of sounds that distinguish them from one another are removed, then we will be left with only one sound, i.e., Om or NaadBrahm. So all the various sounds are unified into one, and i.e. Om or Naadbrahm.

CONCLUSION

Music has been a powerful form of entertainment for ages. In ancient times, no electronic means of entertainment or modern equipment existed. Even then, music connected mankind together. Even if people do not know other people's languages, civilizations and cultures, the language of music is such a universal language that everyone understands and which everyone enjoys. History is witness to the fact that politicians have sent musicians and artists to other countries to improve diplomatic relations. It signifies the universal power of music. Music not only entertains human beings but also has a positive effect on the nervous system of man, which helps a lot in the treatment of serious diseases. The attention of scientists has been on its life-giving potential. There are continuous experiments to use music to remove physical and mental disorders. The trend of music therapy has delivered promising results, and its healing power is now contemplated as the solution to mental disorders.

Many types of research have been done all over the world regarding the effect of music on humans. Doctors have used music therapy on people suffering from severe diseases and found that music positively affects such patients. That is why in the medical world, too, such music is being composed, songs are being written, and musical instruments are being included to help in the diagnosis of diseases, which are proving to be very helpful in the diagnosis of these diseases. This contribution of music toward human welfare is undoubtedly unforgettable. It has been observed that it had a very positive effect on treating diseases with which the help of music therapy was taken. With the help of music therapy and holistic medical treatment, the healing process of the patients got accelerated. Music has a positive effect on patients physically and emotionally, which helps a lot in recovery. Even after being completely cured, doctors advise patients to be associated with music to remain healthy in the future. This not only improves health but also improves the quality of life.

We are very stressed in our daily life. Excess of work at the workplace, rush to reach everywhere on time, doing household chores smoothly and rush to fulfil social obligations make us very tired. In such a situation, music makes us feel like cold rain on the warming earth. After listening to melodious music for some time, all our fatigue goes away, and music removes all negative energy and fills life with positive energy. In the field of education, it has been found that if good music is played to the students who are not able to concentrate on studies, then not only their focus on studies but also their interest in subjects increases. Such students complete the given task in relatively less time.

From a commercial point of view, music has taken the form of a big industry today. Today, instrumentalists, musicians, lyricists, and singers are not only earning money through music but are also earning fame. The increasing influence of technology and communication tools has brought music to the masses. Music has that power that develops our inner potential. It reveals the power of our self-realization. Through this, we can focus our spiritual energy and move forward on the path of spirituality. A set of abilities is hidden inside us, but we do not recognize



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them. Music introduces us to them. Practically, through music, we can channel our abilities in a more creative direction by being filled with positive energy.

It is clear from the above facts that if a scientific approach is adopted toward music, its results can be very positive. It is not that there was no scientific approach to music in the past. As mentioned above, there was a vibrant music tradition in India, and scholars have studied it in detailed and scientific ways. Our scriptures are a vivid example of this. However, due to the dust of time, they were lost from our sight. Today, we need to remove this dust wisely and study its true nature from the present perspective. Music which can lead us to the path of salvation, can definitely make our daily life meaningful, capable, successful, and bright. It is necessary that we recognize its power.

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Effectiveness of Dry Needling and Myofascial Release on Pain, Range of Motion, Disability and Quality of Life in Patients with Tension-Type Headache

Avantika Arora^{1*} and Manu Goyal²

¹MPT Student, Maharishi Markendeshwar Institute of Physiotherapy and Rehabilitation, Maharishi Markendeshwar (Deemed to be University), Mullana Ambala, Haryana, India.

²Professor, Maharishi Markendeshwar Institute of Physiotherapy and Rehabilitation, Maharishi Markendeshwar (Deemed to be University), Mullana- Ambala, Haryana India.

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*Address for Correspondence

Avantika Arora

MPT Student,

Maharishi Markendeshwar Institute of Physiotherapy and Rehabilitation,

Maharishi Markendeshwar (Deemed to be University),

Mullana Ambala, Haryana, India.

E.Mail: avantikaarora475@gmail.com



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ABSTRACT

The aim of the study was to compare the efficacy of dry needling (DN) and myofascial release (MFR) on pain, range of motion, disability and quality of life in patients with tension-type headache (TTH). A two-group pre-test and post-test experimental study conducted. A total of 78 patients were included in the study with the age of 20-40 years. Patients were randomized into group A (DN) and group B (MFR). DN and MFR were given for 3 days in a week for 2 weeks. Assessments were measured in both groups before the intervention and 2 weeks after the intervention. Significant differences in decrease in pain intensity and improved pain threshold were found in both groups ($p < 0.001$). Moreover, effect size was more reported in DN group as compared to MFR group with respect to reducing pain and improving pain pressure threshold. In both cases, intervention showed similar behaviour with respect to range of motion and improving QOL. Both the interventions have found to be effective in improving outcomes in patients with TTH. However, DN is found to be more effective as compared to MFR. Therefore, it should be incorporated in the clinical practice to get additional benefits.

Keywords: Adult, Human, Range of motion, Pain threshold, Superficial back muscles



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INTRODUCTION

Headache is explicated as discomfort in the head and cervical area [1]. The International Headache Society (IHS) exemplify headache as a sharp, twitching, ascetic pain in the occipital region in conjunction with subsequent soreness and sensory deviance [2]. Primary headaches have no ostensible inherent natural disease mechanism whereas secondary headaches are acquired by an inherent fundamental illness and are a symptom of a typical disease progression[3].TTH is a headache consistently recounted as “a band encircling the head” [4]. The International Classification Headache Diagnosis (ICHD-I) coined the phrase “tension-type headache” in 1988 and have been maintained by ICHD II in 2004.[5] TTH is commonly referred to as muscular contraction headache [5], [6]. TTH is one of the most prevalent primary headache condition that typically causes pain that radiates from forehead to occiput [7], [8].

TTH is 50% - 66% prevalent in the adult population worldwide. In the United States, headache is 33% more frequently reported in women. Women experience headaches 5%–9% of the time, whereas males experience them 1%–3% of the time [4], [9]. TTH reported to be greater amid women due to the effect of female hormone levels especially estrogen [10]. Headache problems are the sixth greatest reason of disability globally and the third major cause of impairment among those aged between 15 to 49 [11]. TTH is characterized by recurrent episodes that last between 30 minutes and 7 hours each, happen at least 10 times annually, and take place on less than one day each month[7], [8]. According to (ICHD-II), TTH are classified as either meager periodic (prevailing of headache for at least one day each month), frequent continuous (headache that persuade for a minimum period of fifteen days) and chronic (persistence of headache for duration of longer than fifteen days once trimonthly) [12].

Stress plays an important role in instating, worsening and persistence of headache. The probable physiology of TTH has been linked to myofascial trigger points. The pericranial musculatures are thought to be anticipating the TTH trigger sites. Excessive pericephalic muscle spasms can cause ischemia and the production of the noxious chemical P, which can cause further pain [13].DN is a skilled intercession for the alleviation of chronic pain syndromes. This method aims to eliminate TrPs without the use of an anesthetic by puncturing the epidermis, tissue underneath the skin, muscle and fascia with a fine, sterile needle [13]. MFR is the manual application of a low-load, prolonged stretch to the myofascial complex where integrated muscle and soft tissue inhibition is administered to extend tightened fascia, balancing the length and sliding properties of myofascial tissues to relieve tension from pain-sensitive structures, enhance pain perception, improve the pain threshold and restore normal movement of joint [14], [15]. There is a paucity of literature on addressing tension-type headache for therapists contemplating a several modes of multidisciplinary strategies for diagnosis and treatment planning. This randomized clinical study compares the efficacy of DN and MFR in individuals with TTH. The TTH management regime may benefit from TrPs inactivation. To the best of our understanding, there is no literature comparing these two treatments for tension-type headache. Thus, there was need of the study to implement and contrast therapeutic strategies DN and MFR in managing individuals with TTH. The study aimed to compare the efficacy of DN and MFR on pain pressure threshold, range of motion, disability and quality of life in patients with tension-type headache. The study hypothesized that the effectiveness of DN and MFR may or may not be significantly different in patients with TTH.

MATERIALS AND METHODS

Study Design

A Two group pre-test and post-test experimental design was conducted from November 2021- March 2023. Ethical approval was obtained from Institutional Ethical committee (Approval number IEC2215). The study was registered under ctri.nic.in on 14 September, 2022 (ID: CTRI/2022/09/045495).



**Avantika Arora and Manu Goyal****Participants**

Eligible participants were recruited from tertiary care hospital and the written informed consent were given to the subjects in bilingual language (Hindi and English). Sample size estimation was done from G* Power software 3.1.9.7 and the following input data was used with α error rate of 0.05, power of 0.80 (80%) with effect size of 0.65. The required effect size was calculated on basis of previous study (with digital algometer as main outcome measure) [16]. from which effect size obtained 0.62 and the effect size 0.65 employed for the current study. Based on this obtained data sample size (n)= 78 calculated for current study. Inclusion criteria were patients with age (20-40 years [17], BMI (18.5-29.9 kg/m²), pain pressure threshold of <15 on bilateral upper trapezius and suboccipital muscles, VAS<7[18], Headache disability Index (HDI) 50-68 and HIT-6 (40-50). Exclusion criteria were any history of trauma (fractures) of Cervical spine, Temporomandibular disorders, Needle phobia, any history of Systemic lupus arthritis Fibromyalgia and any inflammatory pathology, malignancy, previous spinal surgery, history of orthopaedic, neurological and cardiovascular disorder.

Randomization, Allocation and Blinding

A number of 78 suitable respondents enlisted and randomly allocated by computer generated random numbers into 2 groups with Group 1 (DN) has 39 patients and Group 2 (MFR) group also has equivalent number of participants. The intervention was performed subsequent the initial evaluations and standardized examinations. No blinding was done during the assessment and intervention period. Participants in DN group received three sessions lasting ten minutes every week for subsequent two weeks and participants in MFR group received three 3 sessions per week for subsequent two weeks. Primary outcome measures were Digital algometer and Visual analogue scale .Secondary outcome measures used were Goniometer, HDI and HIT-6.

Measurement of Pain pressure threshold

Digital Algometer (Orchid Scientific ALGO-DS) was used for determining the pain pressure threshold with digital pressure algometer placed at the site of the greatest mechanical hyperalgesia. When the patient feels pain or discomfort, pressure was increased on the point being evaluated, and the highest pressure obtained was electronically recorded. The intraclass coefficients range from (0.90 to 0.95) [19], [20].

Measurement of Pain

Participants were requisitioned to evaluate the extent of agony by making a mark on a 100-mm VAS. The antithesis of the VAS was labelled as "worst possible pain" and "least possible pain" and were placed horizontally. All paired VAS ratings had an ICC of 0.97 (95% CI: 0.96 to 0.98) [21].

Measurement of Range of motion

The standard goniometer (double-arm, 12-inch plastic goniometer with a semi-circular gauge) was used. Inter-rater reliability ranges from 0.79 to 0.92, and intra-rater reliability has intraclass correlation coefficients (ICC) between 0.83 and 0.98 [22].

Measurement of disability and Quality of life

HDI and HIT-6 were used. HDI was a 25-item rating with two subscales that asses both emotional and functional levels. Every question has one of three possible responses with No- (zero points), Sometimes- (two points) and Yes (four points). HDI scores range from 10-72 % with more percentage of scores indicating more disability[23]. HIT-6 also evaluates the degree of pain in the head. HIT-6 values range from 36 to 78 with higher values signifying more effect on life. Test-retest reliability is within the 0.77 [24].

Procedure**Group 1 Intervention - Dry needling (DN)**

DN for suboccipitalis muscle- Patient in prone position with placing forehead on forearm so that spine remains in neutral position. Label the prohibited triangle (triangle between 2 mastoids). Needles utilized were 0.26x13mm-



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40mm. Introduction of needle with 1 finger space tangentially towards the direction of marked trigger point in star like fashion. Introduction of needles to 5-10mm at angle of 45- and 60-degree [25] deep to a MTrPs is maintained 10 min (static needling) for 3 days in a week for 2 weeks [17].

DN for Upper Trapezius

Patient in prone position with arm abducted. Needles utilized were 0.30x25mm. Uplift belly of upper trapezius muscle aside from the lung area and grasp muscle with thumb, index and middle finger and insert needle in laterally and cranial direction[25] and maintained for 2-3 minutes for 3 days in a week for consecutive 2 weeks[17].

Group 2 Intervention –Myofascial Release (MFR)

Myofascial Release for Suboccipitalis Muscle-Assessment of the trigger points was made by digital algometer along the suboccipital muscles mainly rectus -capitis- posterior- major and minor, obliquus- capitis- superior and inferior in prone position. Patient lies supine with therapist seated at the head end of the treatment table. Suboccipital region was positioned beneath the therapist finger pads. Pressure was applied slowly and softly by the therapist for two to three minutes ventrally, superiorly and obliquely into the tissues[26]. Procedure was done for 3 days in a week for consecutive 2 weeks.[14], [27] Myofascial Release for Upper trapezius muscle - Patient in supine lying and therapist applies ischemic compression with the thumb to trigger point for 60 to 90 seconds.[28] With patient in supine lying the therapist put one hand on shoulder and other hand on back of head and therapist would perform passive neck flexion movement followed by contralateral side flexion movement[29].

STATISTICAL ANALYSIS

Application SPSS version 20 was used for data analysis. Paired t tests and Wilcoxon signed rank tests were used for the within-group analysis of parametric and non-parametric outcome variables. Independent t test and Mann-Whitney U test were used for between-group comparisons of parametric and non-parametric outcome variables respectively. The Significance level was set at 0.05.

RESULTS

A total of 78 participants were included during the study period among which 14 participants were male and 64 participants were female. A total of 8 Male patients and 31 female patients were enrolled in DN group and 6 male patients and 33 females patients were enlisted in MFR group. Demographic descriptives for not normal distributed data were expressed in form of Median (IQR) and demographic descriptives for normal distributed data were expressed in form of Mean \pm SD with categorical variable expressed in percentage as depicted in Table1.

DISCUSSION

The study objective was to contrast the efficiency of MFR and DN in addressing individuals suffering from headaches of the tension type in terms of pain pressure threshold, range of motion disability with effect on quality of life. The study findings showed that both interventions were statistically and clinically significant for patients with TTH but more improvement of dependent variables was seen in the DN group than in the MFR group denotative in terms of effect size demonstrating that DN was more clinically effective in decreasing pain (Effect size DN= 3.02) and (Effect size MFR=1.77), improving pain pressure threshold (Effect size DN= 4.66) and (Effect size MFR= 2.32) and enhancing standard of living (Effect size DN= 2.77) and (Effect size MFR= 0.43). Thus, we reject the null hypothesis. Between group comparison revealed that both the interventions were more beneficial in decreasing pain and improving pain pressure threshold with lessened benefits on range of motion and Quality of life. One of the most prevalent sub-categories of headache, tension type headache was experienced by more than 40% of adults



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worldwide. The diagnosis standard for TTH was mild to moderate pressure on both sides without any other observable symptoms.

Men and women experienced this condition at roughly equal rate. Muscle contraction headache might be caused by nociceptor cells in the pericranial myofascial tissues. According to several studies, people with chronic tension type-headache had heightened sensitivity to electrical, and thermal and pressure stimuli in the pericranial myofascial tissue and might experienced pain from even usually innocuous stimuli [30]. This present study was assassinated to resolute the impact of two interventions DN and MFR on pressure sensitivity, mobility and standard of living. As people with TTH endured from hyperalgesia, reduced mobility with impaired independence level. In accordance with the findings of prior study[31], individuals who experienced headache had significantly lower HR-QoL. Another study aimed to treat headaches more effectively while minimizing their frequency, severity, and duration. In accordance with this study, women account for more than 65% of cases of TTH. The condition (CTTH) develops from (ETTH). TTH could be stressful and interfered with everyday activities. Inappropriate treatment might exacerbate symptoms such as comorbid analgesia abuse headache. Efficient administration was critical to avoid further issues and aid in regaining functionality.

The research found inadequate backing for medication treatments for CTTH. Nonsteroidal anti-inflammatory medication (NSAID) use that continues for more than two days per week might result in chronic headache symptoms and has lessened the efficacy of preventative care [32]. The study discussed about the growing interest in nonpharmacologic therapy alternatives because of the high prevalence of tension-type headache (TTH) and the negative effects of analgesic pharmaceutical treatment. Nonpharmacologic treatment options are becoming more popular due to the high prevalence of TTH and the negative side effects of analgesic pharmaceutical therapy [33]. A study has been carried out to assess the effectiveness of manual therapy with different types of physical therapy. One of the main treatments for TTH was believed to be manual therapy. It was founded on the biomechanical analysis of muscles and joints as a means of treating neuromusculoskeletal dysfunction. TTH was a condition that significantly lowers quality of life and has a negative socioeconomic effect. Both CTTH and ETTH in adult individuals were included. In accordance with this study findings combined manual therapy was helpful in treating TTH [33]. A study was conducted to evaluate the effectiveness of trigger point dry needling in reducing headache frequency, intensity, and duration in patients with chronic tension-type headaches as well as in enhancing health-related quality of life. 168 subjects in total were randomly divided to 2 treatment groups (DN) and sham (DN). In the experimental group, active trigger points in the head and neck musculature were treated with needles and in the sham group, active trigger points were not treated with needles. The following outcome metrics were used: frequency, length, intensity, and effect on daily activities. The research found that after treatment headache intensity, frequency, and duration significantly decreased in the (DN) group ($P < .05$) [34.] A randomized research study was conducted for the purpose of determining the MTrPs for recurring tension-type headaches.

MTrPs imitate the pain patterns of tension headaches and induce head pain. 56 TTH patients were randomized to either a placebo or 12 rounds of massage treatment over the course of six weeks. As result indicators, the PPT at MTrPs in the upper trapezius and suboccipital muscles as well as the self-reported intensity of headache pain were used. In comparison to the control group, the massage group reported a greater symptomatic reduction of headache and PPT improved in all muscles in the massage group when compared to the placebo group ($P=0.002$) [35]. Another study conducted to demonstrate the efficacy of myofascial-release therapy for which 48 individuals randomly divided into 3 groups. PSQI and NDI were utilized to evaluate efficacy of intervention before and after it was administered twice a week for four consecutive weeks. In individuals treated with MFR and MFR-Ex, there was a noticeable improvement in the ATrPs, Neck ROM, NDI, and PSQI, with MFR-Ex having a greater impact on improving neck mobility. According to the experiment results, MFR improved neck mobility and sleep quality more effectively in individuals with persistent tension headaches[1]. The research aimed to evaluate the effectiveness of DN and friction massage on TTH patients. Myofascial trigger points are treatable in a number of ways. 44 people in all were studied for the research. The intensity and incidence of headaches, pain threshold under pressure, and neck ROM were employed as outcome variables. The findings demonstrated a substantial reduction in headache



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frequency and intensity with both treatment modalities, as well as an improvement in pain threshold at the trigger points. However, there was no discernible difference in range of motion, with the exception of extension, which was improved in the DN group. However, cervical range was unaffected by either therapy [36]. Limitations of the study were that the study was a single centred design with abbreviated time period with no follow up employed after the intervention and no masking was done during allocation and treatment. The strength of the study was that there were no potential serious adverse effects reported during the intervention period with zero percent dropout rate and the study was therapeutically and statistically consequential amid all the outcome measures used in this present study. Future studies can address some another age category to comprehend the most beneficial result in both groups with subsequent follow-up period with a larger-scale study employing a multi-centre approach with including other additional headache subtypes to ascertain the effectiveness of the intervention.

CONCLUSION

DN and MFR both are equally consequential among individuals with tension-type headache. This current study unveils that there is more improvement of outcome variables in DN group clinically in contrast with MFR group. It was concluded that DN is more effectual than MFR in ameliorating pain pressure threshold, decreasing pain with both groups having equivalent substantial benefit increasing mobility, lowering disability, and boosting quality of living.

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Table 1- Demographic descriptives of all participants

| S. No | Demographic Characteristics | Median IQR | 95%CI | p-value |
|-------|-----------------------------|-----------------------------|-------------------------------|-------------------------|
| 1 | Age(years) | 25(21,38) | 25.1 to 26.5 | .002 |
| 2 | Height (cm) | 160(152,185) | 159.3 to 162.9 | <.001 |
| 3 | BMI (kg/m ²) | 22(19,25) | 22.0 to 22.6 | <.000 |
| 4 | Gender % | Male Female | 14(17.9) 64(82.1) | |
| 5. | Weight(kg) | Mean± SD 59.3±6.2 | 95% CI 22.0 to 22.6 | p-value .200* |

m- centimetre, kg/m²- Kilogram per square metre, %- percentage, kg-kilogram

Table 2- Within group analysis of Outcome variables of DN and MFR group

| Outcome measure | Group | p- value |
|-----------------------|-----------------------|----------|
| VAS | DN group MFR group | <0.001 |
| Trapezius Right | DN group MFR group | <0.001 |
| Trapezius Left | DN group MFR group | <0.001 |
| Suboccipital R1 | DN group MFR group | <0.001 |
| Suboccipital R2 | DN group MFR group | <0.001 |
| Suboccipital L1 | DN group MFR group | <0.001 |
| Suboccipital L2 | DN group MFR group | <0.001 |
| Cervical Flexion | DN group MFR group | <0.001 |
| Cervical Extension | DN group MFR group | <0.001 |
| Lateral Flexion Right | DN group MFR group | <0.001 |
| Lateral Flexion Left | DN group MFR group | <0.001 |
| Rotation Right | DN group MFR group | <0.001 |
| Rotation Left | DN group MFR group | <0.001 |
| HDI | DN group MFR group | <0.001 |
| HIT-6 | DN group MFR group | <0.001 |

DN- Dry needling, MFR- Myofascial release, VAS- Visual analogue scale, HDI-Headache disability index, HIT-6- Headache impact test, Suboccipital R1- Rectus capitis posterior major, Suboccipital R2- Obliquus capitis superior, Suboccipital L1- Rectus capitis posterior minor, Suboccipital L2- Obliquus capitis inferior





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Table 3 - Between Group Analysis of Outcome Variables of DN and MFR group

| Variables | p-value |
|-----------------------|---------|
| VAS | <0.001 |
| Trapezius R | <0.001 |
| Trapezius L | <0.001 |
| Suboccipital R1 | <0.001 |
| Suboccipital R2 | <0.001 |
| Suboccipital L1 | <0.001 |
| Suboccipital L2 | <0.001 |
| Cervical Flexion | .098 |
| Cervical Extension | .083 |
| Lateral Flexion Right | .069 |
| Lateral Flexion Left | .025 |
| Rotation Right | .662 |
| Rotation Left | .485 |
| HDI | .426 |
| HIT-6 | .542 |

Suboccipital R1- Rectus capitis posterior major, Suboccipital R2- Obliquus capitis superior, Suboccipital L1- Rectus capitis posterior minor, Suboccipital L2- Obliquus capitis inferior

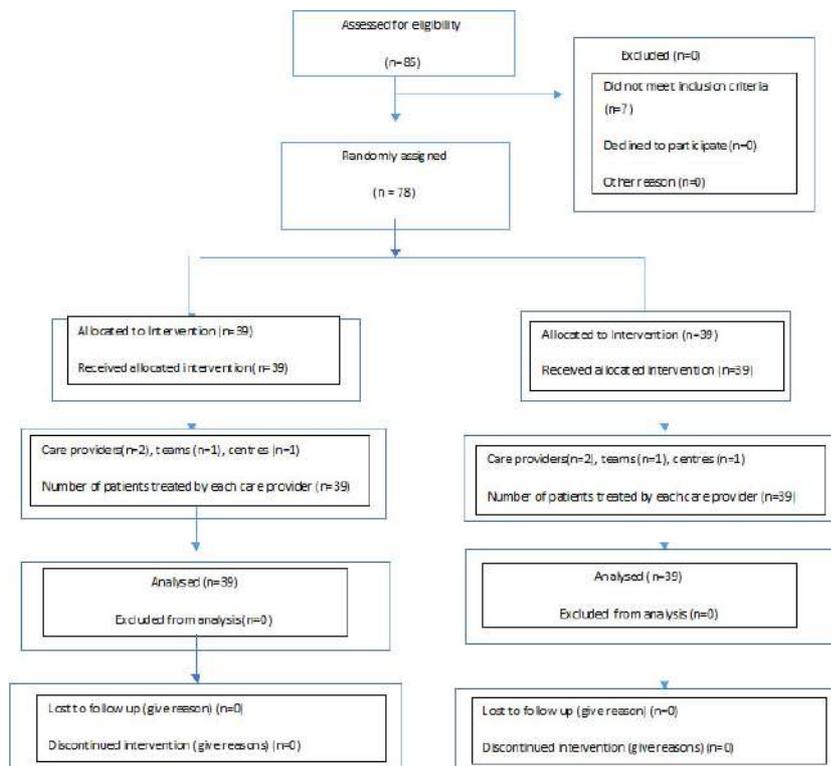


Fig.1- CONSORT flowchart for the study Outcome Measures





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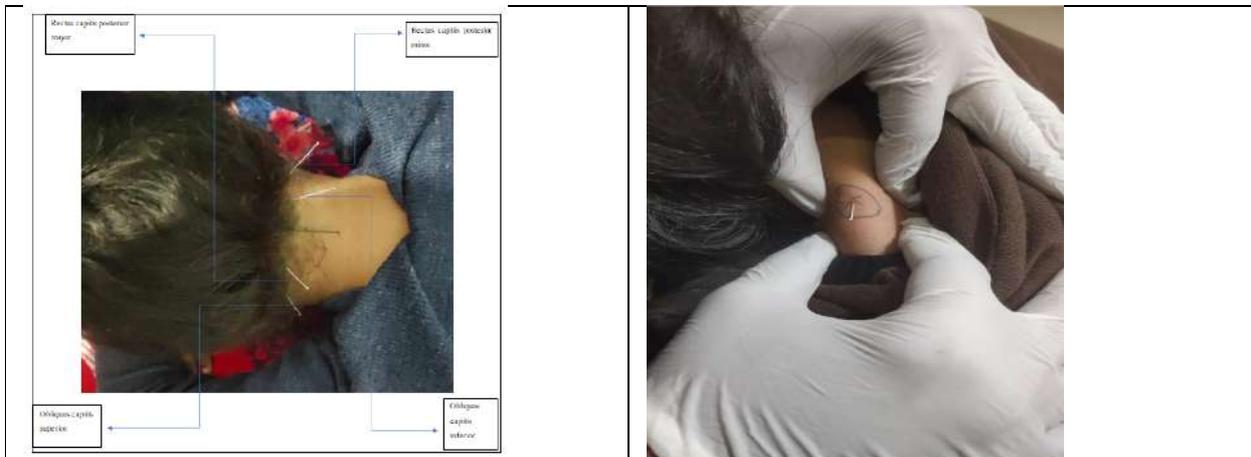


Fig. 2- Dry Needling of Suboccipitalis muscles

Fig.3- Dry needling of upper trapezius muscle



Fig.4 Myofascial release of Suboccipitalis





Hierarchical Agglomerative Cluster Analysis of Air Pollution over Andhra Pradesh and Telangana

Polisetty Venkateswara Rao^{1*} and Nannaparaju Vasudha²

¹Associate Professor, Department of Physics, Vasavi College of Engineering, Hyderabad, Telangana, India.

²Associate Professor, Department of Mathematics, Vasavi College of Engineering, Hyderabad, Telangana, India.

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*Address for Correspondence

Polisetty Venkateswara Rao

Associate Professor,
Department of Physics,
Vasavi College of Engineering,
Hyderabad, Telangana, India.
E.Mail: kishansetty@gmail.com



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ABSTRACT

The present study aims to adopt a method to categorize the air pollution monitoring stations in the states of Andhra Pradesh and Telangana based on air pollution levels and measure the contribution of the season-wise on Air Quality Index (AQI). The results of the study can be used to diminish air pollution by adopting new strategies. Hierarchical Agglomerative Cluster Analysis (HACA) was used to sort various stations based on air pollution level in all three seasons (Summer Winter and Monsoon). The results were validated using Discriminant Analysis (DA). With reference to AQI standards of Central Pollution Control Board (CPCB), monitoring stations were sorted and season-wise clusters were formed. AQI levels between 0 to 50; 51 to 100 and 101 to 250 are considered as low, moderate and high pollution. The contribution of each pollutant in a cluster was calculated using Factor analysis (FA). Our findings show few unusual facts like Amaravati, Bollaram, Pashmylaram and Patencheru falls in the range of High Pollution (HP), during winter, contributing 31.3% of total air pollution which may be due to the Fossil fuel combustion of agricultural systems at Amaravati and chemical smog at other stations. However, Tirupati and Vishakhapatnam were found in Low pollution (LP) during winter and it may be due to temperature inversion layer.

Keywords: Air Quality Index, Hierarchical Agglomerative Cluster Analysis, Discriminant Analysis, Factor Analysis.





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INTRODUCTION

Since the start of industrialisation, air pollution has been a significant health hazard. Pollution can reduce lifespans and cause lung cancer and respiratory tract illnesses (Kan et al., 2012; Kim et al., 2013; Hoek et al., 2013). Studies have demonstrated that gaseous pollutants including CO, SO₂, NO₂, and O₃ as well as particulate matter like PM_{2.5} and PM₁₀ are hazardous to human health. Over the past few years, pollution in Andhra Pradesh and Telangana has been rising alarmingly. Between July and October, Andhra Pradesh and Telangana are subject to the southern monsoon, which produces torrential rain. Most of the states' yearly rainfall occurs at this time of the year. It describes a seasonal wind pattern with wet summers and dry winters in India. Winds travelling across the Indian Ocean and southward toward the subcontinent are associated with strong cyclonic activity during the summer monsoon, which causes heavy rainfall for almost four months.

LITERATURE SURVEY

The study's findings (Balogun and Orimoogunje, 2015) revealed that the relationship between seasonality and air pollution concentration differs greatly. The independent sample T-test found a significant difference between the dry and wet seasons in the mean variation for particulate matter concentration. All land used in the research area have environments that are classified as "moderately dirty" and "dirtier" by values for PM (0.3-10µm). The WHO standard of 90 ppm for CO values for 15 minutes was not exceeded. Except for CO, mean values of all sizes of particulate matter were typically greater in the dry season than in the rainy season. In their study (Roger et. al., 2005) estimated the time-varying effects of air pollution on daily mortality using a Bayesian semiparametric hierarchical model. To improve the accuracy of seasonal relative rate estimations, the model incorporates data from many cities. At both lag 0 and lag 1, we discovered seasonal variations for the national average effect of PM₁₀. Seasonal trends vary by geographical area, with the Northeast exhibiting a robust pattern for lag 1. The lack of seasonal change in the southern portions of the US was also intriguing.

The paper by (Wang and Lu, 2006) presents a thorough investigation of the changes in main air pollutants and the daily air pollution index (API) in the Causeway Bay area between 1999 and 2003. The early morning period (00:00–5:00 am) with low pollution levels, the afternoon phase, and the daily fluctuations of SO₂, NO₂, CO, and RSP levels are based on statistical analysis. During the examined time, the monthly fluctuating processes of the major pollutants show various patterns, but summer has lower levels and other seasons experience higher levels. ARMA (1,1) and ARMA are the best ARMA models for the summer and other seasons (2,2). In comparison to the related observations, both models can yield results that are reliable and satisfactory.

From January to December 2014, the variance in particle mass and particulate types (PM_{2.5} and PM₁₀) related to local/regional meteorology was examined for an urban area across the middle Indo-Gangetic Plain (Murari et al., 2017). Large mass loading with unique seasonal impacts was evident in both coarser and smaller particles. In addition, 56% (for PM_{2.5}) to 81% (for PM₁₀) of monitoring events showed that national air quality guidelines were not met, particularly during the winter. Additionally, there were seasonal fluctuations in the particle types, with the highest PM_{2.5} loading occurring in the winter (0.62), as opposed to the summer months (0.38). In terms of a dry summer, local meteorology has distinct patterns that are easy to spot (March to June).

MATERIALS AND METHODS

Study Sites

Visakhapatnam, the port city is the jewel on the eastern coast of India. It is one of the largest industrial corridors and IT hubs and it is the fast-growing city in Andhra Pradesh. The city has spoon-shaped topography spread over three sides and the sea on one side. It is often known as the bowl area with concern to the assessment of environmental impact as it is surrounded by the Eastern Ghats on three sides of the city and the cost of Bay of



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Bengal. The research region is characterised by sloping topography, with the Bay of Bengal on the eastern side and hill ranges (320–504 m above mean sea level) on the northern, southern, and western sides. While the major and minor industries are spread out on the northern, southern, and western sides, much of the population is concentrated on the eastern side. The central pollution control board identified Visakhapatnam as one of the most polluted cities. There is a drastic deterioration of air quality due to the presence of various industries like HPCL, ZINC, Steel factories, power station, and fertilizer plants. It lies between latitudes 17°45' N and longitudes 83°10' E covering 160 sq km at an altitude of 15ft above sea level. In general, the climate of the city is humid and semi-arid and rainfall usually occurs during the southwest monsoon (June – September).

The seventh most populous city in the state of Andhra Pradesh is Rajahmundry, also referred to as the cultural capital of the state. It serves as the East and West Godavari districts' commercial centre and is renowned for its floriculture, economy, tourism, agriculture, and cultural legacy. There are no distinct seasons due to the tropical environment, which is hot and humid in general. The weather at Rajahmundry is hot and humid, with a tropical climate. Usually, April to June is the hottest season whereas December and January are the coolest months. The city is an industrial and petrochemical hub housing two paper mills and two power plants, and it is the headquarters of ONGC and GAIL. This provides huge employment which results in an increase in population in recent years. Geographically located at the centre of Godavari Districts with latitude and longitude 16°59'N 81°47'E at an average elevation of 14 meters (46 ft).

Amaravathi, a town on the bank of Krishna River in Guntur District with a population of around 1 lakh is the capital of the newly formed state Andhra Pradesh with a huge construction activity due to developments in academic, educational, residential, business sectors in recent years after separation. The terrain of the region and the local weather patterns can both have a significant impact on the levels of urban pollution. a great many. During various phases of construction operations, such as excavation, foundation work, material transportation, and superstructure construction, a significant amount of air pollution will be produced. The principal pollutants during construction are respirable dust and suspended particle matter (SPM), with excavation and transportation activities producing most of the dust. A significant amount of dust becomes windborne and is probably blown away. The workers who are directly exposed to the fugitive dust may experience rapid effects. At the same time, the dust moves farther and can settle in the neighbourhood.

Tirupati (13.65°N 79.42°E), referred to as a temple town with a population of 6.8 Lakhs, is one of the upcoming urban cities in Andhra Pradesh. It lies at the foot of the Seshachalam Hills of Eastern Ghats, pre-dominant in the Western Region and they gradually bend towards the sacred hills of Tirupati. Tirumala Venkateshwara Temple is the most notable temple for being the world's richest temple which attracts many pilgrims every day. Tirupati experience an extreme seasonal variation in the perceived humidity. For a year the temperature varies between 16°C and 41°C with wet season hot and dry season sweltering, muggy, and partly cloudy.

Hyderabad, the capital of Telangana, is one of the fast-developing metropolitan cities in India, located on the banks of the Musi River at a mean altitude of 542m above mean sea level. It extends an area of 650Sq. Km and has a population of approximately 10 million with a raise of 2.7% from 2019, which makes it the 6th most crowded urban agglomeration in India. It is a tropical metro city with dry and wet climatic conditions. The monthly mean temperatures fall in the range of 21 – 33°C. The summer months are hot and humid with temperatures frequently surpassing 40°C and winter with the lowest temperature rarely falling below 10°C. The air quality in Hyderabad often exceeds the NAAQ standards, which may be due to rapid growth in IT and industrial sectors which may also result from vehicular exhaust, road dust, and industrial emissions. The major factors for the pollutant gases are the two-wheelers, causing air pollution and most of the three-wheelers run on adulterated fuel, which may also cause air pollution in Hyderabad.





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Data collection

The air pollution data was taken from publicly available data at cpcb.nic.in maintained by Centre Pollution Control Board (CPCB) under the Ministry of Environment, Forest and Climate Change, Government of India. The air pollutant variables such as PM_{2.5} (µg/m³), NO₂ (µg/m³), NO_x (ppb), SO₂ (µg/m³), CO (mg/m³), O₃ (µg/m³), Benzene (µg/m³) and metrological variables as RH (%), WS (m/s), WD (deg), SR (W/mt²) and AT(deg C) were selected from 2019 from January to December to investigate how local characteristics and the seasons affect air pollution. To do the statistical analysis for this study, daily data from 120 data sets (12 data per station x 10 stations) with 1440 observations (12 data per station x 12 variables x 10 stations) were used. Daily data were used to create a monthly average. The monthly data was separated season-wise into summer (March to June), monsoon (July to October) and winter seasons (November, December, January and February). Later the season wise data was further used for analysis.

Hierarchical Agglomerative Cluster Analysis (HACA)

The data are not immediately sorted into a certain number of classes or clusters in a hierarchical categorization. Instead, the classification is divided into several groups that can range from one cluster that contains each individual to 'n' clusters that each contain one individual. Two subcategories of hierarchical clustering approaches are agglomerative methods and divisive methods. Agglomerative methods progressively combine the 'n' people into groups, whereas divisive methods gradually split the 'n' individuals into smaller groupings. Both the agglomerative path and the divisive path can produce hierarchical classifications, which can be represented by a two-dimensional diagram known as a dendrogram. It shows the fusions or divides made at each stage of the study.

The most widely used hierarchical approaches are agglomerative techniques. They divide the data into a sequence of partitions, the first of which consists of n "clusters" with a single member, and the last of which consists of a single group that includes all n people. At each stage, the people or groups who are closest to one another are combined (or most similar). There are several ways because they define the separation (or similarity) between an individual and a group of persons, or between two groups of individuals, differently.

In 1963, Ward developed Ward's method (Murtagh and Legendre, 2014) a unique form of agglomerative hierarchical clustering. Clusters having the least within-cluster variance are created using Ward's approach. Instead of using distance measures, it takes an analysis of variance method to clustering. The method is based on the calculation of the error sum of squares (ESS), which is the sum of the squared distances between each point and the cluster mean.

$$ESS_j = \sum_{i=1}^{n_j} \|X_{ij} - \bar{X}_j\|^2$$

Where X_{ij} is the i th observation in the j th cluster. The total of all clusters' ESS_j values, or the error sum of squares for all clusters, is as follows:

$$ESS = ESS_1 + ESS_2 + \dots + ESS_k$$

where k is the number of clusters.

The procedure begins by creating a total of 'n' clusters, where 'n' is the number of observations, each containing a single element. Each of these one-element clusters has a mean that is the same as that single observation. Two elements are combined into one cluster in the algorithm's initial stage such that the erroneous sum of squares, or ESS, grows as little as possible. Merging the dataset's two closest observations is one approach to achieving this. The merging, however, causes the least rise in ESS as each stage moves forward, as can be seen. This reduces the separation between the observations and the cluster centres. The procedure is repeated until a single cluster contains all the observations.





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Discriminant Analysis (DA)

A statistical method known as discriminant analysis is used to analyse data when the dependent variable is categorical, and the independent variables are interval variables. We can estimate the discriminant function coefficients after determining the analysis sample. There are two major strategies available. To include all the predictors at once in the direct method, the discriminant function must be estimated, regardless of how efficient a variable's ability to discriminate is. The stepwise method is an alternate strategy. Based on their ability to group discrimination, the predictor variables are entered successively in stepwise discriminant analysis (Huberty, 1975).

Factor analysis (FA)

We presume that the variable is derived from a factor when performing a factor analysis (Fruchter, 1954). Allowing for p variables and $m < p$ factors represented by f_1, f_2, \dots, f_m . Then for a variable $y_i, i = 1, 2, \dots, p$, the model is

$$y_1 - \mu_1 = \lambda_{11}f_1 + \lambda_{12}f_2 + \dots + \lambda_{1m}f_m + \varepsilon_1$$

$$y_2 - \mu_2 = \lambda_{21}f_1 + \lambda_{22}f_2 + \dots + \lambda_{2m}f_m + \varepsilon_2$$

$$y_p - \mu_p = \lambda_{p1}f_1 + \lambda_{p2}f_2 + \dots + \lambda_{pm}f_m + \varepsilon_p$$

Factor λ_{ij} indicates the factor j importance to variable i . The unknown factors are considered as random variables, and we have $E(fi) = 0, \text{Var}(fi) = 1, \text{Cov}(fi, fj) = 0$ assuming the factors to be independent. Each variable $y_i, i = 1, 2, \dots, p$ variance is

$$\text{Var}(y_i) = \lambda_{i1}^2 + \lambda_{i2}^2 + \dots + \lambda_{im}^2 + \psi_i$$

RESULTS AND DISCUSSIONS

By methodically examining the historical values of air quality variables seasonally, HACA was utilised to categorise the air quality stations based on their homogeneity degree. Based on the F-value of air pollution and metrological variables in ANOVA table of K-means cluster algorithm we choose 3 clusters. Higher F more is a strength of variables in contribution. It was observed from Table 1 that the contributions of all the variables were not significant. Silhouette distance is the separation distance between the resulting clusters. The higher the Silhouette value between the neighbouring clusters indicates the clusters are well apart from each other and are distinguished. Based on maximum silhouette distance three clusters were identified to perform HACA. Application of HACA resulted in three clusters formed using Ward's method employing city Block distance. Generated three clusters from the clustering method were depicted in Figure 1 as Dendrogram. Following the clustering, three clusters were created in a very convincing manner, like the stations in the homogeneity traits are present in all these groups. The commonly perceived seasons have diversified study areas into three significant groups of regions, which are the low pollution levels (LPL), moderate pollution levels (MPL), and high pollution levels (HPL). Cluster 1 corresponds to the LPL due to less air pollution with the range between 0 to 50 of AQI. Cluster 2 corresponds to the MPL with the range between 51 to 100 of AQI. Cluster 3 corresponds to the HPL with the range between 101 to 250 of AQI with reference to Indian standard of AQI. Season wise cluster consolidation of study areas was presented in Table 2.

After HACA clustered the monitoring stations into three significant clusters, DA was applied to analyze the air quality data. The seasonal variation among the various regions can be explored by stepwise DA. The three significant groups (LPL, MPL and HPL) were treated as dependent variables, whereas the air quality parameters were considered independent variables. The accuracy of spatial variation of sampling stations concerning seasonal dependent pollution level is 84.6% in LPL, 83.3% in MPL and 100% in HPL. The Box's M test resulted in a significant level (p) of 0.704, $p > 0.01$ signifies that multivariate normality is met.



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Wilk's lambda measures how well each level of an independent variable contributes to the model in discriminant analysis. The range of the scale is 0 to 1, with 1 denoting complete lack of differentiation (Brown and Wicker, 2000). Smaller values of Lambda in Table 3 emphasize the significant difference of each level. Chi-Square statistic F with significance level < 0.01 concludes that the corresponding function explains the group membership of each level well. To assess the seasonal patterns among the investigated air quality indicators and to identify the factors that influence each of the detected levels, FA with varimax rotation was applied to the air quality data set (LPL, MPL and HPL) and the results are depicted in Table 4. Four Principal components were extracted for LPL and MPL and five for HPL levels with Eigen values greater than 1.

The consolidation of contributed air pollutants and metrological variables to the varimax-rotated principal components are shown in Table 5. From Table 2 it was identified that the pollution levels at study sites are more in winter than compared to other seasons. Same was reported by Weif Ding and Yaqian Zhu (2022). As LPL and MPL are less hazardous to the human health, the present study focussed on study areas which falls in HPL during winter and the air pollutants responsible for it. Our research reveals a few unusual facts, such as the fact that Amaravati, Bollaram, Pashmylaram, and Patencheru fall within the HPL range and contribute 31.3% of the total air pollution during the winter due to the pollutant's NO_x , NO_2 and CO. In Amaravati cotton and chilli are the main crops grown during kharif seasons, resulting in stubble burning and consumption of fossil fuels during winter months thereby increasing pollution. However, at Bollaram, Pashmylaram and Patencheru falls in HPL due chemical smog emitted by pharmaceutical industries which does not get dispersed during winter. Vasudha and Venkateswara Rao, 2022 reported that the above said areas are highly polluted due to the presence of pharmaceutical industries. NO_2 , NO_x , Benzene contributed 28.2% to the total variability in HCU, Bollaram, Pashmylaram and Patencheru due to vehicular pollution in MPL. Similar results were stated by Khandar and Kosankar, 2014.

Tirupati and Vishakhapatnam were found in the LPL during the winter unlike other stations whose pollution levels were found to be high. This may have been caused by a temperature inversion layer. A temperature inversion layer is a region where the air above and below is at different temperatures. From a few hundred feet above the earth to thousands of feet up in the atmosphere, inversion layers can form. High AQI readings result from the confinement of pollutants to a shallow vertical layer caused by a severe inversion, while a weak inversion will lead to lower AQI levels.

CONCLUSIONS

This study aims to serve as an introduction to the problem of air pollution in Andhra Pradesh and Telangana highlighting the numerous roles played by the sources of emissions, the states' seasonal and geographical characteristics, and their interconnections. The results were more clearly established the impact of seasons through the effective use of statistical tools and mapping software. All the pollutants reach their peak concentration during the winter, according to a study that looked at the seasonal variation of air pollution.

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Table1: F-values of all air pollution and metrological variables using ANOVA

| ANOVA | | | | | | |
|---------|-------------|----|-------------|----|--------|------|
| | Cluster | | Error | | F | Sig. |
| | Mean Square | df | Mean Square | df | | |
| PM2.5 | 1659.372 | 2 | 296.245 | 27 | 5.601 | .009 |
| NO2 | 734.096 | 2 | 154.440 | 27 | 4.753 | .017 |
| NOX | 418.832 | 2 | 83.159 | 27 | 5.036 | .014 |
| SO2 | .583 | 2 | 26.072 | 27 | .022 | .978 |
| CO | .052 | 2 | .029 | 27 | 1.760 | .191 |
| O3 | 541.649 | 2 | 199.578 | 27 | 2.714 | .084 |
| BENZENE | 1.963 | 2 | 3.326 | 27 | .590 | .561 |
| RH | 389.081 | 2 | 87.567 | 27 | 4.443 | .021 |
| WS | 2.729 | 2 | 2.112 | 27 | 1.292 | .291 |
| WD | 13274.466 | 2 | 854.161 | 27 | 15.541 | .000 |
| SR | 22201.719 | 2 | 776.284 | 27 | 28.600 | .000 |
| AT | 55.618 | 2 | 8.976 | 27 | 6.196 | .006 |





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Table2: Season-wise clusters of sampling stations depending on AQI levels

| Seasons/Levels | LPL | MPL | HPL |
|----------------|---|---|---|
| Summer | Amaravati, Tirupati, ZOO | Bollaram, HCU, Patencheru, Pashmylaram, Rajahmundry, Sanathnagar, Visakhapatnam | |
| Monsoon | Amaravati, Bollaram, HCU, Patencheru, Tirupati, Sanathnagar, Visakhapatnam, ZOO | Pashmylaram, Rajahmundry | |
| Winter | Tirupati, Visakhapatnam | Rajahmundry | Amaravati, Bollaram, HCU, Patencheru, Pashmylaram, Sanathnagar, ZOO |

Table3: Wilk’s Lambda and Chi-square statistic F of three AQI levels

| Wilk's Lambda | | | | | | | | | |
|---------------|---------------------|--------|-----|-----|-----|-----------|-----|--------|------|
| Step | Number of Variables | Lambda | df1 | df2 | df3 | Exact F | | | |
| | | | | | | Statistic | df1 | df2 | Sig. |
| 1 | LPL | .429 | 1 | 2 | 27 | 17.983 | 2 | 27.000 | .000 |
| 2 | MPL | .158 | 2 | 2 | 27 | 19.662 | 4 | 52.000 | .000 |
| 3 | HPL | .116 | 3 | 2 | 27 | 16.097 | 6 | 50.000 | .000 |

Table4: Loadings of variables on varimax rotated principal components of the air and metrological data from LPL, MPL and HPL of Andhra Pradesh and Telangana

| | LPL | | | | MPL | | | | HPL | | | | |
|-----------------|-------------|-------------|-------------|-------------|-------------|--------------|--------------|-------------|-------------|-------------|-------------|-------------|-------------|
| | F1 | F2 | F3 | F4 | F1 | F2 | F3 | F4 | F1 | F2 | F3 | F4 | F5 |
| PM25 | .744 | .564 | .004 | .075 | .459 | .692 | -.395 | .003 | .702 | .125 | -.247 | -.248 | .017 |
| NO2 | .914 | .239 | -.117 | -.183 | .956 | -.026 | -.003 | -.156 | .889 | -.322 | .043 | .101 | -.274 |
| NOX | .825 | .255 | -.109 | -.436 | .958 | .085 | -.003 | -.003 | .923 | -.218 | .179 | .050 | -.226 |
| SO2 | .018 | .236 | .869 | -.039 | .548 | .459 | .053 | .312 | -.174 | .834 | .292 | -.008 | .343 |
| CO | .381 | .836 | .231 | -.016 | .183 | .391 | -.186 | .841 | .972 | .167 | -.126 | .097 | -.049 |
| O3 | .898 | .013 | .159 | .092 | -.283 | .912 | .133 | .125 | .035 | .028 | -.152 | .982 | .005 |
| BENZENE | .386 | .843 | .011 | -.028 | .884 | -.067 | .430 | -.003 | .203 | .004 | .921 | -.269 | .015 |
| RH | -.574 | -.001 | .218 | -.745 | -.309 | -.314 | -.133 | .791 | -.042 | .823 | -.357 | .355 | .174 |
| WS | -.209 | -.058 | .901 | .289 | -.049 | -.178 | -.865 | -.181 | -.206 | .080 | -.123 | -.006 | .957 |
| WD | -.295 | .191 | -.607 | .428 | -.117 | -.968 | -.081 | .111 | .671 | .119 | .443 | -.460 | .352 |
| SR | -.207 | .707 | -.103 | .389 | .016 | -.003 | .420 | .597 | -.299 | -.180 | .897 | .033 | -.158 |
| AT | -.263 | .177 | .226 | .881 | .191 | -.105 | .891 | -.303 | .128 | .840 | -.213 | -.279 | -.351 |
| Variability (%) | 31.2 | 20.6 | 17.9 | 16.6 | 28.2 | 23 | 17.8 | 16.4 | 31.3 | 19.4 | 18.8 | 12.8 | 12.2 |
| Cumulative (%) | 31.2 | 51.8 | 69.7 | 86.3 | 28.2 | 51.2 | 69 | 85.4 | 31.3 | 50.7 | 69.5 | 82.3 | 94.5 |





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Table5: Consolidation of contributed air pollutants and metrological variables to the varimax-rotated principal components

| Factors | LPL | MPL | HPL |
|---------|--|---|--|
| F1 | PM _{2.5} , NO ₂ , NO _x , O ₃ | NO ₂ , NO _x , Benzene | NO ₂ , NO _x , CO |
| F2 | CO, Benzene | O ₃ , WD | SO ₂ , RH, AT |
| F3 | SO ₂ , WS | WS, AT | Benzene, SR |
| F4 | AT | CO, RH | O ₃ |
| F5 | | | WS |

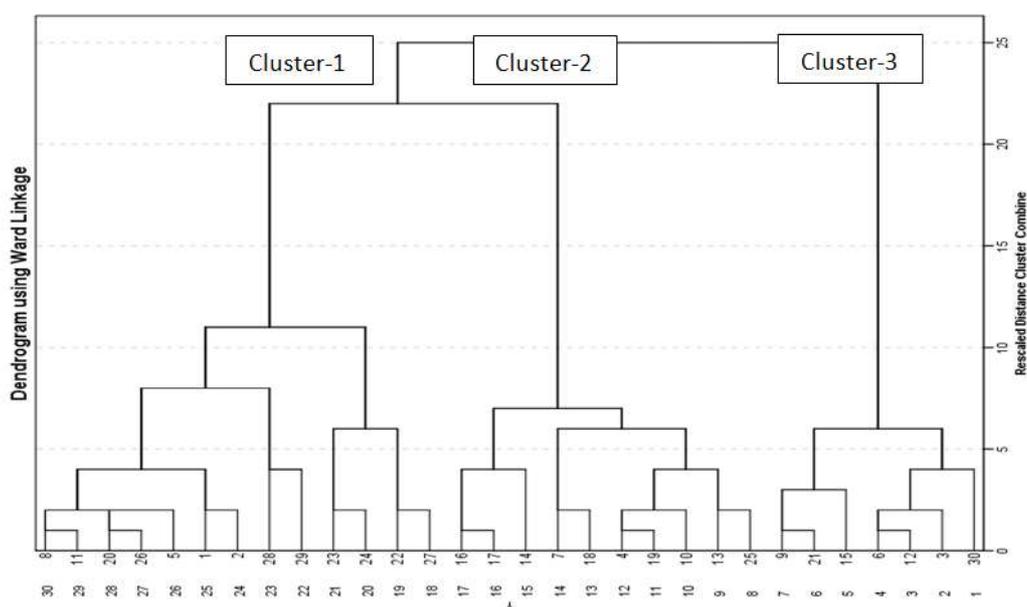


Figure 1: Dendrogram showing season-wise clusters of sampling stations





Formulation of Low Weight Floating Micro-Spheres of Mebeverine Intended as a Treatment to Functional Abdominal Pain

G.Chandra Sekhara Rao^{1*}, V.Sai Sirisha² and Y.Srinivasa Rao³

¹Professor, Vignan Institute of Pharmaceutical Technology, Beside VSEZ, Duvvada, Visakhapatnam-530049, Andhra Pradesh, India.

²PG Student, Vignan Institute of Pharmaceutical Technology, Beside VSEZ, Duvvada, Visakhapatnam-530049, Andhra Pradesh, India.

³Principal, Vignan Institute of Pharmaceutical Technology, Beside VSEZ, Duvvada, Visakhapatnam-530049, Andhra Pradesh, India.

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*Address for Correspondence

G.Chandra Sekhara Rao

Professor,

Vignan Institute of Pharmaceutical Technology,

Beside VSEZ, Duvvada,

Visakhapatnam-530049,

Andhra Pradesh, India.

E.Mail: gonuguntac2@gmail.com



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ABSTRACT

Mebeverine hydrochloride is an antispasmodic drug with a direct action up on the smooth muscles of the human stomach. The current study is to develop a new Mebeverine micro-spheres formulation; which is intended to be used in conditions like functional abdominal pain in children. The current study is to prepare as well as to evaluate oil-entrapped floating microspheres of Mebeverine hydrochloride to favorably improve the gastric residence nature of the drug in the stomach. The micro-spheres were formulated by ionotropic-gelation method using mahuva oil and various polymers like hydroxy propyl methyl cellulose and sodium starch glycolate of varying concentrations. A total of twelve formulations were designed and the respective micro-spheres were prepared in the laboratory. The developed micro-spheres were thoroughly tested for floating properties, flow property and drug entrapment efficiency. The shape and surface morphology were evaluated with the aid of optical microscopy method. The micro-spheres were also subjected to *in-vitro* drug release studies. In the case of formulation F9 the drug release was extended upto 12 hours and floating of micro-spheres remained for more than 12 hrs. Mahuva oil was found to be a suitable low density material in the formulation of floating micro-spheres.

Keywords: Functional abdominal pain, Mebeverine, hydroxy propyl methyl cellulose, Sodium starch glycolate, Mahuva oil, floating micro-spheres.



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INTRODUCTION

Floating drug delivery systems (FDDS) or the hydro dynamically balanced systems are employed to improve the gastric residence time (GRT) of drugs in the body so as to avoid frequent drug administration [1-4]. The dosing interval is prolonged which enhances the patient compliance in comparison to other extended release dosage forms. Gastric emptying is an extremely uncertain process where the gastro retentive drug delivery systems play a crucial role in prolonging and controlling the emptying time [5]. The floating drug delivery systems includes two types of dosage forms i.e., single unit dosage forms and multiple unit dosage forms. Though the single unit sustained release dosage forms are most popular, they have a drawback of 'all/nothing' emptying process from the stomach, leading to high inter-individual variability of drug absorption [6-7]. In order to overcome such problems multiple unit dosage forms are developed to decrease the variability of subjects due to the presence of individual differences among them and to reduce the dose dumping probability [8-9]. These multiple unit dosage forms get widely distributed uniformly, leading to long lasting and uniform drug release from the dosage form. It also reduces the inter subject variability among subjects and can also avoid local irritation [10]. Based on various principles, many multi-unit systems have been developed like porous carrier based micro-particles, hollow micro-spheres or micro-balloons, oil entrapped gel micro-spheres [11].

Alginate microspheres are made up of residues of L-glucuronic acid and d-mannuronic acid which are linear copolymers, biodegradable and non-toxic. They are widely used in pharmaceutical as well as in food industries. In the presence of calcium ions, calcium alginate is formed rapidly by gelation reaction of alginic acid. Floating beads from the alginate beads were developed as early as 1980 in order to prolong the GRT [12]. Alginate micro-spheres are widely used as a unique way for preparing the FDDS in the recent years [13]. The ideal size of the micro-spheres are less than 300mm where the drug is dispersed or dissolved through particle matrix having ability to attain controlled release of drugs. The solubility decreases in case of sparingly-soluble and generally insoluble drugs; thereby the dissolution time becomes less adequate making transit time significant affecting the drug absorption. In such conditions floating micro-spheres provides continuous, controlled drug release at the absorption site. In case of drugs having small half-life, flip-flop pharmacokinetics is observed due to effective drug concentration in systemic circulation because of sustained release of drug into GIT. So as to prevent such conditions in case of short half-life drugs, micro-spheres exhibit a good therapeutic effect [14].

Mebeverine HCl is a musculotropic agent which acts as smooth muscle relaxant and bowel regulator. It treats the abdominal pain induced due to smooth muscle spasms and various intestinal disorders [15]. There are several different types of abdominal pain like functional dyspepsia associated with nausea and vomiting, diarrhea, constipation [16]. In the present work it is used to treat functional abdominal pain in children which is more common in children and adolescents seen by gastroenterologists. It has shorter biological half-life of 2.5 hours with plasma protein binding of 75% attaining peak plasma concentration within 1-3 hours of administration [17]. The current work is aimed to formulate and evaluate oil-entrapped floating micro-spheres of Mebeverine to improve the gastric residence time of the drug. The micro-spheres are prepared by ionotropic-gelation method using mahuva oil. Till now no work was reported employing mahuva oil as a low density material in the formulation of floating micro-spheres.

MATERIALS AND METHODS

Mebeverine HCl was obtained from Yarrow Chem Products, Mumbai. Hydroxy propyl methyl cellulose (HPMC-K100), Sodium starch glycolate (SSG) were obtained as a gift samples from Lee Pharma Pvt. Ltd. Sodium alginate was obtained from BMR chemicals, Hyderabad. Calcium chloride and hydrochloric acid were procured from Rankem, Gurgaon. Mahuva oil was procured from the local market. All the chemicals employed in the present research work were meeting analytical grade.





Preparation of floating micro-spheres

For the preparation of sodium alginate micro-spheres, the required quantity of sodium alginate and Mebeverine HCl were dispersed in sufficient quantity of distilled water with constant stirring. To this the polymer HPMC K100 was added and mixed thoroughly to get homogeneous mucilage. Mahuwa oil was added drop wise to this homogenous mixture and mixed well. Meanwhile 10% w/v calcium chloride solution was separately prepared and taken into a beaker which acts as gelation medium. Now the uniformly mixed alginate mucilage was removed using an 18 G syringe needle into the calcium chloride solution drop wise. To attain round shaped micro-spheres the distance between the needle and curing solution was maintained at 10cm distance. The mucilage dispersed into the gelation medium as small globules were kept still for 30 min to attain micro-spheres. Later, they were filtered, washed thoroughly and oven dried at temperature of 50°C for 4 hours [18].

EVALUATION OF FLOATING MICROSPHERES

Fourier Transform Infrared Spectroscopic (FTIR) Studies

The infrared spectroscopy was used for the identification of incompatibility between pure drug and the polymers used in this work. The FT-IR technique was followed by the KBr pellet method on the drug and polymer. The microspheres were compressed under the hydraulic press in order to form a compact and transparent pellet. Then the pellet was scanned in the spectrophotometer and the obtained peaks were identified.

Micrometric properties

To determine the flow property of the floating micro-spheres, various micrometric properties like bulk-density, tapped-density, angle of repose; compressibility-index and Hausner's ratio were studied.

Particle size

The size of the floating micro-spheres of Mebeverine HCl was usually evaluated by using an optical Debro DMM 11 microscope along with an eyepiece micrometer calibrated earlier. The average particle size was determined by using the following equation:

$$d_{\text{mean}} = \frac{\sum nd}{\sum n}$$

Where, n = No. of microspheres counted,

d = Average/mean particle size range

Percentage yield

The percentage yield of the floating micro-spheres was determined by the actual weight of the micro-spheres to the sum total weight of non-volatile components used in the preparation of floating micro-spheres [19].

Percentage yield = (Actual weight of the product/ Total weight of drug and excipients) × 100

Drug entrapment efficiency (DEE)

Micro-spheres equivalent to 135 mg were taken in a mortar and dissolved in 10 ml of methanol. Later the prepared samples were diluted up to the required volume and analyzed by using UV spectrophotometer at a wavelength of 263 nm. The drug entrapment efficiency was then calculated by using the following simple equation:

DEE = (Practical drug content/Theoretical drug content) × 100

Swelling property

This phenomenon determines the swelling property of the employed polymers. The micro-spheres obtained were taken and subjected to drying at 50° C overnight. The dry micro-spheres were weighed and the value was noted. Now these dry micro-spheres were introduced into 0.1N HCl until they begin to float. Now the floating micro-spheres were separated, collected and weighed [20]. Then the swelling property was calculated by the following equation:



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Swelling property = (Weight of wet micro-spheres/Weight of dry micro-spheres) ×100

Floating properties

Floating lag time is the interval of time between the addition of micro-spheres to the medium and their buoyancy in the upper one-third of the beaker. The micro-spheres were taken in a beaker containing 100ml of 0.1N HCl solution and observed for the lag time of floating. The time elapsed for the micro-spheres to rise to the surface was noted from the stopwatch. The total time for which the micro-spheres float is known as total floating time^[21].

Drug release studies

Mebeverine HCl microspheres were dispersed in 900 ml of 0.1 N HCl for 12 hours at a stirring speed of 100 rpm and temperature of 37± 0.5°C using dissolution apparatus of USP type I basket technique. Accurately weighed quantities of the micro-spheres equivalent to 135mg of drug were added into the dissolution beakers for every formulation. At particular time intervals the samples of volume of 5ml each were collected from the beakers with the replacement of equal amounts of the blank solution. The samples collected were further diluted with the buffer and were measured for absorbance at a wavelength of 263 nm. The % of drug release at various time points was calculated.

RESULTS AND DISCUSSION

FTIR of pure drug and the optimized formulation were shown in figure 1. Mebeverine hydrochloride and its formulation exhibited characteristic peaks at 1459 cm⁻¹, 1326 cm⁻¹, 1096 cm⁻¹ and 729 cm⁻¹ due to the presence of methyl, nitro, C=O and alkene groups respectively. This indicates that the drug is very much compatible with the formulation components. In the present study 12 formulations were designed using 12%v/v mahua oil as a low density floating material. The micro-spheres obtained were found to be small and almost spherical in shape. The results of micrometric properties are shown in table 2. The obtained results showed excellent flow property. Formulation F9 showed the highest yield. All these results were represented in table 2. The swelling properties of all the formulations were also studied. The first three formulations did not show any significant swelling, which might be due to lack of polymers in those formulations. The results of floating time were presented in table 2. F1, F2 and F3 formulations did not float in the medium, which might be due to the absence of swelling polymers in these micro-spheres. From this study, it was found that in addition to the oil, swellable polymer is also required for the micro-spheres to float. For the subsequent formulations the floating lag time ranges from 30 seconds to 248 seconds, whereas the duration of buoyancy was more than 12 hours. Thus incorporation of mahua oil in combination with the polymer showed excellent floating property for the micro-spheres.

Tables 3 and 4, Graphs shown in figure 2 and 3 represent comparative dissolution profiles of F4 to F7 and F8 to F12 in a detailed manner. Formulations from F4 to F7 contain SSG which exhibited faster drug release compared to formulations containing HPMC K 100. Among the formulations containing HPMC at different concentrations, F9 was found to be exhibiting maximum drug release of 99.85% within 12 hours. Hence F9 was found to be the optimized formulation. Coming to the drug release, the prepared formulations followed first order kinetics. Based on the 'n' value (0.45 according to korsmeyer-peppas model) obtained for F9, the drug release mechanism was found to follow fickian diffusion.

CONCLUSION

From the detailed results and discussion, it was found that Mebeverine floating micro-spheres can be prepared employing ionic-gelation method. It can be viewed as novel formulation, having many advantages compared to the traditional immediate release formulations. Mahuva oil was found to be a promising buoyancy inducing agent for the micro-spheres prepared in the present study. The composition of oil and polymer is a critical criterion to be optimized for effective floating and drug release. Formulation with code F9 was found to be the best one among all others studied.



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Table 1: Formulation of Mebeverine floating alginate micro-spheres

| Ingredients | F ₁ | F ₂ | F ₃ | F ₄ | F ₅ | F ₆ | F ₇ | F ₈ | F ₉ | F ₁₀ | F ₁₁ | F ₁₂ |
|----------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-----------------|-----------------|-----------------|
| Mebeverine HCl (gm) | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 | 2.7 |
| Sodium alginate (gm) | 2.7 | 1.85 | 0.95 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 | 1.85 |
| Mahua oil (ml) | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| SSG (gm) | - | - | - | 0.25 | 0.5 | 0.75 | 1 | - | - | - | - | 0.5 |
| HPMC K-100 (gm) | - | - | - | - | - | - | - | 0.25 | 0.5 | 0.75 | 1 | 0.5 |

Table 2: Micrometric properties of various formulations

| Formulations Code | Mean particle size (µm) | Percentage yield | Drug entrapment efficiency | Angle of repose (θ) | Tapped density (g/ml) | Carr's index (%) | Hausner's Ratio | Duration of Buoyancy (hrs) |
|-------------------|-------------------------|------------------|----------------------------|---------------------|-----------------------|------------------|-----------------|----------------------------|
| F ₁ | 500±0.50 | 80.45±0.16 | 78.15%±0.30 | 25.92±0.15 | 0.538 | 13.38 | 1.15 | - |
| F ₂ | 640±0.32 | 84.07±0.28 | 91.28%±0.33 | 24.48±0.04 | 0.564 | 16.61 | 1.2 | - |
| F ₃ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - |
| F ₄ | 500±0.50 | 90.14±0.24 | 75.21%±0.19 | 24.23±0.02 | 0.542 | 18.61 | 1.22 | 9 |
| F ₅ | 610±0.45 | 80.01±0.14 | 73.33 ±0.26 | 24.48±0.04 | 0.526 | 10.6 | 1.18 | 8 |
| F ₆ | 650±0.41 | 88.60±0.10 | 77.15%±0.30 | 24.28±0.04 | 0.634 | 10.57 | 1.13 | 7.5 |
| F ₇ | 689±0.54 | 91.30±0.16 | 86.66%±0.23 | 25.18±0.33 | 0.581 | 9.81 | 1.10 | 6.1 |
| F ₈ | 450±0.41 | 84.04±0.20 | 92.13%±0.10 | 24.48±0.04 | 0.513 | 11.76 | 1.13 | 10 |
| F ₉ | 400±0.28 | 95.35±0.35 | 93.66%±0.30 | 23.93±0.19 | 0.512 | 11.32 | 1.12 | ≥ 12 |
| F ₁₀ | 420±0.52 | 90.13±0.21 | 90.20%±0.12 | 23.31±0.04 | 0.514 | 13.61 | 1.16 | ≥ 12 |
| F ₁₁ | 485±0.15 | 91.01±0.90 | 92.28%±0.33 | 23.93±0.19 | 0.514 | 10.34 | 1.11 | ≥ 12 |
| F ₁₂ | 450±0.18 | 89.03±0.25 | 92.13%±0.10 | 24.72±0.15 | 0.512 | 13.28 | 1.15 | 10 |

Table 3: Cumulative percent released versus time profiles for F4 to F7

| Time (Hrs) | F ₄ | F ₅ | F ₆ | F ₇ |
|------------|----------------|----------------|----------------|----------------|
| 0 | 0 | 0 | 0 | 0 |
| 0.5 | 9.21±0.15 | 12.46±0.23 | 14.59±0.11 | 16.28±0.31 |
| 1 | 19.26±0.26 | 23.77±0.32 | 28.16±0.14 | 30.15±0.17 |
| 2 | 35.2±0.05 | 40.15±0.23 | 44.91±0.22 | 48.71±0.14 |
| 3 | 50.4±0.02 | 59.7±0.54 | 60.94±0.26 | 63.53±0.28 |
| 4 | 63.93±0.28 | 74.8±0.36 | 76.83±0.37 | 78.55±0.15 |
| 5 | 75.88±0.15 | 86.47±0.97 | 87.36±0.27 | 89.13±0.64 |
| 6 | 85.62±0.94 | 93.2±0.43 | 96.21±0.39 | 99.59±0.49 |
| 8 | 92.89±0.34 | 99.81±0.41 | 99.26±0.18 | 100 |
| 10 | 97.63±0.16 | 100 | 100 | 100 |
| 12 | 100 | 100 | 100 | 100 |





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Table 4: Cumulative percent released versus time profiles for F8 to F12

| Time (Hrs) | F ₈ | F ₉ | F ₁₀ | F ₁₁ | F ₁₂ |
|------------|----------------|----------------|-----------------|-----------------|-----------------|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 0.5 | 19.73±0.14 | 21.85±0.36 | 9.43±0.22 | 7.63±0.23 | 18.15±0.21 |
| 1 | 27.96±0.32 | 29.03±0.58 | 18.96±0.28 | 16.35±0.28 | 36.22±0.36 |
| 2 | 37.25±0.87 | 38.54±0.46 | 29.7±0.28 | 25.057±0.52 | 52.42±0.12 |
| 3 | 48.34±0.36 | 49.78±0.61 | 37.34±0.52 | 34.25±0.18 | 61.49±0.44 |
| 4 | 56.98±0.27 | 52.28±0.91 | 45.07±0.29 | 42.26±0.15 | 69.49±0.54 |
| 5 | 65.64±0.84 | 67.13±0.46 | 54.92±0.29 | 49.25±0.23 | 78.49±0.33 |
| 6 | 72.82±0.24 | 75.46±0.96 | 62.63±0.22 | 57.49±0.32 | 86.13±0.12 |
| 8 | 79.91±0.49 | 81.49±0.78 | 68.3±0.341 | 63.71±0.25 | 94.17±0.21 |
| 10 | 99.71±0.54 | 88.99±0.84 | 74.61±0.25 | 68.96±0.23 | 98.99±0.28 |
| 12 | 100 | 99.85±0.99 | 88.42±0.25 | 78.92±0.23 | 100 |

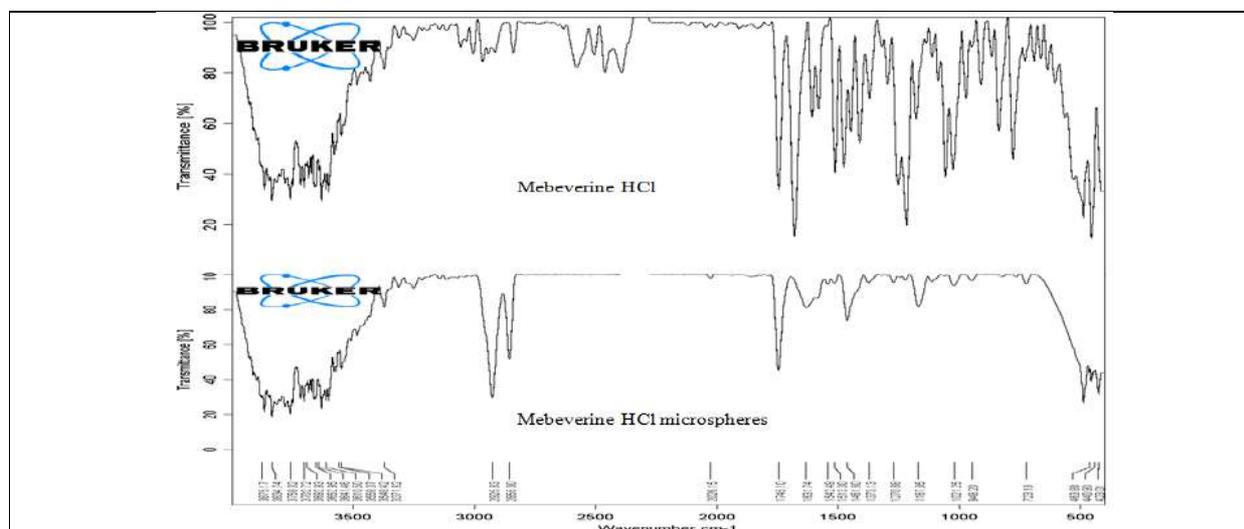


Figure 1: FTIR of Mebeverine hydrochloride pure drug and micro-spheres containing it

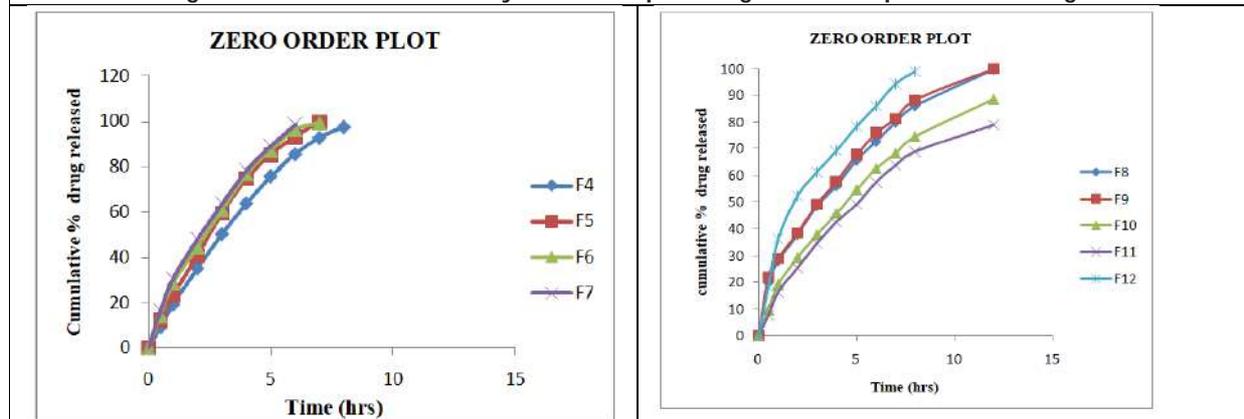


Figure 2: Drug release profiles of formulations F4 to F7

Figure 3: Drug release profiles of formulations F8 to F12





Question Quality Assessment in Online Diagnostic Examinations

Bharathi.B^{1*}, Saranya.S², G.Sneha³, Shrinithi Radhakrishnan³ and Shrisathvika V.A³

¹Associate Professor, Department of Computer Science and Engineering, Sri Siva Subramaniya Nadar College of Engineering, Kalavakkam, Chennai - 603110, Tamil Nadu, India.

²Research Scholar, Department of Computer Science and Engineering, Sri Siva Subramaniya Nadar College of Engineering, Kalavakkam, Chennai - 603110, Tamil Nadu, India.

³Undergraduate Student, Department of Computer Science and Engineering, Sri Siva Subramaniya Nadar College of Engineering, Kalavakkam, Chennai-603110, Tamil Nadu, India.

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*Address for Correspondence

Bharathi.B

Associate Professor,

Department of Computer Science and Engineering,
Sri Siva Subramaniya Nadar College of Engineering,
Kalavakkam, Chennai - 603110, Tamil Nadu, India.

E.Mail: bharathib@ssn.edu.in



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ABSTRACT

Education nowadays has become highly dependent on digital technologies, changing the way students learn across the world. Diagnostic questions do play a crucial role in this change. With the help of the answers that the students give to these questions, we can discern favorable about the exact nature of misconceptions that the students may hold. Students' answers for these diagnostic questions when analyzed, can help in developing and formulating learning curriculum recommendations and play a vital role in understanding students' ability and how they learn. Thus it is really crucial that the diagnostic questions are of good quality in revealing students' misconceptions. Manually assessing huge number of such diagnostic questions in real time is not pragmatic. Thus in this paper, with the help of real world students' answer records for multiple choice questions provided by an e-learning platform, we will assess the questions' quality using discrimination and difficulty metrics. Using these metrics as ground truth, we train a regressor capable of assessing newly generated questions using only the text of the question and text of possible choices. In our experiments we compared performance of several trained regressors and found that random forest gives the best results with anrmse of 0.063 and 0.023 for difficulty prediction and for discrimination prediction respectively

Keywords: Difficulty and Discrimination index, Random Forest Regressor, RMSE, Quality assessment





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INTRODUCTION

Background and Impact

Online education system has become increasingly ubiquitous and affordable which in turn makes high quality education available to a broader audience across the world. These platforms educate a lot of students with their interactive instructional videos, reading course materials, and useful discussions with teachers and peers in learning forums that are instrumental in giving the student a wholesome learning environment. In order to check if and how much a student has really understood a concept, many of these platforms include an assessment. By help of the data collected by these assessments, we can extract appropriate educational information such as how students have been learning, and thereby suitably recommend applicable learning interventions to improve their learning outcomes. However, how good the derived insights are is largely dependent on the quality of the questions asked in these assessments.

It is to be noted that in the past few years, several online educational platforms have started crowd-sourcing a great deal of questions and exercises. The biggest challenge with these huge volumes of crowd-sourced questions is deciding what can be used and what cannot which in turn differs with respective to each student. The first crucial step is to primarily understand the quality and difficulty levels of the questions. This would help us in assessing the questions before being used to evaluate students. Assessment of questions is either manually done by human experts or it is pretested with students (introducing a long delay in question generation). Both these methods are quite challenging and time-consuming. Thus in this proposed work, we look at the text of the question and the text of the possible choices to assess newly generated multiple-choice questions.

Problem Statement

The aim of this research work is to estimate the quality of a mathematical multiple-choice question, given its text and the possible choices. In this proposed work, given students' answer records for multiple choice questions, we will focus on the evaluation of the questions' quality and difficulty. There are many challenges involved in this work. Firstly, handling huge online educational data is difficult; both the number of questions and the number of students are extremely large. Secondly, the severe missingness existing in the data poses an obstacle as each student could only answer quite a tiny fraction of all available questions. Lastly, designing objectives to quantify and thus derive desired insights such as question quality and difficulty should be handled too. Another issue is the cold start problem. As we already mentioned before pretesting a new question item leads to a long delay between question creation and being able to use it in tests and assessments, and this would also lead to an increase in development costs. An alternative solution to this problem would be to set latent trait labels manually by human experts as this method facilitates the prompt usage of newly created questions in assessments, but this is highly subjective and thus introduces a high uncertainty in the question evaluation. Therefore, to deal with this cold start problem our model should also be able to carry out an initial evaluation of newly created questions for which there are no prior student responses so that it can be immediately used in tests and decrease the number of questions that have to be discarded after pretesting because of poor quality.

Related work

Large-Scale Educational Question Analysis with Partial Variational Autoencoders [3] is one of the very few papers which has dealt with real-world data in the multiple choice questions' difficulty assessment. The framework that they have developed in this paper combines partial variational auto-encoder (p-VAE) which effectively processes partially observed, large-scale educational datasets, and formulates a strategy for automatically producing a suite of meaningful insights about multiple-choice questions. This paper describes in detail about imputing missing values in large-scale datasets using partial Variational Auto Encoders. With reference to this paper, we have used partial Variational Autoencoders (p-VAEs) to deal with the extreme sparsity of our data. However, this paper does not deal with the assessment of newly generated questions.



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Past research has mostly focused on estimating the difficulty of open-ended questions. In [1, 6], they addressed the problem of estimating question difficulty in Community Question Answering(CQA) services. They have developed a regularized competition model for question difficulty estimation in community question-answering services. With their proposed methodology, their analysis shows that the text of question descriptions reflects the question difficulty i.e, a question's difficulty level primarily relies on the text of the question.

However, these questions did not require expert knowledge as they were generic in nature. Under Some related works that have a closer problem statement to ours, The authors of [2] primarily uses NLP to estimate the difficulty of question items. This paper involves predicting the difficulty of multiple-choice questions in a high-stakes medical exam wherein they have presented an approach for predicting the construct-relevant difficulty of multiple-choice questions. In order to do this they developed three features namely: embeddings like ELMo and Word2Vec, linguistic features, and IR features for estimating the difficulty of question items. This method is completely based on features and does not require any responses to the questions. Thus a feature-based difficulty estimation is implemented.

In L. Benedetto, A. Cappelli, R. Turrin, and P. Cremonesi (2020) R2DE: an NLP approach to estimate Item Response Theory (IRT) parameters of newly generated questions [4, 5], the authors use item response features to evaluate the discrimination and difficulty of multiple choice questions with the help of text of the questions and of the given possible choices. In specific, this model quantifies discrimination and difficulty of question items as defined in Item Response Theory. However in all these works, they haven't dealt with large scale real-time data like we do here and some haven't done discrimination estimation nor considered newly generated questions. The authors of [17], explain a cognitive diagnosis model based on multi-level attribute score is designed to model students' resource mastery level according to existing answers and the relevance of knowledge points. Stochastic pooling network is used in [18]. The work done in all the above mentioned papers is summarized in Table 1.

Proposed system

Architectural Design

In reference to the architecture depicted in Fig. 1, we start with the student responses data collected from the Diagnostic Question Challenge of NeurIPS2020 which is provided by the online learning platform Eedi. Then we formulate a matrix of student responses with rows of given questions and columns of students who have answered it. Respective entries represent if the student's answer to that question is correct(1) or not(0), but as only 30% of the entries are observed, we perform matrix completion by passing it to a partial Variational Auto Encoders (p-VAEs). With the predicted student responses, we perform Item analysis Process to derive difficulty and discrimination index. Now to deal with newly generated questions, these estimated indices are the target variables for the next set of dataset along with text of each question and their possible choices. The questions dataset is split in 80:20 ratio for training and testing the regression model. The trained regression model will now give us the difficulty and discrimination indices of questions with no prior student responses.

The proposed system is composed of the following modules, which are implemented in a sequential manner for finding the quality metrics of a question.

- Data Collection
- Data matrix formulation
- Student response prediction using p-VAE
- Item analysis process
- Regressor model for question quality assessment

Data Collection

The first step is data collection where we use the dataset which was available online. The dataset used for the proposed work was provided as part of the Diagnostic Question Challenge of NeurIPS2020, by an online education platform Eedi, used in thousands of schools across the world. The data consists extensive student responses to



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multiple-choice questions with four answer choices focusing on mathematics, dating from 2018 September to 2020 May. This educational platform caters to students roughly aged between 7 and 18 years old who would be in primary to high school. It is to be noted that most of the questions in this platform are crowd sourced. Hence our data is composed of a set of question ids, answer ids and a student's response to a given question. The records primarily consist of the answers given by students, whether it is correct or not and the correct choice to the question.

Data Matrix Formulation

With the data we have, it is observed that only a small subset of the questions are answered by a student since different set of students respond to different questions, thereby making the available data partially observed. We are handling this sparsity using a model that predicts the unobserved data with the help of matrix completion or missing data imputation. The data considered for constructing the boolean matrix are 'QuestionId', 'UserId' and 'IsCorrect' field, which represents whether or not a student with an id 'i' has answered a question with id 'j' correctly. Thus the 'IsCorrect' field contains a Boolean value of 0 or 1, where '1' indicates a correct response and '0' indicates an incorrect response. With this inference, a data matrix X of size N by M is generated, where M would be the total number of questions and N would be the total number of students. Each entry x_{ij} is a binary value which represents the 'IsCorrect' value. The matrix is observed to be partially observed. Hence, the missing entries are predicted as accurately as possible in a probabilistic manner. A data matrix is constructed to ease the handling of data which has to be fed into partial Variational Auto encoders for missing value imputation. The data matrix generated can be visualised in Fig. 2.

Student response prediction

From Fig. 2, it is clear that the data contains missing responses. i.e, all student responses are not recorded for a given question, which makes it difficult to accurately estimate its quality metrics (difficulty and discrimination indices). Thus these missing entries have to be determined in a probabilistic manner in order to effectively compute the metrics that define the difficulty and discrimination level of a question. Thus the aim is to fill the unobserved data in the matrix. The unobserved part of the matrix is filled by predicting the student responses to the remaining unanswered questions. This is done using partial.

Variational Autoencoders (p-VAEs). Said to be a deep latent variable model, p-VAEs extend traditional VAEs. It is to be noted that VAEs work on the assumption that the data matrix (X) is created from the local latent variables z_i while the p-VAE model uses the fact that x_i is fully factorized, given z_i . Thus, the inferred z_i 's are now used to predict the missing data entries.

Partial Variational auto encoders work by taking the partially observed dataset as an input, identify highly valuable data and using the inferred information, the missing values are imputed. A Markov chain Monte Carlo method is used to perform this imputation with the trained p-VAE model. The following are the steps involved in generating the fully observed data using p-VAE.

1. Let missing values be replaced with random ones in the data.
2. give the following data to trained p-VAE:
 - (a) Sample from the output of the encoder or the latent variable distribution (for generating Z given X).
 - (b) Sample from the output of the decoder or the reconstructed data distribution (for generating X' given Z).
3. Use reconstructed values to replace missing values.
4. Let the observed values' reconstruction error be computed.
5. Stop process if iteration limit was reached or the reconstruction error was below a given threshold.
6. Else go to step 2.



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Accurately predicting students' answers and estimating uncertainty lies at the core of educational data mining and adaptive testing. p-VAE achieves both with state-of-the-art performances which is a necessary prerequisite to the computation involved in the remaining modules for assessing the question quality. Fig.3 depicts the overall model of the partial variational auto encoder that deals with interaction matrices.

Item analysis process

Item analysis is a statistical process[5, 7, 8] that is broadly used to estimate the efficacy of assessment items. The interaction matrix X with the predicted student responses is what help us in this process for determining the quality of a question. Quality of a question is defined using two factors (difficulty and discrimination level).

Item Difficulty

We use difficulty index to estimate difficulty of assessment items[9, 11–13]. This is done by statistically calculating the percentage of students who gave the test that answered the question/item correctly. Difficulty index' value ranges from 0 to 1 wherein values closer to one indicates that the item/question is easy, while values closer to zero indicates that the item/question is difficult.

Item Discrimination

Item Discrimination is used to measure of how well a question/item distinguishes between those with more understanding and skill from those with less[15, 16]. This is done by separating students on basis of their total test score into two groups. The discrimination index is then calculated as the percentage of students in the high score group who answered the question correctly minus the percentage in the low score group who answered correctly. Its' value ranges from -1 to +1.

Regressor model for question quality assessment

The difficulty and discrimination indices obtained from the above step are treated as a separate feature(Targets) of the questions dataset which has to be fed into the regressor to train the model. This method resolves the cold start problem since the difficulty and discrimination indices has to be computed from the text of the question whenever a new question arises. The questions dataset is split in such a way that 80 percent of it is used for training and remaining 20 for testing. Before feeding the questions to the model, the text of questions is concatenated with the text of choices and are processed.

Questions are provided as images in the dataset from which the question text along with the choices are extracted using an Optical Character Recognition(OCR) Tool. The extracted text concatenated with the choices is preprocessed by removing the stop words and a vector is generated. This vector is fed into the regressor for further processing. We have two parallel regressors, one for discrimination index estimation and another for difficulty index estimation. For both the metrics, we test a set of different algorithms and choose the one that performs better. The quality metrics of a question is predicted using a set of four regressors namely,

- Random Forest Regressor
- Linear Regression
- Decision Trees
- Support Vector Machine

We also use a voting ensemble to fit these four regressors. The results produced by these algorithms are analysed in order to select the best algorithm for the proposed problem.





Experimental setup

Dataset

The first step in determining the quality indices of questions is to collect data, which comprises of students' response to questions. The data utilized for our system is collected from the Diagnostic Questions Challenge posted by NeurIPS 2020. This is a real-time data collected from an online educational platform called EEDI, which records students' answers to multiple choice questions, with every question having exactly four choices from which a student has to choose one. The questions considered for quality assessment are from mathematical domain. The dataset is available in two formats i) A csv file containing records of students' response to each question. ii) A set of images representing the actual question text along with the choices. The 80% of the samples are used for training and 20% of the samples are used for testing. The data in csv format gives information on student's response to a question. This csv file contains the following columns:

- QuestionId: Denotes the ID of the question which was answered by a student.
- UserId: Denotes the ID of the student who answered the question.
- AnswerId: Unique identifier for the (QuestionId, UserId) pair.
- IsCorrect: Binary value(0 or 1) to indicate if a student's response to a question is correct or not. Value 1 indicates a correct response and 0 indicates an incorrect response.
- CorrectAnswer: The correct answer option (1,2,3,4) for the given question.
- AnswerValue: Answer given by the student to the multiple-choice question (1,2,3,4).

Our area of interest is 'QuestionId', 'UserId' and 'IsCorrect' field since it provides all the necessary information required to determine the quality metrics.

Table 2 is an illustration of format of the data. Since every student is not likely to answer every question, the constructed data matrix is truly sparse. However, the dataset we use consists of questions with at least 50 student responses. In total, the dataset consists of 948 questions, 4918 students and 1382727 answers. The training dataset hence contains over a million records making it very difficult for manual analysis. Thus we provide a machine learning solution to assess the quality of every mathematical question in the platform with recorded student responses. The following sections describe in detail the modules implemented and the tools/algorithms used for estimating the quality indices of a question.

Missing Student Response Prediction using p-VAE

The partial Variational Auto Encoder model [10] is implemented in python. The model is developed to impute missing values in the data. We split the dataset into train and test set in our code. Random test set entries are used for imputation quality evaluation. The developed code will save the trained model to an output directory. To impute new data (test dataset in this case), we can load the pre-trained model. The code also computes the test RMSE for imputed missing data and the result is printed on the console. Possible Arguments are listed below.

- epochs: number of epochs. value: 3000
- p: upper bound for artificial missingness probability. For example, if set to 0.9, then during each training epoch, the algorithm will randomly choose a probability smaller than 0.9, and randomly drops observations according to this probability. If original dataset already contains missing data, p is set to 0.
- batch size: batch size for training. value: 100
- iteration: iterations (number of mini batches) used per epoch. set to -1 to run the full epoch.
- data dir: Directory containing the dataset.
- output dir: Directory where the trained model will be stored and loaded.

Item Analysis Process

Item Analysis estimates the difficulty and discrimination indices of each question. It is a statistical approach which is defined as follows. Difficulty of the item is a measure of the proportion of students who answered the question correctly. In order to compute the difficulty index, the questions in the dataset are grouped according to the question





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ids and a separate dataframe with these grouped questions is created. From this dataframe, we extract the count of students who answered the question correctly(n_c) and divide this count by the total number of students(n).

$$\text{Difficulty index } (q) = \frac{n_c}{n} \quad (1)$$

n_c =No.of students who gave correct response for the question q

n =Total no. of students

For computing the discrimination index, we classify the entire set of students into two groups based on a criteria named User Correct Rate. For every student, User Correct Rate defines the number of questions correctly answered by the student among all the questions. The users are split into two groups based on the median of the UserCorrectRate value. Students having a score higher than the median value fall under the higher group and those having a lower score than the median value fall under the lower group. For finding the difficulty index, questions are grouped by question ids and for each question, the number of students in the higher and lower group who have answered the question correctly is determined. With this information, the discrimination index is calculated by,

$$\text{Discrimination index}(q) = \frac{n_h - n_l}{n} \quad (2)$$

where,

n_h = No. of students in higher group who answered the question q correctly

n_l = No. of students in lower group who answered the question q correctly

The computed metrics are evaluated using the evaluation toolkit provided in NeurIPS Challenge 2020 which requires ranking the questions based on the computed metrics. The proposed ranking is evaluated against the ranking defined by a set of four manual evaluators and eventually an overall score is assigned. For our model, the overall score was 0.76.

Question Dataset Formulation

As part of the NeurIPS2021 Diagnostic question task, we were provided student response data set to nearly 948 MCQ questions. The question meta data that they provided had the 948 MCQ questions in the form of JPG images. Thus we had to extract the text of question and possible choices from these given images in order to work with them quickly. We use Optical Character Recognition (OCR). The scanned image and text, graphics is changed into bitmap, that is a matrix of black, white dots. Brightness, contrast is adjusted when the image goes through pre-processing. Image is split into zones. The text is broken into lines, words, characters and the software will be able to match characters by comparison and detection algorithms.

This step may not be 100 percent accurate and will require human intervention to correct elements. But this might take considerably less time as was done in our case rather than manually typing out the text by looking at images. In this proposed work for extracting text from the given question images, we used python tesseract. Opencv is used to read the set of available images in the dataset. The image to string function in pytesseract module is used to extract the text from the images. After extracting the text, it is passed to a preprocessing function that preprocesses the text as follows;

We use the Natural Language Toolkit that has libraries, programs for symbolic and statistical NLP. We make use of tokenize, stem and corpus packages in NLTK.

- **Tokenisation:**
Using NLTK tokenize. regexp() module, extraction of tokens from string using RegexpTokenizer() is done.
- **Lemmatization:**
Extracting the root words of all tokens using WordNetLemmatizer(). For example, the word 'squaring' has a root word 'square'.
- **Stop words removal:**





After lemmatization, insignificant tokens (stop words) like prepositions, adjuncts etc. are removed. The question text along with the respective difficulty and discrimination indices obtained from Item Analysis process are written into the csv file.

Regressor Implementation

The questions dataset which contains the text of the question along with the choices are given to a regression model for predicting the quality metrics of a question. The difficulty and discrimination indices are the target values that has to be predicted by the regression model on taking the question as an input. A set of four regression models and a voting ensemble for them have been implemented to test and analyse their performance and select the one which yields better results. These models are implemented using python's sklearn module and the text vectorization is performed using three different vectorizers namely CountVectorizer, TfidfVectorizer and HashingVectorizer. It is to be noted that Random Forest Regression model yielded good results with an RMSE of for difficulty index prediction being 0.067 and that of discrimination index prediction being 0.06. The regression models implemented are:

- Random Forest Regressor
- Linear Regressor
- Decision Trees
- Support Vector Machine

Voting Ensemble

In addition to the regression models, we use a Voting ensemble in order to combine the achieved results. We make use of VotingRegressor class in sklearn.ensemble module to fit the four regressors namely Decision Tree, Support Vector Regressor, Linear Regressor and Random Forest. We then use root mean squared error scoring to decide if voting ensemble gives better results.

Performance analysis

Evaluation metrics

As mentioned earlier, we used four regression models namely are Random Forests (RF), Decision Trees (DT), Support Vector Regression (SVR), and Linear Regression (LR). The performance of these models are evaluated on two metrics, Root Mean Square Error (RMSE) and Mean Absolute Error (MAE). Tables 2,3 and 4 shows the results of all the chosen models. Root Mean Squared Error(RMSE) RMSE measures the error of a model in predicting quantitative data. Root mean squared error (RMSE) is the square root of the mean of the square of all of the errors. It is formally defined as follows:

$$rmse = \sqrt{\frac{1}{n} \sum_{i=1}^n (y_i - x_i)^2} \quad (3)$$

where,

n=No. of observations

y_i =Observed value

x_i =Predicted value

Mean Absolute Error (MAE) Absolute Error is the difference between the observed value and the predicted value. This measure gives an estimate of the amount of error that has occurred during prediction. Mean Absolute Error(MAE) is the average of all absolute errors. The formula is as follows:

$$mae = \left(\frac{1}{n}\right) \sum_{i=1}^n |y_i - x_i| \quad (4)$$

where,

n=No. of observations

y_i =Observed value

x_i =Predicted value



**Bharathi et al.,****Results Comparison**

Table 3 presents the Root Mean Square Error (RMSE) and the Mean Absolute Error (MAE) for difficulty estimation and discrimination estimation on using use Count Vectorizer. Table 4 presents the Root Mean Square Error (RMSE) and the Mean Absolute Error (MAE) for difficulty estimation and discrimination estimation on using use Tfidf Vectorizer. Table 5 presents the Root Mean Square Error (RMSE) and the Mean Absolute Error (MAE) for difficulty estimation and discrimination estimation when on using Hashing Vectorizer.

Thus, it is to be noted that among all regressors including voting ensemble, Random Forest specifically when used with count vectorizer seems to give the best results. In the processed system, the sparse matrix is reduced by using partial variational auto encoder (which are explained in section 3.4. The p-VAE is used to reduce the dimensionality of the data matrix formed, there by complexity of the vectorization is reduced. The validity of the model developed using the performance metrics such as root mean squared error, mean absolute square which are shown in section 5.1. The proposed system can be used to assess the questions quality of any online examinations. The input the given in the format which are explained in section 4.1. If the questions are and responses are given in the mentioned format, the is can be generalizable for any online examination.

CONCLUSION AND FUTURE WORK

In this work, we built a model that could estimate difficulty and discrimination index for newly generated multiple-choice questions by looking at the text of the question and answer choices. This initial evaluation of newly generated MCQs can be later fine-tuned when we get more student responses, which as seen before is much more efficient than pretesting with limited students. This model reached a RMSE of 0.023 for difficulty estimation and a RMSE of 0.063 for discrimination estimation. Future work might include exploration of advanced embedding for question text encoding, adapting the model for time series data and analyzing effects of features like word embeddings and latent semantic analysis on quality index estimation.

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Table 1. Related Work

| Paper | Workdone | Limitations |
|----------------------|--|--|
| Wang, et al [14] | Question difficulty estimation of MCQs in real time data | Does not deal with discrimination indices or newly generated question estimation |
| Huang, et al [6] | Predicting difficulty of reading models with the help of neural models | Does not deal with MCQ's |
| Benedetto, et al [4] | IR features for estimation of difficulty and discrimination from the question text | Does not deal with large real time data |
| Qiu, et al [9] | Estimation of wrongness in MCQ using deep neural networks | Does not deal with discrimination prediction nor considers estimation of newly generated MCQ's |

Table 2. Data Format

| Question Id | User Id | Answer Id | Answer Value | Correct Answer | Is Correct |
|-------------|---------|-----------|--------------|----------------|------------|
| 2045 | 8976 | 4563 | 3 | 3 | 1 |
| 6078 | 2359 | 9879 | 4 | 2 | 0 |
| 9887 | 5643 | 8761 | 3 | 3 | 1 |
| 1342 | 3217 | 4231 | 1 | 4 | 0 |





Table 3. Experiments for choosing model using count vectorizer

| Regression model | Difficulty Estimation | | Discrimination Estimation | |
|------------------|-----------------------|-------|---------------------------|-------|
| | MAE | RMSE | MAE | RMSE |
| RF | 0.017 | 0.023 | 0.044 | 0.06 |
| DT | 0.023 | 0.033 | 0.056 | 0.081 |
| SVR | 0.023 | 0.031 | 0.072 | 0.08 |
| LR | 0.054 | 0.076 | 0.119 | 0.168 |
| Voting | 0.026 | 0.037 | 0.069 | 0.094 |

Table 4. Preliminary experiments for choosing sample model using TfIdf vectorizer

| Regression model | Difficulty Estimation | | Discrimination Estimation | |
|------------------|-----------------------|-------|---------------------------|-------|
| | MAE | RMSE | MAE | RMSE |
| RF | 0.021 | 0.03 | 0.048 | 0.061 |
| DT | 0.025 | 0.038 | 0.057 | 0.083 |
| SVR | 0.021 | 0.029 | 0.075 | 0.085 |
| LR | 0.032 | 0.044 | 0.102 | 0.151 |
| Voting | 0.021 | 0.030 | 0.056 | 0.072 |

Table 5. Preliminary experiments for choosing sample model using hashing vectorizer

| Regression model | Difficulty Estimation | | Discrimination Estimation | |
|------------------|-----------------------|-------|---------------------------|-------|
| | MAE | RMSE | MAE | RMSE |
| RF | 0.021 | 0.029 | 0.064 | 0.076 |
| DT | 0.028 | 0.038 | 0.073 | 0.102 |
| SVR | 0.025 | 0.034 | 0.072 | 0.082 |
| LR | 0.024 | 0.033 | 0.071 | 0.082 |
| Voting | 0.029 | 0.021 | 0.065 | 0.077 |

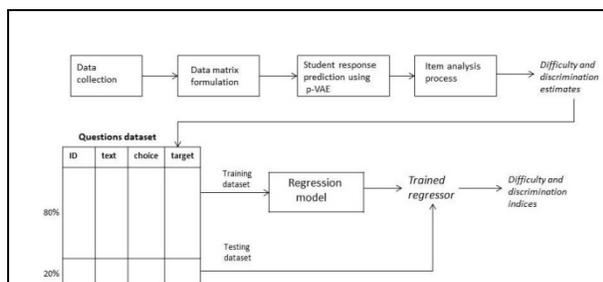


Fig. 1. Detailed design of the proposed system

| QuestionId / StudentId | 1 | 2 | 3 | 4 | 5 |
|------------------------|---|---|---|---|---|
| 101 | 1 | 0 | | 0 | |
| 102 | | 1 | 1 | 0 | |
| 103 | 0 | | 0 | | 1 |

Fig. 2. Boolean Data Matrix representing the correctness of students' response





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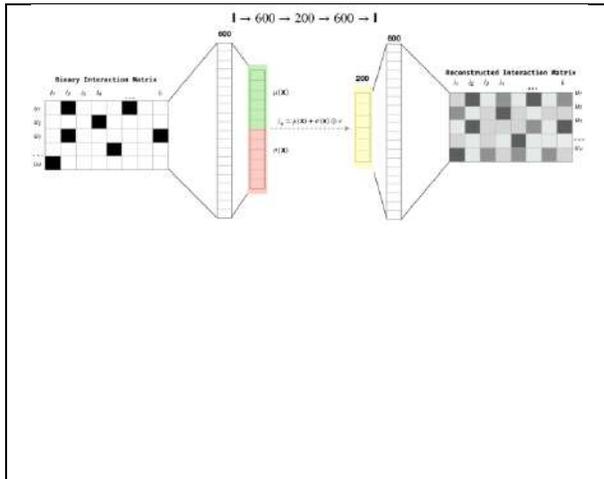


Fig. 3. Partial Variational Auto encoder

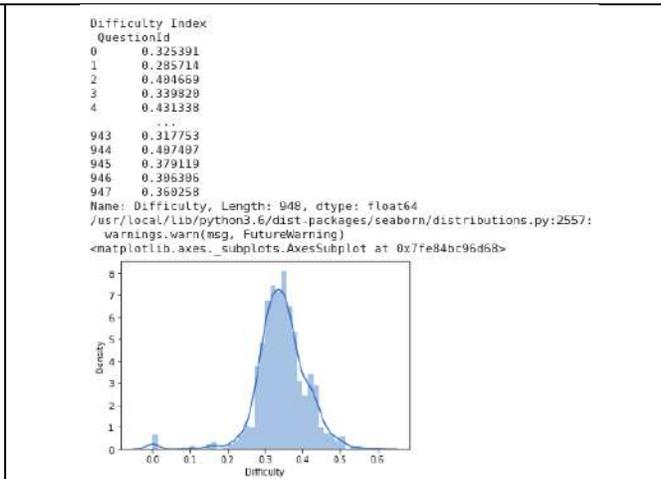


Fig. 4. Difficulty index

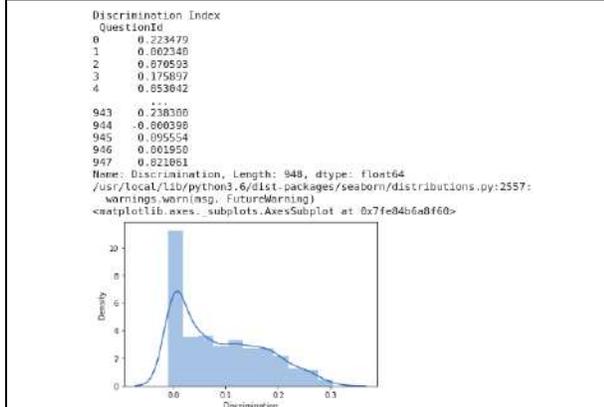


Fig. 5. Discrimination index

```
qd=pd.read_csv('questionData.csv')
qd['Difficulty']=difficulties
qd['Discrimination']=discriminations
qd.to_csv('questionData.csv')
qd.head()
```

| Unnamed: 0 | QuesId | QuestionText | Difficulty | Discrimination |
|------------|--------|---|------------|----------------|
| 0 | 0 | 0 if you multiply a square number by 9 you get a... | 0.325391 | 0.223479 |
| 1 | 1 | 1 how much bigger is 3 than 2 1 4 24 24 11 wtn | 0.285714 | 0.002340 |
| 2 | 2 | 2 which calculation is equal to 20 2x 2 x4 e 2... | 0.404669 | 0.070593 |
| 3 | 3 | 3 what number belongs in the box | 0.339829 | 0.175897 |
| 4 | 4 | 4 abcd is a trapezium where ab is parallel to cd... | 0.431338 | 0.053042 |

Fig. 6. Questions Dataset with difficulty and discrimination indices





Evaluation of Effective Pollinator of *Elettaria cardamomum* in the End of the Flowering Season

M. Ranjithkumar^{1*}, S. David Noel² and V. Tamizhazhagan³

¹Assistant Professor, PG Department of Microbiology, Syed Ammal Arts and Science College, Ramanathapuram, Tamil Nadu, India.

²Department of Biology, School of Science, Gandhigram Rural Institute - Deemed to be University, Dindigul, Tamil Nadu, India.

³Assistant Professor, PG Department of Zoology, Syed Ammal Arts and Science College, Ramanathapuram, Tamil Nadu, India

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*Address for Correspondence

M. Ranjithkumar

Assistant Professor,

PG Department of Microbiology,

Syed Ammal Arts and Science College,

Ramanathapuram, Tamil Nadu, India.



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ABSTRACT

A study was undertaken to identify the effective insect pollinator on the cash plant *Elettaria cardamomum* of the family Zingiberaceae in The Spice Vila Cardamom Plantation at Anavilasam, Kumily (Idukkidist, Kerala, India) for four months from December 2021- February 2022, at different time intervals for three hours (0900h - 1000h, 1100h - 1200h and 1200h - 1300h) during end of the flowering season by calculating their visitation frequency and visitor activity index. *Apis cerana*, *Apis dorsata* and *Trigona iridipennis* were the three social bee pollinators found to exhibit forage activity. *A. cerana* was found to carry pollen and nectar and its visit peaked from 0900h - 1300h whereas the visit of *A. dorsata* was recorded to reach its peak from 1100h - 0100h that also carried pollen and nectar. *Trigona iridipennis* visited the flowers only for pollen that peaked from 1200h - 1300h. Our investigation demonstrated that *A. cerana* has a greater visitor activity index and visitation frequency than the other two pollinators.

Keywords: *Elettaria cardamomum*, *Trigona iridipennis*, Pollination





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INTRODUCTION

Agricultural production forms one of the most important economic sectors. The quantity of most crop species is increased by pollination [1]. Pollination has a role in the economic sector owing to the improvement of quality and quantity [2]. Of the 1,400 crop plants grown around the world, i.e., those that produce all of our food and plant-based industrial products, almost 80% require pollination by animals. Nearly 90% of flowering plant species depend on animal pollinators for pollen transport [3]. In many plants that depend on animal pollination seed set increases with increasing pollinator visitation rates [4].

Pollination by insects is a critical ecosystem service that is necessary for production of most crops, including those providing essential micronutrients, and is thus essential for food security [5]. Honey bees are considered significant pollinators due to their effectiveness and wide availability. The number of visits and the aggregate effects of various bee species influence not only the quantity of crops produced but also their quality, which is important mainly from an economic perspective [6] the quantity and/or quality of pollen and nectar produced may affect the visitation of flowers by bees. Nectar and pollen are sources of energy, protein, and lipids for bees, and other pollinators [7] and bees are drawn to plants to provide food for their young [8]. Honey bees search for flowers with larger amounts of nectar [9]. Therefore during the peak of flowering season, honey bee or social bee exhibit higher visitation rate and Foraging activity. Flowers attracts pollinators by distinct stimuli such as color, shape, size and scent [10] whereas during end of the flowering season, all such Foraging activity, visitation frequency and pollination percentage declines that will affect the crop production adversely. Foraging activity, visitation frequency and pollination percentage of insects depends on the presence of male and female flowers [11]. Pollination will be a limiting factor if the crop plant's pollination threshold is not met (i.e. the number of pollen grains deposited is sufficient for maximum fruit production under ideal growth conditions) [12]. Research on pollinator limitation with reference to pollination threshold has been lacking for the crop plant *Elettaria cardamomum* (Zingiberaceae family). Kerala is the major contributor of cardamom in India having a share of around 88% in total production, and has around 50,000 hectares under cardamom cultivation with the annual average production around 20,000 tonnes. It is estimated that around 35,000 families accounting for over 60% of production Hence it is need of the hour to study the floral visitors, their forage activity and identify the effective pollinator that play a vital role in reproduction of this cash plant [13]. Therefore present study was carried out to reveal the visiting frequency and foraging activity of social bees for *Elettaria cardamomum* and to identify the effective bee pollinator.

MATERIALS AND METHODS

The present study was carried out in The Spice Vila Cardamom Plantation in Anavilasam, Kumily (Idukkidist, Kerala, India). The floral visitors of *Elettaria cardamomum* were observed and recorded for four months from December 2021 to February 2022 at different time intervals. The investigation was carried out at the end of flowering season. One panicle from the selected plants were marked and observed for three hours (0900h-1000h, 1100h-1200h and 1200h-1300h). The flowers last for one day. The flowers open at 0600h and foraging activity declined after 1300h. Three different types of bee pollinators were reported in cardamom and the present study deals with the foraging behavior of such bee pollinators *Apis cerana*, *Apis dorsata* and *Trigona iridipennis*. The visiting time of the pollinators was recorded from 0900 h soon after the dehiscence of anther. It was recorded that the bees took more time to collect pollens rather than nectar. The one bee itself visits the same flowers more than one time [14]. In order to study the foraging habit of the pollinators, flowers were collected soon after the visits of the pollinators and the stigma were observed under stereomicroscope to detect the presence of pollen grains. The pollinators that visited the flowers were also captured and immobilized using ethyl acetate and observed under stereomicroscope. Based on these observations Visitation frequency and Visitor Activity Index were calculated. Visitation frequency can be calculated by [15]

$$\text{Visitation frequency} = \frac{\text{Total number of visits to flowers in the habitat per dominant pollinator}}{\text{Total number of flowers} \times \text{Observation time in the habitat}}$$





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Visitor Activity Index can be calculated based on the five criteria [16] using the formula

$$\text{Visitor Activity Index} = [(A \times B \times C) + (A \times B \times D \times E)]/2[16]$$

Where,

A is the abundance of pollen transferred (which is one (1) for abundant, 0.5 for scarce and zero (0) if no pollen is transferred).

B, C and E where the criteria covered given by Ramírez, 2004[16] (which is one (1), if the criteria is covered and zero (0), if not covered).

(A×B×C) indicates the pollen transferred and (A×B×D×E) indicates the flower visitor adaptation.

RESULTS

Our results revealed that the end of the flowering season witnessed only three species of social bee such as *Apis cerana*, *Apis dorsata* and *Trigona iridipennis* visited *E. cardamomum* plantation at our study site and even no other insects were spotted during this investigation. For this study the presence of pollen on the stigma of the flower was examined using stereomicroscope (fig: 1.C). All the three pollinators were found to carry pollen load on the upper part of the body (fig: 2). The pollinators released the pollen grains loaded on its head and thorax into the stigmatic cup. Among the pollinators *Apis cerana* and *Apis dorsata* were the dominant visitors. *A. cerana* was found to visit the flowers frequently since anthesis and carried a greater quantity of pollen and nectar and deposited pollen on the stigma per visit compared to other two pollinators and its visit peaked from 0900h- 1300h whereas for *A. dorsata*, that carried pollen and nectar reached its peak of visits from 1100h- 0100h whereas *Trigona iridipennis* visited the flowers only for pollen that peaked from 1200h- 1300h. Visitor Activity Index is used to identify the effective pollinator based on the five quantitative criteria. Out of all the three pollinators studied, our results showed that *A. cerana* was identified as effective pollinator. Values of visitor activity index for *A. cerana* were significantly different from zero and subsequently followed by *A. dorsata* and *Trigona iridipennis*.

DISCUSSION

Domestication of crop plants such as cardamom led to the wide range of morphological and physiological traits. Domesticated plant tend to produce large number of branches and ultimately more clumps which attract lot of insects and social bees. In domesticated cardamom social bees are the effective pollinator than solitary bee. The pollination efficiency of social bees varies seasonally in terms of flowering and locality. *A. cerana* was identified as an effective pollinator among the three visitors based on visitation frequency and visitor activity index. From our results it was confirmed that *A. cerana* has visitation frequency (2) and is the frequent visitor of cardamom flower. Similar result was recorded by Chaudhary and Kumar (2000). [14], and Parvathy et al. (1993) [17]. It was observed that after 0100 h the activity of *A. cerana* declined. This may be attributed to the fact that the lower performance and availability of pollen [14]. Our investigation demonstrated that *A. cerana* has greater visitor activity index (1) than the other two pollinators. It may be due to the following facts that *A. cerana* and *A. dorsata* harvest pollen and nectar while *Trigona iridipennis* harvests only pollen [18] as it may not be a co-evolved pollinator of cardamom. Foraging activity of *A. cerana* for nectar could also be a factor for it to be an effective pollinator. It was also supported by Kishore et al. (2012) [19]. Locality and flowering season may be the reason for *A. cerana* to be the effective pollinator than other two pollinators in the present investigation at the study site. Similar observation was observed by Kuriakose et al. (2009) [20]. High visitation frequency (2) and high pollen load maybe the factors for *A. cerana* to make it an effective pollinator in this investigation. Similar results were observed Kishore et al. (2012) [19].

CONCLUSION

Our study gains importance as it was carried out in an economically important cash plant *E. cardamomum*. During the end of the flowering season, the insect visitors declined except the visitation of three different bee species such as *A.*





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cerana, *A. dorsata* and *Trigona iridipennis*. Of late there is a dip in honey bee population in cardamom plantation in Kerala due to radiation from mobile towers, global warming, climate change and deforestation. This drop would become a matter of serious concern if such least number of pollinator visitation recorded in our present study is subject to further devastation. From our investigation it is concluded that *Trigona iridipennis* might be eliminated in competition among other honey bee species. Further, indiscriminate use of pesticides such as phorate, DDT, fungicide etc which are the volatile chemicals that remain in the air for a long time may also cause further decline of pollinator visitation of flower at the end of flowering season.

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Table 1: Visitation frequency and Visitor Activity Index of *A. cerana*, *A. dorsata* and *Trigona iridipennis*

| S.No | Floral visitors | Visitation Frequency | Visitor Activity Index |
|------|----------------------------|----------------------|------------------------|
| 1 | <i>Apis cerana</i> | 2 | 1 |
| 2 | <i>Apis dorsata</i> | 1.27 | 0.43 |
| 3 | <i>Trigona iridipennis</i> | 0.6 | 0.09 |

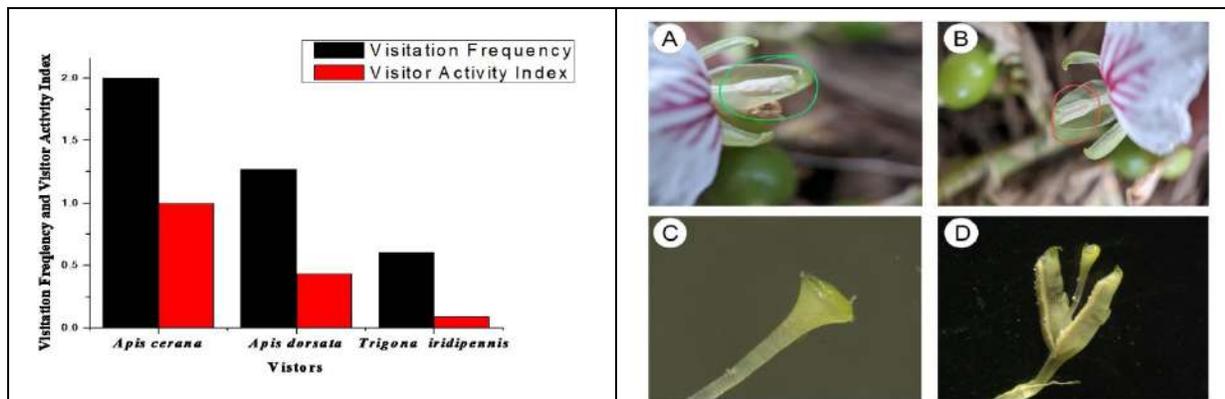


Fig 1: Visitation frequency and visitor activity index of all the three pollinators.

Plate 1: A- Anther with pollen, B- Anther without pollen, C- Stigma with pollen, D- Style and Stigma



Plate 2: Transfer of pollen grains from anther to stigma through pollinators





A Short Review on *Cichorium intybus*

M.K.Vijayalakshmi^{1*}, N.Jayaramakani², K.Snega³, S.Showbharnikhaa³, T.Akshaya³ and J.Nadhiya³

¹Associate Professor, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India.

²Assistant Professor, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

³Final Year B.Pharm Student, Faculty of Pharmacy, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

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*Address for Correspondence

M.K. Vijayalakshmi

Associate Professor,

Faculty of Pharmacy,

Bharath Institute of Higher Education and Research,

Chennai, Tamil Nadu, India.

E. Mail: vijiprathudass@gmail.com



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ABSTRACT

The Asteraceae genus *Cichorium* has six species that are mostly found in Asia and Europe. *Cichorium intybus*, another name for chicory, is a commonly used herb in treating various conditions, including diabetes and wounds. Chicory is very well recognized as a substitute for coffee. Although this plant has a lengthy anecdotal history of use, the pharmacological potential of several of its constituents has not been investigated. Presently, there is a severe dearth of toxicological information regarding *Cichorium intybus*. Up until now, chicory has been predominantly utilized in feed for livestock, but it has also been used in the food industry in a variety of ways, such as a raw ingredient in coffee, a salad ingredient, a tea and tea blends ingredient, and a coffee addiction and also for the manufacturing of inulin. Chicory is increasingly being used to manufacture additional food products and supplements. Numerous compounds found in chicory, including sesquiterpene lactones, inulin, polyphenols, and oligofructose, have the potential to act as carriers of dietary functionality. The pharmacological research and taxonomic analysis of chicory are the main topics of this review article.

Keywords: *Cichorium intybus*, Pharmacological research, Taxonomic analysis





INTRODUCTION

Species: *Cinchona intybus*

Family: Rubiaceae

The name *Cichorium intybus* L. of the common chicory plant is most likely a combination of numerous Greek and Latin words. The Latin word "field," *cichorium*, has an intybus translation in both languages. The Greek translation means "to cut," and it is based on the leaf morphology. The Latin word *tubus*, which means "(a) tube," describes the stem's shape. While the genus name speaks to a plant's habitat, the species name describes the plant itself [1,2,3]. The name *Cichorium* comes from the Latinization of the Greek word *chorion*, which appears frequently in the writings of ancient physicians. It is a tracheophyte with roots in Turkey, Iran, and the Mediterranean. Although the plant has a long history of use that is nearly global, its medical history is especially fascinating because it dates back to the Stone Age. In ancient Rome, Greece, and Egypt, it was used to enhance digestion and metabolism. The plant was used as a vegetable and a pasture plant. Theophrastus of Eresos, Pedanios Dioscorides, Pliny the Elder, and Avicenna all used chicory concoctions to treat digestive and eyesight issues, and there are numerous reports of its medical advantages [4,5]. Avicenna used chicory root decoctions to treat gout discomfort as well. The Unani medical system has also made considerable use of (*Cichorium intybus*) for a very long time. In addition to the aforementioned uses, medieval Europeans used chicory to treat malaria or even jaundice. According to their intended use, various cultivars of chicory can be divided into the following categories:

- (1) Chicory that is referred to as "industrial" or "root" is mostly grown in Chile, India, South Africa, and northwest Europe. Inulin can be extracted from the plant or made into a coffee replacement using the plant's taproot.
- (2) "Brussels" or "witloof" chicory is the most common industrial cultivar in Europe. Its buds turn white when they are stored in a dark place.
- (3) "Leaf" chicory, which is eaten raw or cooked;
- (4) "Pasture" chicory, which is a variety of wild chicory that grows beside highways and in waste areas. Due to the extensive commerce of its specific parts, it is frequently utilized in traditional medicine[2,4,5].

Traditional Uses

Many societies still rely on native medicinal plants to take care of their basic medical needs, despite the fact that the medicinal plants have been utilized for ages as remedies. Ancient Egyptians were the first to employ chicory as medicine, and it has a long history of therapeutic use both where it is native and where it has been introduced. The numerous common names or regional names given to this plant can be attributed to its frequent use by diverse folklore groups [6,7,8].

These plants formulations are used to treat a variety of symptoms and diseases (Table 1). According to legend, the juice helps to treat tumours and uterine cancer. Although chicory is considered a common weed in South Africa, the leaves, stems, and roots are used to make a tea that is used to treat jaundice and as a tonic and purifying treatment for babies. The leaves are used to make an ointment for wound healing in Turkey. A decoction is a preparation that is created by boiling plant material with cold water, letting it simmer for 5-10 minutes, and then straining[7,8].

The European literature status states that one of the root traditional uses is to treat moderate digestive complaints, such as temporary appetite loss and feelings of abdominal fullness, gas, and poor digestion. Aqueous root extracts were used as a light-sensitive plant cure for malaria in Afghanistan, according to folkloric traditions. This conventional wisdom has been supported by the discovery of the light-sensitive sesquiterpene lactones in lactucopicrin as the anti-malarial components of *C. intybus* roots[8,9,10,11].

Chemical Composition

All of the chicory's morphological components, including the roots, herbs, flowers, and leaves, contain a variety of different chemical compounds. The primary constituent of the methanolic extract of chicory has been found as being chicoric acid. Terpenoids are slightly less prevalent in the plant than aliphatic chemicals and their derivatives.





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Methoxy coumarin cichirine, Saccharides, essential oils, flavonoids, and anthocyanins, which give the perianth its blue color, can all be found in chicory flowers. Among other things, the roots of chicory contain sap and bitter sesquiterpene lactones including guajanolides and germacranolides[12,13]. The roots contain between 40 and 60% of insulin, 9–15% reducing sugars, and 0.01–0.02% of bitter intybin glycoside. However, chicory roots are devoid of starch. Chicory leaves also include phenolic compounds, Na, Mg, K, Cu, Mn, Ca, Fe, Zn, and vitamins A, B1, B2, and C. Gum, choline, quinic acid, tannins, phytosterols, mucus, tannins, copper, latex, saponins, flavonoids lipids, proteins, P and K vitamins, amino acids, terpenoids, and anthocyanins are just a few of the components found in chicory plants. The main volatile components have been identified as octane, n-nanodecane, pentadecanone, hexadecane, and an unidentified molecule [14].

Pharmacological Effects

Antimicrobial Activity

Chicory (*Cichorium intybus*) has the potential herbal remedy for treating bacterial infections caused by Gram-negative and Gram-positive bacteria. Organic acids such as Butanoic acid (succinic acid), 3,4,5-Trihydroxycyclohex-1-ene-1-carboxylic acid (shikimic acid), Ethanedioic acid (oxalic acid), 1,3,4,5-Tetrahydroxycyclohexane-1-carboxylic acid (quinic acid) from the aqueous root extract of chicory reduce the bio film formation and bacteria attachment to the cells, the biofilm disruption are created and dead cell detachment cultured substratum. Chicory root extract (CRE) is in polyvinyl alcohol (PVA)/chitosan (CS) nanofibers (NFs) as a safe solution effective against *Escherichia coli* and *Staphylococcus aureus*. The organic seed extract of chicory is effective against pathogenic bacteria such as *Escherichia coli*, *Candida albicans*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*[15,16].

Anthelmintic Activity

Condensed tannins and sesquiterpene lactone-rich extract of cinchory have anthelmintic activity. By using the larval migration inhibition assay condensed tannins and sesquiterpene lactone are effective against GI nematode larvae, larvae of deer lungworm and *Dictyocaulus viviparus*. Condensed tannins reduce the GI nematodes and the larval motility in lungworm; sesquiterpene lactone in cinchory *intybus* inhibits *Haemonchus contortus* egg hatching [17,18].

Antimalarial Activity

Lactucin and lactucopicin are bitter in taste and are used as an antimalarial agent in Afghanistan. It inhibits the HB3 clone of strain honduras-1 of *Plasmodium falciparum*. Lactucin and lactucopicin also have an analgesic effect[18].

Antioxidant Activity

A significant number of anthocyanins are present in the seeds of *Cichorium intybus*, and these anthocyanins may exhibit antioxidant activity by scavenging ROS. Chicory contains natural antioxidants that activate the body's antioxidant defense system and prevent the hepatocellular damage brought on by CCl₄ in the liver¹⁹. The aqueous alcoholic extract has antioxidant activity. It inhibits chelation of ferrous ions with α -diphenyl- β -picrylhydrazyl (DPPH) and inhibits the Hydrogen peroxide and inhibits the Xanthine oxidase [18].

Hepatoprotective Activity

Oxidative stress is a major contributor to both acute and long-term liver damage because free radicals from carbon tetrachloride (CCl₄) decrease the activity of antioxidant enzymes like Glutathione (GSH)-dependent antioxidant enzymes and Glutathione (GSH) of phase 2 detoxification enzymes. *Cichorium intybus* has been described as a key hepatoprotective plant in the Unani medical system, and this herb is extremely helpful in hepatic-related diseases like hepatitis and jaundice [19,20]. Rats given 4-tert-octylphenol (potential environmental pollutants which produce a toxic and estrogenic effect on mammalian cells) had significantly higher levels of Gamma-glutamyl transpeptidase (GGTP), Alkaline phosphate (ALP), Aspartate aminotransferase (AST) and Alkaline transaminase (ALT) in the liver and also the levels of Glutathione (GSH) and SOD had reduced. The reduction of Thiobarbituric acid reactive substances (TBARS) and considerable improvement of the examined biochemical and antioxidant markers show that CSF extract is successful in modulating these 4-tert-octylphenol-related abnormalities. The negative impact of 4-tert-



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OP and the protective impact of CFR extract on liver toxicity were corroborated by histopathological data, as well as by observations of PCNA and DNA fragmentation[6].

Antidiabetic Activity

It has been demonstrated that a Natural Chicoric Acid Extract (NCRAE) from *Cichorium intybus* root increases glucose absorption by muscle cells and insulin release by pancreatic β -cells. Two key substances, caffeic acid, and chlorogenic acid, have been shown to be effective anti-diabetic medicines because they boosted glucose absorption in muscle cells and were discovered to be capable of triggering the release of insulin from islets of Langerhans and insulin-secreting cell lines[17].

Antitumor Activity

On melanomatic C32 cell lines, chicory leaves were also found to have an ant-proliferative effect. However, a root-derived compound called magnolialide was effective in inhibiting a number of tumor cell lines and started the process of differentiating human leukemia cells (u-937 and HL-60) into macrophage or monocyte-like cells[3].

Anti-Inflammatory Activity

Plant extracts obtained from *Cichorium intybus* indicate strong anti-inflammatory action, as the alcoholic extract of this plant appeared anti-inflammatory potential in treating pyorrhea inflammation. Chicory root extract decreased TNF- and interleukin levels[20].

Clinical Trials Of Cinchory

Two scientifically substantiate research support the use of chicory root. However, because these are only primary studies, both are inadequate to provide time-honored suggestions for using chicory plant.

Phase-1: Osteoarthritis patients took part in a non – randomized, double-blind, dose-escalation study to assess the risks and benefits of a specialized bioactive chicory root extract (OA). Osteoarthritis treatment is well accepted. The most severely affected person who took the most prescribed dose of chicory had to stop the drug because of its negative side effects[21].

Phase-2: 27 healthy adults consumed 300 ml of chicory tea every single day to see if it benefited the cardiovascular system. Platelet coagulation, tubular viscosities, and blood potential all beat through chicory espresso. They found that consuming 300 ml of chicory tea every day for one week caused unique platelet aggregation, based on the dilution used in the aggregation test. Whole blood and tube density, in addition to serum MIF situations, were significantly reduced one week after consuming chicory espresso. Based mainly on currently available research, *Cichorium intybus* root is used in the EU as “a standard natural restorative product for the relief of signs, symptoms, and signs associated with mild digestive problems (including fullness, flatulence, and negative digestion).” Appetite” [25]. 36 patients with cirrhosis diagnosed by the Tehran Liver Institute participated in a 6-month study comparing the results of herbal medicines containing Tamarix gallica, Mandurbasma and Capparis Spinosa, *Cichorium intybus* with Liv-52 and Solanum with placebo. New childhood conditions – edema, aspartate aminotransferase (AST), total bilirubin, albumin, prothrombin time, platelets and white blood cells – were growth criteria. The instructions were referred to in all cases before starting drug or placebo and 6 months after. We concluded that Liv-52 has a hepatoprotective effect in cirrhosis. Its protective effect may be due to the antioxidant, diuretic and immunomodulatory properties of the sauces used to make the Livfifty two sauce[22, 23,24,26,27].

CONCLUSION

The consumption of *Cichorium intybus* is widespread throughout the world. In the past, the ancient Egyptians used chicory as an agricultural product, a coffee replacement, a medicinal plant, and even as animal fodder. High levels of minerals, carbohydrates, and proteins can be found in this versatile plant. Because it influences biochemical and





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biological processes, inulin from chicory roots is regarded as a functional dietary element because it improves health and lowers the risk of numerous disorders. Chicory is still a genetically accessible plant with a wide range of uses, and there is interest in genetically modifying it to increase yields and unlock new potential. Chicory's medicinal properties have been the subject of documented traditional knowledge, and studies into its biological activity and botanical compound isolation have corroborated this information. However, many of its constituents' pharmacological potential has not been explored, and further research is necessary to fully comprehend the phytochemicals' protective properties against a variety of illnesses. Since chicory penetrates the food chain, it should be utilized with caution. Recent research recommends using *Cichorium intybus* as a biomonitor for heavy metals. The apparent bioactivity *Cichorium intybus* has shown in preclinical study both in vitro and in vivo attests to its historical use in traditional medicine.

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Table 1: *Cichorium intybus* - Traditional medicinal uses

| Country | Traditional use(s) | Plant part(s) | Preparation(s) |
|------------------------|---|-----------------------------|-----------------|
| Afghanistan | Malaria | Root | Aqueous extract |
| Bosnia and Herzegovina | Diarrhea, strengthening the prostate and other reproductive organs, pulmonary cancer, hangover, and purification of biliary tract | Aerial part, flowers, roots | Not stated |
| | Liver disorders, spasmolytic, cholesterol, antiseptic | Aerial | Decoction |
| Bulgaria | Cholagogue stimulant for gastric secretion, hypoglycemic | Roots, aerial parts | Decoction |
| India | Liver disorders | Seeds | Not stated |
| India | Diabetes | Whole plant | Not stated |
| | Jaundice, liver enlargement, gout, and rheumatism. | Root | Decoction |
| | Cough relief | Root | Not stated |
| Iran | Choleretic, Eueptic, stomachic, depurative, laxative, hypotension, tonic, and antipyretic | Whole plant | Not stated |
| Italy | Blood cleansing | Leaves | Not stated |
| | High blood pressure | Leaves | Decoction |





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| | | | |
|--------------|--|----------------------|----------------------------------|
| | Arteriosclerosis, Blood purification, Antiarthritis, Antispasmodic, Digestive. | Leaves/roots | Decoction |
| | Depurative. | Whorls | Decoction |
| | Choleretic, hepatoprotectivea gainst jaundice, mildlaxative, hypoglycemic | Leaves | Decoction, squashed fresh leaves |
| Jordan | Internal hemorrhage, sedative in typhoid | Whole plant | Cooking |
| Morocco | Renal disease | Aerial/roots | Not stated |
| | Kidney disorders, diabetes | Whole plant | Decoction |
| Pakistan | Diabetes | Roots | Decoction |
| Poland | Digestive complaints and lack of appetite | Roots | Tea |
| Serbia | Diarrhea | Flower | Infusion |
| | Diuretic, digestive, laxative, anti-inflammatory, liver complaints, reducing blood sugar | Roots | Decoction/tea |
| | Cholagogue, digestive, hypoglycemic | Aerial part/root | Not stated |
| South Africa | Jaundice, tonic | Leaves, stems, roots | |
| Turkey | Cancer, kidney stones | Roots | Decoction |
| | Wound healing | Leaf | Ointment |
| | Hemorrhoids, urinary disorders | Aerial | Tea |

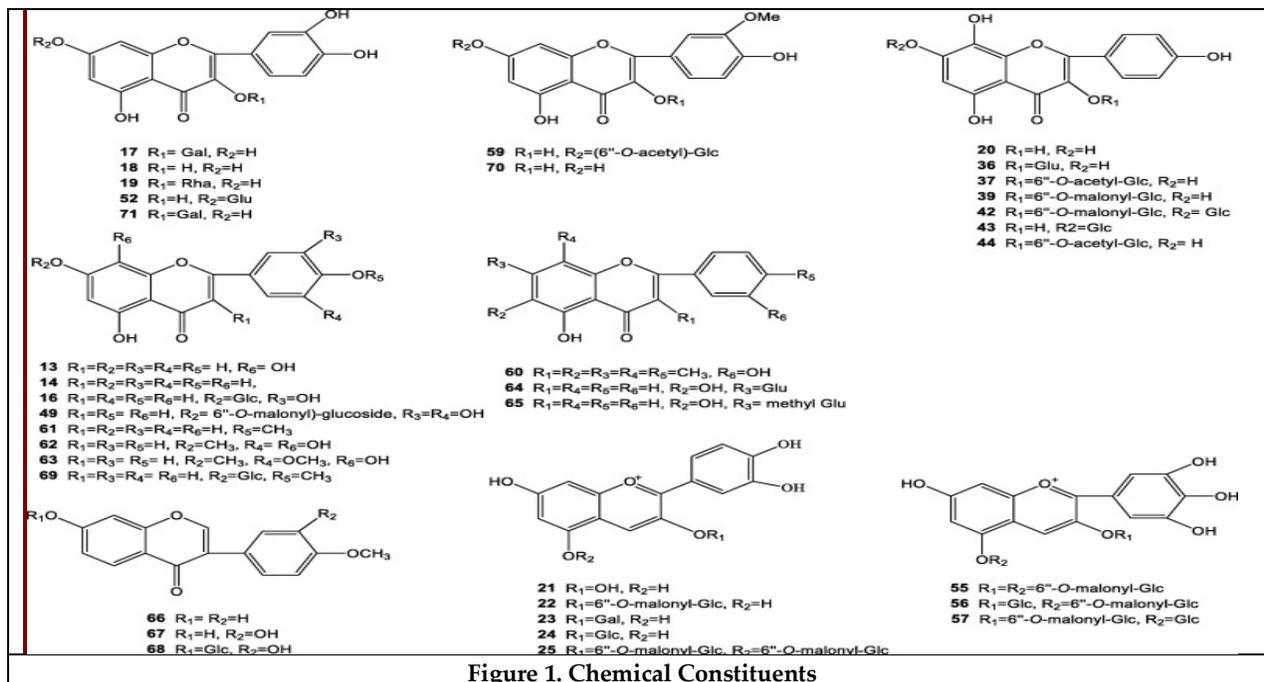


Figure 1. Chemical Constituents





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Fig. 2. *Cinchona intybya*





Formulation, Sensory, Nutritional and Microbial Properties of Nutrient Dense Ready to use Therapeutic Food (RUTF)

Karthika. S¹, Shiny Lizia .M^{2*} and Hemamalini A.J³

¹Postgraduate Student, Department of Clinical Nutrition, Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu, India.

²Lecturer, Department of Clinical Nutrition, Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu, India.

³Professor and Head, Department of Clinical Nutrition, Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (DU), Porur, Chennai, Tamil Nadu, India.

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*Address for Correspondence

Shiny Lizia M.

Lecturer,

Department of Clinical Nutrition,

Faculty of Allied Health Sciences,

Sri Ramachandra Institute of Higher Education and Research (DU),

Chennai, Tamil Nadu, India.

E. Mail: pgpublication2023@gmail.com



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ABSTRACT

Ready-to-use therapeutic food (RUTF) is a popular option for home-based rehabilitation since it does not need to be cooked, possesses a long shelf life, and contains adequate energy to sustain weight loss and growth. Peanuts are the most commonly used ingredient in ready to use therapeutic foods, however, using alternative indigenous ingredients for RUTF formulation high in energy and nutritional density could prove helpful in combating malnutrition. The nutrient, sensory and microbial quality of the product was analyzed using standard procedures. The outcome of the study indicated that the product yielded 524.4 Kcal of energy, 18.2grams of protein, 28.3grams of fat, 49.1grams of carbohydrate, 1.6grams of moisture, 0.41grams of fiber, 253 milligrams of calcium, 9.4 milligrams of iron, 16.7 milligrams of vitamin E, and 75 milligrams of magnesium per 100 grams. The shelf life of the product was found to be more than one month when stored at 2 to 8 degrees Celcius, in a cool and dry place. The present study concludes that indigenous ingredients such as almonds are safer alternatives to peanuts for the production of an acceptable Ready-to-use therapeutic food as recommended by United Nations International Children's Emergency Fund (UNICEF) standards. Also, the expenses associated with using imported ready-to-use therapeutic food will be significantly decreased.

Keywords: therapeutic food, Malnutrition, Almonds, Sensory, Nutritional





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INTRODUCTION

Severe acute malnutrition (SAM) can be defined as extremely low weight-for-height/length (Z-score below -3 SD of the median WHO child growth criteria), a mid-upper arm circumference of 115 mm, or the appearance of nutritional edema and it contributes to both social and medical disorder. Severe Acute Malnutrition is caused by a number of reasons, including inadequate exclusive breastfeeding, delayed supplemental feed introduction, providing diluted feeds with insufficient nutrients, recurrent respiratory and enteral illnesses, ignorance, and poverty [1].

There are two possible phases in the treatment of severe acute malnutrition in children: stabilization and rehabilitation. Children who have dehydration, electrolyte imbalances, concurrent infections, or other problems are treated during the first stabilization phase. The major goal of the rehabilitation phase is to catch up with growth and the recommended calorie and protein needs are substantially greater (Applicable to children presenting with straightforward SAM or those with difficult SAM after issues have been overcome.) Home-based treatment might be preferable for children with SAM whose rehabilitation in hospitals is not always acceptable or feasible, especially in remote areas. RUTF, or Ready to Use Therapeutic food, is popular for at-home rehabilitation[2].

RUTF fulfills all of a child's nutritional needs while recovering from SAM, especially in low-income environments where food security may be a serious problem. Additionally, it is practical to pre-position in a warehouse due to its longer shelf life of about two years. It is easily digestible and has pleasing flavor children may eat it straight from the packet without needing to prepare it. French doctor Andre Briend created "PlumpyNut", the first and best-known RUTF, in 1996. In a foil packet, the plumpy nut is a peanut-based paste. It could be consumed by infants who were not yet ready for solid meals and was portable and non-perishable. However, UNICEF issues a supply alert regarding price increases for ready-to-use therapeutic food (RUTF) constituent ingredients and packaging materials that account for about 75% of the costs of the finished products, as well as related issues with international shipping, transport, and freight costs that result in longer lead times for delivery. And in the last beside the treatment for SAM considering peanuts are most commonly contaminated with aflatoxin and consist of vegetable oil in production thus quality control measures are needed to be of a higher standard[3]. Therefore, to accelerate the treatment of severe acute malnutrition, the goal of this study was to formulate a Ready to Use Therapeutic Food (RUTF) using indigenous ingredients.

MATERIALS AND METHODS

Ethical Approval

The study was approved by the Institutional Ethical Committee of Sri Ramachandra Institute of Higher Education and Research (DU), Porur(REF NO:CSP/23/JAN/120/31).

Description of the Study

The study involved the development of a nutrient dense ready to use therapeutic food for severely acutely malnourished children under the age of five to fulfill the heightened demands of nutrients and reduce the risk of diseases. The study was carried out at Sri Ramachandra Institute of Higher Education and Research for a period of one month.

Formulation

Selection of Ingredients

In this study, the ingredients used for the development of nutrient dense RUTF include almonds as the source of macronutrients, sunflower seeds for the micronutrient, and brown sugar to improve palatability. Almonds were chosen as the macronutrient source because they are regarded as a healthy snack and are a rich source of protein, monounsaturated fatty acids, and dietary fiber (insoluble/soluble fiber at a ratio of 4:1)[4], and the reason sunflower seeds were chosen as a source of micronutrients is that they are rich in minerals and vitamins, including calcium,





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copper, iron, magnesium, manganese, selenium, phosphorous, potassium, sodium, and zinc, as well as vitamin E, B, folate, and niacin[5].

Procedure

Indigenous ingredients such as almonds and sunflower seeds were sorted and washed immediately after purchase. The cleaned almonds and sunflower seeds were roasted in a separate pan from low to medium heat for five minutes to improve the final product's flavor and neutralize the anti-nutrient factors present in them.

Formulation of Ready to Use Therapeutic Food

The formulation was based on the traditional Ready to Use Therapeutic Food recipe of UNICEF[6]. In order to give a similar nutrient profile in a manner that required no further preparation, Ready to Use Therapeutic Food was based on the F100 formula (100 calories, 3.8 g protein/100ml) nutrient profile was prepared, with roasted almonds, roasted sunflower seeds and brown sugar as ingredients. Each ingredient was weighed and grounded using an electric mixer at medium speed until the desired consistency is reached.

Sensory Analysis

The formulated RUTF was subjected to consumers to rate their degree of liking for appearance, taste, color, smell, flavor, texture and overall acceptability on a nine-point hedonic scale. With, 9 Standing for strongly agree (extremely liked very much) 8- standing for very much liked, 7- standing for moderately liked, 6 standing for slightly liked, 5 standing for neither liked nor disliked, 4 standing for slightly disliked, 3 stands for moderately, 2 stands for very much disliked and 1 stand for strongly disagreeing (disliked it extremely).

Inclusion Criteria Adults in the age group of 20 and 60 years, who offered informed consent to participate in the study.

Exclusion Criteria People with mouth ulcers and those who undergo any treatments interfering taste sensitivity. A total 10 adults were chosen as participants to perform the sensory analysis of the product, out of which five were nutrition experts and the other five were non-nutritionists.

Nutrient Analysis

Nutrient analysis of the RUTF was evaluated using the Indian Food composition tables as well as following the proximate principles of nutritive analysis in a standard laboratory.

The analysis was carried out for the following nutrients: Energy, Protein, Fat, carbohydrates, Moisture, Calcium, Iron, Vitamin E, fiber, and Magnesium.

Nutrient Analysis Using AOAC Method.

AOAC METHOD: Association of Official Analytical Chemists is used by government agencies concerned with the analysis of foods, feeds, and other materials related to agriculture, health and welfare, and the environment. AOAC methods are also used by the industry to check compliance with products.

Nutrient Analysis for Energy, carbohydrates, Iron, and Vitamin E was Carried Out By Using ALPL Test

ALPL test method: It is the standard operating procedure used for the determination of carbohydrates, Iron, and Vitamin E content in the food.

Nutrient Analysis for Calcium and Magnesium was Determined By IS:5949: 1990 Method

IS:5949: 1990 test method is used for volumetric determination of Calcium and Magnesium using EDTA.

Microbial Analysis

The shelf life analysis of the Ready to Use Therapeutic food was assessed using Accelerated shelf life testing.





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Accelerated Shelf Life Testing (ASLT) is a means of indirectly assessing how long they will last. The items with the longest shelf life are the ones that are most often submitted to ASLT. In ASLT, goods are kept in increased stress environments to hasten physiochemical changes and increase their rate of disintegration. The most typical acceleration factor employed in these studies is temperature. ASLT shortens the time needed for product testing due to the exacerbated circumstances. With the use of this capability, evaluating the food's shelf life might be completed in half the time or even less than that required for real-time shelf-life testing.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel. Data was coded, tabulated, and analyzed using descriptive statistics and were expressed as Mean and Standard deviation.

RESULTS AND DISCUSSION

Results

Sensory Analysis

Ten consumers evaluated their liking of the products for the sensory attributes of appearance, taste, flavor, colour and texture using nine point hedonic scale. The formulated product was evaluated by ten consumer panellist, five of them are experts in the field of nutrition and five of them are not, and as a result the formulated product have a high likelihood of sensory acceptance.

Nutrient Analysis of the Formulated Ready to Use Therapeutic Food

The formulated ready to use therapeutic food was analyzed for its Energy(Kcals), Protein(g), Fat(g), Carbohydrate(g), Moisture(g), Calcium(mg), Iron(mg), Vitamin E(mg), Fibre(g) and Magnesium(mg) using AOAC method, ALPL test, IS:5949: 1990 test method and using IFCT data.

Microbial Analysis

The test results of the microbial evaluation showed that total plate count was 810 CFU/g at the end. Escherichia coli, yeast and mold were absent till the end of microbial analysis. The product completed a stability test for the therapeutic food that is suitable for usage. When the product's shelf life was verified, it was determined that the therapeutic food that has been prepared and is ready to use with a predicted shelf life of more than one month when stored between 2 and 8 degrees Celsius in a cold, dry environment.

Cost Analysis

The ingredients needed to make this formulation were less expensive than the ingredients used to make commercial RUTF. Each ingredient's cost was assessed on the local market, and the overall cost—including overhead cost is shown in Table 6. The cost of ready to use therapeutic food is Rs. 34 per 100 gram which is economic compared to other commercial RUTF

DISCUSSIONS

Sensory Analysis

An important element in food acceptability and choice is a food product's appearance. In the current study, all of the consumers expressed a preference for the product's appearance, which may have been influenced by its appealing product color and outstanding physical attributes[7]. The primary limiting element determining the consumer panel's acceptance of the product is the flavor and aroma. The product's flavor and scent received good marks. By roasting the food and adding sugar, the product's palatable scent, and flavor may have been improved[8]. Consumers approved of the product's textural features. This might have been influenced by the sample mixture's composition, which gave the finished product a smoother texture. Consumer approval of the texture characteristics of ready-to-use supplemental diets has previously been investigated. Consumers particularly preferred ancillary





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products with a smoother texture[9]. Consumers generally received the items favorably, which could have been due to their familiarity with the readily available local ingredients utilized to make them. It has been shown that utilizing components that are often accessible in homes to create novel supplementary food formulations and boosts the acceptance of goods[10].

Nutrient Analysis

In comparison to the 92 g sachet of Plumpy'Nut, which is frequently used for nutrition intervention, the formulated product had a significantly higher carbohydrate, protein, and energy content and other parameters, such as fat, calcium, iron, vitamin E, fiber, and magnesium, also fairly met the requirements of ready-to-use therapeutic food.

CONCLUSION

The formulations tested in this study were created based on a standard UNICEF recipe that uses readily accessible ingredients such as RUTF Almonds, Sunflower Seeds, and Brown Sugar. Due to the relative flavor and scent that roasting enhances, these formulations were well accepted by consumers and have a high likelihood of being accepted for human consumption in India to treat severe acute malnutrition. They might be used to help rehabilitate severely malnourished children since they were successful in lowering malnutrition and encouraging children's growth. They can also be utilized in programs that support nutrition through supplemental feeding and other means. The demand for RUTF is increasing enormously for the treatment of severely acutely malnourished children in the rehabilitation phase as a result there is an urge to interest in non-peanut-based RUTF, particularly from countries where peanuts are not a staple food in local diets. UNICEF encourages the validation and access to alternative RUTF formulations.

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Authors Contribution

Karthika S. designed and carried out the whole study and drafted the manuscript. Shiny Lizia guided and reviewed the manuscript and Hemamalini and Shiny Lizia reviewed and approved the manuscript.

Conflicts Of Interest

The authors declare no conflict of interest.

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Table1:UNICEF Recipe of RUTF

| INGREDIENTS | WEIGHT% |
|---------------|---------|
| Full fat milk | 30 |
| Peanut | 25 |
| Vegetable oil | 15 |
| Sugar | 28 |
| Mineral mix | 1.6 |

Table 2: Ingredients used in the Formulation of Ready to Use Therapeutic Food

| INGREDIENT | WEIGHT% |
|-----------------|---------|
| Almonds | 50 |
| Sunflower seeds | 30 |
| Brown sugar | 20 |

Table3: Sensory Characteristic Response of Ready to Use Therapeutic Food

| S.No | Criteria | Sensory scores of Nutrition experts | | Sensory scores of Non nutritionists | |
|------|-----------------------|-------------------------------------|--------------------|-------------------------------------|--------------------|
| | | Mean | Standard deviation | Mean | Standard deviation |
| 1 | Appearance | 6.8 | 3.4 | 8.4 | 1.2 |
| 2 | Taste | 8 | 3.4 | 8.3 | 0.5 |
| 3 | Flavor | 7.4 | 3.3 | 8.3 | 0.4 |
| 4 | Colour | 6 | 3.2 | 8.2 | 1.2 |
| 5 | Texture | 7 | 3.2 | 8.1 | 0.8 |
| 6 | Overall Acceptability | 7.8 | 2.9 | 8 | 0.4 |

Table4: Comparison of nutrients in ready to use therapeutic food in laboratory analysis report and IFCT.

| READY TO USE THERAPEUTIC FOOD | | | | | | |
|-------------------------------|-----------|-------|-------|-------------|-------|--------------------|
| NUTRIENT | UNIT | LAB | IFCT* | Differences | Mean | Standard deviation |
| Energy | Kcal/100g | 524.4 | 539.5 | -15.1 | 532.0 | 10.7 |
| Protein | g/100g | 18.2 | 15.4 | 2.8 | 16.8 | 2.0 |
| Fat | g/100g | 28.3 | 42.5 | -14.2 | 35.4 | 10.0 |
| Carbohydrate | g/100g | 49.1 | 24.2 | 24.9 | 36.7 | 17.6 |
| Moisture | g/100g | 1.6 | 5.7 | -4.1 | 3.7 | 2.9 |
| Calcium | mg/100g | 253 | 184.7 | 68.3 | 218.9 | 48.3 |
| Iron | mg/100g | 9.4 | 4.9 | 4.5 | 7.2 | 3.2 |





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| | | | | | | |
|-----------|---------|------|------|-------|-------|-----|
| Vitamin E | mg/100g | 16.7 | 17.7 | -1 | 17.2 | 0.7 |
| Fibre | g/100g | 0.41 | 4.9 | -4.49 | 2.7 | 3.2 |
| Magnesium | mg/100g | 75 | 290 | -215 | 182.5 | 152 |

***IFCT – Indian Food Composition Tables**

The above table gives the nutritive values based on laboratory analysis and values from IFCT.

Table 5: Microbial Analysis of Ready to Use Therapeutic Food

| S.NO | PARAMETERS | UNIT | RESULT |
|------|-------------------|-------|--------|
| 1 | Total plate count | CFU/g | 810 |
| 2 | Escherichia coli | CFU/g | <10 |
| 3 | Yeast&mould | CFU/g | <10 |

Table 6: Comparison of the Prices Used in RUTF Formulation and Commercial RUTF

| COMMERCIAL READY TO USE THERAPEUTIC FOOD | |
|--|-------------------------|
| BRAND | PRICE PER 100g (Rupees) |
| Safari land brand | 115 |
| Nido brand | 614 |
| Miksi brand | 246 |
| KCC brand | 164 |
| Formulated RUTF | 34 |





The Influence of Ecological Science In Gary Snyder's Poetry: An Analysis

R.Manimuthukumar¹ and C.Santhosh Kumar^{2*}

¹UGC-NET- SRF, Ph.D. Research Scholar, Department of English, Annamalai University, Annamalai Nagar -608002, Chidambaram, Tamil Nadu, India.

²Professor and Director i/c, DDE, Annamalai University, Annamalai Nagar -608002, Chidambaram, Tamil Nadu, India.

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*Address for Correspondence

C.Santhosh Kumar

Professor and Director i/c,

DDE, Annamalai University,

Annamalai Nagar -608002,

Chidambaram, Tamil Nadu, India.

E. Mail: santhoshc_2008@yahoo.com



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ABSTRACT

Gary Snyder writes that a poet is a "harvester of words and thoughts" in each of his poetry collections. From the linguistic and mythological ruins that their forebears left behind, the poet unearths uncommon thought components. He recycles and repurposes language in order to capture the vital energy that propels the reader forward both socially and spiritually. This is a common theme in both the prose and poetry of Gary Snyder. In the same way "dead growth recycling" promotes the health of a natural climax system that results in immortality of nature. Snyder asserts that the poem acts as a catalyst and passes information, or intelligence and wisdom, from the higher order of energy to humans. Through the medium of the poets, it is made possible in this universe. There are many other mediums that also cater to this process, but we exclusively speak only of poetry here. Poems, according to Snyder, are "little energies." A poem energizes a person when he is weak and at his lowest point of life and boosts him to lead his life with power and enthusiasm. It is like transfer of energy from the highest order to the lowest. The readers can feel the energy of the poem when they read it. This paper discusses the fundamentals of ecological science and its impact on Gary Snyder's poems. The researcher has taken the poetry collection "Earth House Hold" by Gary Snyder as the subject of this article.

Keywords: Ecology, Consciousness, Spiritual, Energy, Science



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INTRODUCTION

Gary Snyder is considered by many to be the greatest living poet in America and one of the most influential writers of our time. He says that science, especially ecological science, has been a rich source of inspiration for him as well as an important source of ideas about society and art. Snyder says that he has been influenced by writers of science such as Alfred Russel Wallace, John Muir, Jane Jacobs, Edward Abbey, Rachel Carson, and Paul Shepard. Snyder is also greatly influenced by Buddhist writing and tradition and considers the works of Dogen and Basho to be some of the finest writing on earth. Wallace described rainforests as the “regenerators of life on earth,”(25). Snyder believes that poetry can help to preserve these forests and give them space to heal the damage that humans have done to them.

A few of his major concerns are the destruction of natural habitats and species, especially those in tropical rainforests; the lack of a proper appreciation of wilderness and wildlife; the problem of sustainability in human life and culture; overpopulation as an obstacle to individual freedom and ecological well-being; the centrality of place in human lives; and issues related to science and technology. Snyder was concerned about what he saw as the negative effects of scientific reductionism, as exemplified by Rene Descartes' dictum “Cogito ergo sum”(para.1). In his introduction to *The Real Work*, Snyder explains that his ecological writings demonstrate his belief that “the truth of the world is in its wildness, its places and creatures, not in human systems” (66). Snyder's works address the same themes again and again: the conservation of endangered species; preservation of wilderness; mutual respect between human and non-human.

To support his argument, Snyder cites the English Romantic poet John Keats, who maintained that poets “know nothing of botany,” yet they still “...gain (ed)... access to the universal”(Foundation, art.1). Snyder asserts that all it takes to produce excellent poetry is to know the world and one's own mind; a knowledge of natural history will help one understand that world, while the use of scientific observations will help one depict it truthfully. For example, he analyses Thoreau's famous assertion of his essay “Walking” that “In Wildness is the preservation of the World”(192). It is only within the confines of civilization that humankind can destroy nature, but not in the wildness. Snyder also feels that only by understanding the full human and natural history of a place can one write beautiful poetry about it. By embracing nature, he hopes to inspire people not to feel threatened by it but rather to realize that human beings are a part of the natural world and cannot survive without the environment.

Snyder owes his understanding and intellectual knowledge of nature's functions to a number of famous and fascinating scientists and their works. The most well-known of them is Eugene Odum, who was called “the world's foremost ecologist” in a review of his influential work, *Fundamentals of Ecology*(1953). Additionally, Howard T. Odum, the brother and colleague of Eugene Odum, is often cited by Snyder. Snyder was influenced by Howard Odum's controversial work, *Environment, Power, and Society* (1970), not just for the facts it contained about the natural world but also for its conceptions about politics and religion, especially in the way he explains the trichotomy between nature, society, and technology. The Spanish scientist Ramon Margalef is a third environmentalist who had a significant impact on the poet during the time when Snyder wrote his first major poem, “Eleven Essays.”

Snyder and the Odum brothers regularly make reference to Margalef's *Perspectives on Ecological Theory* (1968) and *Patterns of Culture* (1934) in several of their essays and poems. These three wildlife biologists address fundamental ecological ideas while concentrating on energy flow in ecosystems and the intricate relationships between species, the physical environment, and human activity. The Odums, Snyder, and Margalef challenge the “dominant evolutionary paradigm,”(*The Gary Snyder Reader*, bk.1)as ecologist David Gibson states in his comprehensive introduction to *The Gary Snyder Reader* (2008).

Snyder often brings up succession, a second well-acknowledged and important ecological theory. In this theory, the basis for all existence is described in terms of a pyramid that stretches from simple cells and organisms all the way up to complex plants and animals.





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He uses not only examples from Mendota but also many others to support his idea that ecological succession is the result of changes in the environment and behaviour of species. The long-term changes in the composition of a community of species through time may be interpreted as a change from instability to stability, which eventually leads to an ecological climax.

It has been pointed out that an ecological climax is not stability but rather a maximally stable situation in which all species have adjusted to each other and their environment. The Odum brothers regard Margalef as the source of their knowledge of these concepts. According to Margalef, this process is described as an “accumulation of information” and as a “process of energy transfer”(Margalef 67). Most authors agree that the eventual goal of succession is to arrive at a climax ecosystem. A mature ecosystem has “wisdom” as a result of the knowledge that biomass carries. Ecologists describe a mature ecosystem as being more complex and self-regulating than less stable ones. Snyder rewrites Eugene Odum, who rewrites Margalef as follows:

Life biomass. . . is stored information; living matter is stored information in the cells and in the genes. ... There is more information of a higher order of sophistication and complexity stored in a few square yards of forest than there is in all the libraries of mankind.(Margalef 72). Ecosystems that are mistreated become unstable because the wisdom of the natural system is lost. Any ecosystem becomes vulnerable when its natural wisdom is lost or ignored. Many of the things we consider “natural” are, in fact, beneficial systems that require constant upkeep to keep them in good working order. The use of what Howard Odum refers to as “loop circuits” helps to reduce the amount of instability and loss that might occur.

It is important that humans make sure to build these loop circuits into their relationships with the ecosystems around them in order to prevent any further loss of nature’s wisdom. Energy is returned to its source through a feedback loop, which “rewards its source by passing necessary materials back to it” (McClintock 80). Odum gives the example of animals returning minerals to the soil, which then enables plants to thrive and provides animals with food. Without these loop circuits present, there is an enormous amount of risk involved with the depletion of resources, as evidenced by the human extraction of Earth’s resources, which will ultimately leave humans without life-giving materials. Snyder learnt about succession from Eugene Odum and Ramon Margalef. As we have been over taking from the natural system, we have in fact created a dangerous imbalance in ecological systems that will lead to destruction(Snyder, 80). It is clear from Snyder’s poem “Old Rotting Tree Trunk Down,” which he wrote in 1983, that he sees the potential for poetic beauty in this kind of feedback loop in an ecosystem:

... If “meditation on decay and rot cures lust”
 I’m hopeless:
 I delight in thought of fungus,
 beetle larvae, stains
 that suck the life still
 from your old insides...
 Corruption, decay, the sticky turnover-
 Death into more of the
 Life-death same,
 A quick life:
 and the long slow
 feeding that follows-
 the woodpecker’s cry.(Snyder, *Axe Handles* 17–38)

Snyder is drawn to these biological-ecological viewpoints not only because they are accounts of phenomenal nature, but also because of their implications for society, politics, and culture. The “vital materialism” of deep ecology offers him a paradigm for radical ecological politics. Deep ecology embraces biocentrism, the view that life forms are ends



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in themselves, independent of their usefulness to humans. Snyder looks to such eco-centrists as the deep ecologist ArneNaess, an early contributor to the field who recognized the significance of non-human nature for humans and posited biocentrism in its place. According to Naess, individuals “have the right to live and blossom in their own specific way.” For Snyder, this theory put him to focus of environmental politics on defending nature rather than on improving human life.

Opposing the liberal tradition’s emphasis on anthropocentrism, which encourages us to see ourselves as radically separated from the rest of nature, deep ecology argues that we cannot divorce our fate from that of the natural world. Deep ecology emphasizes the non-human in ways that leave less space for the importance of gender, race, class, or other kinds of social difference. Deep ecology proposes that we need to rethink the relationship between humans and nature, and it challenges the western philosophical tradition’s assumption that human beings are radically separated from nature. Eugene Odum writes in *Ecology, the Link Between the Natural and the Social Sciences* that “ecology has grown from a division of biological science to a major interdisciplinary science that links together the biological, physical, and social sciences” (128).

Odum also argues that this new and wide-ranging scientific discipline will cause people to see a strong connection between human societies and the natural world. Environmental issues like pollution and the depletion of resources as well as conventional social issues are of concern to these ecologists. It is not yet clear how deep ecology will affect western science and philosophy, but it is clear that the people who are concerned with these issues want to connect their observations of the natural world with their way of life. Howard Odum (1971) makes the claim that “energy language” can be used to “consider the pressing problems of survival in our time” and that “energy analysis can help answer many of the questions of economics, law, and religion, already stated in other languages”(93).

Snyder is also willing to draw direct parallels between scientific and non-scientific language. Odum suggests that the flow of energy or material through an ecosystem can be described by a simple equation: “Flow in = Flow out”(95). Odum’s equilibrium model explains how new energy inputs in an ecosystem always generate higher energy output. His equation states that the total amount of energy or matter (or its material equivalent) entering a system is equal to the total amount of energy or matter leaving that system. When “ecologists talk about ecology of oak communities,” he writes, we must recognise that “they are communities,” and that oak and human communities share similar characteristics (McClintock 81).

Snyder uses the idea of succession to analyse social change and environmental history. Stable and diverse communities are hallmarks of mature societies. They encourage the exploration and experimentation necessary for new ideas and technologies to emerge, but once this occurs, innovation gives way to increased reliance on status quo institutions. He uses American Indian cultures as examples of these mature cultures, whereas industrialized nations serve as examples of unstable cultures:

In Dr. Eugene Odum’s terms, what we call civilization is an early succession phase; immature monoculture system. What we call the primitive is a mature system with deep capacities for stability and protection built into it. (*The Old Ways* 61). Snyder fights against “monoculture” and advocates for “biological diversity and the integrity of organic evolution on this planet.” The key to preserving biological diversity is for people to realize the necessity and urgency of making changes in their current ways of life. (McClintock 81). According to Snyder, we should recognize plants, animals, “non-human” and “a variety of wild life” as “citizens” in a broader sense of political society. Snyder argues that this recognition should extend beyond humans to include other species in the community and that the survival of other species is equally important to the survival of human beings (*The Old Ways* 65).

He goes on to say that we have “within our deepest powers... to change our culture” (*The Old Ways* 65) through a more inclusive sense of community. When we look at the state of our culture today, it is apparent that this broadened sense of community needs to be further developed and deepened in order for us to survive. Snyder has argued for many years that the way to achieve this is to “re-inhabit” a place, which means integrating knowledge of the





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ecological aspects of a region with the cultural wisdom and understanding of the Indian tribes that formerly lived there. What the Earth teaches us to do is to be sure that our habits are harmonious with ecological realities. And the habits should be productive in terms of human needs and our capacity for happiness. We must make space in our lives and in our communities for spiritual and ethical relations with the natural world.

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Transferosomes: A Promising New Vesicular Carrier for both Lipophilic and Hydrophilic Drug Delivery for Effective Transdermal Drug Delivery

Sopan Suryawanshi^{1*}, Madhu B.K², Manishdev.M¹, Shinde Mahesh³, Kiran Kumar.G.B⁴ and Prakash Goudanavar⁵

¹M.Pharma, Dept. of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar, Karnataka, India.

²Assistant Professor, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar, Karnataka, India.

³Dept. of Pharmaceutics, SVET Pharmacy College, Humnabad, Karnataka, India.

⁴Associate Professor, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar, Karnataka, India.

⁵Professor and HoD, Dept. of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar, Karnataka, India.

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*Address for Correspondence

Sopan Suryawanshi

M.Pharma, Dept. of Pharmaceutics,
Sri Adichunchanagiri College of Pharmacy,
Adichunchanagiri University,
B G Nagar, Karnataka, India.
E. Mail: sopansuryawanshi007@gmail.com



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ABSTRACT

In recent years, interest in transdermal delivery systems has increased because to its advantages over traditional oral and parenteral delivery methods. The vesicular system has assumed a prominent role in a sustained drug delivery system. Transferosomes are vesicular drug delivery devices that increase a drug's ability to penetrate in a non-invasive way. In order to fit through a small pore, they can shrink themselves by a factor of 5 to 10 of their own diameters. Medications with low or high molecular weights can be successfully delivered both superficially and deeply across the skin using transferosomes. Transferosomes are prepared using a variety of techniques, including ethanol injection, freeze-thaw, reverse phase evaporation, vortex/sonication, and film rotary evaporation. The system can be evaluated in vitro for vesicle size and shape, entrapment effectiveness, degree of deformability, and quantity of vesicles per cubic mm, stability study and skin permeation study. Using a particular dissolution media, in vitro release tests must be carried out. They act as a delivery system for drugs with both low and high





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molecular weights, including painkillers, an aesthetics, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin.

Keywords: Transferosomes, Transdermal drug delivery, Transport mechanism, Phospholipids.

INTRODUCTION

Fungus is among the most typical infections that can harm the epidermis, pores, and nails.[1]. This disorder, which impacts toenails significantly more severely than fingernails, affects 5% of the world's population[2]. Areas like the stratum corneum and subcutaneous regions are always affected since they are hot, sweaty, and wrinkled[1]. According to reports, 20% to 25% of people have skin fungal infections. Skin fungal infections are categorized as superficial, cutaneous, and subcutaneous based on the extent of penetration into the tissue [3]. The development of cutaneous fungal infection is caused by parasitic fungus invasion into deeper epidermal skin layer. This illness, also known as dermatomycoses, may also affect the skin's hair and nail follicles.[3]. Many techniques have been used to improve the transdermal distribution of medications, including iontophoresis, micro emulsions, electrophoresis, chemical permeation enhancers, micro emulsions, sonophoresis, and the use of vesicular systems such liposomes, niosomes, ethosomes, and transferosomes. Vesicular systems have drawn attention as a method of delivering drugs in a controlled or sustained manner for many years.

Vesicles are self-improved aggregates with an incredibly adaptive layer, and these called transferosomes. Both low and high molecular weight drugs can be delivered transdermally using a novel type of carrier system called a transferosome, such as leptins, cross-protein components, albumin, anaesthetics, painkillers, corticosteroids, sex hormones, cancer-preventative medications, etc. The word "transferosome" derived from the Latin terms "transfere" and "soma," which mean "to carry across" and "body," respectively. "Specially designed, highly deformable [ultra flexible], lipid supramolecular aggregates called transferosomes can pass through undamaged mammalian skin.[4]. They are metastable forms that produce the incredibly flexible vesicular membrane, which can readily be deformed and squeezed through pores in the stratum corneum. [5]. Professor Cevc from the Technical University of Munich first proposed the transferosomes system, which shows that the membrane has a high degree of deformability. During skin touch, the material's composition is stable and it is not deformable. According to Zellmer, complete transferosomes have exceptional advantage for transdermal administration in blood circulation.[6]. The medications travel from the epidermis to the dermis of the skin before entering lymphatic and blood arteries, where they have a therapeutic impact.[2].

The characteristics of nano-vesicles are influenced by edge activator types like non-ionic surfactants [Span 80 and Tween 80], which have various HLB values [4.5 and 15, respectively]. Due to the hydrocarbon chains' high flexibility and lack of bulk, the vesicles made with Tween demonstrated greater deformability with lower entrapment efficiency. In contrast, the span that contained the vesicle displayed the least deformability and the highest level of entrapment effectiveness.[7]. The structure of the membrane is altered locally and in the opposite direction when transferosome forced against or attracted to a tiny pore. The highly adaptable transferosome molecules exhibit high concentration and high membrane deformation, whereas the less stable molecules are diluted in areas of extreme stress. Lipophilic medications, which have a high catching capability [nearly 90 percent more]. They protect the encapsulated drug from oxidative breakdown.[8]. The main difference between standard and ultra-deformable liposomes is the former's greater hydrophilicity, which allows the elastic membrane to expand more than the latter's conventional lipid bilayer. These vesicles are more resistant to aggregation and fusion under osmotic stress than typical liposomes, which is a worry because of their high membrane hydrophilicity and elastic nature. It was shown that as compared to Tween 80, sodium cholate and sodium deoxycholate produced smaller vesicles with a higher zeta potential. Ultra deformable liposomes made with a 95%:5% [wt/wt]. [Phosphatidylcholine: Edge



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activator]. ratio displayed entrapment efficiency in the following order, according to readings of the associated edge activator's hydrophilic-lipophilic balance: Na cholate > Na deoxycholate > Tween 80 > Span 85.[9].

Lipid-based formulations have the benefit of concurrently encapsulating both lipophilic and hydrophilic active medicinal ingredients within the concentric bilayers and central core. When exposed to an aqueous liquid in direct contact at a temperature above their phase transition temperature, phospholipids self-assemble form vesicles[37]. Thus, Transferosomes function as a warehouse that slow and steady releasing of its contents. Both systemic and topical medication administration are possible with these. [8].

Composition Of Transferosomes [10].

First, the primary component, an amphipathic component [for example, soy phosphatidylcholine, egg phosphatidylcholine, etc.], which is the vesicle-forming component that creates the lipid bilayer and can be a mixture of lipids. The second ingredient is the most widely used edge activators in transferosome preparations, which include sodium cholates, sodium deoxycholate, Tween and Span [Tween 20, Tween 60, Tween 80, Span 60, Span 65, and Span 80]. and dipotassium glycyrrhizinate. These renewable bilayer-softening compounds increase vesicles' ability to transport molecules. Finally, the hydrating medium includes 3-10% alcohol [methanol or ethanol]. and either contains water or a salt pH buffer. [pH 6.5–7].

In a watery setting, phospholipids like phosphatidylcholine self-assemble to create a lipid bilayer and then constrict to create vesicles. An "edge activator," or nontoxic surfactants, a bilayer softening component, is introduced to increase the vesicle's transparency and lipid elasticity. The lipid bilayer is typically destabilised by a single chain surfactant, which increases the flexibility and elasticity of the membrane. Mixing the right surface active substances in the right proportions could change how flexible the membrane of transferosomes is.[11]. As a result, transferosomes are naturally activated to the osmotic gradient through the epidermis upon application in a nonocclusive manner. In conclusion, the size, form, and flexibility of the vesicles, as well as their softness, all have an effect on these vesicles' ability to increase penetration.[10].

Transdermal Drug Delivery System

Transdermal medication delivery has many benefits compared to conventional drug distribution, including the ability to reduce side effects, eliminate hepatic first pass metabolism, and increase patient compliance.[12]. The stratum corneum's permeability properties, which restrict drug transport and often render this mode of intake insufficient for medical use, are a significant barrier to dermal and transdermal drug delivery.[13]. The flexibility required for liposomes is added by nonionic surfactants, creating a novel structure known as a nanotransferosome.[14]. Transdermal drug delivery uses the skin as a point of entry to transfer medication molecules to the body. The goal of the transdermal drug delivery system [TDDS], which falls under the category of controlled drug delivery, is to dispense the medication through the skin at a predetermined and monitored rate[36].

Mechanism of Penetration of Transferosomes

By stretching along the stratum corneum's interior binding lipids, skin penetration is made possible by transferosomes. It is yet unknown how to improve the distribution of active ingredients into and across the skin. There have been two suggested modes of action-[15].

1] Transferosomes act as penetration boosters by dissolving the stratum corneum's tightly packed interfacial lipids, which facilitates the drug molecules' entry into and passage through the stratum corneum.

2] Drugs are transported by transferosomes, which continue to function after penetrating the epidermis.

Colloidal particles, or vesicles, are aqueous compartments surrounded by concentric bilayers consisting of molecules with amphiphiles. They are effective vesicular drug delivery systems that transport aqueous pharmaceuticals housed in the interior water region while covering hydrophobic pharmaceuticals in the lipid bilayer.[16]. Phospholipids like phosphatidylcholine self-assemble to form a lipid bilayer in an aqueous environment before contracting to form vesicles. An "edge activator," or biodegradable surfactants, a bilayer softening component, is introduced to improve the vesicle's porosity and lipid flexibility. The lipid bilayer is typically destabilised by a single chain surfactant,





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increasing the flexibility and elasticity of the membrane. Mixing the right surface active substances in the right proportions could change how flexible the membrane of transferosomes is.[11]. Due to their extreme deformability, transferosomes work with EAs to create a transepidermal osmotic gradient, then squeeze between the cells of the stratum corneum and transport drugs throughout the entire skin.

Features of Transferosomes

- By mixing hydrophobic and hydrophilic moieties in their structure, transferosomes are able to bind to medicinal substances with a variety of solubilities.[17].
- Transferosomes' great deformability allows them to penetrate vesicles more effectively.[18].
- Transferosomes are able to bend and squeeze through openings that are 5–10 times smaller than their own diameter without experiencing any discernible loss. Intact vesicles can penetrate more easily due to the great deformability.[19].
- Drugs, with both low and high molecular weights, such as anaesthetics, corticosteroids, sex hormone, anticancer, insulin, gap junction protein, and albumin, may be transported by them[32].
- As liposomes, they are biocompatible and biodegradable due to their inherent phospholipid composition[33].
- They can be used for systematic and transdermal medication administration, and they have a high entrapment efficiency upto 90% in the case of lipophilic medications[34].
- Simple procedures that don't require a long process, unnecessary use, or additions that aren't allowed in medicine make them simple to scale up[35].

Methods Of Preparation

Rotary Evaporation Sonication/Thin Film Hydration Technique

This method, which was first created by Bangham, is also referred to as the hand-shaking procedure.[20].

- An appropriate [v/v]. ratio of a volatile organic solvents [Chloroform:Methanol]. combination is used to dissolve the phospholipids and edge activator [the components that create vesicles]. in a round-bottom flask.
- At this moment, the hydrophobic medication may be added. In order to produce a thin layer, it evaporates organic solvent using a rotary vacuum evaporator above the lipid transition temperature [40-45°C]. under decreased pressure.[16].
- To completely eliminate any solvent residue, keep it under vacuum. The overall solvent evaporation was accomplished by keeping this thin film overnight [keep it under vacuum].
- After the thin film has been produced, it is hydrated with a buffer solution that has the right pH [for example, pH 7.4]. by spinning it for an hour at the right temperature. [Above the phase transition temperature of phospholipids incorporated like for SPC and DMPC at 30°C, Whereas for HSPC at 60°C]. Drugs that are hydrophilic may be introduced at this time.
- At the ambient temperature, the ensuing vesicles are distended and small vesicles are produced by sonicating the resultant vesicles for 30 minutes at 4°C with a bath or probe sonicator. [21].
- Compression via a sandwich of polycarbonate membranes and the sonicated vesicles are homogenised throughout a width range of 200 nm to 100 nm.[16].

Vortexing Sonication Method

- The medication, edge activator, and phospholipids are mixed in a phosphate buffer. A milky transferosomal suspension is then obtained by vortexing the mixture.
- It is then extruded through polycarbonate membranes after being sonicated for the appropriate amount of time in a bath sonicator at room temperature [example: 450 and 220 nm].[10].
- This technique has also been used to set cationic transferosomes. Cationic lipids, like DOTMA, are mixed with PBS at a concentration of 10 mg/ml before being counted using sodium deoxycholate [SDC].
- The mixture is then vortexed, sonicated, and extruded through a polycarbonate [100 nm]. filter.[20].

Reverse Phase Evaporation Method [21].

- The round bottom flask is filled with phospholipids that have been dissolved in an organic solvent.





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- Under nitrogen purging, edge activator [surfactant]. is introduced to water.
- The solubility of the medicine will determine whether it dissolves in water or an organic solvent.
- After being sonicated, the mixture ought to maintain its homogeneity for at least 30 minutes. It is sonicated repeatedly until the mixture is homogenous.
- The organic solvent is then released under decreased pressure, resulting in its transformation into a viscous gel-like substance and the formation of vesicles.
- Finally, Unencapsulated particles and non-residual solvents are eliminated using centrifugation or dialysis.

Modified Handshaking Method [21].

- The necessary quantities of the drug, surfactant [an edge activator]., and lecithin [a phospholipid]. were placed in the first beaker. The necessary amounts of ethanol and chloroform were transferred to the second beaker in a 1:1 ratio, respectively.
- The first beaker's materials are then mixed with the second beaker's contents.
- In order to removal of organic solvents, the mixture is then allowed to remain for vaporization at a temperature above the lipid transition point.
- The thin lipid layer formed by vaporization is kept overnight to enable the full evaporation of the organic solvent.
- After adding buffer to the thin film, it is gently shaken for 15 minutes.
- The suspension is then further hydrated for one to two hours at 80 °C.

Ethanol Injection Method [21].

- At a constant temperature, the drug is continually mixed into the aqueous medium.
- To a water-based media, ethanolic phospholipid and surfactant solution are introduced drop by drop.
- Lipid molecules precipitate when ethanolic mixes come into contact with aqueous solutions, forming a bilayer foam.

Characterisation Of Transferosomes

Vesicle Size, Size Distribution, Size Diameter, Polydispersity Index and Zeta Potential [22–24].

- Using dynamic light scattering technology, the Malvern Zeta sizer was used to determine vesicle size, size distribution, and zeta potential.
- Applying transmission electron microscopy, the vesicular form is analyzed.
- The diameter of the vesicle is revealed by the photon correlation spectroscopy or dynamic light scattering [DLS]. approach.
- The light scattering method is used to examine the vesicle's size and the distribution of its sizes.
- In order to evaluate the typical particle size and PDI of transferosomes, photon correlation spectroscopy was employed. All samples were appropriately diluted and examined with a He-Ne laser in a thermostatic room set to 25 °C.

Drug Entrapment Efficiency Assessment [17].

An ultracentrifuge was used to spin down transferosomal suspensions for 30 minutes at 20,000 rpm and 10°C. Following this, a UV-Vis spectrophotometer was used to measure intensity after 1 millilitre of the supernatant had been dissolved in saline solution [pH 7.4]. The efficiency of drug entrapment was determined as follows-

$$DEE = \frac{[WT - WF]}{WT} \times 100\%$$

Where WT is the total amount of medication in transferosomal suspensions and DEE is the medication entrapment efficiency. The drug's free concentration, or WF, was discovered in the supernatants.

Drug Content



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Based on the pharmacopeial medication's analysis method, one of the instrumental analytical techniques, such as modified high performance liquid chromatography [HPLC]. using a UV detector, column oven, auto sample, pump, and computerised analysis programme, can be used to determine the drug concentration.[25].

In a volumetric flask, 1 ml of transferosomal preparation was combined with phosphate buffered saline pH 7.4. Phosphate buffer was used to create the volume. Filtered samples were used. After appropriate dilution, the drug content was spectrophotometrically measured at a particular absorbance. Three duplicates of each experiment were carried out.[17].

Penetration Power

Fluorescence imaging can be used to evaluate transfersome penetration.[26].

Turbidity Measurement

A nephelometer can be used to measure the turbidity of a medication in aqueous solution.[27].

Surface Charge And Density

To ascertain the charge density and surface density of transferosomes, apply the zetasizer.

Vesicles per cubic millimetre number:-

This parameter can be used to optimise the transferosome composition and other process variables. 0.9% sodium chloride is used to dilute unsonicated transfersomal compositions five times. This sample is examined using an optical microscope and a hemacytometer. The transferosomes with vesicles bigger than 100 nm can be observed under an optical microscope.

Degree of Deformability or Permeability Measurement [18].

The particle size and size distributions are recorded using Dynamic light scattering [DLS]. measurements following each pass. The deformability testing is conducted against pure water as a baseline.

Studies on *In vitro* Drug Release [28].

Research on the release of drugs *in vitro* is carried out to determine the penetration rate. The formulation is optimised using data from *in vitro* studies, for instance, the amount of time required to reach steady state permeation, the steady-state permeation flux, the information, before more costly *in vivo* tests are conducted. Transferosomes solution is incubated at 32°C for the purpose of evaluating drug release. Samples are collected at various intervals, and the free drug is separated by micro column centrifugation. Using the initial amount [100 percent entrapped and zero percent released]. and the quantity of drug entrapment at zero times, the amount of drug released is then calculated implicitly.

***In-Vitro* Skin Permeation Studies**

- Franz diffusion cells, which resemble diffusion cells, were utilised in the study.
- It has a 50 ml receiver compartment with an effective diffusion area [2.50 cm²]. Goatskin was used in the investigation, which was done in phosphate buffer with a pH of 7.4.
- Goat abdomen skin was procured from the source.
- Hydrated in 0.9% sodium chloride solution, a saline solution.
- A cotton swab was used to rub the fatty layer off.
- The skin was then preserved by being stored in an isopropyl alcohol solution at a temperature of 0 to 40°C.
- The stratum corneum was retained towards the donor end, and the epidermis was connected to the receptor compartment end.
- A magnetic stirrer is used to stir 50ml of phosphate buffer, which is heated to 37°C, at 100 RPM, in the receiver chamber.
- As the investigation was conducted, formulation was applied to the skin and the top of the diffusion cell.





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Physical Stability [29].

In enclosed glass ampoules, formulations were kept, and the efficiency of their early absorption was evaluated. Ampoules were stored for at least three months at 37°F [body temperature], 25°C [room temperature], and 4°C [frozen]. Formulations had their entrapment effectiveness assessed after storage and compared to their initial values.

Application of Transferosomes

- These are respectable drug delivery methods for treating cutaneous cancer that can penetrate the skin's layers.[30].
- It is feasible to deliver high molecular weight medications through mucosal layers.[30].
- Due to the integration of phospholipids, transferosomes have the potential to increase the stability of labile pharmaceuticals and provide a regulated release of the supplied medication.[28].

Insulin Supply [8].

Transfer pharmaceuticals are a useful method for the therapeutic and non-invasive application of these powerful molecular medications to the skin. Insulin is typically given through a painful subcutaneous procedure. These two issues are resolved by transfersulin [insulin encapsulation]. Transfersulin was applied to the unaffected skin, and depending on the carrier substance, the first indications of systemic hypoglycaemia appeared 90 to 180 minutes later.

Delivery of Interferons and Interleukin [24].

Interleukin-2 and interferon- [IL-2 and INF-], preparations containing transferosomes with vesicle sizes smaller than 25 nm are used for transdermal delivery for potential application. They provide an appropriate concentration of IL-2 and INF- for immunotherapy when encased in transferosomes.

Delivery of Corticosteroids[31].

In 2003 and 2004, Cevc and Blume examined the biological properties and biological activity of transferosomes loaded with the halogenated corticosteroid triamcinolone acetonide. The researchers discovered that transferosomes enhanced biological potency, prolonged the impact, and decreased therapeutic dosage.

Delivery of Anticancer Drugs[31].

In a study published in 2018, Jiang et al. investigated the topical treatment of melanoma using paclitaxel-containing oligopeptide hydrogels transferosome-embedded by the thin-film dispersion method. Phosphatidylcholine, Tween 80, and sodium deoxycholate transferosomes have been shown to effectively enter tumour tissues.

Delivery of Herbal Drugs[24].

Drugs made from herbs typically have high molecular weights. Transferosomal vesicles are effective for transdermal delivery of herbal medications. To solve this problem, Xiao-Ying et al. created transferosomes of capsaicin, which exhibit improved topical absorption compared to pure capsaicin. The stratum corneum can be penetrated by transferosomes, which can then deliver nutrients locally to support the stratum corneum's functions and maintain epidermis.

Delivery of NSAIDS[21].

Numerous GI adverse effects of NSAIDS have been reported. Transdermal delivery with the use of ultra deformable vesicles can get rid of them. Efficacy tests for some drugs, such as diclofenac and ketoprofen transferosome formulation, have already been conducted and the Swiss regulatory authority has already allowed the use of this formulation.

Delivery of Protein and Peptides[8,21].

Larger macromolecules like proteins make it challenging to inject them into the body; also, they cannot be taken orally because they would entirely breakdown if they did. When proteins are given via transferosomes, their bioavailability is equivalent to that of proteins injected subcutaneously. After numerous skin challenges and counterpart injections of proteo-transferosomes, as well as after repeated administration of epicutaneous substances





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like adjuvant immunogenic serum albumins, the transferosomal preparations of this protein have produced powerful immune response.

CONCLUSION

When compared to conventional vesicular systems, transferosomes are ultra-deformable carries that are more efficient at transporting a variety of drug compounds through the skin barrier. With its decreased toxicity, biocompatibility, stability and extended controlled distribution of a medication, transferosomes is one potentially innovative drug delivery technique. When a substance is delivered topically or transdermally, these characteristics frequently play a key role. Transferosomes are 1500 times smaller than water, but they can almost as easily travel through pores that are 100 nm wide. They can integrate both hydrophilic and lipophilic medicines with large molecular weights due to their high deformability. They can be utilised to increase the permeability of both high and low molecular weight medicine. To enter the skin through pores, the elastic vesicles self-deform. Compared to high temperature, transferosomes are more stable at low temperature. Drug release in this type of delivery can also be regulated in accordance with needs. As a result, this strategy can fix the issues that arise with traditional methods.

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Table 1: Different Additives Used In Formulation Of Transfersomes

| SR.NO | Class | Examples | Functions |
|-------|-----------------------|--|--------------------------------------|
| 1 | Phospholipids | Soyaphosphatidyl- choline, egg phosphatidyl-choline, di-palmitoyl phosphatidyl choline | Component which forms the vesicles. |
| 2 | Surface active agents | Tween-80, Span-80, sodium cholate, sodium deoxycholate, and Tween-20. | Compound which forms the vesicles. |
| 3 | Solvents | Chloroform, isopropyl alcohol, methanol and ethanol. | Component which serves as a solvent. |
| 4 | Buffering agents | Saline phosphate buffer [Ph 6.4], phosphate buffer pH 7.4 | Good medium for providing hydration. |
| 5 | Dye | Rhodamine-123 RhodamineDHPE Fluorescein-DHPE Nile-red | Component for conducting CSLM. |

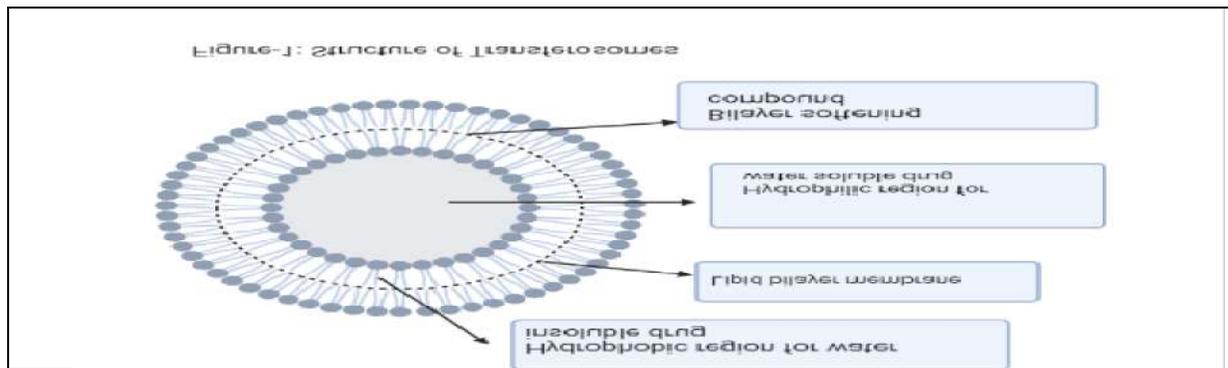


Figure-1: Structure of Transfersomes

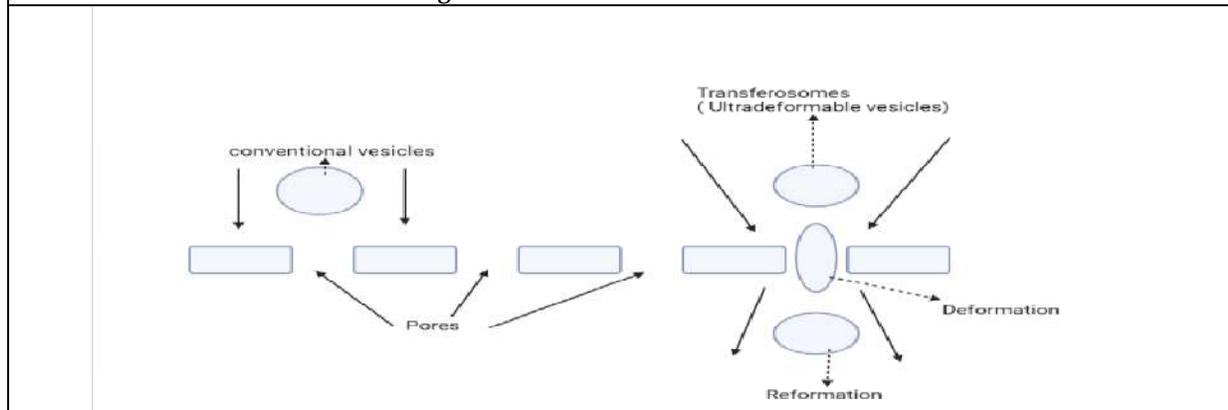


Figure-2 Mechanism of Action





Nanoethosomes for Loading Drugs, Phytochemicals and Peptides in Wound Healing: Formulation Strategy

K.Mekala¹ and S.M.Shaheedha^{2*}

¹Research Scholar, Crescent School of Pharmacy, B. S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur-600048, Chennai, Tamil Nadu, India.

²Associate Professor, Head of Department of Pharmacognosy, Crescent School of Pharmacy, B. S. Abdur Rahman Crescent Institute of Science and Technology, Vandalur-600048, Chennai, Tamil Nadu, India.

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*Address for Correspondence

S. M. Shaheedha,

Associate Professor,

Head of Department of Pharmacognosy,

Crescent School of Pharmacy,

B. S. Abdur Rahman Crescent Institute of Science and Technology,

Vandalur-600048, Chennai, India

E. Mail: shaheedhashabudeen@gmail.com



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ABSTRACT

The intricate process of wound healing involves a series of meticulously coordinated biochemical and cellular events aimed at restoring the integrity of the skin and subcutaneous tissue. Recognized for their diverse active components, accessibility, and minimal side effects, certain plant extracts and their phytoconstituents hold great promise as alternative agents for promoting wound healing. The application of nanotechnological techniques presents an opportunity to augment the effectiveness of herbal treatments and products. In this regard, we delve into the utilization of nanoethosomes as a means of delivering drugs to the skin for the treatment of wounds. Through our review, we explored the use of nanoethosomes as carriers for delivering medications such as peptides, plant extracts, and their phytochemicals. This approach enables improved bioavailability, controlled release via sustained delivery systems targeted at the wound site, and enhanced permeability of these therapeutics to deeper layers of the skin – all of which are crucial factors for facilitating the healing process. Leveraging nanoethosomes loaded with bioactive substances provides a promising avenue for advancing wound healing. By harnessing the potential of these nanocarriers, we can optimize the efficacy and delivery of therapeutic agents, ultimately contributing to the restoration of damaged skin and subcutaneous tissue.

Keywords: Ethosomes, Nanoethosomes, Drugs, Phytoconstituents, Peptide and Formulation Strategy.





INTRODUCTION

Nanoethosomes [1, 2], a type of nanotechnology, have been recently as a potential method of treating wounds. Nanoethosomes are tiny vesicles that can deliver healing cells or chemicals to the location of a wound. Compared to other forms of nanoparticles, nanoethosomes have a larger surface area and lower surface tension, which makes it easier for them to permeate tissue and provide therapy. Because they are more flexible and can fit into tighter gaps than typical nanoparticles [3,4], in vitro tests have demonstrated that these vesicles are better able to target cells and deliver their payloads. Additionally, the cost of manufacturing nanoethosomes is far lower than that of manufacturing with other materials, making this technology more widely available to individuals in underdeveloped nations with strong demands for accessible, cheap healthcare. The advent of nanoethosomes may be an effective way to combat one of the world's most persistent problems wound infection.

Medicinal Plants and Wound Healing

The threat from wounds to the world's economy and health is growing. Among chronic wounds, surgical and diabetic wounds are the most challenging to cure. Additionally, a patient's quality of life may suffer significantly if acute wounds take longer to heal. Loss of mobility and function could result from it. Wound care products are in high demand in both Europe and the USA. Chronic leg ulcers account for the majority of chronic wounds. A wound is the interruption of the cellular, anatomical, and functional continuity of a living tissue. An open wound is one that has been sliced, ripped, or perforated. On the other hand, burn wounds are caused by heat, fire, corrosives, radiation, electricity, or even sunshine, whereas closed wounds are caused by blunt force trauma resulting in a bruise. In order to restore structural and functional tissue integrity and increase the strength of injured tissues, wound healing entails a complicated and detailed series of cellular and biochemical activities. This includes continuous interactions between cells and between cells and the matrix, enabling overlapping phases to take place such inflammation, wound contraction, reepithelialization, tissue remodeling [5], and the creation of granulation tissue with angiogenesis. Usual, the stages of wound healing advance predictably and promptly. If they don't, improper healing may result in a chronic wound called a 'venous ulcer'[6] or a pathological scarring known as a 'keloid scar'[7] The goal of wound care is to promote wound healing effectively in the quickest amount of time with the least amount of pain and scarring for the patient. It must take place in a physiological setting that supports tissue regeneration and repair.

Many natural substances found in medicinal plants have been shown to have wound-healing[8] abilities. Traditional herbal remedies have long been used to treat various skin conditions and promote wound healing. These treatments could often work by cleaning the area, removing dead tissue, and creating an atmosphere that will promote the healing process as it progresses naturally. It's interesting that a large portion of the global population still relies on traditional medicines to manage wounds, even if this is a less expensive choice. Alkaloids, phenolics, tannins, terpenes, steroids, flavonoids, glycosides, and fatty acids are only a few of the useful families of phytochemical chemicals discovered via studies of historically used medicinal plants. These phytochemicals may be useful in the treatment of wounds by promoting blood clotting, preventing infection, and hastening wound healing. A possible method to increase the effectiveness of medicinal plants for wound care is to nanosize them or include them into nanoscale carrier vesicles, all of which are referred to as nanoethosomes. Due to their small size and high surface-to-volume ratio, nanomaterials[9] have distinctive properties, and the nanosizing of medicinal plants can lead to changes in their physical and chemical properties.

Peptide for Wound Healing

The prevalence of chronic wounds [10] associated with diabetes, which is on the rise in the western world, remains a key area of interest for wound care research. Additionally, more than 50% of chronic wounds show symptoms and signs that are in line with localised bacterial biofilms that are at the root of severe infections that cause tissue loss, sluggish wound healing, and other grave problems. The majority of contemporary biomedical strategies for





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advanced wound care focus on giving the open wound antibacterial protection together with a matrix scaffold (typically made of collagen) to aid in the regrowth of skin tissue.

Small Molecules for Wound Healing

Modulation of important signalling pathways involved in tissue repair is the foundation of newly developed small-molecule treatments for wound healing. Wnt proteins, which control embryonic development and cell fate and have also been connected to mammalian cutaneous wound healing, are signalling molecules with a high degree of conservation. They are, Connective tissue growth factor (CTGF)[11], small interfering RNA (SiRNA), vascular endothelial growth factor (VEGF)[12], connective tissue growth factor (CTGF) and Transforming growth factor beta (TGF-β)[13]. Hence, Nanoethosomes used for small molecules to targeted delivery to above signaling pathways in wound healing.

Ethosomes

Touitou et al., 1997 created ethosomes, another unique lipid carrier made of ethanol, phospholipids, and water. They are said to enhance the skin absorption of several medications. An effective permeation enhancer, ethanol is thought to work by interfering with the stratum corneum's intercellular area. These soft vesicles are make a novel vesicle carriers for improved skin absorption. The ethosomes' vesicles can range in size from a few nanometers to many microns.

Nanoethosomes

Nanoethosomes are ethanol-rich, lipid-based vesicular carriers with a small size that are utilised to deliver bioactive compounds deeper into the skin. Phospholipids, ethanol, and water make up the majority of Nanoethosomes. High levels of ethanol in their structure set them apart from other vesicular systems and aid in the release of encapsulated substances into the bloodstream and basal skin layer. Touitou developed ethosomes for the first time in 1996 in order to improve skin penetration. Nanoethosomes' structural details are shown in Fig. 1. A small amount of ethanol may also inhabit the bilayered region of the phospholipid bilayer along with water and the medication molecule. Nanoethosomes are naturally pliable and flexible. The size of nanoethosomes is in the region of nanometers, while the exact size depends on the phospholipid content that is utilised. Another reason why Nanoethosomes are smaller than liposomes made under the identical conditions is that they contain more alcohol. On the surface of the vesicle, ethanol imparts a net negative charge that encourages size decrease. As shown in Fig. 2, nanoethosomes enter the stratum corneum [14] by an intercellular channel. The intracellular space between corneocytes expands as a result of fluidization brought on by ethanol.

Various Formulation Ingredients of Nanoethosomes and Their Role

Nanoethosomes primarily consist of phospholipids, ethanol, and water, as depicted in Table-1. Phospholipids play a vital role in the formation of bilayers due to their hydrophilic head and hydrophobic tail. Commonly employed phospholipids like phosphatidylcholine (PC) [15], soybean phosphatidylcholine (Phospholipon 90), and phosphatidylethanolamine (PE) contribute to the production of nanoethosomes. The distinctive characteristic of the nanoethosomal system is its inclusion of alcohol, which grants it a distinct identification as a vesicular system. Ethanol, abundant in the stratum corneum, greatly influences the malleability and flexibility of nanoethosomes, enabling them to traverse the minute gaps created by fluidization in the stratum corneum. The concentration of alcohol within the vesicular system impacts its diameter, as it imparts a net negative charge to the vesicle's surface, leading to shrinkage. For the development of stable ethosomes, an optimal ethanol concentration range of 30-40% is recommended, while reducing the ethanol concentration to 20% may enhance vesicular size.

Effects of Materials Used on Nanoethosomal System

Ethanol

Ethanol plays a crucial role as an effective penetration booster in ethosomal systems. It imparts distinctive qualities to vesicles in terms of size, Zeta-potential, stability, entrapment efficiency, and enhanced skin permeability. Ethanol



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concentrations within ethosomal systems typically range from 10% to 50%. Several studies have indicated that increasing the ethanol concentration results in a reduction in the size of ethosomes. For instance, Bendas and Tadros found that an ethosomal formulation containing 40% ethanol had a mean vesicle width that was 44.6% smaller than a traditional liposomal formulation without ethanol [17]. However, exceeding the optimal ethanol concentration can lead to increased vesicular leakage, a minor increase in vesicle size, and a significant reduction in entrapment efficacy. High ethanol concentrations can also cause the solubilization of vesicles. Research suggests that ethanol penetrates the ethanol hydrocarbon chain at elevated concentrations, reducing the thickness of the vesicular membrane and consequently decreasing vesicle size. Some researchers propose that ethanol affects the net charge of the systems, resulting in steric stabilization and a reduction in mean vesicle size.

Vesicular charge is a critical factor influencing vesicular characteristics, including stability and vesicle-skin interaction. In ethosomes, the high concentration of ethanol shifts the vesicular charge from positive to negative. Dayan and Tuitou observed that increasing ethanol concentration corresponded to an increase in the negative charge of empty ethosomes. Ethanol acts as a source of negative charge on the surface of ethosomes, preventing aggregation through electrostatic repulsion. Additionally, ethanol possesses stabilizing properties. The efficacy of the ethosomal system is significantly influenced by ethanol, and generally, higher ethanol concentrations enhance entrapment efficiency. This effect holds true for molecules with varying levels of lipophilicity. Ethanol enhances the solubility of lipophilic and amphiphilic drugs, thereby increasing drug loading. This relationship was found to be linear within the range of ethanol concentrations from 20% to 40%. However, at very high concentrations, phospholipids can readily dissolve in ethanol, leading to increased permeability of the ethosomal membrane, which significantly reduces entrapment efficacy. Therefore, optimizing ethanol concentration is crucial during the formulation process, as low concentrations may result in minimal entrapment efficacy.

Phospholipids

The selection of phospholipid type and concentration in the formulation plays a critical role in establishing the ethosomal system, as it directly impacts the size of the vesicles, entrapment effectiveness, Zeta-potential, stability, and penetrating properties. Studies conducted on the synthesis of ethosomal systems for alfuzosin hydrochloride [19] and tolterodine tartrate[20] using different phospholipid types (Phospholipon 90H, 80H, and soy phosphatidylcholine) revealed that the phospholipid type had a significant effect on ethosomal size, but not on entrapment efficiency. Shen et al. found that Lipoid S100 or SPC50 resulted in smaller transethosomes compared to those formed by Lipoid E80, and phospholipids with higher phosphatidylcholine content produced more stable vesicles. In some formulations, cationic ethosomal vesicles were generated using a cationic lipid such as DOTAP, while highly negatively charged ethosomal vesicles were created by incorporating DPPG (1,2-dipalmitoyl-sn-glycero-3-phosphatidylglycerol) in the ethosomal formulation (1,2-dioleoyl-3-trimethylammonium-propane [chloride salt]). In ethosomal formulations [21], phospholipid concentrations typically range from 0.5% to 5%. Increasing the phospholipid concentration can lead to slight or significant growth in vesicle size, but it significantly enhances entrapment efficiency. This relationship holds true up to a certain concentration, beyond which further increases in phospholipid concentration do not have a significant impact on trapping effectiveness.

Cholesterol

Including cholesterol in ethosomal systems enhances the stability and effectiveness of drug entrapment due to its rigid steroid molecular structure. In addition to preventing leakage, it lessens vesicular fusion and permeability. It is typically used at a concentration of 3%, however in some formulations it has been utilised up to 70% of the formulation's total phospholipid concentration. According to a number of studies, cholesterol made ethosomal systems' vesicles larger. When 25.87 mM of cholesterol was employed in the formulation, López-Pinto et al. discovered that the ethosomal size rose from 13642 nm to 23027 nm. According to another study, the rise in cholesterol concentration from 0% to 0.15% w/w led to an increase in vesicular size from 10213 nm to 15212 nm. Cholesterol, however, was found to have no stabilising effects on the ethosomes in only one research. In the formulation, the authors utilised 15% ethanol and phosphatidylethanolamine[22]. According to López-Pinto et al., adding cholesterol to the formulation of the ethosome boosted vesicular rigidity and stability. The enhanced rigidity





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of these multilamellar vesicles (MLVs) prevented them from passing through the stratum corneum, making it harder for the medicine to penetrate the skin, according to in vitro research utilising Franz diffusion cells and confocal laser-scanning imaging. Other researchers also noted that the addition of cholesterol enhanced the rigidity (i.e., decreased elasticity) of the ethosomal vesicles.

Dicetyl phosphate

To avoid vesicle aggregation and improve the stability of the formulation, dicetyl phosphate[23] is frequently employed. Within the ethosomal formulation, it is utilized in varying amounts, typically ranging from 8% to 20% of the total concentration of phospholipids. All ethosomal formulations including dicetyl phosphate, according to Maestrelli et al., produced vesicles with strongly negative zeta-potential. It is still unknown how dicetyl phosphate affects other ethosomal system characteristics.

Stearylamine

In two separate experiments, the positive-charge agent stearylamine was employed in ethosomal formulations. The first experiment utilized an ethosomal system containing mycophenolic acid with a molar ratio of 2:1:1 for phosphatidylcholine, cholesterol, and stearylamine. When stearylamine was added to the ethosomal formulation, several noticeable changes occurred. These included a considerable enlargement of the vesicles, a reduction in the effectiveness of drug entrapment, a shift from a negative to a positive zeta-potential charge, and the aggregation of vesicles within a week. Scientists attributed these effects to the incompatibility between the positive charge of stearylamine and the negative charges present in soy phosphatidylcholine and mycophenolic acid. In the second investigation, the in vitro permeation of two types of ethosome-encapsulated vancomycin hydrochloride [24], with and without stearylamine, was compared. It was observed that negatively charged ethosomes exhibited a higher quantity and transdermal flow of the medication delivered after 12 hours compared to positively charged ethosomes. The reduced permeability of positively charged ethosomes was attributed to the shielding effect of stearylamine and the competition between positively charged stearylamine and positively charged vancomycin hydrochloride. Since stearylamine has a lower molecular weight (296.5 Da) compared to vancomycin hydrochloride (1,458.7 Da), it can easily penetrate the skin. Consequently, stearylamine penetrates the skin more rapidly than vancomycin hydrochloride.

Other Alcohols

Along with ethanol, other alcohols as PG and IPA are also employed in the formulation of binary ethosomes.

Propylene glycol

A common penetration enhancer is Propylene Glycol [25]. It influences the ethosomal features of size, entrapment effectiveness, permeability, and stability when utilised in the manufacture of binary ethosomes at concentrations between 5% and 20%. When PG is incorporated into ethosomal systems, the particle size is further reduced compared to ethosomal systems that do not contain PG. When PG content was increased from 0% to 20% v/v, a noticeable reduction in particle size was achieved, going from 103.70.9 nm to 76.30.5 nm. Zhou *et al.* found that when PG was added to the ethosomal system with an ethanol to PG ratio of 1:1 (total alcohol 45%), the amount of sophoridine, matrine, sophocarpine, and lehmanine that could be captured dramatically increased. According to other researches, the inclusion of ethanol and PG in ethosomes improves the solubility of pharmaceuticals, increasing the effectiveness of drug trapping and enhancing drug distribution within the vesicle. Tacrolimus deposition in the stratum corneum was not significantly different between classical ethosomes and binary ethosomes, according to in vitro drug-permeation tests utilising Franz diffusion cells. Additionally, it was noted that the amount of tacrolimus deposition in the epidermis dropped from 2.230.10 g/m² to 1.480.04 g/m² when the PG concentration in ethosomes was increased from 0% to 20% v/v. Additionally, tacrolimus distribution in the epidermis would not be considerably increased by vesicles containing solely PG at a concentration of 30% v/v. In contrast, the maximum drug disposition in the epidermis was found in ethosomes that only contained ethanol at a 30% v/v concentration.





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As a result, this showed that ethanol had more impacts on stratum corneum permeability than PG. The effects of various weight ratios of PG to ethanol—10:0, 9:1, 7:3, 5:5, 3:7, 1:9, and 0:10—on the in vitro skin deposition of terbinafine hydrochloride ethosomes were examined by Zhang et al. The ethosomal system had 45% v/w of ethanol and/or PG overall. They noticed that raising the PG percentage in the ethosomes reduced the amount of terbinafine hydrochloride that was deposited on skin. This might have happened as a result of the ethosomes' reduced ethanol concentration, which impacted the solubility of phospholipid bilayers. The ethosomes with ethanol:PG at a 7:3.13 ratio had the maximum terbinafine skin deposition. Therefore, it can be inferred that in binary ethosomes, the ethanol:PG ratio should be tuned in order to get better drug permeability. When kept at 4°C, binary ethosomes were discovered to be more stable than traditional ethosomes. Consequently, it is theorized that PG enhances viscosity and anti-hydrolysis properties, thereby enhancing the stability of ethosomes.

Isopropyl Alcohol

Dave *et al.* conducted a study to investigate the effects of a diclofenac-loaded ethosomal system on skin penetration and entrapment effectiveness. Three different formulations were developed: binary ethosomes with 20% IPA and 20% ethanol, a vesicular system containing 40% IPA, and classical ethosomes with 40% ethanol. The findings revealed that the binary ethosomes exhibited lower entrapment efficiency (83.8%) compared to the vesicular system with 40% IPA (95%). In terms of in vitro drug release within 8 hours, the vesicular system demonstrated the lowest release rate (83.2%) in contrast to the binary ethosomes (85.4%) and classical ethosomes (93%). Additionally, the vesicular system exhibited the lowest transdermal drug-flux values through mouse skin (146 g/cm²/h), while the binary ethosomes (159 g/cm²/h) and classical ethosomes (226.1 g/cm²/h) showed higher values. These results indicate that IPA had a notable influence on entrapment effectiveness but had minimal impact on drug release. Further research is necessary to evaluate the effects of IPA or other alcohols on various aspects of ethosomal system characteristics.

Edge Activators or Penetration Enhancers

Since edge activators and penetration enhancers have a significant impact on the ethosomal system's characteristics, choosing the right one is an important step in the formulation of transethosomes.

N-Decylmethyl Sulfoxide and Dimethyl Sulfoxide

A nonionic surfactant called N-Decylmethyl Sulfoxide has been shown to increase the permeability of several amino acids and the pentapeptide enkephalin over the skin of hairless mice. N-decylmethyl sulfoxide was added by Ainbinder and Touitou to a 5-fluorouracil ethosomal system, giving it the designation Tumorep DS (tumor-repressive delivery system). The transethosomes that were created were spherical MLVs with dimensions of 222.218.1 nm. Using the skin of a pig ear, the dermal delivery of 5-fluorouracil from transethosomes [26] and traditional ethosomes was studied. Transethosomes (7.98%) produced a higher level of drug accumulation in the skin of the pig than did traditional ethosomes (3.39%). The skin delivery of transethosomes and the commercially available skin product Efadex were compared in an in vitro investigation (Valeant Pharmaceuticals, Bridgewater, NJ, USA). Efadex and Tumorep DS both had 5% w/w 5-fluorouracil in them. According to the findings, 5-fluorouracil from transethosomes accumulated three times as much in the skin (10.17%) as compared to Efadex (3.75%). A well-known penetration enhancer used in topical medicinal formulations is dimethyl sulfoxide. Instead of using 1% w/w N-decylmethyl sulfoxide in Tumorep DS, Ainbinder and Touitou used 10% dimethyl sulfoxide in transethosomes containing 5-fluorouracil. The in vitro study's findings indicated that compared to conventional ethosomes (38.886 g), transethosomes had 2.3 times lower drug accumulation in the skin and 2.9 times higher drug permeability (109.23 12.35 g) through the skin.

Tweens and Spans

In the ethosomal system, Tween 80 is used at concentrations that vary between 10% and 50% of the total phospholipid concentration. Tween 80 has been found to minimise vesicular size, improve system stability, and improve skin-permeation qualities in ethosomal systems. Tween 80 has an impact on the ethosomal system, mostly because of its solubilizing ability and ability to impede vesicle fusion. In order to create transethosomes of artesunate



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and febrifugine, Shen et al. used Tween 60. The formulations, however, were unstable since after 5 days, needle crystals started to develop in the mixture. Bragagni et al. added Tween 20 to a celecoxib ethosomal system at a concentration of 15% of the total phospholipids. In comparison to transethosomes containing Tween 80 or sodium cholate, it was discovered that those containing Tween 20 had smaller vesicles (258.43.3 nm), higher entrapment effectiveness (54.4%), and improved ex vivo skin penetration into human skin. Tween 20 was added to a formulation in another trial to create an unstable one. Transethosomes that were homogeneous and stable could not be created with spans 80, 60, and 40. In the successful synthesis of transethosomes of caffeine and vitamin E, only Span 20 was utilised.

Oleic Acid

Oleic acid [27] plays a significant role in influencing vesicular size, elasticity, zeta potential, and skin permeation properties by enhancing the fluidity of the stratum corneum. In a study conducted by Song et al., it was observed that transethosomes of voriconazole, containing oleic acid as a penetration enhancer at a dose of 0.5%, exhibited smaller and more elastic characteristics compared to transethosomes containing Tween 80 or sodium taurocholate. Furthermore, transethosomes with oleic acid demonstrated an increased skin permeation rate, negative zeta potential, and improved drug distribution in both the epidermis and dermis. Another study by Ma et al. revealed that transethosomes containing oleic acid (85.38% ± 1.4%) exhibited significantly higher entrapment effectiveness for imiquimod compared to conventional ethosomes (41.93% ± 2.1%). These findings highlight the role of oleic acid in enhancing the properties of transethosomal systems and their effectiveness in drug delivery.

l-Menthol

Ascorbic acid transethosomes were given a 5% l-menthol addition as a penetration booster. Transethosomes containing l-menthol released more drugs cumulatively (36.5%) after 24 hours through a human skin cadaver than traditional ethosomes (33.55%), according to in vitro tests. The medication and l-menthol [28] combined to form a eutectic mixture, which improved drug solubility and changed the stratum corneum's barrier characteristics, according to the scientists.

Sodium Stearate

In the formulation of a 5-aminolevulinic acid ethosomal system, phosphatidylethanolamine, cholesterol, and sodium stearate [29] were combined in a ratio of 2:1:2.5, with the addition of sodium stearate serving to enhance system stability and increase the negative charge on the vesicular surface. The inclusion of sodium stearate resulted in three notable effects: Firstly, the vesicles exhibited a reduction in size from 132.6 ± 1.9 nm to 126.4 ± 2.8 nm, indicating the incorporation of sodium stearate carbon chains into the lipid bilayers. Secondly, the negative surface charge of the vesicles increased from -53.0 ± 0.8 mV to -70.06 ± 1.9 mV. Lastly, the entrapment efficiency of 5-aminolevulinic acid improved, rising from 9.09%. These findings highlight the beneficial impact of incorporating sodium stearate in the ethosomal system, resulting in smaller vesicles, increased negative charge, and enhanced entrapment efficiency for 5-aminolevulinic acid.

Bile Acids and Salts

Deoxycholic acid, a bile acid [30] used in the production of mycophenolic acid transethosomes, has been found to have a significant impact on vesicular size and entrapment efficiency. It has been observed that the inclusion of deoxycholic acid leads to an increase in vesicular size, ranging from 258 nm to 546 nm, along with improved entrapment efficiency. This effect may be attributed to the high interbilayer distance resulting from electrostatic repulsion. Furthermore, deoxycholic acid reduces vesicular aggregation and enhances the stability of the ethosomal system, potentially due to an elevation in the negative surface charge of the vesicles. In the case of transethosomes loaded with imiquimod, sodium deoxycholate bile salt was used at a concentration of 0.8% w/v. This resulted in a substantial reduction in ethosomal size, from 190.6 ± 10 nm to 92.7 ± 8.7 nm, while increasing entrapment efficiency and zeta potential values from -19.9 ± 2.7 mV to -282.4 mV and 41.93% ± 2.1% to 70.86% ± 1.2%, respectively. Sodium cholate was employed in the formulation of transethosomes containing vitamin E and caffeine. The system, with a soy phosphatidylcholine:sodium cholate [31] molar ratio of 3.75:1, resulted in an enhancement of the vesicles'



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negatively charged zeta potential, indicating improved stability. However, another study utilizing a concentration of 0.66% experienced the development of physically unstable vesicles within 24 hours. When preparing voriconazole-loaded transethosomes, sodium taurocholate was used at a concentration of 0.53%. In comparison to conventional ethosomes, conventional liposomes, and transfersomes, the inclusion of sodium taurocholate led to higher negative zeta potential, improved formulation stability, enhanced vesicle elasticity, and increased in vitro permeability through hairless mouse skin. These findings demonstrate the significant impact of bile acids, such as deoxycholic acid, sodium deoxycholate, and sodium taurocholate, on the characteristics and performance of transethosomal systems for various drug compounds.

Polyethylene Glycol 4000

Phosphatidylcholine, cholesterol, and polyethylene glycol 4000 were combined in transethosomes of mycophenolic acid [32] in only one study at molar ratios of 2:1:1 and 6:2:1. The authors claimed that it enhanced vesicular size, but no evidence was provided for its effects on entrapment effectiveness, permeation characteristics, or ethosomal system stability.

Hexadecyltrimethylammonium Bromide

Hexadecyltrimethylammonium bromide [33], a cationic surfactant, was examined by Meng et al. for its impact on the transethosomes of testosterone propionate. They discovered that raising this surfactant's concentration from 0% to 1% w/w reduced vesicle size and changed the charge of the vesicles from negative (-1.220.89 mV) to positive (9.391 mV). It was discovered that changing the zeta-potential of the ethosomal system required only the addition of 0.2% weight per weight of hexadecyltrimethylammonium bromide. They hypothesised that this positively charged zeta-potential would improve the ethosomal system's skin-permeation capabilities as a result of attraction to the negatively charged skin surface.

Cremophor

The trademark name for a group of nonionic polyethoxylated detergents is Cremophor [34]. Cremophor EL-35 was employed in a testosterone propionate ethosomal system at concentrations ranging from 0.5% to 1.5% w/w. It was discovered to decrease vesicular size and boost drug solubility and entrapment effectiveness. Shen et al. used Cremophor RH-40 to simulate the transethosomes of artesunate and febrifugine, however after 5 days, needle crystals had formed because the vesicles were unstable.

Skin-Penetrating and Cell-Entering Peptide

Short interfering RNA (siRNA) [35] and streptavidin are delivered into the skin via direct chemical conjugation by the skin-penetrating and cell-entering (SPACE) [36] peptide, a skin-penetration enhancer identified through phage display. 71 Hyaluronic acid and siRNA were delivered by transethosomes using this penetration enhancer. The transethosomes were given the term SPACE ethosomal system by the authors. Hyaluronic acid transethosome penetration into human skin in vitro was 7.8 x 1.1 times more than buffer solution, 5.9 x 2.5 times greater than buffer-ethanol solution, and 3.2 x 0.6 times greater than classical ethosome penetration. The concentration of the SPACE peptide in the recipe affected how well the SPACE-peptide transethosomes penetrated the skin.

Transethosomes using siRNA were given the name SPACE peptide-decorated cationic ethosomes (DOTAP-SPACE ethosomal system). A cationic lipid (DOTAP), cholesterol, SPACE-conjugated lipids (SPACE peptide - POPE), free SPACE peptide, and SPACE-conjugated siRNA were all included in the formulation and suspended in a 45% buffer-ethanol solution. Three states of the SPACE peptide were present: free form, direct conjugation to siRNA, and direct conjugation to the vesicular surface. Studies on the penetration of siRNA transethosomes (i.e., the DOTAP-SPACE ethosomal system) through pig skin in vitro revealed that this was 6.3–1.7 times more than the penetration of siRNA aqueous solution. An anionic surfactant called sodium dodecyl sulphate [37] was used in the creation of imiquimod and ketoconazole transethosomes at a concentration of 0.8% w/v. The findings demonstrated that sodium dodecyl sulphate dramatically decreased the size of ethosomal systems, improved their ability to penetrate skin both in vitro and in vivo, increased their entrapment efficiency, and increased their zeta potential. The formulation of



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nanoethosomes also incorporates skin permeation enhancers such as polyols (e.g., propylene glycol) and transcucol. Additionally, a small amount of cholesterol is added to maintain the stability of the ethosomal membrane [16].

CONCLUSION

A promising method for delivering of biological molecules, small molecule such as soluble as well as insoluble bioactive compounds and phytoconstituents is the creation of ethanol-based vesicular carriers like Nanoethosomes for wound healing. Drug-related and physiological issues including first pass effect, short half-life, GIT irritation, reduced penetration, etc. can be concealed by ethanol-based carriers. Comparing nanoethosomes to standard liposomes or hydroalcoholic solution, they have demonstrated a higher transdermal flow of several bioactive compounds. For ethanol-based carriers, increased stability is an important factor to take into account because these materials deteriorate as a result of lipid/phospholipid content oxidation. The ideal storage temperature for them is between 4 and 8 °C. The viscosity of ethanolic vesicular carriers may be improved through the formulation of gel, which would lengthen their stay at the application location, such as the skin. Therefore, ethanolic vesicular carriers may be used in nanomedicine [46] to deliver medications with solubility/permeability issues via a transdermal route.

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Table 1: Formation of Various Lipid Vesicular Carrier Systems

| S. No | Additives | Liposomes | Ethosomes | Transethosomes | Examples | Uses |
|-------|--------------|---|---|---|-----------------------|--|
| 1 | Phospholipid | Phosphatidylcholine, phosphatidylethanolamine, etc. | Phosphatidylcholine, phosphatidylethanolamine, etc. | Phosphatidylcholine, phosphatidylethanolamine, etc. | Drug encapsulation | Drug delivery, gene therapy, cosmetics, vaccine delivery |
| 2 | Polyglycol | Polysorbate, polyethylene glycol (PEG) | Polysorbate, polyethylene glycol (PEG) | Polysorbate, polyethylene glycol (PEG) | Doxorubicin liposomes | Enhanced drug delivery, solubilization of |





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| | | | | | | |
|---|-------------|---|---|---|---------------------------|--|
| | | | | | | hydrophobic drugs |
| 3 | Alcohol | Ethanol, isopropyl alcohol | Ethanol, isopropyl alcohol | Ethanol, isopropyl alcohol | Amphoteric in B liposomes | Transdermal drug delivery, topical applications |
| 4 | Cholesterol | Cholesterol | Cholesterol | Cholesterol | Paclitaxel liposomes | Increased stability, improved drug encapsulation |
| 5 | Vehicle | Water, saline solution, aqueous solutions | Water, saline solution, aqueous solutions | Water, saline solution, aqueous solutions | Liposomal doxorubicin | Intravenous drug delivery, targeted therapy |
| 6 | Surfactant | Tween 80, sodium deoxycholate, cetyltrimethylammonium bromide | Tween 80, sodium deoxycholate, cetyltrimethylammonium bromide | Tween 80, sodium deoxycholate, cetyltrimethylammonium bromide | Amikacin ethosomes | Enhanced skin penetration, transdermal drug delivery |

Table-2: Examples of nanoethosome formulation

| Drug | Formulation | Excipients | Evaluation |
|--|----------------------------------|--|---|
| Dimethyl Fumarate [38] (DMF) | Transethosomes (TET) | Soy phosphatidylcholine, polysorbate 80. And ethanol | The results of this investigation showed that TET0.9-DMF vesicles have the right physicochemical characteristics for effective DMF loading and transdermal penetration. Surprisingly, in vitro research revealed that TET0.9-DMF vesicles can enter HaCaT cells and release DMF, improving wound closure. The patch test proved that TET0.9-DMF10-gel, which was especially created based on cell viability research, may be used on the skin safely. These results open the door for more ex vivo and in vivo research targeted at confirming TET0.9-DMF's applicability for the treatment of chronic illnesses like diabetes mellitus or peripheral vascular disease. |
| hydrocortisone 17-butyrate [39] (HB17) | Nanoethosomes, Iontophoresis[40] | Lecithin [41], ethanol and cholesterol | The hot method was used to create nano-ethosomal HB17, which contained lecithin (2.5–20 mg), ethanol (10 ml), cholesterol (0.125–1 |





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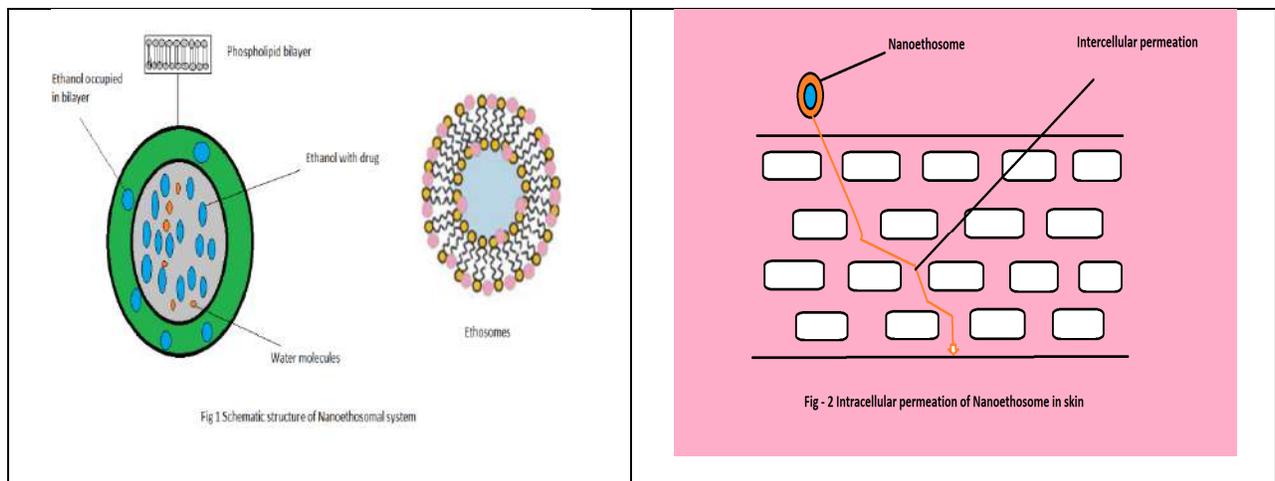
| | | | |
|---|---------------|--|---|
| | | | mg), and water (25 ml). When the formulation was utilised to treat the rat skin with H17B as opposed to free H17B, the findings of the ex vivo test showed a mild increase in the speed of H17B penetration (7.98 g/cm ² in 120 min) (zero at the same time). The combination of ethanol, surfactant, vesicles, and skin lipids works synergistically to speed up the penetration of ethosomes into the skin. |
| <i>Achillea millefolium</i> L. Extract[42] | Nanoethosomes | Methanol, ethanol, n-Hexane, and Propylene glycol, 1,1-Diphenyl-2-picrylhydrazyl and TRITON X-100 Phospholipid | The AM ethanolic extract shown substantial phenolic and flavonoid concentrations of up to 123 mg GAE/g and 42 mg QE/g, respectively, and 88% free radical scavenging activity. The optimised nanoethosomes had a surface charge of about 31.1 mV, were spherical in shape, and had a high entrapment effectiveness of 90% when enclosed in AM extract (240 nm). Furthermore, during the course of the investigation, the chosen topical gel remained steady. The maximum release percentage of 79.8% was observed in the Exvivo permeation investigation of ethosomal gel. |
| Intranasal In Situ Gel of Apixaban-Loaded Nanoethosomes | Nanoethosomes | Cholesterol (Chol), Potassium dihydrogen orthophosphate anhydrous [43], dipotassium hydrogen orthophosphate anhydrous, Standard cellophane membrane (molecular cutoff range = 12,000), Poloxamer 407 [44] (P407) and Poloxamer 188 (P188) Carbopol 934 | The nasal delivery of the anticoagulant medication apixaban's ethosomal thermoreversible in situ gel. Lecithin, cholesterol, and ethanol were combined to generate ethanosomes utilising the thin-film hydration approach. Using P407 and P188 as thermoreversible agents and carbopol 934 as a mucoadhesive agent, the ethosomal formula was integrated into gel. The influence of independent variables on gelation temperature, mucoadhesive strength, and in vitro cumulative percent medication released at 12 hours was studied using a box-Behnken design. The formulation, which was tested for compatibility, ex vivo permeation, nasal mucosa histopathology, and in vivo research, was spherical with an |





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| | | | |
|------------------------------|---------------|--|---|
| | | | average size of 145.1 nm, a zeta potential of -20 mV, an entrapment efficiency of 67.11% nm, and an in vitro% release of 79.54% nm. The pH and medication concentration of gel formulations were acceptable. For gelation temperature, mucoadhesive strength, and in vitro percent released, the ideal gel gave 32.3°C, 1226.3 dyne/cm ² , and 53.50%, respectively. Ex vivo penetration of in situ gel through the nasal mucosa was greater (1.4990.11 g/cm ² h) than that of pure apixaban gel. According to a histopathological analysis, ethosomal gel did not cause any necrosis or tearing of the nasal mucosa. |
| DOTAP-SPACE ethosomal system | Nanoethosomes | A cationic lipid [45] (DOTAP), cholesterol, SPACE-conjugated lipids (SPACE peptide - POPE), free SPACE peptide, and SPACE-conjugated siRNA | For wound Healing |





Common Risk Factors Involved in Pathogenesis of Alzheimers and Age Related Macular Degeneration

Aiswarya G Nambiyar¹ and Brindha Durairaj^{2*}

¹Research Scholar, Department of Biochemistry, PSG College of Arts and Science, Coimbatore- 641 014, Tamil Nadu, India.

²Professor and Principal, Department of Biochemistry, PSG College of Arts and Science, Coimbatore- 641 014, Tamil Nadu, India.

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*Address for Correspondence

Brindha Durairaj^{2*}

Professor and Principal,
Department of Biochemistry,
PSG College of Arts and Science,
Coimbatore- 641 014, Tamil Nadu, India.
E.Mail: publicationbiochemistry@gmail.com



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ABSTRACT

Age-related macular degeneration and Alzheimer's disease are two neurodegenerative conditions that are frequently seen in older people. Macular degeneration causes aberrant vision, but Alzheimer's disease causes cognitive repair. They share clinical and pathological characteristics, such as oxidative stress, mitochondrial dysfunction, lysosomal dysfunction, and protein aggregation, although having diverse etiologies. It is believed that the presence of amyloid protein in the brain and drusen makes the two diseases related, however no correlation has been found beyond the clinical and pathological characteristics but the presence of one condition is found to worsen the other. As a result, a review of risk factors that frequently contribute to this condition is presented in light of the literature.

Keywords: Alzheimer's Disease, Age Related Macular Degeneration, Genetic Risk Factors, Oxidative Stress, Mitochondrial and Lysosomal Dysfunction, Other Risk Factors.

INTRODUCTION

Cognitive abilities deteriorate as a result of Alzheimer's disease, a degenerative and irreversible brain condition. Alzheimer's disease was initially identified in 1906 when clinical psychiatrist Dr. Alois Alzheimer discovered aberrant protein deposits in the brain [1]. The leading factor in adult-onset dementia is thought to be Alzheimer's disease. It is found to effect 10% of individuals aged 65 or over [2]. Both tau and amyloid protein, which form tangles



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inside the brain and plaques surrounding it, are the types of protein deposits that are discovered there. Alzheimer's disease is thought to be characterized by plaques and tangles in the brain. The gene that codes for the amyloid protein is located on human chromosome 21. It is a tiny fragment of a membrane-associated glycoprotein. Another symptom is the brain's loss of connections between nerve cells, or neurons, which first manifests in the hippocampus, the area of the brain that is crucial for creating memories. Additional brain regions are also impacted as neurons die. As Alzheimer's progresses, the Brain severely shrinks and there is an extensive damage. Age-related macular degeneration is an eye condition that primarily affects older people. In those between the ages of 65 and 70 (9 to 25%) and those beyond 70 (>80%), it may cause severe or irreversible eyesight loss [3]. The core region of the retina known as the macula is where the issue manifests itself. Although it seldom results in blindness, macular degeneration, a gradual loss of the macula, may cause significant vision problems. The dry and the moist forms of age-related macular degeneration are the two basic types. Yellow deposits known as drusen are frequently found in macula in dry form. A increase in drusen can exacerbate the situation by weakening the light-sensitive cells and causing them to eventually perish. In the wet form, blood vessels develop from beneath the macula and may leak blood and liquid into the retina, eventually developing a scar that results in a loss of central vision that is irreversible. Images that are vague, distorted, or dark may result from macula damage. The most prevalent risk factor for both AMD and AD is ageing. Both AMD and AD have oxidative stress and inflammation as common clinical characteristics in their development. In cells like retinal pigment epithelial and neuronal cells, protein aggregation can be triggered in response to these conditions. Clearance of damaged cellular proteins within cells gradually decreases during aging.

RISK FACTORS IN AMD AND AD**Genetics Association in AMD and AD**

Genetic risk factors associated with AMD and AD have been reported in several studies. Numerous populations exhibit correlations between AMD and AD by Y402H polymorphism in the complement factor H gene[4]. AMD has been associated with the high temperature requirement factor A1 (HTRA1) in the general population [5]. But HTRA1 polymorphism has not been associated to AD, according to reports [6]. The important lipid transporter apolipoprotein E even helps to heal brain damage. The pathophysiology of two illnesses is influenced by various apolipoprotein E alleles through various mechanisms. People who possess the Apo E4 allele are more likely to get Alzheimer's disease, whereas people who carry the Apo E2 allele have a lower chance of developing the condition. The presence of Apo lipoprotein E alleles in macular degeneration occurs in a different way, such that Apo E4 allele carriers are in lower risk compared to the individuals carrying Apo E2 allele [7]. A recent study found that beyond the structural variations of ApoE it is also a important risk factor in AMD[8]. The presence of Complement Factor (H1277C allele) in the patients may predispose them for comorbidity in AD and AMD[9]. However, many genetic polymorphism can be noticed in AD, of which the polymorphism of Apo E, PICALM and CLU genes show the highest risk in AD[10]. The Apolipoprotein B bind to LDL and act as ligands in the receptor mediated endocytosis of LDL. The LDL degraded by lysosomes release the cholesterol. A central role of Apolipoprotein B is found in atherosclerosis. Under the retina 37 to 44% of the esterified cholesterol is found in drusen[11]. Many studies have shown that development of pathogenesis in AD is related to non genetic or environmental factors which trigger at faster rate. Based on a study conducted individuals at the advanced stage of AMD have shown an increased risk to AD, but by adjusting the risk factors the risk can be decreased[12]. These findings suggest that AD and AMD demonstrate similar mechanism of pathogenesis mechanism but vary in the genetic risk factors.

Oxidative Stress

One of the major risk factor in AMD and AD is oxidative stress. It is caused due to the imbalance between free radicals and antioxidants. During any diseased condition, an increase in the reactive oxygen species leads to oxidative stress. Oxidative stress plays a major role in aging and this has been reported as the major risk factor in the pathogenesis of AD and AMD. The cells with high oxygen demand are more prone to hypoxia, which increases oxidative stress. The cerebral blood flow during AD and AMD is decreased by 20%[13]. Increase in oxidative stress leads to the activation of β amyloidase pathway which decreases in the α -amylase pathway (non amyloid pathway) leading to the excessive production of amyloid β protein. An increased plasma malondialdehyde and nitric oxide



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levels in AMD, act as an indicator of oxidative stress[14]. Reports have shown that a decrease in superoxide dismutase I leads to pathology of AMD[15].

Mitochondrial Dysfunction

Neurons and the retinal epithelial cells have a high energy requirement hence any injury or defect in mitochondria can lead to the cell death. Mitochondrion plays a major role in the iron/sulfur cluster, amino acid synthesis and fatty acid metabolism. Mitochondria is the primary site of ATP synthesis through oxidative phosphorylation which plays a major role in many biochemical pathways and are also involved in ion homeostasis. The electron transport performed by macromolecular proteins and their subunits are made up of both mitochondria and nuclear DNA. And the main complexes for respiratory chain are complex I and III. The two important mechanisms that maintain mitochondria's growth, shape, structure and distribution of mitochondria are mitochondrial fission and fusion. In mitophagy and apoptosis a significant role of mitochondrial fission and fusion can be found[16]. Although mitochondrion is involved in synthesis of ATP, it produces ROS like factors that are produced as byproducts which might increase mitochondria dysfunction. Many findings have shown that impairment in calcium influx, altering mitochondrial membrane potential, deficiency in mitochondrial oxidative phosphorylation and DNA are significant changes that occurs in cells during the late neurodegenerative disease[17]. Similar to AD, mitochondrial dysfunction can also lead to Retinal Pigment Epithelial cell death. It has been reported that a decrease in oxidative phosphorylation in the individuals with AMD as compared to that of normal individuals[18]. A bioenergetic crisis is the major factor driving the AMD pathology[19]. Identifying compounds that would modulate mitochondria dysfunction can be better methodology for testing both AMD and AD.

Drusen Vs Amyloid Plaques

In neurons, there is a transmembrane protein termed APP, which upon proteolysis produces A β peptides. AD may result from an APP mutation. In most cases, the α -secretase cleaves the APP leading to the non amyloidogenic pathway, but in a few rare cases, cleaves the APP leading to the amyloidogenic pathway, which causes the production of amyloid plaques, or clusters of protein, in the brain in AD. In AMD, drusen, which are extracellular protein deposits, are present in the retinal pigment epithelium and bruch membrane[20]. Acute phase proteins, proteoglycans, complement elements, apolipoprotein, proteases, metal ions, lipids, cholinesterase, polysaccharides, and subunits of ATP synthase make up this substance[21]. The presence of APP were found using immunoblotting in drusen which indicate the relationship [22]. Drusen were discovered to have more esterified cholesterol, indicating problems in cholesterol metabolism, a risk factor for Alzheimer's disease [23]. The non fibrillar oligomers are the amyloid type most frequently detected in drusen [24]. Drusen were shown to include complement components, acute phase reactants, immune modulators, and other inflammatory mediators that are present in amyloid deposits [25]. Amyloid plaques exhibit tinctorial characteristics such as Congo red and Thioflavin T staining[26]. Even though drusen stain Thioflavin T and congo red staining, the apple green birefringence are not observed in congo red stained amyloid fibrils [27].

Complement system in AMD and AD

Enzymes and regulators involved in the innate immune defence mechanism make up the complement system. Complements are produced by numerous endothelial, monocyte, epithelial, neuronal, and glial cells as a defensive mechanism. The complement performs a variety of biological functions, such as regulating inflammatory and chemotherapeutic responses, removing immunological complexes, activating cells, and defending against microbes. There are three main pathways involved in complement activation: the classical, lectin, and alternative pathways, that lead to the activation of the third component of the complement system, C3, starts the creation of terminal complexes called c5b9, which eventually results in the lysis of the target cell. An essential regulatory protein known as complement factor H is involved in the aetiology of AMD [28]. It prevents C3 activation and binds to CRP, which may prevent tissue damage caused by CRP-dependent activation. High risk of AMD is observed in complement factor H (Y402H) polymorphism[29]. Role of complement system seen increased during AD with risk factors and the neuronal death[30].



**Aiswarya G Nambiyar and Brindha Durairaj****Other risk factors**

The oxidant released from the mitochondria diffuses into lysosomes, which are abundant in cysteine and redox active ions, contributing to lipofuscin production. Different iron-containing proteins that are degraded in the photoreceptor outer segment disc and from which lysosomal iron arises[31]. The hydroxyl radicals are created when lysosomal iron and mitochondrial hydrogen peroxide combine. In the lysosomes, certain oxidation products polymerize and transform into lipofuscin, which is not degradable[32]. Lipofuscin, which can incorporate transition metals, is thought to be cytotoxic. Their presence can, however, modify cellular components and enhance radical production.

By the process of oxidation, the structural and functional integrity of the proteins are lost. During this process the proteins expose their hydrophobic molecules towards the aqueous environment which results in the accumulation and aggregation of oxidized proteins. The presence of aggregated proteins is observed in both AD and AMD pathogenesis. During aging the damage or mutation to proteins can lead to malfunction of proteins. The ubiquitin proteasome system removes the improperly folded proteins and certain chaperons directly or indirectly and interacts with proteasome to remove certain proteins under stress condition. Even under chronic oxidative stress Retinal Pigment Epithelium cells have the ability to thrive due to vitamin E derived visual cycle metabolism. During aging the capacity of normal processes and the functioning of proteosomal activity are decreased leading to cell damage[22].

CONCLUSION

This review discusses similarities of risk factors in AD and AMD. The APP, PSEN1 and PSEN2 genes' mutations, together with a number of other risk factors trigger the pathology of Alzheimer's disease. Although the exact cause of age related macular degeneration is unknown, various risk factors are found to be associated, which includes smoking, obesity, family history, diet, high cholesterol and high blood pressure. One similarity between the two is the prevalence of aging. In comparison to genetic risk factors, no similarities were found in both diseases but at advanced stage of AMD increased risk of AD are noticed due to the alteration of other risk factors. In addition managing risk factors can decrease the prevalence of AD. The dysfunctions of mitochondria, lysosomes and protein function and clearance have shown increase in risk of AD and AMD. The oxidative stress and other risk factors like complement pathway, presence of amyloid etc are found to work in a similar pathological pathway increasing the risk of diseases.

CONFLICT OF INTERESTS

The authors declare no conflict of interest in this study

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None

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Utilization of Diets Amended Probiotics for the Growth Performances and Immune Responses to Infection with *Vibrio harveyi* in *Litopenaeus vannamei*

Venkatesan. M^{1*}, Sudhakar.S² and Jayaprakash. R¹

¹Research scholar, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608502, Tamil Nadu, India.

²Research Guide, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai – 608502, Tamil Nadu, India.

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*Address for Correspondence

Venkatesan. M

Research scholar,

Centre of Advanced Study in Marine Biology,
Faculty of Marine Sciences, Annamalai University,
Parangipettai – 608502, Tamil Nadu, India.

E.Mail: eshaayy2013@gmail.com



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ABSTRACT

The experiment was conducted to examine the effects of dietary administration of commercially available probiotic, Zymetin (ZY) on growth performance and immune responses of white shrimp (*Litopenaeus vannamei*). Shrimp (0.31 ± 0.04 g) was fed with basal diet (control without challenge test (-) and control with challenge test (+)), supplemented with 2.5%, 5.0%, 7.5%, and 10.0% Zymetin was encapsulated with commercial feed (CP Aqua) for 30 days. Five shrimp were sampled from each tank after the feeding experiment to determine bacterial levels and immunity. Finally all the shrimp were challenged by *V. harveyi* infection for 7 days. Shrimp week growth rates and food conversion ratios were significantly better in the ZY treatment after 30 days than in the control group. A higher Total Haemocyte Count (THC) and phenoloxidase activity were also measured in all ZY treatments than in the control group. Following a co-infection challenge test, probiotic treatments had higher PO, THC, and Respiratory Burst (RB) activity than the control (+). Then, the lowest survival rate of shrimp was observed in the control (+), whereas higher survivability was observed in Zymetin treated groups.

Keywords: Shrimp culture; *Litopenaeus vannamei*, Probiotics, Zymetin, *Vibrio harveyi*.





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INTRODUCTION

Aquaculture production has increased dramatically in recent years, with Penaeid shrimps being one of the most significant cultivated species worldwide, particularly in Asia, due to their high economic worth and exportability [1]. More than 5 million metric tonnes of prawns are produced each year, but the current global demand for both wild and farmed prawns is more than 6.5 million metric tonnes per year [2]. Nowadays, despite high levels of shrimp production by culture, shrimp farmers have suffered significant economic losses due to disease problems that have plagued the industry. The continuous outbreak of the White Spot Syndrome Virus (WSSV) disease in *Penaeus monodon* culture has led to a loss of shrimp culture in India. As a result, farmers are actively seeking alternative shrimp species for culture. In 2008, the Coastal Aquaculture Authority (CAA) of India introduced *Litopenaeus vannamei*, a new shrimp species, as an alternative Penaeid species for culture and export. *Litopenaeus vannamei* exhibits a fast growth rate, and its culture period is significantly reduced compared to *Penaeus monodon*. Therefore, *Litopenaeus vannamei* has been established as an alternative to *Penaeus monodon* for shrimp farming in several countries, including East, Southeast, and South Asia [3].

The spread of infectious disorders, especially those brought on by viruses and bacteria, is one of the issues with white prawn intensive farming. *Vibrio harveyi*, a bacterium, was the source of the bacterial sickness that affected the white prawns [4]. Around the world, *Vibrio harveyi* has been identified as a dangerous infection for a variety of aquaculture organisms [5]. The condition has been managed using a variety of strategies. The use of antibiotics or other chemicals in aquaculture may increase the danger of infections that are resistant to antibiotics [6]. In order to prepare prawns for innate immunity by boosting their resistance to infections, probiotics were administered as a disease-controlling alternative. Probiotics are seen of as a live microbial food supplement that enhances the host's and their culture environments' microbial balance and wellness [7]. Probiotics significantly enhance disease resistance and modulate gastrointestinal microbiota in shrimp [8], lobster [9], and sea cucumber [10], parrot fish [11] and koi [12].

The present study aimed to determine the most optimum concentration of probiotic, Zymetin on the growth performance, immune response and disease resistance to infection with *V. harveyi* of shrimp (*L. vannamei*).

MATERIALS AND METHODS

Probiotic and Diet preparation

Commercially available probiotic, Zymetin (ZY) was used in this study. Two hundred milliliter of the ZY with rice bran, tapioca flour, sugar and yeast were added to 200 L of freshwater and left overnight with vigorous aeration. After fermentation, the slurry was applied evenly in the ponds. The dosage of the probiotic was increased as the culture days increased. four experimental diets were prepared with different concentration of probiotics supplemented with 2.5%, 5.0%, 7.5%, and 10.0% was encapsulated with commercial feed for 30 days. Diets were then stored in clean plastic bags at 4°C until use.

Culture condition

A commercial hatchery in Sirkazhi, Tamil Nadu, India provided the experimental post-larval vannamei (PL) prawn, which was reared there for 21 days before testing. Shrimp with a mean initial body weight of 0.33 g and a density of 15 per tank were randomly placed in triplicate tanks (30 35 40 cm; volume 0.3 m³). The shrimp were fed four times a day until they reached their target weight, with 10% of the total biomass in each feeding. Water temperature was maintained at 27-29°C; salinity ranged from 29-32‰; pH 7.4-7.5 and dissolved oxygen (DO) between 4.5-6.5 mg/L as well as ammonia nitrogen levels less than 0.016 mg L⁻¹ inside culture vessels





Growth and feed conversion ratio

After 1 month, the total number of prawns was counted and the mean body weight was calculated. Based on this weight of shrimp and the quantity of shrimp counted, the Daily Growth Rate (DGR) and Feed Conversion Ratio (FCR) were determined using the following equations:

$$DGR(\%) = (\sqrt[t]{W_t / W_o} - 1) \times 100 \quad FCR = \frac{F}{B_t + B_m - B_o}$$

where,

DGR is the specific growth rate in weight (% g),

W_t and W_o are shrimp weight at current time (t) and at the beginning of the experiment (o), respectively,

t is the number of rearing days (day).

FCR is food conversion ratio,

F is the total dry food consumed (g) and

B_t , B_m and B_o is the total biomass of the shrimp (g) at current time (t), due to mortality of shrimp (m) and at the beginning of the experiment (o), respectively, whereas t is the number of rearing days (day).

Challenged test

The bacteria *Vibrio harveyi* was procured in CAS in Marine biology, Parangipettai, Tamilnadu, India. For the challenge test; ten shrimp were injected with a dose of live *V. harveyi* bacteria equal to 10^3 CFU per tank from a 24 h culture grown in ZMA medium at 29°C. The shrimp mortality rate was observed for 7 days following injection

Immune parameter assay

Using a syringe filled with anticoagulant solution, hemolymph was drawn from the ventral sinus. The identical samples obtained from the five prawns kept in the same tank were combined and utilized as a single batch. The anticoagulant solution for haemolymph (10 mM EDTA, 340 mM NaCl, 30 mM trisodium citrate, and pH 7.0). A 1:2 (v/v) anticoagulant solution was extensively incorporated into the haemolymph. Shrimp haemolymph samples were taken twice, once after 30 days of feeding (before the challenge test) and once after the challenge test with *V. harveyi* was completed (7 days later). The THCs were determined using a haemocytometer at 100 times magnification. Cells were counted on both sides of the grids. THCs were calculated using the following equation:

$$THC = \text{Cells counted} \times \text{dilution factor} \times 1000 = \text{Volume of grid (0.1 mm}^3\text{)}$$

According to Liu and Chen [13], phenoloxidase activity was measured. Briefly, 50 L of haemolymph were combined with 50 L of 0.1% trypsin in CAC buffer, which was incubated at 25°C for 10 min. After that, 50 L of L-DOPA (0.3% in CAC buffer) were added, mixed, and the ideal density was assessed at 490 nm. An increase of 0.001 min/mg protein in absorbance was used to measure one unit of enzyme activity.

The method of Song and Hsieh [14] was used to evaluate the Respiratory Burst (RB) activity of haemoglobin by reducing NBT (nitrobluetetrazolium) as a gauge of superoxide anion (O_2^-). After being incubated for 30 minutes at room temperature, a total of 300 mL of the haemolymph-anticoagulant combination was centrifuged for 20 minutes at 3000 rpm, with the supernatant being discarded. After that, 100 mL of NBT (HBSS solution containing 0.3% NBT) was added, and stand for 2 hours at room temperature. The mixture was centrifuged at 3000 rpm for 10 min, the supernatant was collected, and 100 mL of 100% methanol was added. This was followed by another 10 min at 3000 rpm centrifugation, with the supernatant being discarded. Then, two times, 70% methanol was used to rinse the formed pellets. The next 120 mL KOH (2 M) and 140 mL of dimethyl sulfoxide (DMSO) was added to dissolve the pellet. Insoluble pellet was then inserted into the microplate to measure Optical Density (OD) using a microplate reader at a wave length of 630 nm. Respiratory burst expressed as NBT reduction per 10 μ L haemolymph.





Statistical analyses

Results are presented as Mean \pm SD (standard deviation of means). Data analysis was done with statistical analysis methods at a 95% confidence interval ($\alpha = 0.05$). Statistical analyses were performed using a statistic program SPSS (version, 17.0). One-way analysis of variance (One-way ANOVA) was used to determine significant differences between the treatments followed by Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Growth and feed conversion ratio

Supplementation of ZY appears to increase the WGR and decrease the FCR as compared to the controls. The highest DGR (9.94 \pm 0.18%) and the lowest FCR (1.42 \pm 0.05), respectively observed in 7.5% ZY treatment and was significantly different than control groups ($p < 0.05$; Fig. 1). The 2.5% ZY treatment has no significant difference in growth performances compared with the control groups ($p > 0.05$). Similar findings were made for the European lobster (*Homarus gammarus* L.), which when fed a probiotic diet comprising a combination of *Bacillus* spp. and MOS showed considerably higher weight gain, SGR, and FCR [9]. *Bacillus subtilis* and fructo oligosaccharide (FOS) were shown to improve growth performance in juvenile large yellow croaker (*Larimichthys crocea*) by Ai et al. [15].

Immune response

Total Haemocyte Count (THC) of shrimp fed on different doses of probiotic is shown in Fig. 2. After 30 days of culture, THC increased significantly in the group treated with probiotic 7.5% (C) compared with control groups. THC of shrimp from the groups fed ZY supplemented with 2.5%, 5.0%, 7.5% and 10.0% were higher ($p < 0.05$) compared control (+). Shrimp fed with the control positive diet produced the lowest THC. According to Rodriguez and Le Moullac [16], Smith et al. [17], and Hauton [18], the haemocyte produces inflammatory-type reactions such as phagocytosis, haemocyte clumping, generation of reactive oxygen metabolites, and release of microbial proteins. After the challenge test, THC levels in all prawn treatments decreased. The body's defence mechanisms, including phagocytosis activity, encapsulation, nodule formation, and the degranulation process for system prophenoloxidase activation (ProPO), had an impact on the reduction of cells' hemocytes following the challenge test [17].

Phenoloxidase (PO) activities in haemolymph showed increasing tendency with the increasing doses of ZY in diets (Fig. 3). The shrimp in treatment 7.5% ZY and 10% ZY produced the highest PO activities with, followed by shrimp fed with treatment 5.0% ZY and they were significantly higher than the control groups (Fig. 3; $p < 0.05$). After being given the challenge test with *V. harveyi*, PO activities in control (-), control (+) and all treatments showed an increase. According to data, PO activity of shrimp before challenge test known to be positively correlated with the value of THC. Hemocytes of shrimp function within the production and release of PO into hemolin in the form of inactive pro-enzyme called ProPO [17]. After being given a challenge test all treatments showed an increase. Increased activity of PO indicates a high immune response of shrimp. Lesmanawati [19], stated that an increase in PO activity occurs on the fifth day post-infection IMNV and shrimp on synbiotic treatment resulted in an increase in PO was higher than the control.

In this study, respiratory burst activities (RB) for all treatments were presented in Fig. 4. After feeding trial for 30 days, RB activity in Haemolymph was not significantly different between probiotic treatments and controls (Fig. 4; $p > 0.05$). In contrast, RB activity after challenged with *V. harveyi* significantly increased and was significantly different with controls (Fig. 4; $p > 0.05$). According to Rodriguez and Le Moullac [16], respiratory burst (RB) is an oxygen-dependent killing mechanism used by phagocytic cells to rid themselves of foreign particles. According to the findings, probiotic therapy does not boost RB activity; rather, *V. harveyi* infection is to blame for the rise. Numerous investigations [11, 20] revealed that the treatment of probiotics had no discernible effect on RB activity.





Survival

Shrimp survival after the 30 days of experimental period was similar in all experimental groups. Shrimp survivals after challenged with *V. harveyi* significantly increase as the dose of dietary probiotic in diet increased (Fig. 5; $p < 0.05$). Shrimp fed with the control positive diet produced the lowest survival and were significantly different ($p < 0.05$) from the groups fed synbiotic supplemented with ZY 2.5%, 5.0%, 7.5% and 10.%. This probiotic had significantly reduced mortality of the shrimp challenged by infection with *V. harveyi* and also stimulate immunity of the shrimp. In the previous study, probiotic SKT-b had been reported that probiotic may enhance resistance of shrimp (*L. vannamei*) to *V. harveyi* [21]. Similarly, the result has been reported that SKT-b is known to inhibit the growth of *V. harveyi* pathogens effectively both *in vitro* and *in vivo* on tiger shrimp (*Penaeus monodon*) larva [22]. The high resistance of survival shrimp might be caused by enhanced of innate immunity. Results of this study showed that probiotic, Zymetin (ZY) in shrimp diets can significantly improve growth and disease resistance by enhancing immunity, as well as presumably modulating microflora in the shrimp's gut. The results showed that the most optimum dose of dietary probiotic in this study was shrimp 7.5% ZY.

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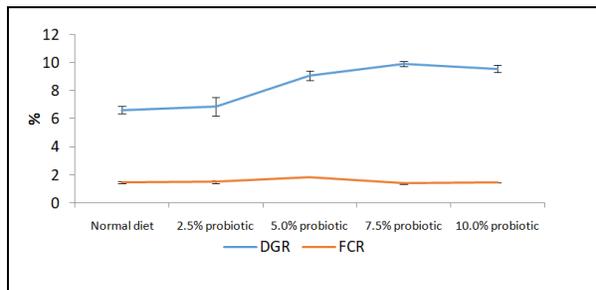


Fig 1: Daily growth rate and feed conversion ratio of *L. vannamei*

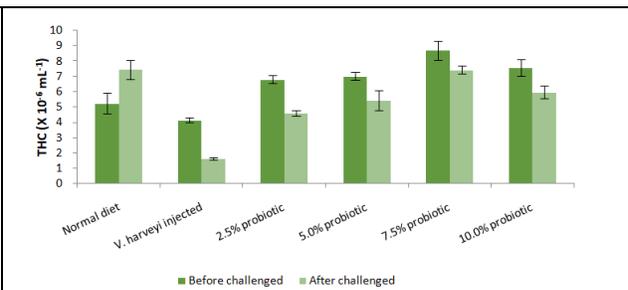


Fig 2: Total Haemocyte Count of *L. vannamei*

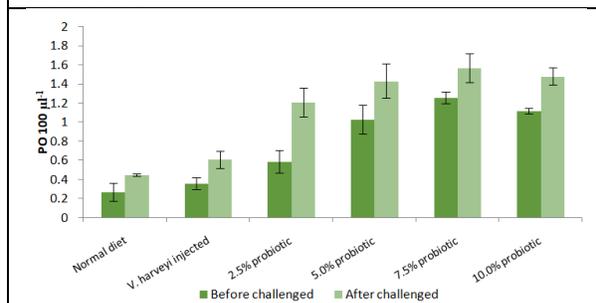


Fig 3: Phenoloxidase activities in haemolymph of *L. vannamei*

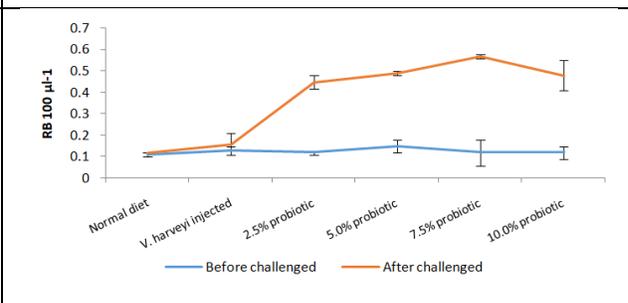


Fig 4: Respiratory burst activities of *L. vannamei*





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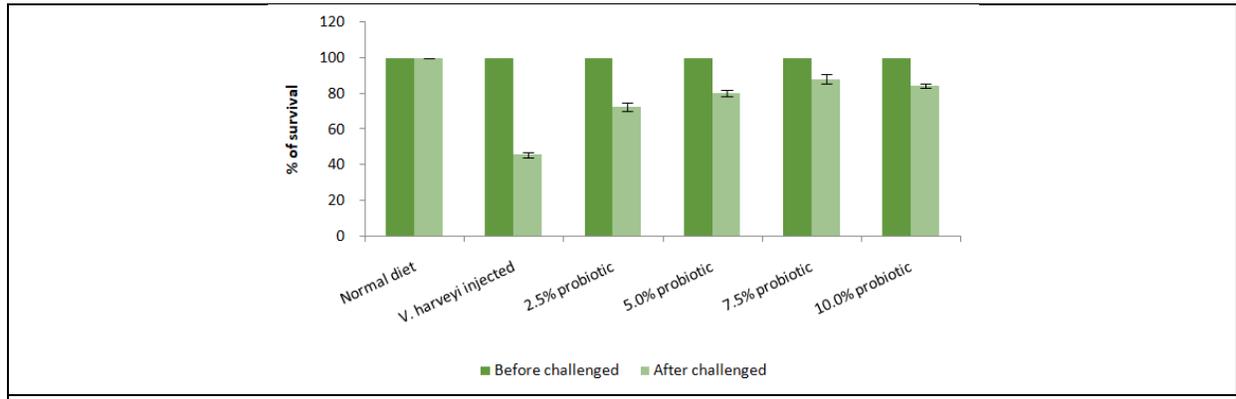


Fig. 5: Survival rate of *L. vammamei*





Isolation and Analyzing of Plant Growth Hormone Production by Phosphate Solubilizing Bacteria

Rajan L Fradlin Singh¹ and G. Kumaresan^{2*}

¹Ph.D. Research Scholar, Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

²Assistant Professor, Department of Agricultural Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

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*Address for Correspondence

G. Kumaresan

Assistant Professor,

Department of Agricultural Microbiology,

Faculty of Agriculture, Annamalai University,

Annamalai Nagar-608002, Tamil Nadu, India

E.Mail: microsen1974@gmail.com



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ABSTRACT

Phosphorus is one of the major plant nutrient required in optimum amount for proper plant growth and development. Phosphorus has the ability to involve many functions in the plant growth and metabolism. The use of Phosphate solubilizing bacteria as bio inoculants has increased phosphorous uptake and increased the growth and yield of crops. In our study, ten locations of Cuddalore district were selected for the collection of brinjal rhizosphere soil samples and for the isolation Phosphorus solubilizing microorganisms. From these samples ten isolates were isolated and designated as BPB-1 to BPB-10 and subjected to various biochemical characterization and plant growth hormone production test. Among the ten isolates, the isolate BPB-5 performed well in IAA production (70.90 µg/ml), Siderophore production (Catechol type-8.73 molmL⁻¹, Salicylate type-9.10 molmL⁻¹), Gibberellic acid production (8.20 µg/ml) and showed maximum production of ACC deaminase activity (+++), HCN (+++). The ten isolates also screened for phosphatase activity, organic acid production and available phosphorous and the result revealed that isolate BPB-5 performed excellent in production of Phosphatase activity (26.4 µ mole/ml/hr), organic acid production (13.0) and available phosphorous (45.90 ppm). The isolate BPB-5 found be the best performer in terms of plant growth hormone production, phosphatase activity, organic acid production and available P and this isolate is identified as *Bacillus megaterium* by 16S rRNA sequencing.

Keywords: Phosphorus solubilizing bacteria, IAA, Gibberellic acid, ACC Deaminase activity, molecular analysis





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INTRODUCTION

Brinjal, also called eggplant belongs to the family of "Solanaceae". Brinjal is the rich in carbohydrates, protein, vitamins, minerals, fat, fiber content and elemental salts. Solanaceous vegetables requires major nutrients like nitrogen, phosphorus and potassium in larger quantity. The cost of inorganic fertilizers has been increasing marginally and the use of inorganic fertilizers creating environmental imbalance and pollution in soil. The important index of soil is the fertility which can be improved by the application of bio inoculants. Phosphorus (P) is the second limiting nutrient required for plant growth and development and it's involved in important metabolic pathways like nutrient uptake, biological oxidation, and energy metabolism (Nesme *et al.*, 2018). The total P in soil accounts roughly for 0.04–0.1% (w/w), only a very tiny proportion of P (soluble H_2PO_4) can directly be assimilated by plants (Chen *et al.*, 2008), as the large portion of P in soils exists in inorganic insoluble form and organic insoluble/soluble form (e.g., phytate and nucleic acid) (Neal *et al.*, 2017). The input of P to soil is mainly via fertilization, and both abiotic substance (mainly inorganic P mineral) and organic P compounds (e.g., animal, plant, and microbes residues and wastes) are widely used in agricultural ecosystems (Fraser *et al.*, 2015), and these extraneously added P including inorganic and organic P get converted into salts and become insoluble by bounding to Ca, Al, Mg, Mn, and Fe (Reddy *et al.*, 2015). Insoluble inorganic phosphorous especially $Ca_3(PO_4)_2$, $AlPO_4$, and $FePO_4$, and insoluble/soluble organic phosphorous especially phytate taking up 80% of soil organic phosphorous by phosphorus-solubilizing microbes (PSM) to transform into orthophosphate which can be absorbed by plants and microbes (Fraser *et al.*, 2015).

Biofertilizers are the products containing selected and beneficial living microbes which are added to soil as microbial inoculants. As they are ecofriendly, non-hazardous, and nontoxic in nature. Biofertilizers are gaining importance in agriculture *viz.*, Cyanobacteria, *Azolla*, *Rhizobium*, *Azospirillum* and phosphate-solubilizing microorganisms are presently being used as biofertilizers (Kannayan *et al.*, 2004). Approximately 95 to 99% soil phosphorous which are present in the form of insoluble phosphates and hence the phosphorous source cannot be utilized by plants. Phosphorous-solubilizing microorganisms is playing a major interest because of their potential use as biofertilizers, which could reduce the use of chemical fertilizers that pollute and modify the structure and microbial community of soils (Ingle and Padole 2017). The objectives of the present study to isolate and characterize various phosphorous solubilizing microorganisms from brinjal rhizosphere soil samples collected from different location of Cuddalore district.

MATERIALS AND METHODS

Isolation of Phosphate solubilizing organisms

Brinjal rhizosphere soil samples were used for isolating phosphate solubilizing bacterial strains which are collected from different locations of Cuddalore district of Tamilnadu. Serial dilution plating method is used to isolate Phosphate solubilizing organisms by using NBRIP (National Botanical Research Institute Phosphorus) agar medium containing 10 g glucose, 5 g $Ca_3(PO_4)_2$, 5 g $MgCl_2 \cdot 6H_2O$, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.2 g KCl, 0.1 g $(NH_4)_2SO_4$ (Nautiyal, 1999). The inoculated plates were incubated for 5 days at 30°C in incubator. The colonies which showed clear halo zones were identified as phosphate solubilizing bacterial colonies. Identified colonies were further purified by streaking on NBRIP agar plates and incubating at 30°C. The identified isolates were designated as Brinjal Phosphobacteria (BPB-1 to BPB-10).

Bio chemical characterization Phosphate solubilizing isolates

Phosphate solubilizing isolates were characterized by subjecting them to the following biochemical tests: Gram reaction, Hydrogen sulfide production test, Gelatin liquefaction, Methyl red test, Voges prausker's test, Citrate utilization test, starch hydrolysis and Nitrate reduction test.



**Rajan L Fradlin Singh and Kumaresan****Gram reaction**

Gram staining was carried out as per the Hucker's modification (Rangaswami, 1975)

Starch hydrolysis (MacFaddin, 2000)

Sterile starch agar plates were streaked with overnight culture and incubated at $28 \pm 2^\circ\text{C}$ for 24-48 hours. After incubation period, the plates were flooded with an iodine solution. The formation of a transparent zone around the colony was taken as a positive reaction to the test.

Gelatin liquefaction (MacFaddin, 2000)

Overnight isolated cultures were inoculated on sterilized nutrient gelatin deep tubes and incubated for 24 hours at $28 \pm 2^\circ\text{C}$. The tubes were kept in the refrigerator for 30 minutes at 4°C . The isolates showing liquefied gelatin were taken as positive and those which resulted in the solidification of gelatin on refrigeration were recorded as negative for the test.

Methyl red test (Crown and Gen, 1998)

Sterilized glucose-phosphate broth tubes were prepared and inoculated with isolated culture and incubated at $28 \pm 2^\circ\text{C}$ for 48 hours. After incubation period, five drops of methyl red indicator were added to each inoculated tube and gently shaken. Red color production in the broth tube was taken as positive and yellow color production was taken as negative for the test.

Voges prausker's test (MacFaddin, 2000)

On the pre-sterilized glucose-phosphate broth tubes, isolated cultures were inoculated and incubated at 37°C for 48 hours. After incubation ten drops of Barritt's reagent-A was added and gently shaken followed by the addition of 10 drops of Barritt's reagent-B. The development of pink color in the broth was taken as positive for the test

Hydrogen sulfide production test (Cowan and Steel, 1970)

Tubes containing SIM agar medium were sterilized and the sterilized tubes were stabbed with the test cultures. The tubes were incubated for 48 hours at $28 \pm 2^\circ\text{C}$. After incubation period the development of black color along with the line of the stab was observed and considered as positive for the test.

Citrate utilization test (James and Natalie, 1992)

Bacterial isolates were streaked on sterilized citrate agar slants containing bromothymol blue as an indicator and incubated overnight at room temperature. After incubation slants were observed for the formation of blue color in the medium and it was taken as positive for the citrate utilization test.

Nitrate reduction

Nitrate agar slants were inoculated with bacterial cultures and incubate the tubes at the optimal temperature 37°C for 24 hours. Then observe for the reaction (color development).

Phytohormone production by Phosphobacterial isolates:**ACC deaminase activity**

The ACC deaminase activity was determined by culturing the bacterial isolates on nitrogen-free medium supplemented with 3 mM ACC as a nitrogen source. ACC deaminase activity of bacterial isolates was quantitatively estimated UV spectrophotometrically in terms of α -ketobutyrate production at 540 nm by comparing with the standard curve of α -ketobutyrate.

Production of indole acetic acid (IAA)

Indole acetic acid (IAA) production was detected as described by (Brick *et al.*, 2004). Phosphobacterial isolates were grown for 72 hr on their respective media at 37°C and 28°C . Fully grown cultures were centrifuged at 3000 rpm for 30 min and the supernatant (2ml) was mixed with two drops of orthophosphoric acid and 4ml of salkowski reagent (50



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ml, 35% of per chloric acid, 1ml 0.5 m FeCl₃ solution). Development of pink color indicates IAA production. After 2 hours of the color development the optical density was measured at 530 nm.

Siderophore production

Siderophore production by the phosphobacterial isolates was estimated by the method described by Reeves *et al.*, (1983). Nutrient broth was prepared and dispensed in 100 ml quantities in 250 ml Erlenmeyer flasks and sterilized. One ml of standard inoculum of bacterial isolates was added into each flask and incubated at 37°C for 7 days. After the incubation period, the culture broth was centrifuged at 10,000 rpm for 20 min. The supernatant was used for estimation of siderophore production. Twenty ml of culture supernatant was taken and the pH was adjusted to 2.0 with dilute HCl and from this suspension, 20 ml of ethyl acetate was added and extraction was done twice. Hathway reagent (one ml of 0.1 M ferric chloride and 1 ml of 0.1 N HCl was added to 100 ml of distilled water and to this 1 ml of 0.1 M potassium ferric cyanide was added) was prepared. Five ml of the assay solution was added with five ml of Hathway's reagent and absorbance was determined at 560 nm with sodium salicylate as standard for the estimation of salicylate type of siderophore.

To measure the catechol type siderophore, five ml of the assay solution was added with five ml Hathway reagent and absorbance was determined at 700 nm with 2, 3-dihydroxy benzoic acid (DHBA) as a standard. From the absorbance value of the sample, the quantum of siderophore produced was calculated and expressed as µg ml⁻¹ of culture filtrate.

Gibberellic acid production

Gibberellic acid was estimated spectrophotometrically by the method of Mahadevan and Sridhar (1982). Two ml of zinc acetate solution were prepared and added to the dissolved residue. After two min, two ml of potassium ferro cyanide solution was added and the mixture was centrifuged at 10,000 rpm for 10 min and the supernatant was collected. Five ml of culture supernatant was added to 5 ml of 30 per cent hydrochloric acid and incubated at 20°C for 75 min. The blank was prepared with 5 per cent hydrochloric acid. The absorbance was measured at 254 nm in spectrophotometer.

Estimation of organic acid production

Organic acid produced by PSB in the culture was estimated following the method of Sperber (1958). Ten phosphobacterial strains were inoculated in the NBRIP broth and allowed to grow for 7 days and after incubation period the culture was centrifuged. Two ml of culture filtrate was taken in a flask and titrated against 0.01 N of sodium hydroxide. The total titrable acidity was expressed by ml of 0.01 N NaOH consumed.

Estimation of phosphatase activity:

Phosphatase activity was estimated by method described by Eivazi & Tabatabai (1977). The ten isolates were centrifuged 10,000 rpm for 10 minutes and the pellet was suspended in 5 ml of sterile distilled water. 1 ml of the sample was taken in a 50 ml conical flask and added 0.25 ml of toluene, 4 ml of modified universal buffer, 1 ml of p-nitrophenyl phosphate solution and incubated at room temperature for 1 h. After incubation, 1 ml of 0.5 M of calcium chloride and 4 ml of 0.5 M sodium hydroxide were added and filtered the content through a filter paper. The absorbance was measured at 660 nm. The phosphatase activity was expressed µ moles of PNP released ml⁻¹ of filtrate h⁻¹.

Estimation of available P

The available phosphorus in the soil filtrate was estimated by following the method of Olsen *et al.* (1954). Air dried soil (5 g) was extracted with the use of extractant with a pinch of phosphate free activated charcoal and shake on a horizontal reciprocating shaker for 5 min and the extract were filtered through Whatman No.1 filter paper. One ml of culture was pipetted out into a 25 ml standard flask and add 10 ml of distilled water and shake well. 5 ml of freshly prepared ascorbic acid and ammonium molybdate solution were added and made up to 25 ml. Absorbance was





measured at 882 nm after half an hour. A standard curve was prepared using 0, 1, 2, 3, 4 and 5 ml of 5 ppm standard P solution.

Molecular identification

PCR of the 16S rRNA gene with universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5' TACGGYTACCTGTACGACTT-3'). A single discrete PCR amplicon band of 1500 bp was observed when resolved on agarose gel. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with forward primer and reverse primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. 16S rRNA gene sequence was used to carry out NCBI-Basic Local Alignment Search Tool database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program ClustalW multiple alignment. Distance matrix was generated and the phylogenetic tree was constructed by using MEGA 11 software.

RESULT AND DISCUSSION

Bio chemical characterization

All the ten isolates were examined for biochemical characterization *viz.*, starch hydrolysis, gelatin hydrolysis, hydrogen sulfide production, citrate utilization test, MR-VP test and nitrate reduction and the results were presented in Table.1. All the phosphate solubilizing organisms were positive for starch hydrolysis except the isolates BPB-6, BPB-8, BPB-9. In gelatin hydrolysis test all the isolates were tested positive. In vogues proskauer test the isolates BPB-6, BPB-8, BPB-9 showed negative result. . For methyl red test the isolates BPB-2, BPB-5, BPB-7, BPB-10 showed positive result. The isolates BPB-1, BPB-2, BPB-5, BPB-7, and BPB-10 showed negative result for citrate utilization test. All the isolates from BPB-1 to BPB-10 showed the positive for nitrate reduction test. For hydrogen sulphide test the isolates BPB-6, BPB-8, BPB-9 showed negative result and remaining isolates showed positive result. Based on biochemical characterization the isolates were tentatively *Bacillus* sp, *Bacillus megaterium*, *Bacillus subtilis*, *Bacillus polymyxa*, *Pseudomonas fluorescence*, *Pseudomonas* sp

Screening of Phosphobacterial isolates for Plant growth hormone production

All the ten isolates were screened for plant growth promotion activity *viz.*, IAA, siderophore, gibberellic acid, HCN production and ACC deaminase activity and the results were presented in Table-2. Among the ten isolates, the isolate BPB-5 showed maximum IAA (70.90 µg/ml) production and the isolate BPB-9 recorded lowest production of 10.00 µg/ml. Similarly Rajat Maheshwari *et al.*, (2020) observed highest and lowest IAA production of (79.04 ± 0.78 µg.mL⁻¹) and 38.36 ± 1.89 µg.mL⁻¹) on their isolates PJN13 and PJN 17 using Salkowski reagent. On siderophore production, the isolate BPB-5 recorded 8.73 molmL⁻¹ and 8.20 molmL⁻¹ catechol and salicylate type siderophore followed by the isolate BPB-1 (8.10 molmL⁻¹ and 8.72 molmL⁻¹) and the least production was observed in the isolate BPB-9 (3.14 molmL⁻¹ and 4.81 molmL⁻¹). Similarly, Sofia santos *et al.*, (2014) reported siderophore production of 17 g l⁻¹ in *Bacillus megaterium*.

Gibberellic production of Phosphobacterial isolates were studied and the results showed the isolate BPB-5 recorded highest Gibberellic acid production of 8.20 µg/ml and the isolate BPB-9 recorded least production of 2.46 µg/ml. Gusmiaty *et al.*, (2019) found that the isolate JS9 produced the lowest gibberellin of 1.959 mgL⁻¹, the highest of 4.322 mg L⁻¹ in the BB2 isolate. Pandya and Desai (2014) reported that many of the isolates produced gibberellic acid and the production of gibberellin was in the range of 7.50 g ml⁻¹ to 93.93g ml⁻¹. ACC deaminase activity and HCN production of phosphobacterial isolates were studied and the isolate BPB-5 (+++) recorded maximum ACC deaminase activity and HCN production. The isolate BPB-9 (-) recorded no production on ACC deaminase activity and BPB-9 (+) showed very low on HCN production. Similarly, Maheshwari *et al.*, (2020) noticed higher ACC deaminase activity of 160 µM on their isolate PJN13 and lowest ACC deaminase activity of 130 µM on isolate PJN17.



**Rajan L Fradlin Singh and Kumaresan****Screening of phosphobacterial isolates for phosphatase activity, organic acid production & available phosphorous**

All the ten isolates were screened for phosphatase activity, organic acid production, available phosphorous and the results were presented in Table 3. Among the ten isolates, maximum phosphatase activity were showed by the isolate BPB-5 (26.4 μ mole/ml/hr) followed by BPB-1 (26.0 μ mole/ml/hr) and the isolate BPB-9 (18.0 μ mole/ml/hr) showed very low phosphatase activity. Similar results were observed by Dipak Paul and Sankar Narayan Sinha (2013) found that the phosphatase activity of the isolates showed that the strain TPSB-23 had higher activity (31.24 μ moles/g/h) followed by TPSR-21 (27.12 μ moles/g/h). The phosphatase activity was low in TPSB-20 (19.92 μ moles/g/h). The ten phosphobacterial isolates were screened for organic acid production and the results showed that the isolate BPB-5 recorded maximum production of 13.0 and the isolate BPB-9 shows least production 3.0. That is in accordance with Tenzing Baliah *et al.*, (2016) who found that organic acid production of the isolate PSB strains TP1 (7.8) and TP2 (6.5) was good in organic acid production in the presence of tri calcium phosphate. Available phosphorous of the ten phosphobacterial isolates were studied and the isolate BPB-5 recorded maximum available phosphorous of 45.90 ppm followed by the isolate BPB-1 (45.67 ppm) and the isolate BPB-9 showed least production of 38.80 ppm. Tenzing Baliah *et al.*, (2016) also reported that out of 10 strains, the strain CTP2 (46.0 ppm) released more phosphorus in the medium followed by TP1 (40.6) with Tri calcium phosphate. From our present research we found that the isolate BPB-5 performed well in IAA, Gibberellic acid, HCN production, organic acid production, ACC deaminase activity, phosphatase activity and available phosphorous. Hence the isolate BPB-5 was selected for species identification using 16S rRNA sequencing.

Molecular identification

The genomic DNA of BPB-5 was used to sequence the 16S rRNA in order to identify the bacterial isolate. The obtained 16S rRNA sequence of the isolate BPB-5 was submitted to the NCBI GenBank and was assigned Accession No.OQ835609. On the basis of sequence homology and phylogenetic analysis, bacterial isolate BPB-5 was identified as *Bacillus megaterium* and the phylogenetic tree was drawn using MEGA 11 software (Fig I).

CONCLUSION

The phosphorus which applied to soil are not fully used by the plants due to its chemical fixation. Some microorganisms have the characteristics to solubilize the phosphorous and make available to the plants. Several soil bacterial species belonging to the genera *Pseudomonas* and *Bacillus* possess the ability to solubilize the insoluble phosphorous into soluble form. Ten phosphate solubilizing isolates were isolated from brinjal rhizosphere soil. The isolates were subjected to various biochemical tests and plant growth promoting activity *viz.*, IAA, GA₃, Siderophore, Available phosphorous, Organic acid production, Phosphatase activity. From our research findings we concluded that among the 10 isolates the isolate BPB-5 (*Bacillus megaterium*) recorded excellent in plant growth hormone production, phosphatase activity, organic acid production and available phosphorous. Hence, the isolate BPB-5 found to be used for the production of bioinoculants.

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Table 1: Biochemical characterization of Phosphobacterial isolates from ten different locations of Cuddalore district

| Isolate | Motility | Gram reaction | Starch Hydrolysis | Gelatin Hydrolysis | Voges – Proskauer test | Methyl red test | Citrate Utilization | Nitrate reduction | H ₂ S Production | Tentative identification |
|---------|----------|---------------|-------------------|--------------------|------------------------|-----------------|---------------------|-------------------|-----------------------------|---------------------------------|
| BPB-1 | Motile | + | + | + | + | - | - | + | + | <i>Bacillus sp</i> |
| BPB-2 | Motile | + | + | + | + | + | - | + | + | <i>Bacillus megaterium</i> |
| BPB-3 | Motile | + | + | + | + | - | + | + | + | <i>Bacillus subtilis</i> |
| BPB-4 | Motile | + | + | + | + | - | + | + | + | <i>Bacillus polymyxa</i> |
| BPB-5 | Motile | + | + | + | + | + | - | + | + | <i>Bacillus megaterium</i> |
| BPB-6 | Motile | - | - | + | - | - | + | + | - | <i>Pseudomonas fluorescense</i> |
| BPB-7 | Motile | + | + | + | + | + | - | + | + | <i>Bacillus megaterium</i> |
| BPB-8 | Motile | - | - | + | - | - | + | + | - | <i>Pseudomonas fluorescense</i> |
| BPB-9 | Motile | - | - | + | - | - | + | + | - | <i>Pseudomonas sp</i> |
| BPB-10 | Motile | + | + | + | + | + | - | + | + | <i>Bacillus megaterium</i> |

Table2: Screening of Phosphobacterial isolates for plant growth hormone production

| Isolates | IAA Production (µg/ml)* | Siderophore production (molL ⁻¹)* | | ACC Deaminase activity | HCN Production | Gibberellic acid production (µg/ml)* |
|----------|-------------------------|---|-----------------|------------------------|----------------|--------------------------------------|
| | | Catechol type | Salicylate type | | | |
| BPB-1 | 66.23 | 8.10 | 8.72 | +++ | +++ | 7.90 |
| BPB-2 | 17.60 | 4.27 | 5.70 | ++ | ++ | 3.65 |
| BPB-3 | 30.00 | 5.21 | 6.51 | - | + | 4.76 |
| BPB-4 | 44.70 | 6.90 | 7.17 | + | +++ | 6.12 |
| BPB-5 | 70.90 | 8.73 | 9.10 | +++ | +++ | 8.20 |
| BPB-6 | 61.58 | 7.96 | 8.23 | ++ | + | 7.41 |
| BPB-7 | 35.60 | 5.87 | 6.67 | + | ++ | 5.10 |
| BPB-8 | 24.47 | 4.73 | 6.14 | +++ | - | 4.17 |
| BPB-9 | 10.00 | 3.14 | 4.81 | - | ++ | 2.46 |
| BPB-10 | 53.48 | 7.38 | 7.85 | - | - | 6.80 |

*Values of mean of three replications ± SD
(High production +++, Moderate production ++, Low production +, No production -)

Table 3: Screening of phosphobacterial isolates for phosphatase activity (µ mole/ml/hr), Organic acid production and Available P (ppm)

| Isolates | Phosphatase activity (µ mole/ml/hr) | Organic acid production (0.1N NAOH Consumed) | Available P (ppm) |
|----------|-------------------------------------|--|-------------------|
| BPB-1 | 26.0 | 12.9 | 45.67 |
| BPB-2 | 19.4 | 7.0 | 40.28 |
| BPB-3 | 21.9 | 9.0 | 42.35 |





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| | | | |
|--------|------|------|-------|
| BPB-4 | 23.7 | 10.7 | 44.08 |
| BPB-5 | 26.4 | 13.0 | 45.90 |
| BPB-6 | 25.8 | 12.2 | 45.10 |
| BPB-7 | 22.0 | 9.8 | 42.96 |
| BPB-8 | 20.8 | 7.7 | 40.98 |
| BPB-9 | 18.0 | 3.0 | 38.80 |
| BPB-10 | 24.9 | 11.8 | 44.68 |

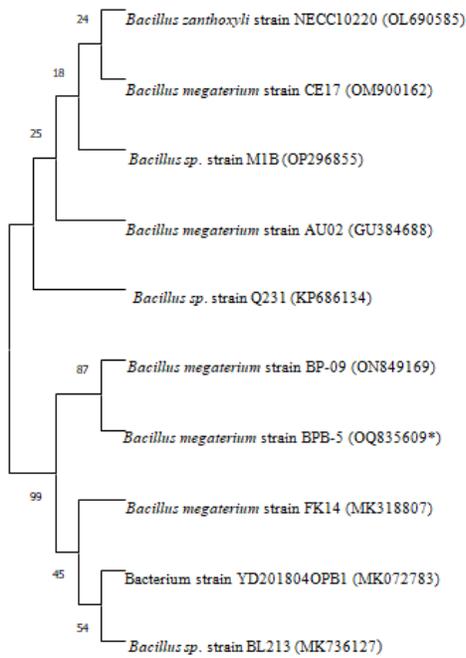


Fig I. Phylogenetic tree showing relationship of isolate BPB-5 (OQ835609*) with sequences from NCBI using MEGA 11 software by neighbour-joining method
 >OQ835609 *Bacillus megaterium* strain BPB-5

TGGACCCGTGGGGGGCTGCTAATACATGCAAGTCGAGCGAACTGATTAGAAGCTTGCTTCTATGACGTTAGCGGCG
 GACGGGTGAGTAACACGTGGGCAACCTGCCTGTAAGACTGGGATAACTTCGGGAAACCGAAGCTAATACCGGATA
 GGATCTTCTCCTCATGGGAGATGATTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCCGGTGCATTAGCT
 AGTTGGTGAGGTAACGGCTCACCAAGGCAACGATGATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGA
 GACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTCCGCAATGGACGAAAGTCTGACGGAGCAACG
 CCGCGTGAGTGATGAAGGCTTTCGGGTCGTAATAACTCTGTTGTTAGGGAAGAACAAGTACGAGAGTAAGTCTCGT
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 TCTGACAACTCTAGAGATAGAGCGTTCCTTTCGGGGACAGAGTGACAGGTGGTGCATGGGTTGTCGTCAGCCT
 CGTGTCTGAGAATGTTT





ECOC for an Adaptive Congestion Control Technique in TCP Layer

M.Dhanasekar^{1*} and R.Rangaraj²

¹Research Scholar, Department of Computer Science, Hindusthan College of Arts and Science, Coimbatore - 641028, Tamil Nadu, India.

²Associate Professor and Head, Department of Computer Science, Hindusthan College of Arts and Science, Coimbatore - 641028, Tamil Nadu, India.

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*Address for Correspondence

M.Dhanasekar

Research Scholar,
Department of Computer Science,
Hindusthan College of Arts and Science,
Coimbatore - 641028, Tamil Nadu, India.



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ABSTRACT

Due to recent developments in health care technology, we are now able to implement a number of home monitoring solutions for the elderly or people with chronic illnesses. WBAN used to monitor, when a patient is immobile and suffering from an urgent medical condition can help with the early detection of irregularities. WBAN experiences a variety of issues, such as those with data quality, traffic, network design, data loss, wireless technology, etc. One of the main issues with wireless body area network (WBAN) applications in healthcare is congestion control. Even if there is congestion, some emergency sensors, such as heart and brain sensors, etc., shouldn't stop providing data. The ECOC algorithm for TCP layer was suggested as a solution to overcome congestion. The novelty of this work is to make sure that all available bandwidth (channel resources) is being used, increase the congestion window setting more quickly. To create a multi-phase method that assesses the bandwidth of each link independently before escalating the number of links till the observed bandwidth.

Keyword: WBAN, Biological Sensors, Congestion Control, TCP, Tahoe, Reno

INTRODUCTION

Due to recent advancements in wireless communication and electronics that enable small and intelligent sensors that can be used on, around, in or implanted in the human body, ubiquitous healthcare is an emerging technology that promises improvements in efficiency, accuracy, and availability of medical treatment (Negra et al., 2016). We now have the ability to adopt a variety of home monitoring solutions for the elderly or persons with chronic diseases



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thanks to recent advancements in health care technology. As a result, people can go about their regular business while continually being under the care of medical professionals. Injury risk is minimized because to the optimum interior environment. Data was provided to the appropriate intervention units in relation to the discovered emergency through alarm triggers and smart algorithms. In a number of settings, such as post-operative care and senior living facilities, it is necessary to continuously monitor a patient's vital biological data, such as ECG, SpO₂, blood glucose, body temperature, etc. When a patient is immobile and under urgent medical conditions, monitoring these measures can aid in the early diagnosis of anomalies. Lower mortality rates can undoubtedly be achieved with early detection of problems and prompt, appropriate treatment (Sharma et al., 2011). WBANs are made up of a variety of diverse biological sensors (as shown in fig. 1). These sensors can be worn or implanted under the user's skin and are put in various body regions. Each of them is employed for various missions and has unique requirements. These tools are used to monitor changes in a patient's vital signs and identify human states like anxiety, worry, and happiness. They communicate with a unique coordinator node, which is typically more energy-efficient and capable of processing more data.

A WBAN's sensor nodes may have various sampling frequencies (Jaramillo et al., 2016). Many high-sampling-frequency instruments, including accelerometers, electrocardiograms, electroencephalograms, and electromyography, are available on the market. WBAN encounters a number of problems, including those relating to data quality, consistency, interoperability, congestion, network architecture, data rates, mobility, data loss, wireless technology, etc.,. Particularly, collisions and congestion-caused packet loss have a substantial impact on quality of service (QoS). It will take more energy to retransmit missed packets during the congestion period due to increased packet loss and latency. Therefore, it is imperative to use effective congestion control methods to reduce congestion (Kiran & B, 2020).

Motivation

To begin the early diagnosis, the erratic health status of people in emergency situations must be immediately communicated (Arefin et al., 2017). Because of this, some emergency sensors, such as heart and brain sensors, etc., shouldn't stop transmitting data even if there is congestion. Controlling congestion is one of the biggest problems with wireless body area network (WBAN) applications in healthcare. Numerous-to-one communication, unpredictable traffic loads, and constrained bandwidth occupancy are a few of the main factors that can contribute to congestion in such systems. This research makes a proposal for an efficient Congestion Control method that can track communication between the mounted or implanted biosensors on the human body to the server.

Contribution

- To handle the congestion control on queue of high data transfers in TCP layer.
- To adjust the window size as per the availability of bandwidth and assigning window size from 1 to increase it by 1 as per the acknowledgement received from the sender.
- To implement ECOC for effective data transfer without overload control.
- To train with a function that gives status information and action guide for the TCP sender.

Related Work

Congestion has detrimental effects on the overall performance of the network, including packet losses, an increase in end-to-end delay, and energy waste from numerous retransmissions. Any lag in the transmission of life-critical signals could result in a patient's death. Thus, creating a method for congestion estimate and control is essential for improving network quality of service (QoS). In (Ghanavati et al., 2015) research, we provide brand-new congestion detection and control strategy for WBAN-based remote patient health monitoring. The proposed method can identify congestion by taking into account regional data such as buffer capacity and node rate and distinguishes between essential messages in cases of congestion and prioritizes them according to their level of significance. As a result, the method proposed offers a higher standard of service for sending really crucial vital indications.



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Serious congestion issues in WBAN are caused by the erratic traffic load, power limitations, constrained bandwidth, and many-to-one communication design. (Kiran & B, 2020) suggests the Network Status Aware Congestion Control (NSACC) technique to address this challenging problem. The proposed algorithm's primary goals are to accurately forecast the level of congestion and to control the transmitting rate in accordance with that level. The suggested technique uses runtime, dynamic characteristics such buffer occupancy level, packet sending rate, and node priority to infer the degree of congestion using the fuzzy controller. Based on the important information, the transmission rate is controlled to reduce packet loss, collision, and congestion. The suggested method distinguishes between essential messages in cases of congestion and prioritizes them according to their level of significance. As a result, the suggested method offers a higher standard of service for sending really crucial vital indications.

Device mobility affects network performance through topology changes, which cause recursive link disconnections, energy waste, packet delays, reduced throughput, and congestion due to excessive control messaging during route repair. This affects user safety in addition to alarming network performance. In (Liu et al., 2021) paper, offer WBAN performance optimization criteria that emphasize increasing energy economy, network throughput, and decreasing end-to-end delay in multiple existence schemes. In addition to node sequence number and hop count as the basic route selection metrics, first propose an alternative routing algorithm whose routing decision depends on a cost function taking into account the parameterized residue energy to node distance ratio, link energy reliability, and specific heat absorption. In order to increase throughput, lessen congestion, and shorten delay, we also apply congestion control adaptation in the medium access control (IEEE 802.11MAC) mechanism. We present a comparison network performance to address the impact of WBAN speed at various hello intervals due to link discontinuities during mobility. In terms of energy efficiency, network throughput, and a decreased end-to-end delay, the comparison research reveals that implementing a protocol using a cross-layer method beats the traditional protocol without MAC adjustments by an average of 0.45%, 2.8%, and 13.7%, respectively.

Packet loss and significant energy usage might result from WBAN congestion. Although the IEEE 802.15.6 Standard supports QoS, it makes no explicit recommendations for congestion control measures. The IEEE 802.15.6 Standard and an energy-efficient and emergency-aware MAC (Medium Access Control) protocol are the foundations of the novel rate management strategy proposed in (Jaramillo et al., 2016) research to alleviate congestion in WBANs. The system responds to emergency situations in any node while maintaining the regular traffic rate. It is context-aware. The suggested remedy boosts the efficiency of the IEEE 802.15.6 Standard and the MAC protocol. Every time a network node experiences an emergency, the hub determines the Rate Control Factor (RCF). Next, in order to reduce the pace of normal traffic and maintain a nearly constant average rate across the whole WBAN, it sends the calculated RCF to all normal nodes. To all regular nodes, the RCF is transmitted during the SRP phase. The hub stays clear of congestion and packet collision in this way.

When it comes to energy, security, routing, load balancing, and other modern applications, Wireless Sensor Network (WSN) technology is crucial. The potential of remote healthcare monitoring systems has substantially expanded in recent years due to the use of Wireless Body Area Network (WBAN). The problems with congestion management and the energy efficiency of WBAN, however, continue to exist. In order to handle congestion control and assess energy efficiency, (C. Srinidhi et al., 2016) paper aims to create a heterogeneous WBAN network. A more thorough examination of congestion control was produced by also computing the control parameters, such as distance and traffic load. With regard to congestion control, graphs of the Cumulative Distribution Function (CDF) and Probability Density function (PDF) vs. traffic load were provided. These graphs demonstrate an improvement in the rate of traffic flow. Moreover, an assessment of WBAN node energy usage is carried out.

TCP- Congestion Control Algorithms

On the Internet, data is transferred from computer to computer via the TCP protocol. The primary distinction between the TCP protocol and other network protocols is that it ensures 100% data delivery and transmission. This implies that if we use TCP to send some data from one machine to another, the recipient will receive the entire amount sent. Many of the network applications we use, including the Internet and email, are built on top of TCP due



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to its very efficient nature. The TCP protocol experiences congestion when the channel cannot handle a lot of transferred data.

Tahoe Algorithm

One of the earliest congestion control methods to be used was TCP Tahoe. Its deployment can be roughly divided into two phases: congestion avoidance and a gradual start. The congestion window (cwnd), which I previously discussed, begins at one during the sluggish start phase and rises by one with each acknowledgement the sender receives. The sender enters "congestion avoidance" whenever the cwnd reaches a configurable threshold known as the "Slow Start Threshold," and the cwnd rises by 1 each time a full window of packets is acknowledged (Abed et al., 2012). This effectively means that the cwnd develops more slowly during the congestion avoidance phase and faster during the slow start. TCP Tahoe uses a "Fast Retransmit" protocol if a packet is lost. The "Rapid Retransmit" method retransmits the lost packet, updates the slow start threshold to half of the current cwnd, and sets the cwnd to 1. It continues until the "Slow Start Threshold (sssthresh)" is reached in the congested window size. The congestion window size (cwnd) is doubled by the slow start algorithm in a single RTT. Ssthresh is initially set to endless(). Then, depending on the packet loss events, it adapts. Slow start ends when cwnd reaches the ssthresh threshold. Takeovers in the AIMD phase follow. When the tentative start ends, it begins. Multiplicative Reduction lowers ssthresh to 50% of cwnd while Additive Increase raises cwnd by 1. Keep in mind that cwnd is 'not' 50% less, but rather ssthresh. It's important to remember that cwnd once more resets the window size at this point (10 in Linux kernel). It is the algorithm for loss detection. Three duplicate acknowledgments set it off. It resets cwnd to initcwnd when packet loss is detected.

Reno Algorithm

The Tahoe features were carried over into the Reno TCP implementation, although the Fast Retransmit procedure was changed to include Fast Recovery [Jac90]. The new technique eliminates the requirement for a Slow-Start to fill the communication conduit ("pipe") after a single packet loss by preventing it from being empty after a Quick Retransmit. Rapid Recovery functions under the presumption that each dup ACK it receives signifies a single packet that has left the pipe. As a result, the TCP sender can estimate the amount of outstanding data during Quick Recovery.

TCP Reno is TCP Tahoe's extension; therefore the slow start and AIMD phase are identical. Both RTO and Fast Retransmit are used. A quick retransmit technique is in use if three repeated acknowledgments cause the packet loss detection to occur. Rapid Retransmit detects packet loss and reduces cwnd by 50% ($cwnd = cwnd/2$). Receiving three duplicate ACKs indicates that the network is functioning well because when ACKs are received, it means that packets are being sent to the receiver. Hence, cwnd is decreased by 50% to enable the network to decongest. Reset the cwnd to initcwnd when a packet loss through RTO is detected. When the RTO timer runs out, the network is severely overloaded. Hence, in order to relieve congestion on the network, the cwnd must be returned to its initial value.

ECOC Algorithm

The proposed ECOC (Enhanced CUBIC overload control Algorithm) works on the parallel processing hybrid approach. There are two key issues that must be resolved for high-bandwidth channels to function properly:

- Raise the congestion window value more quickly to ensure that all bandwidth (channel resources) are utilised;
- Use less rapid window zooming

Based on these issues, we would like to develop a multi-phase technique that evaluates each link's bandwidth individually before gradually increasing this number up to the measured bandwidth.

The "concave" phase (before the inflection point) and the "convex" phase are two characteristics of the ECOC_TCP (Enhanced CUBIC overload control TCP Algorithm) (after the inflection point). When reaching w_{max} , the





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congestion window value increases quickly at first before slowing down. When no packet loss happens, the window around the w_{max} number starts to extend quickly to explore more bandwidth. When a packet is lost, the size of the congestion window is used as the value for w_{max} . The proposed algorithm carried with the given phases of modification for its enhancement.

Congestion Avoidance

During congestion avoidance, ECOC does not continuously widen the congestion window. By tracking changes in RTTs (Round Trip Times) of segments that the connection has previously sent, it regulates $cwnd$. When observed RTTs increase, ECOC detects that the network is getting busy and reduces the $cwnd$ speed. On the other side, ECOC boosts $cwnd$ if RTTs shrink, signaling that the network is no longer congested.

The $cwnd$ is updated as seen equ (1) during the phase of congestion avoidance:

$$Cwnd(t + tA) = \begin{cases} cwnd(t) + 1, & \text{if } diff < \alpha/base_rtt \\ cwnd(t), & \text{if } \frac{\alpha}{base_rtt} < diff < dif \\ cwnd(t) - 1, & \text{if } \beta/base_rtt < dif \end{cases} \quad (1)$$

$diff = cwnd(t)/base_rtt - cwnd/rtt$

Where rtt is an observed round trip time, $base_rtt$ is the minimum value of observed RTTs, and α and β are some fixed values.

Enhanced Overload control

The ECOC assumes a cubic shape; it begins to raise quickly, narrows as it gets closer to where the previous drop happened, and then rises again quickly in an effort to enhance throughput. The product of the delay and the channel's bandwidth is used to determine BDP (bandwidth delay). A channel's latency, also known as its "length," is the time it takes for one packet to go from the sender to the receiver. A channel's bandwidth, also known as its "width," is the quantity of information that can move through it in a single second. The "capacity" of the data being transported can be considered as the BDP. When the sender maintains the quantity of BDP bytes sent at any given time, the channel is utilised at its best. Even with a slightly worse RTT, ECOC still performs marginally better on low BDP channels. Using the ECOC with a High BDP results in a 50% throughput gain without a noticeably higher RTT (round trip delay). Unlike to other algorithms, ECOC increases the window significantly more quickly during packet drops, maximising the use of the available bandwidth.

Modified Fast transmission and Slow-start rate

Three modifications are made to TCP's (fast) transmission strategy by ECOC. It begins by calculating the RTT for each segment sent. Fine-grained clock readings are used as the basis for the measurements. An effective timeout period is calculated for each segment using the fine-grained RTT values. ECOC checks to see if the timeout period has elapsed after receiving a duplicate acknowledgement (ACK). If so, the section is redistributed. Second, ECOC checks for the timer's expiration once more and may retransmit another segment if it receives a non-duplicate ACK that comes either first or second after a fast transmission. Finally, the congestion window is only shrunk for the first fast transmission in the event of multiple segment loss and multiple fast transmissions. To determine when to transition to the congestion avoidance phase during slow-start, a similar congestion detection approach is used. Only every other RTT is the congestion window permitted to grow in order to get accurate comparisons between the expected and actual throughput.

RESULT AND ANALYSIS

Compared the proposed ECOC algorithm with Tahoe and RENO using different evaluation metrics. Threshold (bytes per second), Congestion Window Size and the average RTT are the primary factors used to evaluate an algorithm's effectiveness (ms).



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From fig. 4 that instead of scaling with the β constant, the ECOC algorithm no longer resets the congestion window to 0 once packets are dropped (for which the RFC recommends a value of 0.7). It begins to increase quickly, narrows as it gets closer to where the previous drop happened, and then quickly increases again in an effort to enhance throughput. From fig. 5. it is observe that the ECOC algorithm yields the best results when the BDP value is high. Without a considerable increase in RTT, It observes a 50% improvement in throughput (round trip delay). Contrary to Tahoe and Reno, it is not as far behind during packet drops, and when ECOC expands the window significantly faster, utilizing the available bandwidth far more. From fig 6 the slow start threshold climbs more gradually due to the earlier drops, ECOC is able to attain higher throughput, if it select a high BDP value. ECOC are much smoother and offer more throughput than a Reno and Tahoe sender running by it.

CONCLUSION

The proposed ECOC algorithm for TCP increases the congestion window value more quickly to ensure that all available channel resources (bandwidth) are utilized. It also avoids employing quick window zoom. The issue was resolved by creating a multi-phase method that evaluates each link's bandwidth individually before gradually increasing the value until the detected bandwidth is reached. It shows a constant scaling of congestion window size to avoid setting to 0. If ECOC chooses a high BDP number, it can achieve a higher throughput. Compared to a Reno and Tahoe sender passing past it, ECOC are significantly smoother and provide better throughput. It notices a 50% increase in throughput without a significant rise in RTT (round trip delay).

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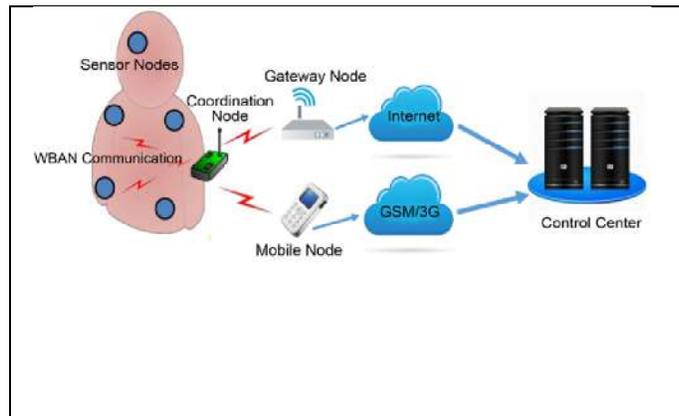


Figure: 1. WBAN Architecture

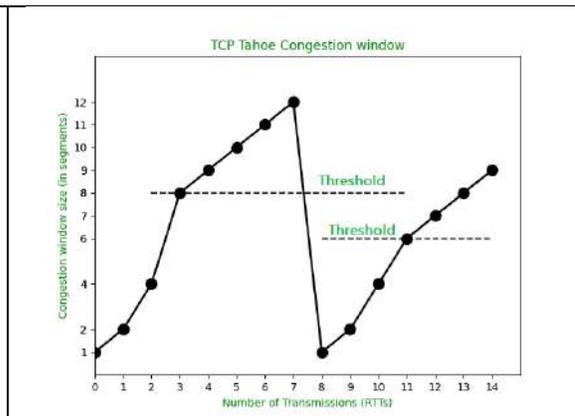


Figure 2. TCP Tahoe

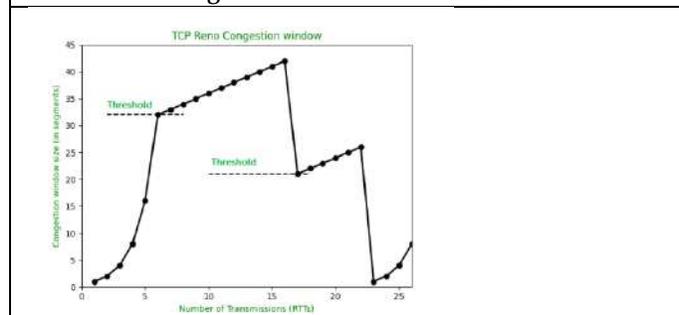


Figure 3. TCP Reno

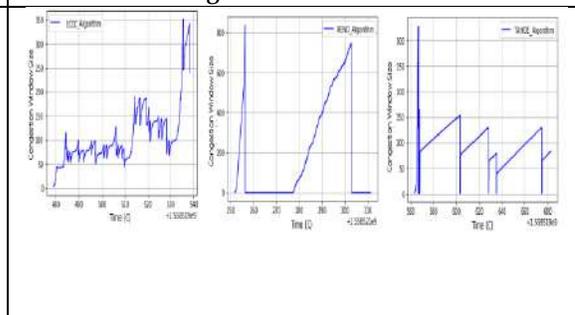


Figure 4. Congestion Window Size Vs Time

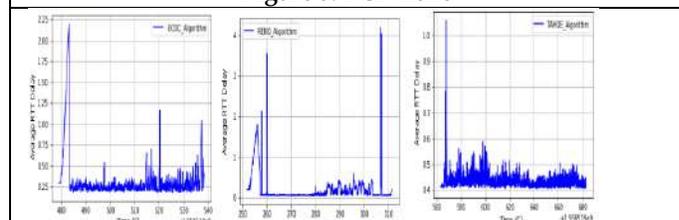


Figure 5. Average RTT Delay Vs Time

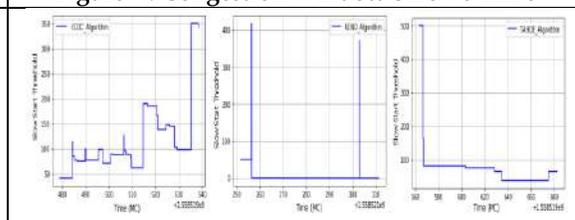


Figure 6. Threshold Vs Time





Impact of Optimism and Life Satisfaction on Prosocial and Aggressive Behaviour

Radhika Shakya^{1*} and Kakul Hai²

¹Masters in Clinical Psychology, Amity Institute of Psychology and Allied Sciences, Amity University, Noida, Uttar Pradesh, India.

²Assistant Professor, Amity Institute of Psychology and Allied Sciences, Amity University, Noida, Uttar Pradesh, India.

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*Address for Correspondence

Radhika Shakya

Masters in Clinical Psychology,
Amity Institute of Psychology and Allied Sciences,
Amity University, Noida,
Uttar Pradesh, India.



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ABSTRACT

Optimism, an optimistic perspective on life, involves anticipating favorable circumstances in the future. Similarly, life satisfaction encompasses various aspects of living and serves as an indicator of overall well-being. Both optimism and life satisfaction have a notable influence on aggression and prosocial behavior. Optimistic individuals tend to perceive things positively, leading to lower levels of aggressiveness and reduced vulnerability to intimidation. On the other hand, people who are dissatisfied and depressed are more prone to engaging in aggressive behaviors. The current study aims to look at how life satisfaction and Optimism helps in influencing the prosocial and aggressive behaviour using Revised Life Orientation Test (LOT-R), Prosocialness Scale, The Aggression Questionnaire and satisfaction with life scale as the tools on sample of 100 adults. The study found that optimism has a minimal impact on prosocial and aggressive behavior, while life satisfaction has a significant impact on both. Regression analysis supports these findings and suggests that other factors may be influencing the outcome variables. The study did not find any significant gender differences in aggression and prosocial behavior.

Keywords: Optimism, Life satisfaction, Aggression, Prosocial behavior, Physical and mental well-being, Happiness



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INTRODUCTION

Optimism is a multifaceted construct that has been variously defined in the empirical literature as positive expectancies for the future a positive inferential style beliefs in personal control and self-enhancing views relative to others. (Kleiman EM, 2018). Optimism is seen as a variable attribute that has been linked to a variety of psychological as well as physical advantages, including improved coping abilities, reduced levels of stress, and improved cardiovascular health. "Life satisfaction has been defined as "a person's cognitive and affective evaluations of his or her life" (Diener, 2002). Life satisfaction is a person's subjective assessment of their life as a whole. It entails a cognitive and emotive evaluation of one's own life situations, such as social connections, financial condition, career, and wellness. Higher degrees of life satisfaction are believed to be linked to a variety of favourable outcomes, including improved physical and mental well-being, enhanced happiness, and longer lifespan.

Aggression is defined as any behavior that is designed to inflict bodily or verbal damage or impairment toward another individual. This may vary from modest verbal aggressiveness like name-calling or taunting to much more extreme physical violence like punching or attacking someone. Aggressive conduct is a significant problem that can have harmful effects on both individuals and society as a whole. Prosocial behavior refers to actions that benefit others without expecting any personal gain or reward. This behavior is characterized by empathy, compassion, and a willingness to help and support those in need. Aggression and prosocial behaviour can be significantly influenced by optimism and life satisfaction. Lower levels of aggressiveness have been connected to optimism, or the inclination to perceive things in a positive way. People who are optimistic have a more positive attitude on life and are less prone to feel intimidated or upset when others become aggressive. This may result in fewer instances of hostile behaviour. People who are dissatisfied with their lives and are depressed are more likely to indulge in aggressive behaviours.

Based on a meta-analysis of existing studies conducted by Raskin, Novacek, and Hogan (1991), there is an inverse relationship between optimism and aggression, indicating that people who exhibit more optimism tend to display less aggressive behavior (Raskin, 1991). Optimism is connected with positive emotions, a hopeful mindset, and a belief in one's ability to cope with adversity, which can decrease feelings of hostility and encourage more constructive methods of conflict resolution (Carver, 2010). Consequently, individuals who have an optimistic outlook may deal with social situations in a more positive manner, which can potentially reduce the possibility of exhibiting aggressive behaviour (Galín, 2014).

The research on the impact of optimism and life satisfaction on aggressive and pro-social behaviour is inconclusive. Some studies suggest that there is a positive correlation between the two variables, while others suggest that there is no significant relationship. However, more research is needed to determine the extent to which optimism and life satisfaction impact aggressive and pro-social behaviour. It should be noted that culture might have an impact on the connection between these factors. People in India prioritise the needs of the collective over their own needs since they live in a collectivistic culture. This can have an impact on the manner in which optimism and life satisfaction affect behaviour towards others. The current study aims to look at how life satisfaction elps in influencing the prosocial and aggressive behaviour. It also aims to look at how optimism affects the display of aggressive and prosocial behaviour.

REVIEW OF LITERATURE

Juan, Concha, and Patricia (2022) did a study in Colombia to investigate the influence of Positive Self-Belief (PsB) on life satisfaction (SWL). Using the Classification Tree approach, they discovered that participating in one or more PsBs (for example, donating money or sharing food) improves SWL and reduces the influence of distressing feelings such as pessimism, indecisiveness, and irritation that have evolved as a result of the COVID-19 epidemic. These findings are crucial because they highlight the value of life satisfaction as an individual's resource for coping with challenging situations and give proof of PsB's advantages to one's well-being.



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Tu, Zhang, Sun, and Wang; (2022) conducted a study among Chinese college students to explore the correlation between life satisfaction and prosocial behavior. The study utilized a self-reported scale to measure prosocial behavior and found a positive correlation between higher levels of life satisfaction and prosocial behavior. The authors postulated that this association could be attributed to individuals with higher life satisfaction being more inclined to feel a sense of belonging and connection to others. This, in turn, could motivate them to engage in helpful and cooperative behaviors towards others. Furthermore, individuals with high life satisfaction may experience reduced levels of negative emotions like anger or frustration, which could lower the likelihood of aggressive or antisocial behaviours

Kaur and Kaur (2021) performed a study on the association between life happiness and prosocial behaviour in teenagers, with an emphasis on gender differences: The study employed a cross-sectional design in which 400 teenagers (200 males and 200 girls) were recruited from schools in Punjab, India, to fill out self-report questionnaires about life satisfaction and prosocial behaviour. The study discovered a positive link between life contentment and prosocial behaviour, implying that greater levels of satisfaction with one's life were related to elevated degrees of prosocial behaviour in both genders. The study did discover, however, that females reported greater levels of prosocial behaviour than boys, even after correcting for variations in life satisfaction.

Zhang and Zhao (2020) explored how the life happiness of urban inhabitants effects their prosocial behavioural intentions in their community. This study uses self-administered questionnaires to obtain data from a web-based survey of Chinese urban inhabitants (N = 765) in China. These findings imply that high degrees of orientation to purpose in life, sense of virtuous humanity, and cognitive reappraisal may assist urban dwellers' communal prosocial behavioural intentions.

Schwaba, Robins, Sanghavi, and Bleidorn(2019) investigated how optimism develops throughout life and how it is impacted by positive and bad life experiences. Researchers investigated the normative trajectory of optimism growth from longitudinal group aged 26 to 71 (N = 1,169) of Mexican-origin couples tested four times over a seven-year period. The analysis of latent development curves revealed that optimism rose across early and middle adulthood until plateauing at the age of 55, with substantial individual variations in change.

Hanniball, Viljoen, and Shaffer (2018) carried out a research to investigate the correlation between life satisfaction and youth violence and offending. They performed a prospective evaluation among a group of community youth (n = 334) and at-risk youth (n = 99) to examine this relationship. The study's results revealed a significant connection between life satisfaction and a decrease in violence and offending in both samples. Furthermore, the study found that life satisfaction contributes additional value in forecasting violent and total offending among community youth, beyond the established risk factors. As a result, the study suggests that enhancing overall life satisfaction could potentially help prevent adolescent offending.

METHOD

Aim The aim of the study was to assess the impact of Optimism and Life satisfaction on prosocial and aggressive behaviour among adults.

Objective

- To find association between optimism and prosocial behaviour
- To find association between optimism and aggressive behaviour
- To find association between life satisfaction and prosocial behaviour
- To find association between life satisfaction and aggressive behaviour
- To examine impact of optimism on prosocial behaviour
- To examine impact of optimism on aggressive behavior



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- To examine impact of Life satisfaction on prosocial behavior
- To examine impact of Life satisfaction on aggressive behavior
- To examine gender differences in Prosocial and Aggressive behaviour

Hypothesis

- There are will be significant association between optimism and aggressive behaviour.
- There are will be significant association between optimism and prosocial behaviour
- There are will be significant association between Life satisfaction and prosocial behaviour
- There are will be significant association between life satisfaction and aggressive behaviour.
- Optimism will be a significant predictor of pro social behaviour
- Life satisfaction will be a significant predictor of pro social behaviour.
- Optimism will be a significant predictor of aggressive behaviour.
- Life satisfaction will be a significant predictor of aggressive behaviour.
- Females will show higher pro social behaviour than males.
- Males will show higher aggressive behaviour than females

Design

A correlational research design was used to assess the impact of Optimism and life satisfaction on prosocial and aggressive behaviour.

Variables

Independent variable- Optimism, Life satisfaction

Dependent variable- prosocial behaviour ,Aggression

Sample

With the aim of quantitative research ,data was collected from 100 respondents using a questionnaire,. The sample consisted of 50 males and 50 females . The respondents were from the age of 21 -30 years, all residing in Delhi and NCR

Tool Used

Using the questionnaires data was collected both offline and online . There were 100 responses to the survey. One questionnaire consisted of a total of 10 questions with multiple choice answers measuring Optimism. The other questionnaire consisted of 5 questions measuring the level of life satisfaction. The other consisted of 34 and 16 questions measuring aggressive and prosocial behaviour respectively. Demographics and personal characteristics-Basic information sheet providing information on the age, ethnicity, and educational credentials of the subject.

Revised Life Orientation Test (LOT-R)-The Revised Life Orientation Test (LOT-R), created by psychologist Michael Scheier and colleagues (1994), is a scale consisting of ten items that assesses optimistic or pessimistic feelings of individuals towards the future. Prosocialness Scale-To measure an individual's level of prosocial behavior, the Prosocialness Scale for Adults consists of 16 items. Each item is rated on a five-point Likert scale, ranging from "never/almost never true" (coded as 1) to "almost always/always true" (coded as 5), allowing participants to indicate how frequently each behavior applies to them. The Aggression Questionnaire-The Aggression Questionnaire (Buss & Warren, 2000) is a well-established self-report instrument used to evaluate aggression. The AQ assesses both an individual's aggressive tendencies and their capacity to channel these tendencies in a positive and safe manner. Due to its brief completion time of 10 minutes, the AQ can be administered efficiently to a large sample size. The Satisfaction With Life Scale-The Satisfaction With Life Scale (SWLS) is a widely-used psychological tool designed to measure an individual's general life satisfaction. The SWLS does not focus on specific areas of life, such as health, finances, or relationships, but rather provides an overall evaluation of one's subjective well-being. The instrument is composed of five statements that assess a person's cognitive and emotional perceptions of their life, Participants rate their degree of agreement with each statement using a seven-point Likert scale that ranges from "strongly disagree" to "strongly agree."



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With the aim of data collection an appropriate tools was selected. The questionnaires were administered on the participants. All the instructions regarding how to administer the instrument were given to the participants . It was then also circulated through the various social media platforms like WhatsApp, Instagram ..etc..The data was obtained, after which the necessary calculation and scoring was done. Further analysis of the data was done, addressing the objectives of the study in a detailed discussion thereafter

ANALYSIS OF RESULT

The results were analysed quantitatively. The data was pooled in the MS Excel 2013, scoring was done and then was analysed using SPSS. The satisfaction with life scale was scored. It has a continuous scoring but also has some cut off benchmarks 31 - 35 Extremely satisfied, 26 - 30 Satisfied ,21 - 25 Slightly satisfied ,20 Neutral ,15 - 19 Slightly dissatisfied ,10 - 14 Dissatisfied ,5 - 9 Extremely dissatisfied .The Aggression Questionnaire (Buss & Warren, 2000), was scored by adding up the responses for each item within its respective subscale. The questionnaire consists of four subscales: Physical Aggression, Verbal Aggression, Anger, and Hostility. The Physical Aggression subscale contains items that assess the tendency to cause physical harm. The Verbal Aggression subscale includes items related to verbal attacks. The Anger subscale assesses angry feelings. Lastly, the Hostility subscale measures negative attitudes towards others.

The Revised Life Orientation Test (LOT-R) was scored on the basis of ten items and is scored using a five-point Likert scale. The test includes both positively and negatively worded statements, and participants are asked to rate their level of agreement or disagreement with each statement using a scale that ranges from "strongly disagree" to "strongly agree."To compute an individual's optimism score, the scores for the four positively worded items are summed, while the scores for the six negatively worded items are reversed and then summed. The total scores for the two sets of items are then added together, resulting in a total score that ranges from 0 to 24. A higher score indicates a greater degree of optimism. To score the Prosocialness Scale for Adults, the responses for each of the 16 items are added up. Participants provide their responses on a five-point Likert scale, where 1 indicates "never/almost never true" and 5 indicates "almost always/always true." The scale aims to measure different aspects of prosocial behavior, including showing care for others, cooperating with others, and providing assistance to others. Scores can range from 16 to 80, with higher scores indicating a greater inclination towards prosocial behavior. The Statistical analysis was carried out with the help of MS Excel 2013 and SPSS. Correlation, mean , standard deviation and regression was used to draw meaningful conclusion.

Pearson's r varies between +1 and -1, where +1 is a perfect positive correlation, and -1 is a perfect negative correlation. 0 means there is no linear correlation at all. Table 1 shows the correlation between prosocial behaviour and optimism . The pearsons r comes out to be .013 which shows a very weak positive correlation. Optimism does have an impact on prosocial behaviour but the impact is very small. The Sig. (2-tailed) is .898 meaning that the correlation obtained is not significant . Table 2 shows the correlation between aggressive behaviour and optimism . The pearsons r comes out to be -0.17 which shows a weak negative correlation. Optimism does have an impact on aggressive behaviour. With the increase in Optimism the aggressive behaviour decreases and vice versa. The Sig. (2-tailed) is .867 meaning that the correlation is not significant. Table 3 shows the correlation between aggressive behaviour and life satisfaction . The pearsons r comes out to be .475 which shows a strong positive correlation. Life satisfaction have an impact on aggressive behaviour. The Sig. (2-tailed) obtained shows that the correlation is highly significant . Table 4 shows the correlation between prosocial behaviour and life satisfaction . The pearsons comes out to be .170 which shows a strong positive correlation. Life satisfaction have an impact on prosocial behaviour. The Sig. (2-tailed) obtained shows that the correlation is highly significant .

Table 5 shows regression between the aggressive behaviour and optimism. The R value is 0.17 showing a weak positive relation between aggressive behaviour and optimism. The linear regression analysis revealed a weak



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positive relationship between the aggressive behaviour and optimism. The model accounted for 17% of the variance in the outcome variable, indicating that a significant portion of the variance is not accounted for by optimism. The low R-squared value suggests that other factors not included in the model may be influencing the outcome variable. The significance value is >0.05 . Therefore the result is not significant and F value is also small. The p-value is 0.867, which is greater than the commonly used alpha level of 0.05. This suggests that the Regression component of the model is not statistically significant, meaning that Optimism is not a significant predictor of Aggressive Behaviour in this particular sample. The coefficient shows the results of a linear regression analysis with Aggressive Behavior as the dependent variable and Optimism as the independent variable. The model includes a constant, which is statistically significant ($B = 62.062$, $t(98) = 7.759$, $p < .001$), indicating that there is a significant amount of variability in the dependent variable that is not explained by the independent variable. However, the coefficient for Optimism is not statistically significant ($B = -0.107$, $t(1) = -0.167$, $p = .867$), indicating that there is no significant linear relationship between Optimism and Aggressive Behavior. This suggests that Optimism is not a significant predictor of Aggressive Behavior in this particular sample

Table 6 provides information on the overall performance of the model. The R-square value is 0.000, indicating that Optimism does not explain a significant proportion of the variability in Prosocial behavior. The adjusted R-square is -0.010, suggesting that the model does not fit the data well. The standard error of the estimate is 21.502, indicating the average distance between the observed values of the dependent variable and the predicted values of the dependent variable. The ANOVA tests the statistical significance of the regression model. The table shows that the regression model explains a statistically non-significant amount of variance in Prosocial behavior. The F-value is 0.017, and the associated p-value is 0.898, which is greater than the commonly used alpha level of 0.05. This suggests that Optimism is not a significant predictor of Prosocial behavior. The coefficients shows the estimates of the regression coefficients. The intercept (Constant) is 94.685, which is the predicted value of Prosocial behavior when Optimism is zero. The coefficient for Optimism is 0.144, indicating that, on average, an increase of one unit in Optimism is associated with an increase of 0.144 units in Prosocial behavior. The t-value for Optimism is 0.129, and the associated p-value is 0.898, indicating that the coefficient is not statistically significant.

The table 7 provides information on the overall performance of the model. The R-square value is 0.226, indicating that Life satisfaction explains 22.6% of the variability in Aggressive behavior. The adjusted R-square is 0.218, suggesting that the model fits the data reasonably well. The standard error of the estimate is 18.923, indicating the average distance between the observed values of the dependent variable and the predicted values of the dependent variable. The ANOVA tests the statistical significance of the regression model. The table shows that the regression model explains a statistically significant amount of variance in Aggressive behavior. The F-value is 28.557, and the associated p-value is 0.000, which is less than the commonly used alpha level of 0.05. This suggests that Life satisfaction is a significant predictor of Aggressive behavior. The coefficients shows the estimates of the regression coefficients. The intercept (Constant) is 58.295, which is the predicted value of Aggressive behavior when Life satisfaction is zero. The coefficient for Life satisfaction is 1.859, indicating that, on average, an increase of one unit in Life satisfaction is associated with an increase of 1.859 units in Aggressive behavior. The t-value for Life satisfaction is 5.344, and the associated p-value is 0.000, indicating that the coefficient is statistically significant. Overall, the results indicate that Life satisfaction is a significant predictor of Aggressive behavior, as the coefficient is statistically significant, and the model explains a significant amount of variance in Aggressive behavior. Higher levels of Life satisfaction are associated with lower levels of Aggressive behavior

The table 8 shows that the correlation coefficient (R) is 0.17, indicating a weak positive relationship between the variables. The R-Square value of 0.029 suggests that only 2.9% of the variance in Prosocial Behavior can be explained by Life Satisfaction. The Adjusted R-Square value of 0.019 indicates that the addition of the independent variable, Life Satisfaction, did not significantly improve the model's ability to explain the dependent variable. The ANOVA shows that the regression model is not significant as the p-value of the F-statistic is greater than 0.05. This indicates that the model does not provide a good fit for the data. The Coefficients indicates that the intercept value is 52.894, which means that when Life Satisfaction is zero, the predicted value of Prosocial Behavior is 52.894. The regression



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coefficient for Life Satisfaction is 0.382, indicating that for every one-unit increase in Life Satisfaction, the predicted value of Prosocial Behavior will increase by 0.382. However, since the p-value of the coefficient is greater than 0.05, we cannot conclude that there is a significant linear relationship between Life Satisfaction and Prosocial Behaviour. The group statistics table 9 shows that the mean score on the Aggressive behaviour for females ($M = 95.54$, $SD = 22.286$) is slightly lower than that for males ($M = 97.38$, $SD = 20.651$). The sample size is equal for both groups with $N=50$. The group statistics shows that the mean score on the Prosocial Behavior measure for females ($M = 59.44$, $SD = 13.042$) is slightly lower than that for males ($M = 62.04$, $SD = 11.439$). The sample size is equal for both groups with $N=50$.

For Aggressive behaviour Levene's test for equality of variances indicates that there is no significant difference in variances between the two groups ($p = .427$), so we can assume that the variances are equal. The t-test for equality of means shows that there is no significant difference in mean scores between males and females, $t(98) = -0.428$, $p = .669$. The mean difference between the two groups is -1.840 , with a standard error of 4.297 . The 95% confidence interval for the mean difference ranges from -10.367 to 6.687 , indicating that we cannot be sure that there is a true difference between the two groups. The results suggest that there is no significant difference in mean scores on the measure of aggression between males and females in this sample. This is further supported by the non-significant p-value and the confidence interval including zero. Therefore, we cannot reject the null hypothesis that there is no difference in mean scores between males and females. For Prosocial behaviour Levene's test for equality of variances indicates that there is no significant difference in variances between the two groups ($p = .531$), so we can assume that the variances are equal. The t-test table 20 for equality of means shows that there is no significant difference in mean scores between males and females, $t(98) = -1.060$, $p = .292$. The mean difference between the two groups is -2.600 , with a standard error of 2.453 . The 95% confidence interval for the mean difference ranges from -7.469 to 2.269 , indicating that we cannot be sure that there is a true difference between the two groups.

In the results suggest that there is no significant difference in mean scores on the Prosocial Behavior measure between males and females in this sample. This is further supported by the non-significant p-value and the confidence interval including zero. Therefore, we cannot reject the null hypothesis that there is no difference in mean scores between males and females.

DISCUSSION

The current research topic “impact of optimism and life satisfaction on prosocial and aggressive behaviour”. Optimism is described as an optimistic perspective or attitude on life that entails anticipating favourable circumstances or occurrences in the future. Optimism is seen as a variable attribute that has been linked to a variety of psychological as well as physical advantages, including improved coping abilities, reduced levels of stress, and improved cardiovascular health. “Life satisfaction has been defined as “a person’s cognitive and affective evaluations of his or her life” (Diener, 2002). Life satisfaction is a person’s subjective assessment of their life as a whole. It entails a cognitive and emotive evaluation of one’s own life situations, such as social connections, financial condition, career, and wellness.

Aggression is defined as any behavior that is designed to inflict bodily or verbal damage or impairment toward another individual. This may vary from modest verbal aggressiveness like name-calling or taunting to much more extreme physical violence like punching or attacking someone. Prosocial behaviour is defined as any behaviour which has the intent to help or assist others without regard for self-gain or profit. The study collected data from 100 participants using questionnaires administered both online and offline. The questionnaires included the Revised Life Orientation Test (LOT-R) to measure optimism, the Prosocialness Scale to measure prosocial behavior, the Aggression Questionnaire to measure aggressive tendencies, and the Satisfaction with Life Scale to measure overall life satisfaction. Demographic information was also collected. The procedures for administering the questionnaires were explained to participants, and the data was analyzed and discussed in detail to address the study’s objectives.



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A very weak positive correlation between prosocial behavior and optimism, indicating that optimism does have an impact on prosocial behavior, but the impact is minimal. On the other hand, Table 2 shows a weak negative correlation between aggressive behavior and optimism, indicating that optimism has an impact on aggressive behavior, but the impact is also not significant. The correlation between aggressive behavior and life satisfaction is a strong positive correlation, indicating that life satisfaction has an impact on aggressive behavior. This result is further supported by the regression analysis where a weak positive relationship is observed between aggressive behavior and optimism, and the model accounted for only 17% of the variance in the outcome variable, indicating that other factors not included in the model may be influencing the outcome variable. Table 6 also confirms that optimism is not a significant predictor of aggressive behavior in this particular sample.

Similarly, a strong positive correlation between prosocial behavior and life satisfaction, indicating that life satisfaction has an impact on prosocial behavior. This is further supported by the regression analysis, where optimism does not explain a significant proportion of the variability in prosocial behavior, and the model does not fit the data well. Life satisfaction explains 22.6% of the variability in aggressive behavior, and the model fits the data reasonably well. This is further supported by the ANOVA table which shows that the regression model explains a statistically significant amount of variance in aggressive behavior, suggesting that life satisfaction is a significant predictor of aggressive behavior.

In summary, the results suggest that optimism has a minimal impact on prosocial behavior and aggressive behavior, while life satisfaction has a significant impact on both prosocial behavior and aggressive behavior. The regression analyses further support these findings and suggest that other factors not included in the models may also be influencing the outcome variables. Based on the results of the t-tests, there were no significant differences in mean scores between males and females on both measures of aggression and prosocial behavior. The confidence intervals for the mean differences included zero, indicating that we cannot be sure that there is a true difference between the two groups. Therefore, the study did not find any evidence of gender differences in aggression and prosocial behavior in this sample. It is important to note that this study had an equal sample size for males and females, which strengthens the validity of the results. However, it is also important to consider that these findings may not generalize to other populations or contexts, and further research is needed to explore gender differences in aggression and prosocial behavior.

To sum up our understanding of how optimism and life happiness impact our behavior in relation to aggression and prosocial tendencies is multifaceted and warrants additional investigation. Nevertheless, it is obvious that optimism and happiness in life are essential elements in shaping human behavior towards oneself and towards others. These positive traits are indicative of a strong connection between personal happiness and favourable behaviour that promotes social connections and cooperative behaviour in society. Conversely, negative emotions like aggression and hostility are less common in those with positive being attitudes. Overall, the connection between happiness, optimism, and behavioural tendencies is intricate and inactive not to the full elucidated. Future research in the field of psychological science should continue to explore this topic to advance our comprehension of positive emotions and their role in shaping human behaviour. While more study is needed to explore the influence of optimism and life satisfaction on aggressiveness and prosocial behaviour in Indian adults, the present data shows that these characteristics are likely to be key predictors of these behaviours.

In addition, the results suggest that optimism and life satisfaction may be especially important for promoting prosocial behaviors in collectivistic cultures. Additional studies are needed to determine whether these effects persist throughout life, as well as whether interventions that promote optimistic thinking could have beneficial effects on aggression and prosocial behavior. Finally, it is important to consider cultural factors when developing interventions that promote optimistic thinking. Several potential limitations of the current study should be considered. The sample size used in the research is relatively small, which may limit the generalizability of the findings. The data used in the research was collected from only 100 participants, which may not be representative of the larger population. The research only examines a limited number of variables that are assumed to impact prosocial and aggressive behavior.





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The study does not consider other factors that may have an impact on these behaviors such as environmental factors, family structure, and socioeconomic status. The study relies on correlation analysis, which does not necessarily imply causation. The study may show a correlation between optimism and prosocial behavior, but it does not prove that optimism causes prosocial behavior. The data collected for the study is based on self-reports by participants. This may lead to bias as participants may not always provide accurate responses, which could affect the reliability and validity of the results. Weak Statistical Significance: Some of the statistical tests performed in the study did not yield statistically significant results, indicating that there may be no relationship between the variables being studied. For example, the weak correlation coefficient between optimism and aggressive behavior suggests that there may not be a significant relationship between these variables. The study does not control for confounding variables that could affect the relationship between the variables being studied. For example, the study does not control for variables such as personality traits or past experiences that could affect prosocial or aggressive behavior.

SUMMARY AND CONCLUSION

The study found that optimism has a minimal impact on prosocial and aggressive behavior, while life satisfaction has a significant impact on both. Regression analysis supports these findings and suggests that other factors may be influencing the outcome variables. The study did not find any significant gender differences in aggression and prosocial behavior. However, the findings may not generalize to other populations or contexts, and further research is needed. Overall, the study highlights the importance of cultivating life satisfaction for positive behaviour.

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Table 1 shows the correlation between Prosocial behaviour and Optimism

| Correlations | | | |
|---------------------|---------------------|----------------------|----------|
| | | Aggressive behaviour | OPTIMISM |
| prosocial behaviour | Pearson Correlation | 1 | .013 |
| | Sig. (2-tailed) | | .898 |
| | N | 100 | 100 |
| OPTIMISM | Pearson Correlation | .013 | 1 |
| | Sig. (2-tailed) | .898 | |
| | N | 100 | 100 |

Table 2 shows the correlation between Aggressive behaviour and Optimism

| Correlations | | | |
|---------------------|---------------------|----------|-----------|
| | | OPTIMISM | PROSOCIAL |
| agressive behaviour | Pearson Correlation | 1 | -.017 |
| | Sig. (2-tailed) | | .867 |
| | N | 100 | 100 |
| optimism | Pearson Correlation | -.017 | 1 |
| | Sig. (2-tailed) | .867 | |
| | N | 100 | 100 |





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Table 3 shows the correlation between Aggressive behaviour and Life satisfaction

| Correlations | | | |
|----------------------|---------------------|-------------------|----------------------|
| | | Life satisfaction | Aggressive behaviour |
| Life Satisfaction | Pearson Correlation | 1 | .475 |
| | Sig. (2-tailed) | | .000 |
| | N | 100 | 100 |
| Aggressive behaviour | Pearson Correlation | .475 | 1 |
| | Sig. (2-tailed) | .000 | |
| | N | 100 | 100 |

Table 4 Shows correlation between life satisfaction and prosocial behaviour

| Correlations | | | |
|-------------------|---------------------|-------------------|-----------|
| | | life satisfaction | PROSOCIAL |
| life satisfaction | Pearson Correlation | 1 | .170 |
| | Sig. (2-tailed) | | .090 |
| | N | 100 | 100 |
| PROSOCIAL | Pearson Correlation | .170 | 1 |
| | Sig. (2-tailed) | .090 | |
| | N | 100 | 100 |

Table 5 shows the regression between Aggressive behaviour and optimism

| model | R | R Square | F | Sig. | Standardized Coefficients Beta | t | Adjusted R Square | Std. Error of the Estimate | Unstandardized Coefficients | |
|--|-------------------|----------|------|-------------------|--------------------------------|-------|-------------------|----------------------------|-----------------------------|------------|
| | | | | | | | | | B | Std. Error |
| Optimism | .017 ^a | .000 | .028 | .867 ^b | -.017 | -.167 | -.010 | 12.335 | -.107 | .641 |
| (constant) | | | | | | | | | 62.062 | 7.999 |
| DEPENDENT VARIABLE -AGGRESSIVE BEHAVIOUR | | | | | | | | | | |

Table 6 shows the regression between Prosocial behaviour and optimism

| model | R | R Square | F | Sig. | Standardized Coefficients Beta | t | Adjusted R Square | Std. Error of the Estimate | Unstandardized Coefficients | |
|---|-------------------|----------|------|-------------------|--------------------------------|-------|-------------------|----------------------------|-----------------------------|------------|
| | | | | | | | | | B | Std. Error |
| Optimism | .013 ^a | .000 | .017 | .898 ^b | .013 | -.167 | -.010 | 21.502 | .144 | 1.118 |
| (constant) | | | | | | | | | 94.685 | 13.943 |
| Dependent Variable: PROSOCIAL BEHAVIOUR | | | | | | | | | | |





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Table 7 Shows regression between Aggressive behaviour and Life satisfaction

| model | R | R Square | F | Sig. | Standardized Coefficients Beta | t | Adjusted R Square | Std. Error of the Estimate | Unstandardized Coefficients | |
|--|-------------------|----------|--------|-------------------|-----------------------------------|-------|-------------------|----------------------------|-----------------------------|------------|
| | | | | | | | | | B | Std. Error |
| Life satisfaction | .475 ^a | .226 | 28.557 | .000 ^b | .475 | 5.344 | .218 | 18.923 | 1.859 | .348 |
| (constant) | | | | | | | | | 58.295 | 7.388 |
| Dependent Variable: AGGRESSIVE BEHAVIOUR | | | | | | | | | | |

Table 8 Shows regression between Prosocial behaviour and Life satisfaction

| model | R | R Square | F | Sig. | Standardized Coefficients Beta | t | Adjusted R Square | Std. Error of the Estimate | Unstandardized Coefficients | |
|---|-------------------|----------|-------|------|-----------------------------------|-------|-------------------|----------------------------|-----------------------------|------------|
| | | | | | | | | | B | Std. Error |
| Life satisfaction | .170 ^a | .029 | 2.924 | .090 | .170 | 1.710 | .019 | 12.157 | .382 | .223 |
| (constant) | | | | | | | | | 52.894 | 4.747 |
| Dependent Variable: PROSOCIAL BEHAVIOUR | | | | | | | | | | |

Table 9 Shows mean, standard deviation and standard error mean for prosocial and aggressive behaviour

| Group Statistics | | | | | |
|----------------------|--------|----|-------|----------------|-----------------|
| | gender | N | Mean | Std. Deviation | Std. Error Mean |
| PROSOCIAL | female | 50 | 59.44 | 13.042 | 1.844 |
| | Male | 50 | 62.04 | 11.439 | 1.618 |
| AGGRESSIVE BEHAVIOUR | female | 50 | 95.54 | 22.286 | 3.152 |
| | Male | 50 | 97.38 | 20.651 | 2.920 |

Table 10 Shows Independent sample t-test for prosocial and aggressive behavior

| | | Levene's Test for Equality of Variances | | t-test for Equality of Means | | | | | | |
|----------------------|-----------------------------|---|------|------------------------------|--------|-----------------|-----------------|-----------------------|---|-------|
| | | F | Sig. | t | df | Sig. (2-tailed) | Mean Difference | Std. Error Difference | 95% Confidence Interval of the Difference | |
| | | | | | | | | | Lower | Upper |
| AGGRESSIVE BEHAVIOUR | Equal variances assumed | .636 | .427 | -.428 | 98 | .669 | -1.840 | 4.297 | -10.367 | 6.687 |
| | Equal variances not assumed | | | -.428 | 97.436 | .669 | -1.840 | 4.297 | -10.367 | 6.687 |





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| | | | | | | | | | | |
|-----------|-----------------------------|------|------|--------|--------|------|--------|-------|--------|-------|
| PROSOCIAL | Equal variances assumed | .395 | .531 | -160 | 98 | .292 | -2.600 | 2.453 | -7.469 | 2.269 |
| | Equal variances not assumed | | | -1.060 | 96.362 | .292 | -2.600 | 2.453 | -7.470 | 2.270 |





Techno Stress in the Age of Artificial Intelligence: Potential Impact of AI on Mental Health and Well-Being

R. D. Padmavathy*

Assistant Professor, Department of Education, Tezpur University (Central), Assam-784028, India

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*Address for Correspondence

R. D. Padmavathy

Assistant Professor,

Department of Education,

Tezpur University (Central),

Assam-784028, India

E.Mail: padmajothi@yahoo.in



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ABSTRACT

This study investigates how, in light of the prevalent phenomenon known as techno stress, artificial intelligence (AI) may impact our mental health and wellness. Given their rapid development and ubiquitous integration into a variety of areas of our lives, it is imperative to look into the potential psychological effects that AI technologies may have. By examining the existing research on technological stress and its underlying mechanisms, this study seeks to shed light on the precise ways in which AI can impair human mental health and well-being. In this research how AI might affect people's psychological health and wellbeing. Also investigate how AI might affect our overall mental health and wellbeing, the potential factors effects of technostress as it relates to AI, as well as how it might affect technostress. In addition to that researcher discusses techniques for dealing with technological stress in the era of AI. This research would be a useful addition about the future of artificial intelligence since it would give readers knowledge of the possible effects of AI on human mental health and wellbeing. It would also be a useful tool for corporations and policymakers as it would provide them advice on how to lessen the dangers of AI and technological stress.

Keywords: Techno stress, Artificial Intelligence, Mental Health and Well-Being

INTRODUCTION

Artificial intelligence (AI) technology have quickly advanced, altering many facets of our lives, including how we work, communicate, and engage with the outside world. While there are many advantages and opportunities provided by AI, there are also special difficulties and potential hazards to our mental health and wellbeing. Techno





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stress, which is defined as the stress and adverse psychological effects brought on by the usage of technology and ICT systems, is one such difficulty (Tarafdar, 2020). Understanding the potential effects of these technologies on our mental health is crucial in the age of AI. This research examines the idea of "techno stress" in relation to artificial intelligence and examines some of the potential ways that technology may affect our mental health and wellbeing. By examining the existing literature, empirical research, and survey-based studies, we hope to shed light on the complex relationships between AI and mental health. In doing so, we seek to draw attention to how important it is to solve these problems in order to ensure a positive and balanced relationship with technology (Ragu-Nathan *et al.*, 2008; Tarafdar *et al.*, 2015). The impact of artificial intelligence (AI) on our way of life and work is growing quickly. Artificial intelligence (AI)-enabled devices are widely accessible and have a big impact on how we live our daily lives. But there is growing worry that the use of AI could increase levels of technological stress. An overview of AI technologies and their rising use in modern society is given in the article's opening paragraphs. After that, it explores the idea of technostress, stressing its sources and effects. The study further investigates the moderating and mediating elements that may affect the association between AI and mental health outcomes, drawing on the various processes through which AI can generate technological stress.

Techno stress

A form of stress brought on by the usage of technology is known as technostress. "The strain and negative psychological impact resulting from the use of technology and information and communication technology (ICT) systems" (Tarafdar, 2020, p. 1195) is how technological stress is defined. Craig Brod (1984) initially used the word "technostress" in his definition of as "a modern disease of adaptation caused by an inability to cope with the new computer technologies in a healthy manner." When people deal with technological tools and systems, they often suffer sensations of anxiety, annoyance, and overwhelm (Ragu-Nathan *et al.*, 2008). Information overload, continual connectedness, and the fear of missing out are just a few of the causes.

Key Contributors To Techno stress In The Context Of AI

Three categories can be made out of the elements that lead to technological stress:

Technology's Use: Technostress can also be influenced by how technology is used. Someone may feel more stressed and anxious if they are continuously checking their email or social media, for instance.

Technology's inherent stressors: Some technologies are by nature more stressful than others. Examples of technology that can be more stressful than others are those that change frequently or demand a lot of multitasking.

The personality and coping mechanisms of the person: Technostress affects some people more than others. Technostress may be more prevalent in, for instance, individuals who are more worried or who struggle with change.

Specific Factors That Can Contribute To Technostress

Information overload: In recent years, the amount of information to which we are exposed on a daily basis has grown dramatically. As a result of this, you could feel stressed and anxious. Information overload is described as "the feeling of being overwhelmed by the sheer volume of information available and the pressure to keep up with it" (Cascio & Shurygailo, 2003, p. 188). Cognitive overload and an inability to efficiently digest and use the information that is available can result from the continuous influx of information from AI-powered systems (Tarafdar *et al.*, 2015). Constant connectivity: Both at work and at home, we are now always plugged into technology. Because of this, it could be challenging to unwind and disengage, which might result in more stress. FOMO (Fear of missing out): One of the main causes of technological stress can be the worry about missing out on something that is happening online. Young people, who are more prone to utilize social media and other online platforms, are particularly affected by this. Cyber bullying: A type of bullying that occurs online or via technological devices is known as cyber bullying. It can have the same negative effects as conventional bullying and raise levels of technostress. Technology addiction: This problem occurs when a person's dependence on technology interferes with their day-to-day activities. This could make you feel anxious, stressed out, or depressed.





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Loss of control: which happens when people feel that they have no control over the technology they utilize. Due to the autonomy of AI systems, people may feel that the technology is controlling their decisions and behaviors rather than their own free will (Tarafdar *et al.*, 2015). The loss of control over technology can lead to feelings of frustration and powerlessness (Ragu-Nathan *et al.*, 2008).

Job insecurity and technological stress are tightly related, particularly in the context of the adoption of AI. People may feel anxious and afraid about the possible effects on their job security and employability when AI technologies automate some tasks (D'Arcy *et al.*, 2017). Increased stress levels and unfavorable psychological effects can result from the worry that AI systems would replace humans (Tarafdar, 2020).

Social isolation: Another aspect of technological stress that results from an increased reliance on technology for social connection and communication is **social isolation**. While AI-powered communication systems make it simple to stay in touch, they may also result in less in-person encounters and a sense of separation from others, which can make people feel lonely and isolated (Amichai-Hamburger & Ben-Artzi, 2003).

Privacy issues related to AI technologies can considerably increase the stress caused by technology. Concerns concerning data security and privacy breaches are raised by the acquisition and use of personal data by AI systems (Harrison *et al.*, 2019). Individuals may experience worry and anxiety as a result of ongoing monitoring and the potential disclosure of personal information, which may have an adverse effect on their general wellbeing (Ragu-Nathan *et al.*, 2008).

Implications Of Techno-Stress On Student Well-Being And Academic Performance

The experience of Techno-Stress can have significant implications for student well-being and academic performance in higher education. Understanding these implications is essential for promoting a healthy and productive learning environment. **Impact on Psychological Well-being:** Techno-Stress can lead to negative psychological outcomes such as anxiety, frustration, and burnout among students (Brod, C., *et al.*, 2005). These stressors can adversely affect mental health and contribute to decreased motivation, engagement, and overall well-being. **Academic Performance and Productivity:** Techno-Stress can hamper academic performance by impeding concentration, cognitive functioning, and information processing (Ragu-Nathan, T.S., *et al.*, 2008). Students overwhelmed by Techno-Stress may experience difficulties in managing their workload, meeting deadlines, or effectively utilizing technology for learning purposes.

Learning Engagement and Persistence: Techno-Stress can negatively impact student engagement and persistence in learning (Brod, C., *et al.*, 2005). Students overwhelmed by Techno-Stress may disengage from technology-mediated activities, exhibit reduced interest in coursework, and may even avoid using technology altogether. **Relationship and Social Connectedness:** Techno-Stress can disrupt social relationships and connectedness among students (Ragu-Nathan, T.S., *et al.*, 2008). Excessive technology use or negative experiences with technology can hinder face-to-face interactions, reduce social support, and contribute to feelings of isolation. Recognizing and addressing the implications of Techno-Stress is crucial for educational institutions and stakeholders. Implementing strategies to manage Techno-Stress and foster a healthy technology environment can enhance student well-being, improve academic performance, and create a positive and supportive learning environment.

Potential Impact Of Artificial Intelligence On Techno Stress

AI has the potential to increase technostress in several ways. AI-powered products, for instance, might make it more difficult to disengage and unwind because we would constantly be available and connected. AI may also result in lost jobs, which can make people feel uneasy and uncertain. Several aspects of technology use contribute to technological stress. Those are **Overwhelmed by technology:** AI-powered systems can automate choices and jobs that were previously completed by humans. Employees may get techno-overloaded as a result of feeling overburdened with the duties and information they need to manage. (Ayyagari *et al.*, 2011) **Techno-invasion:** AI systems have the potential to be intrusive, always watching our actions and offering advice. This can cause a sense of privacy invasion and lack of control, which can add to technostress. (Lee *et al.*, 2016)



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Technological complexity: AI systems can be intricate and challenging to comprehend. Technological complexity may result from this because employees may become anxious and frustrated if they cannot comprehend how the system operates or why certain decisions are being made. (Sundararajan,2017) Techno-insecurity: Since AI systems are frequently assumed to be faultless, this can cause techno-insecurity. Employees may be concerned that AI will replace them or that they will commit errors that the system won't. (Chui,2016) Techno-ambiguity: Given the uncertainty surrounding AI's future, there may be technological uncertainty. Employees can be concerned about how AI will affect their careers, jobs, and society at large. (Brynjolfsson & McAfee,2014) AI can affect technostress in many broad ways in addition to these particular stressors. AI systems, for instance, can make workers feel isolated since they may think they are no longer in charge of their work. AI systems can potentially cause a loss of meaning since workers may believe that their labor has no purpose if a machine can perform it. It's crucial to keep in mind that not everyone will be affected by AI's technostress. However, AI can be a big cause of worry for individuals who are prone to it. It's critical to be aware of the potential risks associated with AI and to take precautions to reduce those risks.

Strategies For Managing Techno Stress In The Age Of AI

In the era of artificial intelligence, there are proactive strategies to reduce the detrimental psychological effects of technology use. These tactics seek to encourage a positive balance between social connection and technological use. The following are some successful tactics backed by current research: Technology use combined with mindfulness and digital detox: According to Rosen *et al.* (2013), techno stress can be minimized by regularly taking breaks from technology, disconnecting, and intentionally blocking off time for it. Limitations of technology: The overuse of technology and consequent technostress can be avoided by setting clear guidelines and limitations. A better connection with technology can be cultivated by designating distinct times and locations for tech-free activities, such as family time or leisure activities (Mark *et al.*, 2018). Self-regulation approaches: According to Lee *et al.* (2012), using time management techniques and setting goals for technology use can both help reduce technological stress.

Take breaks: Throughout the day, take regular pauses from using technology. Your body and mind will get a chance to unwind and rejuvenate as a result. Give AI systems a rest. Get up and move around, or take a little break from your computer. Become trained in the use of AI systems. You'll feel more in control and be able to understand how the systems operate as a result. (Van Der Heijden & De Vries,2006) Improve your time management skills: Improve your time management skills to stop feeling rushed or anxious all the time. Seek expert assistance: Discuss your worries with someone. Talk to a friend, member of your family, or a therapist if you are experiencing stress over AI. Ask for expert assistance if necessary. Speak with a therapist or counselor if you are having trouble controlling technological stress on your own. It may be necessary to seek professional assistance if you are suffering from acute technostress. You can discover the causes of your technostress and create coping mechanisms with the assistance of a therapist. Improve your time management skills: Improve your time management skills to stop feeling rushed or anxious all the time.

Social support and connectedness: Strong social networks and support systems help counteract the harmful impacts of technological stress. The perception of social isolation frequently connected to excessive technology use can be reduced by participating in offline social activities, maintaining face-to-face relationships, and asking for help from friends, family, or support groups (Amichai-Hamburger & Ben-Artzi, 2003).Organizational interventions: Organizations have a significant impact on how employees deal with technological stress. Technological stress in the workplace can be decreased by putting in place rules and procedures that support work-life balance, encourage technology breaks, and offer tools for stress management (Karanika-Murray *et al.*, 2015). Engage in mindfulness. The discipline of mindfulness involves being in the moment without passing judgment. You can unwind and manage tension by doing this. 2014 (Orme-Johnson & Epel). Technology-related mindfulness practices, such as being in the moment and deliberately observing one's technology use, can also help people feel less stressed (Xu *et al.*, 2012). Digital well-being tools and applications: Making use of tools and programs for digital well-being that are especially made for managing and monitoring technology use can help people keep a healthy balance. These solutions give users access to features like usage tracking, screen time limits, and notification management, enabling them to better





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understand their technology usage patterns and make wise decisions (Griffiths *et al.*, 2020). Education and awareness: People may make wise decisions and manage technological stress by being more knowledgeable and aware of the possible effects of AI and technology use on mental health. Information on potential risks, advantages, and methods for healthy technology usage can be found through educational programs and initiatives (Dhir *et al.*, 2019). Consume a balanced diet and exercise frequently. You can combat stress by eating healthy foods since they will provide you the energy you need. Exercise is an excellent strategy to lower stress and enhance your general health. Find healthy stress-reduction techniques, such as exercise, yoga, or meditation, if you discover that you are having trouble handling technostress. Get adequate rest. You can handle stress better when you are well-rested. Technostress is a complicated problem, and there is no one-size-fits-all solution, it is crucial to remember. Depending on the person, different strategies will work best to handle technostress. However, the methods mentioned above can assist in lowering technological stress and enhancing your general wellbeing. Utilizing these tactics can benefit both individuals and companies.

The Ethical Implications Of AI And Technostress

Privacy: AI systems have the potential to gather and keep a significant amount of personal data, which creates privacy concerns. AI systems can be used, for instance, to follow our online activity, keep an eye on our movements, and even forecast our future conduct. We might be the target of discrimination or manipulation based on this information. 2014 (Floridi)

Fairness: Because AI systems have the potential to be biased, they may treat some people or groups unfairly. For instance, AI-based financing or hiring processes may be biased towards particular groups of people. 2017 (Selbst, *et al.*)

Accountability: Holding AI systems responsible for their behavior might be challenging. This is due to the fact that AI systems are frequently complicated and opaque, making it challenging to comprehend how they decide what to do. (2018) (Brundage *et al.*)

Safety: AI systems can malfunction, which could lead to harm to individuals or groups. For example, an AI-powered self-driving car could crash, or an AI-powered medical diagnosis system could make a mistake. (Coeckelbergh, 2017)

Meaningfulness: Because machines can now perform activities that previously required human labor, there may be a loss of meaning in work as a result of AI systems. Feelings of boredom, loneliness, and even melancholy may result from this. (Lee, 2018)

When creating and utilizing AI systems, the above ethical ramifications must be taken into account and must make sure AI technologies are created and applied in a way that upholds human rights and moral principles.

CONCLUSION

Our lives are being significantly impacted by the development of artificial intelligence (AI), both positively and negatively. On the one hand, AI improves the productivity and comfort of our lives. On the other side, AI is also bringing along fresh difficulties like technostress, appear in many different ways, such as worry, depression, restlessness, and difficulties focusing. Technostress can be caused by a variety of things, including as how much time we spend using technology, the kind of technology we use, and our own unique vulnerabilities. It's a complicated topic to consider how AI can affect our mental health and wellbeing. One way that AI might benefit mental health is through the development of fresh cures and treatments. AI, on the other side, as our reliance on technology grows; AI may also contribute to technostress. It's critical to be aware of the potential risks associated with AI and to take precautions to reduce those risks. We can achieve this by utilizing technology mindfully, drawing lines between our personal and professional lives, and taking breaks from it. If we are having trouble controlling technostress on our own, we can also get professional assistance.





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Piezosurgery: A New Dimension in Periodontology and Implantology

Aayushee Gupta¹, Shikha Sharma², Vidushi Sheokand³ and Amit Bhardwaj^{4*}

¹Post Graduate Student, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.

²Senior Lecturer, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.

³Reader, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.

⁴Head of the Department, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India

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*Address for Correspondence

Amit Bhardwaj

Head of the Department,
Department of Periodontology,
Faculty of Dental Sciences,
SGT University, Gurugram,
Haryana, India

E.Mail: amitbhardwaj@sgtuniversity.org



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ABSTRACT

Piezosurgery is known to be one of the recent advancements in the field of dentistry which has a great impact on periodontology and implantology. Moreover, piezosurgery works on the principle of ultrasonic vibrations which allows precise and accurate bone cutting without damaging the nearby soft tissues. It provides better access of the surgical field to the operator and reduces the heat production during bone cutting thus enhancing the healing of the surgical site. So, this review discusses the importance, literature, equipment, broad range of applications, advantages, and disadvantages of the device.

Keywords: Piezosurgery, implantology, a piezoelectric device, Ultrasonics



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INTRODUCTION

In the past few years, with the tremendous advancement in dentistry such as Ultrasonography, CBCT, microsurgery, implants, lasers, etc. we have embarked into the world of painless dental practice. These innovations have been a turning point in day-to-day dental practice to handle some major clinical situations. The main aim of such techniques is to get better results in a minimal period of time, making the procedure easier and more predictable [1]. Various tools such as micro motor handpiece, and airtor that are used in removing enamel, dentin, cementum, and bone are the major factors that decide the success rate of treatment modality as a postoperative outcome of various procedures is controlled by the quality of hard tissue removed [2]. Conventional hand-cutting instruments along with the rotary instruments used in day-to-day dental practice have a major drawback of generating heat while bone cutting, thus requires external irrigation and have been found to exert a great amount of pressure in osseous dentistry. To get the control various limitations discussed above, a novel surgical technique was introduced termed "Piezosurgery". This new innovative invention in dentistry is grounded on the fact "precise and selective bone cutting" [3].

Historical Background

Jacques and Pierre Curie in the year 1880 explained about the piezoelectric effect, according to which certain crystals and ceramics get deformed as the electric current passes across them. Vibrations produced, are amplified and then transmitted to the vibration tip. Slight minimal pressure is applied to bone tissue leading to the cavitation phenomenon [4]. Further in the year 1950, Pohlman was the first one who put in the application of ultrasound to humans for treating neuropathic pain and myalgia [5]. Ultrasonics were firstly used in dentistry in the year 1952 for cavity preparation. In the year 1957, Richman was the first one who used and disclose the use of an ultrasonic chisel in the removal of bone and in apicoectomies without a slurry [6]. Shelley and Shelley in the year 1986 reported the similarity between piezoelectric surgery unit and ultrasonic system. This characteristic provided the ability to cut through bone but not soft tissues.

Prof. Tomaso Vercellotti (Carasco, Italy) invented Piezosurgery which was later introduced by Mectron with an objective to amplifying ability of surgeons to perform bone surgeries more accurately with minimizing the intraoperative and postoperative risk. The main ideology that lies behind the invention of Piezoelectric Surgery depends upon some major concepts of microsurgery. So, first one involves a minimally invasive surgery which enhances the healing of the tissues and minimizes the discomfort to the patient [7].

Piezoelectric unit includes:

The device is consisting of a sterile box that includes all equipment needed during piezo surgery, and a control panel that has two different modes that include BONE and ROOT cutting modes which an operator can select from the display screen. BONE cutting mode, cut the bone based on the type and density of the bone. ROOT cutting mode is used in the debridement, shaping, and smoothening of root surfaces. Further, it has a dynamometric wrench which helps in tightening the insert tips to the hand piece. It has a liquid bag with the irrigation fluid hanging onto the rod which has a peristaltic pump. Peristaltic pump help in the discharge of irrigation at speed of 0-60ml/minute and it is refrigerated at a temperature of 4°C. The device consist of a hand piece connected permanently with a cord and is sterilized with it. Further, it is attached with a foot switch to control the handpiece [8]. The Piezo unit includes a platform, control panel along with the digital display, and keypad of frequency 25–29 kHz, and power of approximately 5 W. There are three different level of power which includes a low power mode (inapico endocanal cleaning and orthodontic surgery), a high-power mode (smoothing the radicular surface and cleaning), and a boosted power mode (for performing osteoplasty and osteotomy).

INSERT DESIGN: Various types of insert designs used in piezoelectric surgery can be classified on the basis of their functional, clinical characteristics and color of the tips:





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Functional Classification [9]

Sharp

Tip with sharp edge that enables gentle and effective cut on mineralized tissue. They are composed of nitride titanium steel and are gold in color. For Example, Design Number IM3P, OT-7, IM2A, EX1 and OP-3

Smooth

These tips include a diamond surface coating which permits the precise and controlled cutting to get accurate boner shape. They are gold in color. For example: Design number OP-4, OT5, OT-1 and OT-4

Blunt

They include tips with rounded end tips which intensify the cut which are in close contact with the soft tissues. They are basically used in atraumatic elevation of sinus membrane during the grafting procedure.

Clinical Classification [10]

1. Osteotomy includes the following tips: OT1, OT2, OT3, OT4, OT5, OT6, OT7, OT7S4, OT7S3, OT8R/L
2. Osteoplasty includes the following tips: OP1, OP2, OP3, OP4, OP5, OP6, OP7
3. Extraction includes the following tips: EX1, EX2, EX3
4. Implant site preparation includes the following tips: IM1, IM2A, IM2P, OT4, IM3A, IM3P
5. Periodontal Surgery includes the following tips: PS2, OP5, OP3, OP3A, Pp1
6. Endodontic Surgery includes the following tips: OP3, PS2, EN1, EN2, OP7
7. Sinus Lift includes the following tips: OP3, OT1 (Op5), EL1, EL2, EL3
8. Ridge Expansion includes the following tips: OT7, OT7S4, OP5 (IM1), IM2, OT4, IM3
9. Bone Grafting includes the following tips: OT7, OT7S4, OP1, OP5
10. Orthodontic Microsurgery includes the following tips: OT7S4, OT7S3

These inserts tips are used in different techniques in combination with each other for different surgical protocols.

Figure: 1

Based on insert Tip Colour [1]

Gold insert tips: They are used in treating bone.

Steel insert tips: They are used in treating soft tissue and other delicate structures.

Based on the coating on insert tips are [10]

Diamond Coating: The diamond cutting tips are adapted to work for the respective treatment.

Titanium Nitride Coating: The coating provide corrosion resistance and increase the working.

Mechanism of Action of piezo surgery

It is typically consisting of

Electric power sources

Piezoelectricity is known to be a phenomenon in which there is a coupling between the electrical and mechanical states of the material. It forms the basis of the piezosurgery unit and is present in certain crystals which are subjected to mechanical charges which lead to electric polarization [9].





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Ultrasonic piezoelectric transducer

It is a device that converts any other energy to ultrasonic vibrations. Most commonly used crystals include quartz, Rochelle salt, and some ceramics. They work at all frequency ranges and output levels [11].

Amplitude booster

The main idea behind using this is the bonding with the host structure. The host structure is known to be a cantilever beam which provides a large number of applications as an energy harvester. It has two basic advantages. Firstly, this combination leads to increased electric power output, and secondly, it also enhances the energy harvester's power density and thus minimizes the stress in the piezoelectric unit [9].

Sonotrode or horn

This sonotrode is intended for dissecting the hard tissue and is molded to be an interchangeable cutting insert formed by steel which further act as oscillating scalpels. The cavitation effect in piezoelectric surgery plays an important role. Cavitation is the process of vaporisation, bubble formation, and eventual implosion into a small portion of its original size as a result of a drop in pressure brought on by ultrasonic vibration. This effect entails low vapor pressure for oscillating tips, maintaining bone temperature and hemostasis, washing off the debris, and cleansing the field with the help of bursting bubbles of water under high pressure, providing better visibility and ease for operation [12].

Application of piezoelectric surgery in dentistry

1. Used in endodontics, removing root canal fillings and helps in retrieval of broken instrument from canals.
2. Used in oral surgery, for tooth extraction, cystectomy, and maxillofacial surgery.
3. Used in orthodontics, for procedures like orthodontic surgery, peizo incision etc
4. The benefits of piezo-based osteotomy can also be used for augmentation procedures prior to implants.

For example, during the sinus lift procedure using piezo surgery unit, there is minimal or no risk of perforation and mucous membrane injury as soft tissues are not affected when using this method [13].

Application of piezoelectric surgery in Periodontology and Implantology

Scaling and Root Planing

The piezoelectric device is used in the removal of calculus both supra and subgingival, stains, and debris. In this insert tips are arranged in a vertically parallel manner to long axis of tooth and moving it continuously [14].

Crown Lengthening/Gingivectomy

It is a treatment modality carried out for modifying extent of supragingival margin by shifting tooth structure apically to marginal gingiva and reducing the supporting bone if required. In contrast to traditional rotary instrumentation, piezo surgery needs minimal hand pressure and allows operator sensitivity and control to a larger extent [15].

Periodontal Surgery

Periodontal surgeries performed with the use of piezosurgery facilitate and improve the manipulation of hard and soft tissues. Once the flap is raised using the traditional method, PS2 or OP3 insert is used for the removal of granulation tissue and involves minimal bleeding due to the cavitation effect [16].

Osteoplasty and ostectomy

Piezosurgery device, develops the positive, physiologic architecture of bone while performing pocket removal surgery. This enables the removal of the particular part of the bone, minimizing the risk of injury to underlying root surfaces. Final smoothing is done using specific ultrasonic insert, for flap closure [17].





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Implant Site Preparation

A piezoelectric device has proved to be a more suitable and effective method of drilling the implant site. According to various studies, it has been concluded that piezoelectric device enhances implant stability. With the help of special tip that provide precise drilling of implant hole and also reduces thermal and mechanical damage of bone. Thus, the proper surgical technique helps in faster healing and reduces tissue damage. Preti *et al* in year 2007 assessed the difference in the use of conventional drill and piezosurgery and showed a tremendous increase in new bone formation [18].

Implant Insertion and Placement

Depending on location and potential severity of bone insufficiency, different options may be considered in the event that the donor site is unsuitable. The use of the piezoelectric device for sinus-floor elevation in the upper jaw is a prime example [19].

Elevation of Maxillary Sinus Floor

Sinus floor elevation is known to be one of the routine procedures in treating vertical defects in the posterior maxilla. The traditional method used (modified Caldwell-Luc technique) has a weaker osteogenic role thus membrane perforation is the major risk factor during window preparation. When the bone wall is thin No.5 osteotomy tip is used and No.1 osteotomy tip in thick bone wall, followed by No.5 osteotomy tip.²⁰

Alveolar decortication and corticotomy and a ridge augmentation

Piezosurgery is used while cutting proximal and crestal facial cortices in a precise and controlled manner. This method enlarges atrophic, anatomically restricted, narrow ridges in preparation for rapid implant insertion. As grafting is not required, this shortens the course of treatment and further eliminates donor site morbidity [21].

Peri-mucositis, peri-implantitis, and calculus removal

Piezo surgery helps in the effective calculus removal from titanium osteo synthetic material quickly. In order to remove the debris and contaminated bone that have accumulated on the implant surface without damaging the implant site, piezo technology is used [22].

Harvesting autogenously bone grafts

The extraction of bone chips from intra-oral sources involves piezo surgery. For the purpose of removing cortical bone and doing away with the necessity for bone traps, two different kinds of surgical tips are used. Additionally, because they create space and encourage growth factors at the recipient site to speed up bone healing, bone chips are essential for bone regeneration [23].²³

Advantages of piezosurgery

1. Precise cutting
2. Excellent control of surgical device
3. Selective cutting.
4. No Bleeding at the surgical site
5. Better healing process
6. No risk of emphysema
7. Minimal post-surgical traumatic stress

Disadvantages of piezosurgery

Extensive operation time.

Piezosurgery inserts quickly lose their effectiveness.

The handpieces' ergonomics dictate that they should have a longer stem and a suitable diameter to reach the bottom of the cavities, preventing the "flattening" phenomenon that is typical of other osteotomy devices.





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Contraindications of peizosurgery

1. Electrical implants such as pacemakers, in either patient or the clinician.
2. Certain systemic diseases such as cardiovascular diseases, diabetes and bone disease or in patients undergoing radiotherapy, all of which can hinder the dental implant surgery.
3. Alterations may or may not be related to systemic diseases, bone structure and vascularization. Verifying the bone structure and the healthy vascular condition of the patient are fundamental elements for good integration and healing.
4. Behaviors such as smoking and excessive drinking [24].

CONCLUSION

Piezosurgery is known to be an innovative invention in the field of dentistry making complex surgical procedures easy to perform. As the traditional hand and rotary, motor-driven instruments which work on the principle of macro vibrations used to perform various bone surgeries have a high risk of injuring adjacent soft tissues, excessive bleeding from the site, high pressure, and copious irrigation was required with them as they produce heat while working. Moreover, various studies have shown delayed or slow healing of the surgical site with these instruments. To overcome these shortcomings, piezosurgery has emerged to be of great importance in dentistry. Piezosurgery works on the principle of cavitation effect allowing clinicians to perform atraumatic surgeries. It is used in various surgical procedures such as osteotomy and osteoplasty, maxillary sinus floor elevation, ridge expansion procedures, grafting procedures, nerve lateralization procedures, and resective surgeries. It provides micrometric, selective, and safe cutting of the bone with less post-operative pain and discomfort to the patient. It provides better visualization as it minimizes the bleeding from the surgical sitemaking it easier to work for the dental surgeon. Moreover, in various histological studies and histo-morphometric analysis, it has been found that the use of piezo surgery enhances bone healing. Thus with a large number of advantages, it has a few limitations that it cannot be used in patients with electrical implants and certain systemic diseases.

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Teacher Performance among Higher Secondary School Teachers of Kashmir Valley in Relation to their Work Culture

Zafarullah Rashid^{1*} and S.Veena²

¹Research Scholar, Department of Education, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India

²Assistant professor, Department of Education, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India

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*Address for Correspondence

Zafarullah Rashid

Research Scholar,

Department of Education,

Annamalai University,

Annamalai Nagar- 608 002,

Tamil Nadu, India

E. Mail: abdulrashidbhat332@gmail.com



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ABSTRACT

The current investigation was undertaken to know the teacher performance among higher Secondary School Teachers of Kashmir valley in relation to Work culture. The investigator had selected 200 teachers from Kashmir valley. Stratified random sampling was used in order to collect the sample. Work culture scale validated and standardized by Manju N. D and Sheela. G was adopted to know the level of work culture tendency among teachers and self constructed scale of teacher performance was also used to know the performance of teachers. Pearson's product moment of correlation was applied to find out the relationship and 't' test was applied to find out the significance of the difference between different variables. The findings of the study revealed that there is a significant and positive relationship between teacher performance and work culture among higher Secondary School teachers of Kashmir valley. The study also found that there is a significant difference in the work culture of male and female teachers, urban and rural teachers.

Keywords: Work culture, Performance, Relation, Teacher, Student



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INTRODUCTION

Teachers play the most important role in education system of enhancing student learning that matter to their future success. Teacher performance has a direct influence on student learning and student progress is the standard by which teacher performance should be assessed (Barbara C.Hunt, 2009). Teacher performance is a complex term and have been defined by different researcher differently but the main theme is the accomplishment or executing of a given task. According to Amin et. al., (2013), teacher performance is defined as their duty in a particular period in the school system for achieving organizational goals. Peretemode (1996) describes that a teacher's performance is determined by the worker's level of participation in the day to day running of an organization. Hanif (2010) added that, the quality of educational process and its product is unquestionably influenced by the teacher's job performance. The entire edifice of education is shaking if the performance of teachers is weak and ineffective. Therefore, effective job performance of teachers is a must for educational improvement, which is striving hard to bring about. Teacher performance may be influenced by several factors like leadership styles, Salaries, recognition, work culture, self directed professional development etc. One of the main factors affecting the performance of teachers is Work culture. Work culture can be defined as the depth and complex part of an organization that will affect organizational members. Most organizations adhere to a long work culture which is considered a prerequisite for organizational culture. Work culture is a philosophy based on the view of life as values that become the nature, habits and driving forces, entrenched in the life of a community group or organization, then reflected from attitudes into behavior, beliefs, ideals, opinions and actions that manifest as "work" or "work" (Triguno, 1995: 3). According to Osborn and Plastrik (2002:252) "Work culture is a set of behaviors and psychological frameworks that are internalized very deeply and are shared by members of the organization". Work culture is a guideline for the behavior of all people involved in the learning process of elementary school students. Work culture is the set of shared, taken-for-granted implicit assumptions that group holds and determine how it perceives, thinks about and reacts to its various environments (Kinicki&Kreitner,2009:36). This has a considerable impact on the performance of teachers.

Need And Importance Of The Study

Teacher Performance plays a significant role in both education and the learning process. In this fiercely competitive society, it has evolved into a predictor of a teacher's bright academic career. It is the primary objective that every teacher in every society is supposed to perform. Teachers learn about their talents, abilities, and competences through their performance, which is a crucial component in developing job satisfaction. Teacher Performance is one of the most crucial results of any educational establishment. Individuals are classified as high, average, or poor Performers based on their degree of Performance. The performance of the teachers depends on the Work culture of the educational establishment or any educational institution. Studies show that there is a positive correlation between Teacher Performance and Work Culture.(Nurbaya et al 2019, A, Lutfah,2019, Daryatmi, 2005).The related literature indicated the evidence regarding the association of Teacher Performance and Work Culture but none of the study was found pertaining to Higher Secondary School Teachers from Kashmir valley. So a need was felt to study Teacher Performance in relation to Work culture of Higher Secondary School Teachers of Kashmir Valley.

REVIEW OF RELATED LITERATURE

The researcher becomes familiar with existing information about the study being investigated after reviewing relevant literature. It lessens the possibility of repetition. It aids the researcher in determining the nature of the variables, the topic of study, the breadth and boundaries of the issue. It provides reassurance to the researcher that the issue under examination has antecedents in published literature and necessitates additional research. It provides the researcher with extensive background information on the correlations between the variables and the results of previous studies that are relevant to the issue under examination. The researches that have been done in the past , it can be said that higher the Work culture, the higher will be the Teacher Performance(A Lutfah, 2019). Nurbaya S (2019) conducted a study on Teacher Job Performance and the mediating role of Work Culture. Findings showed



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that Work Culture has a significant and positive impact on Teacher Performance. S Basir (2020) investigated the influence of interpersonal communication and work culture on teacher performance in Junior High School at Wajo Regency (Indonesia) and it was found that Work Culture has been significant and positive impact on teacher performance. B Sahara(2020) made a correlation study between Teacher Performance and Principal Leadership Style, Teacher Work Culture, Teacher Competency and Teacher Job Satisfaction. Findings of the study depicted that there exists a significant and positive relationship between Work Culture and Teacher Performance of Special School Teachers.

Above given studies are related to the Work culture and its effects on the Teacher Performance. Several studies were conducted to know the impact of Work Culture on the Teacher Performance. Unfortunately, not a single work was found on the Teachers of Kashmir valley. So, this study represents a feeble effort to close the current gap.

Operational Definition

- **Teacher Performance:** Teacher performance refers to the teaching competency, set of attitudes and behaviors that results in learning for students.
- **Work culture:** Work culture is closely linked to the attitude towards work, beliefs about work, common expectations of work, and the perception of the way things have always been done at work

Objectives Of The Study

- To study the relationship between Teacher Performance and Work Culture of Higher Secondary School Teachers of Kashmir Valley.
- To study if any significant difference exists between Work Culture of male and female Higher Secondary School Teachers.
- To study if any significant difference exists between Work Culture of rural and urban Higher Secondary School Teachers.

Hypothesis

- There exists no significant relationship between Teacher Performance and Work Culture of Higher Secondary School Teachers of Kashmir valley.
- There exists no significant difference between Work Culture of male and female teachers.
- There exists no significant difference between Work Culture of rural and urban teachers.

METHODOLOGY

Observational method, case study method, and survey research are the various approaches of the descriptive method. From the different approaches that may be employed in descriptive research, normative survey method was adopted for collecting data relevant for the study, considering the objectives.

Sample

A sample is a portion of the population from which the researcher hopes to extrapolate the findings. For the purpose of choosing the sample, stratified random sampling was used. 200 Higher Secondary School Teachers of Kashmir valley were chosen as a sample out of which 100 Male and 100 Female.

Tool Used

Teacher Performance scale: Teacher Performance scale was constructed and validated by Zafarullah Rashid and S. Veena(2022) in order to know the level of Work Culture of Higher Secondary School Teachers of Kashmir valley. The scale is having 30 items and is based on three dimensions on 5- point likert format, each statement is rated on five-points, (strongly agree=5, agree= 4, undecided= 3, disagree= 2 and strongly disagree= 1).

Work Culture Scale: Work Culture scale was validated and Standardized by Manju N. D and Sheela.G.



**Zafarullah Rashid and Veena****Statistical Technique Used**

In order to determine the difference between two groups, the investigator employed the statistical technique known as the “t” test. Pearson’s product moment of correlation technique were also adopted to study the relationship between teacher performance and work culture of Higher Secondary School Teachers of Kashmir valley.

Analysis and Interpretation

Results were systematically presented in the below mentioned tables.

Hypothesis 1- There exists no significant relationship between Teacher Performance and work culture of Higher Secondary School Teachers of Kashmir valley as shown in Table no. 1.

Hypothesis 2:

There exists no significant difference between Work Culture of male and female Higher Secondary School Teachers as shown in Table no. 2.

Hypothesis 3:

There exists no significant difference between Work Culture of rural and urban Higher Secondary School Teachers as shown in Table no. 3.

FINDINGS

There exists significant and positive correlation between Teacher Performance and Work Culture of Higher Secondary School Teachers of Kashmir valley, which means the higher the Work Culture, the higher will be the Teacher Performance and vice versa. Significant difference was found between male and female teachers of Kashmir valley with respect to their Work Culture. Male teachers are having high level of work culture than female teachers. Significant difference was found between urban and rural teachers of Kashmir valley with respect to their Work. Urban teachers are having high level of work culture than rural teachers.

DISCUSSION OF THE RESULTS

One of the most crucial elements of the educational system and the main determinant of the evaluation of educational excellence is teacher performance. Work culture is one of several factors that affect teacher performance, and it is a significant one. So, the goal of the current study was to draw conclusions about how teacher performance and work culture of Higher Secondary School Teachers in the Kashmir valley relate to one another. Work culture is obviously a significant contributor that is to be emphasized because it has a positive impact on teacher performance. Some characteristics of work culture include collaboration, appreciation, friendly policies and practical guidelines and team building. Hence the teachers with favorable work culture tend to perform their functions in team work and maintain cordial relations with others in the institution. Like Nurbaya S(2019) examined that there is a positive relationship between Work Culture and Teacher Performance. Our result showed the same. As per this study teachers having high Work Culture had good teacher performance than those who are having low work culture tendency in the academic chores. Our findings have also support from different researches which conforms that teacher performance is positively related with work culture. (Nurbaya et Al, 2019; A, Lutfah,2019; Daryatmi, 2005)

CONCLUSION

The study focused on the correlation between work culture and teacher performance. The results revealed that there exists a significant difference between male and female teachers of Kashmir valley with respect to their Work Culture; there exists significant difference between urban and rural teachers of Kashmir valley with respect to their Work Culture. Positive significant relationship was found between teacher performance and work culture of Higher Secondary School Teachers of Kashmir valley.





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Table- 1 showing the variables, r value and their significant level

| SL.No | Category | Teachers | Df (N-2) | R value | P Value | Level of significance |
|-------|---------------------|----------|----------|---------|---------|-----------------------|
| 1 | Teacher performance | 200 | 198 | .534 | 0.000 | Significant |
| 2 | Work culture | 200 | 198 | | | |

Table 1 depicted that the calculated r value is greater than table value at 0.01 level, hence the null hypothesis stands rejected. Thus there is significant positive relationship between Teacher performance and work culture which means that the higher will be the teacher performance, the higher will be work culture and vice versa.

Table -2 : Result of Mean, SD, t- value of male and female teachers with respect to work culture

| SL. No | Gender | Teachers | Mean | S.D. | 't' Value | P value | Level of significance |
|--------|--------|----------|-------|------|-----------|---------|-----------------------|
| 1 | Male | 100 | 85.2 | 13.2 | 2.45 | 0.016 | Significant |
| 2 | Female | 100 | 80.81 | 9.92 | | | |

Table 2 depicted that the calculated 't' value is greater than the table value at 0.05 level. Hence the null hypothesis stands rejected. So we can say that there is a significant difference between work culture of male and female teachers.



**Zafarullah Rashid and Veena****Table – 3: Result of Mean, SD, t- value of urban and rural teachers with respect to their work culture.**

| SL. No | Locality | Teachers | Mean | S.D. | 't' value | P Value | Level of significance |
|--------|----------|----------|------|------|-----------|---------|-----------------------|
| 1 | Rural | 100 | 79.4 | 11.1 | 5.03 | 0.000* | Significant |
| 2 | Urban | 100 | 87.3 | 12.4 | | | |

Table 3 depicted that the calculated 't' value is higher than the table value at 0.01 level. Hence the null hypothesis stands rejected. This shows that there is significant difference between urban and rural teachers of Kashmir valley with respect to their work culture.





A Review on Detection of Pesticides in Fruits and Vegetables by Different Analytical Methods

Manu Jose^{1*}, Jayalakshmi P. M², Aswin biju³ and Fathima Nezhin Mohammed³

¹Associate Professor, Department of Pharmaceutical Chemistry, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India

²Assistant Professor, Department of Pharmaceutical Chemistry, Caritas College of Pharmacy, Kottayam, Kerala, India

³Student, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India

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*Address for Correspondence

Manu Jose

Associate Professor,
Department of Pharmaceutical Chemistry,
Nirmala College of Pharmacy,
Muvattupuzha, Kerala, India
E. Mail: manujose09@gmail.com



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ABSTRACT

The overuse of pesticides has led to a severe problem with food quality. Although pesticides are intended to prevent the invasion of insects, fungus and other pests, regular exposure to them has had a negative impact on people's health. They have a major impact on the human's thyroid, reproduction, nervous system and their continuous exposure can lead to diabetes, cancer and parkinson's disease. Nearly 3.5 million tonnes of insecticides have been applied globally this year. According to Statista Research Department 2023, the US ranks top in the world for pesticide usage, followed by Brazil and China. In the research from the EWG, it is said that "more than 90% of samples of strawberries, apples, cherries, spinach, nectarines and grapes tested positive for residues of two or more pesticides." The estimate of pesticides in food samples has been done using a variety of extraction and detection techniques. The chosen approach must be simple, affordable, quick, and adaptable to other matrices. The application of pesticides on fruits and veggies must comply with FDA, USDA and EPA regulations. No method is 100% effective in completely removing the pesticides from the food samples. Therefore it is important to spread the awareness regarding the health deterioration by the continuous exposure of pesticides to the common people and farmers.

Keywords: Pesticide residues, extraction methods, MRL, analytical methods, toxicity levels, health hazards



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INTRODUCTION

The quality of food has become a serious issue due to the overuse of pesticides. Even though pesticides are used to control the attack of insects, fungi, and other pests, their daily exposure has caused detrimental effects on the human body. Over the past decade, there has been a significant increase in the usage of pesticides worldwide. This is due to the rise in population, thus an increase in demands and sudden urbanization. Since then there is a marked increase in the development of different classes of pesticides belonging to various groups [1]. Hence it becomes very important to aware consumers of the potential risks that develop after the regular intake of pesticides from their regular diet. These include diabetes, headache, dizziness, nausea, respiratory diseases, and in extreme cases can cause coma, convulsions, genetic disorders, spontaneous abortion, and fetal death. Apart from their regular intake, it can also cause serious issues in direct contact with the pesticides for spray workers [2].

Pest control agents are compounds known as pesticides. Pesticides come in a wide variety of forms, each of which is intended to be efficient against a particular pest. The prefix "-cide" is derived from the Latin verb "to kill." Based on their chemical composition, place of manufacture, and intended target organism, pesticides are grouped. Pesticides can be either biological (bio-pesticide) or inorganic synthetic (made synthetically) in nature. Biopesticides are a class of compounds that may be used to manage pests. These chemicals are derived from natural sources including plants, animals, microorganisms, or certain minerals. For instance, canola oil and baking soda applied with insecticides are regarded as biopesticides. Three primary classes of biopesticides may be distinguished. Includes microbial pesticides, biochemical pesticide, plant-incorporated protectants. Microbial pesticides have microorganisms (such as bacteria, fungi, viruses, or protozoa) as its main component. Although each individual active component is somewhat specialised for its target pest(s), microbial pesticides may treat a wide variety of pests. For instance, certain weeds may be controlled by particular fungus, while certain insects can be killed by other fungi. Biochemical pesticides are naturally occurring compounds that use non-toxic ways to control pests. Contrarily, traditional insecticides are often synthetic substances that obliterate or inactivate the pest. Insect sex pheromones and other compounds that prevent mating as well as different scented plant extracts that draw nuisance insects to traps are examples of biochemical insecticides. A special committee would be established by the relevant authorities to make such determinations since it might be challenging to evaluate if a material satisfies the requirements for designation as a biochemical pesticide. Pesticidal compounds known as Plant-Incorporated-Protectants (PIPs) are made by plants from genetic material that has been incorporated into the plant. encoding instance, using the gene encoding the B.t. pesticidal protein, scientists can add the gene to the plant's genetic makeup. Then, rather than the B.t. bacteria, the plant produces the chemical that eliminates the pest [3].

Synthetic pesticides are man-made compounds that are particularly designed to kill or repel pests. They are mostly employed in agriculture, but they are also utilised in other sectors and in the home. Synthetic insecticides are divided into several classes. Organophosphates, carbamates, pyrethroids, and organochlorines are the primary classes. Animals and humans may have acute (short-term) or chronic (long-term) consequences from pesticide exposure, particularly to the reproductive, endocrine, and central neurological systems. DDT, toxaphene, dieldrin, aldrin, and other organochlorines have adverse effects on the immunological, neurological, endocrine, and reproductive systems. Diazinon, glyphosate, malathion, and other organophosphates harm the central nervous system. Carbamates influence the central nervous system and include carbofuran, aldicarb, carbaryl, etc. Fenpropanthrin, deltamethrin, cypermethrin, and other pyrethroids are examples [4]. There are already more than 500 insect and mite species that are immune to particular types of insecticides. Due to the rising resistance, nations have begun to use more goods, mix pesticides, and increase applications.

When a pesticide is applied, spilled, or dumped, it leaves behind deposits of the pesticide's active ingredient, as well as any metabolites or breakdown products. The kind and extent of any chemical pollution in the environment, as well as its persistence, are measured by residue analysis. In order for governments to register pesticides for use legally in certain applications, they must undergo comprehensive effectiveness, environmental, and toxicological





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testing. Human exposure to the administered pesticides and/or their breakdown products may persist as residues in the agricultural products. Selected sample programmes may be used to look at the mobility of pesticides in the environment, their relative rates of degradation, and their residual levels. The types and amounts of residues that are legally allowed to be present on foods are limited by regulatory organisations across the globe by the maximum residue levels (MRLs), sometimes known as "tolerances" in the United States^[5]. Analysis of pesticides provides a measure of the food quality, i.e., how much they have been contaminated by the chemical components in order to avoid the possible dangers to human health. Hence there has been an accepted Maximum Residue Limit (MRL) for different classes of pesticides as decided by the Codex Alimentarius Commission (CAC), European Union Commission (EU), and Gulf Cooperation Council (GCC).

Various extraction and detection methods have been used for the estimation of pesticides in food samples. The selected method should be easy, inexpensive, fast, and applicable to different matrices. Over the past years, gas chromatography was found to be the most extensively used method because of its high selectivity and high separation power. Gas chromatography-mass spectrometry (GC-MS), gas chromatography-ion trap mass spectrometry (GC-ITMS) and gas chromatography-tandem mass spectrometry (GC-MS/MS) has made immense progress in the field of analysis due to the identification capabilities of MS. Apart from GC methods, there are other traditional quantification methods like high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), liquid chromatography-tandem mass spectrometry (LC-MS/MS) and low-pressure gas chromatography-mass spectrometry (LP-GC/MS). The following review mainly focuses on the various extraction and quantification methods used for the analysis of pesticides in fruits, vegetables, and plantation crops which is listed in Table No: 1, pesticides usage by different countries and health hazards caused by the overexposure of pesticides from their constant diet.

Pesticide Usage By Different Countries

Pesticides are extensively used by different countries as it is the most effective and economical way to increase the quality of the crop yield. In the present year, nearly 3.5 million tonnes of pesticides have been used worldwide. The US was ranked first in pesticide usage followed by Brazil and China according to Statista Research Department 2023. In the US, the conventional usage of pesticides every year was found to be 407,780 tons. Brazil trailed in second with 377,180 tons of pesticides consumed. The total production of chemical pesticides in China was found to be around 262,700 tons[7].

Most Pesticides Used Fruits and Vegetables

Any exposure to pesticides is dangerous, concerning children as they can be more harmed by these toxic chemicals than adults. Consumption of fruits and vegetables is critical to a healthy diet and good health. Environmental Working Group (EWG) 2023 Shoppers Guide represents the analysis of the latest fruit and vegetable testing data from the Department of Agriculture and Food and Drug Administration. In this 2023 guide, they have tested around 46569 samples of 46 fruits and vegetables[6]. "More than 90 percent of samples of strawberries, apples, cherries, spinach, nectarines, and grapes tested positive for residues of two or more pesticides," EWG's report states.

Harmful Effects on Pesticide Exposure

Pesticide Exposure and Thyroid Function

The researchers focused mainly on the pesticide used in banana production i.e. mancozeb. This fungicide contains ethylene thiourea (ETU) which is observed to produce thyroid toxicity. It contains manganese which is also commonly found in drinking water and it can be toxic to the human body. It was found that FT4 may be inhibited by ETU, causing a hypothyroidism effect, while excess manganese may stimulate FT4, causing a hyperthyroidism effect [79].





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Children's Environmental Health Study

The researchers have also examined the role of pesticide exposure on respiratory and allergic reactions in young children. Children under the age of 5 are at greater risk of mortality from lower respiratory tract infections (LRTI). They have examined that greater exposure to pyrethroid insecticide has more chances to witness wheezing, asthma, and LRTI. In pregnant cases, the fetus may not be able to break down these chemicals. Symptoms of pyrethroid poisoning include tremors, salivation, headache, fatigue, vomiting, stinging and itching skin, and involuntary twitching [82].

Pesticide Effect on the Nervous System

Organophosphates and carbamates interfere with nerve signal transmission by attacking the brain and nervous system. Symptoms include headaches, nausea, dizziness, vomiting, chest pain, diarrhea, muscle pain, and confusion. In severe poisoning cases, the symptoms can include convulsions, difficulty breathing, involuntary urination, coma, and death.

Cancer

It was researched that significant risks of colon and bladder cancer were observed on exposure to imazethapyr (imidazolinone herbicide), a heterocyclic aromatic amine herbicide according to the prospective cohort study of 57,311 licensed pesticide applicators in Iowa and North Carolina, USA. It was also reported that the alteration in the antioxidant defense system in breast cancer cells was mainly due to the redox imbalance induced by the chlorpyrifos in pesticides. According to a study conducted in the USA, on increased exposure to acetochlor herbicides, there is a high risk of lung cancer compared to non-users.

Parkinson's disease

There was a 2 fold increase in the chance of Parkinson's disorder due to exposure to paraquat or maneb/mancozeb. It was also reported that chronic exposure to organophosphate or organothiophosphate will develop PD at a younger age relative to patients with no family history of the disease.

Diabetes

A higher risk of developing type 2 diabetes and its co-morbidities is suspected in patients with more exposure to pesticides, particularly organochlorines, and metabolites. It was also reported that 4.5% of women exposed to agricultural pesticides are more prone to gestational diabetes mellitus during pregnancy.

Pesticide Effect on The Reproduction System

Majority of pesticide such as organochlorine and organophosphate components exposure induces male reproductive system defects such as reduction of sperm activities (e.g., counts, motility, viability and density), inhibition of spermatogenesis, reduction of testis weights, damaging sperm DNA, and increasing abnormal sperm morphology which suggest that these pesticides may cause hypospadias [84].

Steps To Overcome the Pesticides

The food samples contain dirt, germ and pesticides on them during the time it is grown until it is served on the table. The Food and Drug Administration (FDA), US Department of Agriculture (USDA), and Environmental Protection Agency (EPA) limit the amount of pesticide residue permitted on foods to ensure food quality. The application of pesticides on fruits and veggies must comply with these regulations. No method is 100% effective in completely removing the pesticides from the food samples.

- Initial step is the washing and rubbing of samples with running tap water instead of dunking it. More time taken to wash the sample more will be the result
- Do not use soap and detergents for washing fruits and veggies as they have pores just like the skin and these soap products can get trapped in the pores.





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- FDA does not recommend commercial product wash as these have not been proven to be more effective than water alone.
- Scrubbing firm products like carrot and potatoes with clean brush will help to get rid of more residues.
- Heating will help to reduce the pesticide content but it will also take of the nutrients from it.
- In addition, submerging the samples in salt, turmeric, lemon water and white vinegar solution for sometime will help to reduce the residues to a greater extend.

The best way is to grow the food products in our garden itself or participating in the community garden. This will help to limit the use of pesticides on the food we eat. The Integrated Pest Management will allow to control the garden pest with the least possible harmful effect [80].

CONCLUSION

The increasing health hazards due to agricultural pesticide exposure is a growing concern in our society. From the above information, it is sure that still large amount of pesticides are used in fruits and vegetables. The number of countries on pesticide usage are also increasing by every year. When different analytical methods were applied on fruit samples, their recovery % was also high as shown in the Table No:1 which indicates their presence in the food samples. And no method is 100% effective in completely removing the pesticide residues. This shows that a very small amount of pesticides are taken by the people from their regular diet everyday and this can lead to accumulation of toxins in human body. Hence it is very important to spread the awareness by conducting social healthcare programs regarding the health deterioration by the continuous exposure of pesticides to the common people and farmers.

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Table no 1: different pesticides used in fruits and vegetables along with their extraction method, detection method , recovery%, mrl & acute oral toxicity levels.

| Matrix | Pesticides | Extraction Methods | Detection Methods | Recovery% | Maximum Residue Level (Mrl) | Toxicity (Acute Oral Ld50) |
|------------|--|---------------------------------------|-------------------|---------------|-----------------------------|------------------------------|
| Apple | Chlorpyrifos[8] | Liquid - Liquid extraction | HPLC | 88.9 – 93.3% | 0.05 | 96 – 270 |
| | Methyl parathion[9] | Solid phase extraction | HPLC - UV | 94.03 - 98.9% | 0.1 - 1 | 25 |
| | Methoxy Oxamyl[10] | Ammonium acetate – acetic acid | LC- MS/MS | 84 – 88% | 0.01 0.3 | 37 |
| | Linuron, Imazalil, Ethiofencarb[11] | 20mM Methanol in water | LC- MS/MS | 77 – 85 % | 0.01 2 0.01 | 4000 227 – 343 1322 |
| | Terbutol , Simazine, Etridiazole[12] | C ₁₈ SPE | HPLC | 85 – 107% | 0.01 0.2 0.4 | >500 >5000 945-1141 |
| Mango | Deltamethrin[13] | Chloroform + sodium sulphate | HPLC - UV | 71.3 – 75.8% | 0.01 | 129 |
| | Emamectin benzoate[14] | QuEChERS | HPLC - MS/MS | 72 – 102 % | 0.006 | 2950 |
| | Endosulfan, Carbofuran, Chlordane[15] | Single – drop micro extraction (SDME) | GC -MS | 69 – 119% | 0.5 0.005 0.02 | 160 6-18 150-700 |
| | Clofentezine, Malathione, Azoxystrobin[16] | SPME | GC - MS | 71.6 – 117 % | 0.5 0.2 0.7 | >5000 2800 >500 |
| | Tetraconazole, Trichlorfon, Tebuconazole[17] | Matrix solid phase dispersion | GC - MS | 80 – 146 % | 0.5 0.005 0.01 | 1031 450-650 3766-3710 |
| Strawberry | Chromafenozide[18] | QuEChERS | HPLC – DAD | 99 – 110 % | 15 | 1322 |
| | Pyrifluquinazon[19] | Dispersive solid phase extraction | HPLC – MS/MS | 71.4 – 106 % | 0.3 | 300-2000 |
| | Bifenthrin, Chlorpyrifos, β-endosulfan[20] | Disposable pipette extraction | GC - MS | 93 – 123 % | 0.01 0.05 2 | 375 96-270 18-160 |
| | Methiocarb, Pendimethalin, Iprodione[21] | Dispersive solid phase extraction | GC - MS | 72 – 89 % | 0.07 0.01 0.5 | 13-130 3956 >4400 |
| | Azoxystrobin, Bifenthrin[22] | QuEChERS | GC - MS | 85 – 127 % | 10 0.01 | >500 375 |
| Grapes | Simazine, | Solid phase extraction | HPLC | 82 – 91% | 0.05 | >5000 |





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| | Atrazine[23] | | | | 0.01 | 1780 |
| | Tebuthiuron, Ametryn[24] | Solid phase extraction | GC-MS | 67 – 120 % | 0.01 0.05 | >5000 1162 |
| | Carbosulfan, Dichlorvos, Mevinphos[25] | QuEChERS | GC- μ ECD/NPD | 72 -113% | 0.01 0.005 | 90-250 80 3-12 |
| | Monocrotophos, Flusilazole, Atrazine[26] | Ethyl acetate + Dspe | LC – MS/MS | 70 – 120 % | 0.01 0.5 0.01 | 17-20 674 1780 |
| | Lindane, Vinclozolin, Procymidone[27] | Pressurised hot water extraction (PHWE)+ Macroporous membrane liquid – liquid Extraction (MMLLE) | GC-MS | 10 – 28% | 0.01 1 5 | 200 >5000 27 |
| Watermelon | Pyroquilon, Boscalid, Imibenconazole[28] | Acetonitrile + NaCl +methanol | LC – MS/MS | 70 – 131 % | 0.2 1.5 0.3-1 | 321 >5000 1322 |
| | Metolcarb, Carbofuran, Carbaryl, Diethofencarb[29] | Dispersive liquid - liquid microextraction | HPLC – DAD | 76.2 – 94.5 % | 0.05-1 0.005 0.25 | 498-580 6-18 246-283 1322 |
| | Chlorpyrifos, Diethofencarb, Acetochlor[30] | LLE + DSPE Cleanup | GC -MS -SIM (Gas Chromatography with electron impact mass spectro photometric detection in the selected ion monitoring mode) | 70 – 121 % | 0.05 | 95-270 1322 2953 |
| | Fenvalerate, Deltamethrin, Bifenthrin[31] | Solid phase micro extraction | HPLC-PIF-FD (post column photochemically Induced fluorimetry derivatization & fluorescence detection) | 91 – 110% | 2 0.01 0.01 | 451 31-139 375 |
| | Isocarbophos, Phosmet, Triazophos[32] | Solid – liquid phase microextraction | HPLC | 82.6 – 92.4% | 0.1 10 0.005 | 50 147-316 82 |
| Orange | α -endosulfan, | Ethylacetate | GC – MS + | 82% | 2 | 18-160 |





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| | Imazalil, Dicofof[33] | +anhydrous NaSo ₄ | Electron impact ionization | | 10 0.25 | 227-343 575-960 |
| | Thiabendazole[34] | Ethylacetate +anhydrous NaSo ₄ | LC – MS + Atmospheric pressure chemical ionization (APCI) | 96% | 10 | 3100 |
| | Acephate, Carbendazim, Imazalil[35] | Acetone/Dichloromethane | LC – MS/MS | 70 – 110% | 0.01 1 10 | 980 6400 227-343 |
| | Methomyl, Pirimicarb, Imidacloprid[36] | Acetonitrile + salting out with NaCl | LC – MS/MS | 72 – 97 | 1 3 0.005 | 17 147 450 |
| | Bitertanol, Methiocarb, Pyriproxyfen[37] | Pressurized liquid extraction | LC – IT – MS ³ LC-quadropole ion trap triple stage MS | 88 - 94% | 0.01 0.07 0.5 | >5000 60-100 >5000 |
| Carrot | Haloxypop-methyl, Procymidone, Linuron[38] | Solid-liquid extraction+low temperature partition (SLE -LTP) | GC-MS | 90-110% | 0.01-0.05 5 0.01 | 2179 6800 4000 |
| | Zoxamide, Spinosad A , Triflumizole[39] | QuEChERS | HPLC-HRMS | 96-101% | 0.002 0.005 0.5 | >5000 5000 1057 |
| | Chlorpyrifos, Chlorothalonil, Dieldrin[40] | Disposable pipette extraction | GC-MS | 89-99% | 0.1 7 0.03 | 96-270 242-422 24-167 |
| | Bromophos, Vinclozolin, Cyfluthrin[41] | Acetone + Dichloro methane partitioning | GC + electron capture detection (ECD) | 68-88% | 0.05 1 0.01 | 52 >5000 826 |
| | Aldicarb Ethiofencarb Propoxur[42] | Ultrasonication With methanolic Ammonium acetate – acetic acid buffer | LC – MS /MS | 72-79% | 1 0.01 0.05-0.1 | 1 1322 >500 |
| | Abamectin Amitraz Difenconazole[43] | QuEChERS | HPLC -HRMS (High Resolution Mass Spectrometry) | 80-115% | 0.01 0.05 0.5 | 650 370 1453 |
| | Potato | Thiamethoxam Deltamethrin Rimsulfuron[44] | QuEChERS + MSPE | LC – MS / MS | 77-111% | 0.3 0.2 0.01 |
| Monocrotophos Fenitrothion Methidathion[45] | | stir bar sorptive extraction (SBSE) | Capillary GC with thermionic | 93 – 96 % | 0.01 5 0.02 | 5-14 330 25-44 |





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| | | | specific detection (TSD) | | | |
| | Methoxychlor Ethoprop Dimethoate[46] | Supercritical fluid extraction (SFE) | GC - ion trap MS(GC - ITMS) | 90-98% | 0.05 0.05 0.5 | 1855 62 235 |
| | Metribuzin Quizalofop - p-ethyl[47] | Solid Phase Extraction (SPE) | GC-ECD | 72.9-109.5% | 0.01 0.01 | 1100- 2300 1210 |
| | Thiamethoxam Lambda-cyhalothrin Deltamethrin[48] | MSPD Matrix Solid-Phase Dispersion (MSPD) | HPLC-MS/MS | 70-96% | 0.005 0.01 0.005 | >5000 79 129 |
| Cabbage | Fluridone, Simazine, Flubendiamide [49] | QuEChERS | PD-LVI UHPLC-MS/MS (Pre-column dilution large volume injection ultra-high performance liquid chromatography mass spectrometry) | 63.3-109% | 0.05 0.03 11 | >10000 >5000 >2000 |
| | Dicamba Quinmerac Imazosulfuron [50] | QuEChERS | LC - MS /MS | 80-95% | 0.04 0.1-0.2 0.02 | 1707 >2000 3129 |
| | Bromacil Chlorpyrifos Edifenphos[51] | QuEChERS | GC-MS-SIM (selected ion monitoring mode) | 80-95% | 0.05 1 0.005 | 5200 96-270 100 |
| | Sulfotep Diazinon Ethion[52] | Dispersive Biosurfactant Microdroplets enhanced MSPD | GC-FID (Flame ionization detector) | 85.8-107.9% | 0.01 0.5 0.005 | 10 300-400 13-20 |
| | Indoxacarb Cypermethrin Pirimicarb[53] | QuEChERS | LC -MS/MS | 90-130% | 0.005 0.1 1 | 1867 250 142 |
| Cauliflower | Trichlorfon[54] | Acetonitrile | LC -ESI - MS/MS | 83-101% | 0.005 | 250 |
| | Fipronil[55] | QuEChERS | GC - MS/MS | >80% | 0.02 | 97 |
| | Dimethoate, Isocarbophos, Methyl-parathion[56] | Solid phase extraction | HPLC | 82.5-96.6% | 0.1 0.1 0.05 | 235 50 25 |
| | Chlorpyrifos[57] | Acetonitrile + NaCl+ | HPLC - UV | 99.4% | 0.1 | 96-270 |





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| | | phosphate buffer | | | | |
| | Cypermethrin, Deltamethrin Chlorpyrifos[58] | QuEChERS | LC- MS/MS | 74-110% | 0.1 0.005 0.1 | 250 129 96-270 |
| Ladyfinger (okra) | Fipronil , Difenoconazole[5 9] | Ethyl acetate | LC- MS/MS | 80-107% | 0.01 6 | 97 1453 |
| | Triazophos, Profenofos, Chlorpyrifos [60] | Ethyl acetate | HPLC -UV | >85% | 0.005 0.01 0.05 | 57-82 358 96-270 |
| | Monocrotophos, Quinalphos, Chlorpyrifos[61] | Acetonitrile + DSPE +PSA (1 ^o 2 ^o amines)+GCB(Grap hite Carbon Black) ,c ₁₈ &anhydrous MgSO ₄ | LC-ESI- MS/MS +MRM(Multi ple Reaction Monitoring) | 81 – 106 % | 0.01 0.01 0.05 | 5 26 96-270 |
| | Tebufenozide[62] | Acetonitrile | LC- MS/MS | >72% | 10 | >5000 |
| | Monocrotophos, Methyl parathion, Cypermethrin[63] | MSPD | GC – MS/MS | 96.2% | 0.01 0.3 0.7 | 5 25 250 |
| Mushroom | Diazinon, Deltamethrin, Iprodione[64] | Selective pressurized liquid extraction | GC -MS/MS | 81-103% | 0.7 0.2 0.25 | 300-400 129 >4400 |
| | Heptachlor, Vinclozolin, Methoxychlor[65] | Acetone – n- hexane mixture | GC – AED (Atomic Emission Detector) | 77-95% | 0.05 1 0.05 | 119-320 1320 3460 |
| | Phoxim, Chlorpyrifos, Pyridaben[66] | QuEChERS | UHPLC - MS/MS | 75.5 – 98.4 % | 0.05 0.05 0.1 | 300 96-270 820-1350 |
| | Pyriproxyfen, Avermectins, Diflubenzuron[67] | QuEChERS | UHPLC - MS/MS | 78.1 - 112.5% | 0.2 0.05 0.3 | >5000 >4640 |
| | Cyantraniliprole, Chlorantranilipro le, Cyclaniliprole[68] | QuEChERS | HPLC – MS/MS | 73.5 – 110.2% | 0.6 2 0.1 | >5000 >5000 >2000 |
| Tea | Metamitron, Aldoxcarb, Acephate[69] | QuEChERS | UHPLC- MS/MS | 78 – 95% | 0.1 0.02 0.1 | 1183 500-800 980 |
| | Ethoprophos, Thiometon, Terbufos[70] | SPME (SWCNT) (Single Walled carbon nano tubes) | GC -MS | 71.1 – 118.4% | 0.02 0.5 0.05 | 62 40 29-34 |
| | Bifenthrin[71] | LLE-SPE | GC-ECD | 89-108% | 30 | 375 |
| | Flonicamid, | Ethyl acetate + | LC- MS/MS | 70-120% | 7 | 884-1768 |





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| | Abamectin, Bifenox[72] | cyclohexane + dSPE | | | 0.1 0.05-1 | 650 >5000 |
| | Quintozene, Dimethoate, Carbofuran[73] | QuEChERS | Size Exclusion Chromatography-GC - MS | 85 – 97% | 0.1 0.01 0.01 | 1710 235 6-18 |
| Coffee | Chlorothalonil, Deltamethrin, Fenitrothion[74] | QuEChERS | GC – MS With Nacl (-ve chemical ionization) | 95 – 101% | 0.01 10 0.5 | >10000 129 330 |
| | Carbofuran, Imidacloprid, Tebuconazole[75] | Ultra solvent extraction + LLME | UPLC – MS /MS | 74-81 % | 0.005 0.01 0.01 | 6-18 450 3766-3710 |
| | Bupirimate, Bitertanol, Dimethomorph[76] | QuEChERS | LC- ESI- MS/MS | 73-99% | 0.01 0.01 0.005 | >4000 >5000 3900 |
| | Oxasulfuron, Kresoxim-methyl, Nuarimol[77] | QuEChERS | LC – MS/MS | 70.7-108.8% | 0.01 20 0.2 | - >5000 1250 |
| | Buprofezin, Triazofos[78] | QuEChERS | GC-MS | 85-97% | 0.005 0.005 | 1635-3857 26-82 |

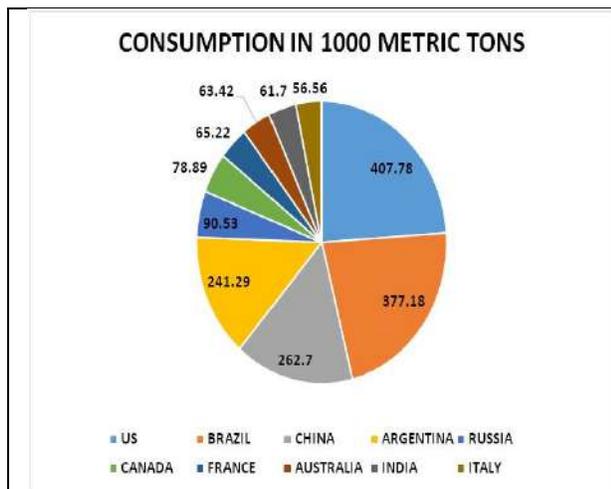


Fig No 1:A pie chart representing usage of pesticide by different countries in 1000 metric tons.

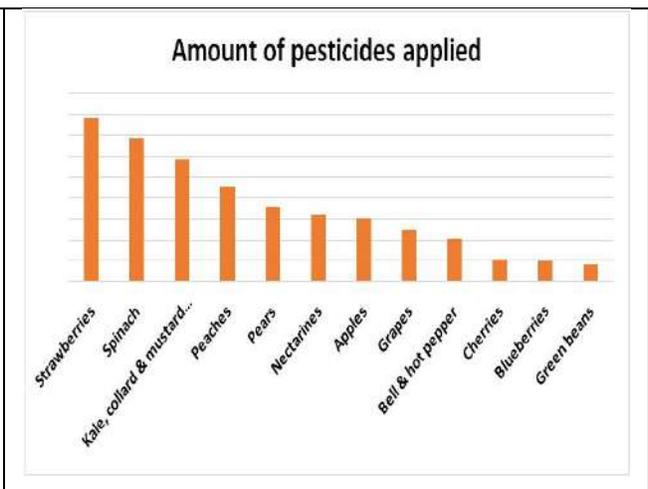


Fig No 2: A graph showing the highest amount of pesticides used in fruits and vegetables.





Benefit Cost - Analysis of Silkworm Rearing Units in Krishnagiri District of Tamil Nadu

T. Lingavarayan^{1*} and R. Selva Kumar²

¹Research Scholar (Agri-Business Management), Department of Agricultural Economics, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India.

²Assistant Professor, Department of Agricultural Economics, Annamalai University, Annamalai Nagar- 608 002, Tamil Nadu, India

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*Address for Correspondence

T. Lingavarayan

Research Scholar (Agri-Business Management),
Department of Agricultural Economics,
Annamalai University, Annamalai Nagar- 608 002,
Tamil Nadu, India.

E.Mail: lingavarayan1996@gmail.com



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ABSTRACT

Sericulture is one of the rural-based agro-industries with global reach. The rearing of silkworms for the production of silk is known as sericulture. Sericulture comprises the cultivation of mulberry, silkworm rearing, and silk reeling activities. On average, 86,311 MT of mulberry silk are cultivated in many parts of the world. Since the Krishnagiri district has top-notch mulberry cultivation and cocoon production. The identification of 120 respondents involved in the mulberry silkworm production activity was done using a multi-stage random sampling procedure adopted for the selection of blocks and villages. The specific objectives of the study were to estimate the cost and return structure of mulberry leaf production and cocoon production. The analysis indicated that the average leaf yield per acre was 25,072 kilogram's, which formed Rs. 53,500 to the total cost. On the other hand, returns generated for cocoons for the gross returns, net returns, and benefit-cost ratio amounted to Rs. 39,805.58, Rs. 21,855.58, and 2.22:1 / 100 DFLs, respectively. Mulberry farming, silk worm rearing, and cocoon production are all issues that are connected to providing appropriate gainful employment to rural people in the study area.

Keywords: Sericulture, Silkworm rearing, Mulberry, Cocoon, Production, Cost, Return, Disease free layings.

INTRODUCTION

Sericulture is one of the rural-based agro-industries with global reach. The rearing of silkworms for the production of



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silk is known as sericulture. Sericulture comprises the cultivation of mulberry, silkworm rearing, and silk reeling activities. The only food for the silkworm (*Bombyx mori*) is the mulberry foliage. It is fully grown under diverse climatic circumstances reaching from temperate to tropical. One of the major economic components of sericulture is the mulberry leaf. The quality and quantity of leaf generated per unit area have a direct bearing on the cocoon harvest. The cocoon production consists of two major economic activities namely, the cultivation of mulberry and rearing of silkworms. The establishment takes six months, garden maintenance has to be done round the year, and the silkworm rearing activity for cocoon production is for 30 to 40 days/cycle. On average, 86,311 MT of Mulberry silk are cultivated in many parts of the world. China was the top producer of Mulberry silk with a production of 46,700 MT in 2022. Next is India, which will produce 25,820 MT in 2022, and India is the World's second-largest Mulberry silk producer. Some studies attempted to find out the economic prospects of sericulture in Karnataka, Andhra Pradesh, Tamil Nadu and West Bengal (Manjunatha *et al.*, 2017; Raju *et al.*, 2018). Tamil Nadu occupies the Third position in terms of area and production, with 49,700 acres under cultivation producing of 2298.70 MT (Department of Sericulture and Central Silk Board, 2021–22). Mulberry is cultivated by 24,427 farmers in Tamil Nadu. Sericulture is extensively practised in Krishnagiri, Dharmapuri, Salem, Erode, Tiruppur, Vellore, Namakkal, Dindigul, Tirunelveli and Theni districts. It is practised on a moderate scale in the other districts except Chennai. The present study attempts to investigate the economic profile as also its costs and returns.

Objectives

- To estimate the various costs associated with mulberry leaf production and to work out the returns from it in the study area.
- To analyze the costs and returns of cocoon production in the study area.

MATERIALS AND METHODS

The research was carried out in the traditional sericulture belt of Krishnagiri district. Since the Krishnagiri district has top mulberry cultivation and cocoon production. The identification of 120 respondents involved in the mulberry silkworm production activity was done using a multi-stage random sampling procedure was adopted for the selection of Blocks and villages. In Krishnagiri district, four blocks were selected namely Schoolagiri, Hosur, Veppanapalli and Kelamangalam where the majority of farmers adopted mulberry cultivation and silkworm cocoon production. The information was gathered via personal contact and standardized surveys. The data was evaluated using a basic tabular manner, with averages and percentages, as well as frequencies, generated to determine the cost and returns of cocoon production. The analytical tools used in the study were cost and return. The problem of this study is to discover issues with mulberry production, cocoon production, and farmer profitability in a specific study region. (Lakshmanan *et al.*, 2000; Balasaraswathi *et al.*, 2010; Elumalai *et al.*, 2019). The field survey of primary data for the study as well as the analytical works was conducted during the months of February to May 2023.

Costs and Returns of Mulberry and Cocoon Production

The cost-return analysis was worked out separately for mulberry leaf production (Rs/acre/year) and cocoon production (Rs/100 DFLs) among the 120 Sample Respondents.

Cost of Mulberry leaf Production

Mulberry is a perennial crop that, after being correctly established over the first six months, will begin to produce in the second year and continue to do so for 15 to 20 years without experiencing a material loss in output. The cost of mulberry farming was calculated based on the variety and planting technique used. Establishment costs (fixed cost) and maintenance costs (variable cost) are included in the cost of cultivation.



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Maintenance Cost of the Mulberry Garden

The cost of the bullock, the equipment and labour used for ploughing and intercultural tasks, the cost of manure and fertilizers, the cost of irrigation, revenue, and the cost of leaf harvesting from the first harvest to the last crop of the year under consideration are all included in the maintenance cost.

Establishment Cost of the Mulberry Garden

By dividing the total establishment cost by the average lifespan of the respondent's particular variety of mulberry, the establishment's proportionate cost was determined. The establishment cost includes the cost of land preparation, the cost of inputs like seed materials, manures and fertilizers, and the human power utilized in plantation, inter-cultivation and irrigation up to the first harvest from the date of planting.

Interest on working capital: On the actual amount expended, the interest rate was calculated at an annual rate of 8 percent.

Interest on fixed capital: On the actual cost expended for the creation of the mulberry garden during a 6-month period, interest was calculated at a rate of 8% annually.

Cost of Silkworm Cocoon Production

Certain special prerequisites are necessary for the development of silkworms, including a separate rearing house, rearing equipment, and inputs such as disinfectants, silkworm seed, etc. The majority of the study's farmers had their own separate rearing houses. In the research region, several chawki rearing centres had been constructed, and almost every farmer was purchasing chawki rearing larvae. The Japanese rotary cardboard mountages, the plastic collapsible montage, and the traditional bamboo chadrike montages were among the most popular types of montages used in the shoot rearing technique of raising. These data were used to determine the cost of producing a cocoon. As shown below, the fixed and variable costs involved in raising silkworms were evaluated.

Variable cost of silkworm cocoon production

This includes the recurring or operational costs of different items used in silkworm rearing, such as chawki worms, mulberry leaf, human labour, mount ages, disinfection, miscellaneous charges, marketing charges, etc.

Interest on the working capital: On the actual costs spent by the respondents, an interest rate of 8% per year was determined.

Fixed cost of silkworm cocoon production

Based on the material's depreciation cost, the fixed cost for every item used in silkworm rearing was calculated and the following table details how depreciation costs for a farmer's equipment, machinery, and rearing house were calculated separately.

$$\text{Annual depreciation} = \frac{\text{Purchase/ Construction value}}{\text{The exoected life span of the assets(s)}}$$

The respondents reported asset's average life span was taken to be considered for computing the depreciation value.

Interest on fixed capital: After deducting the cost of depreciation for the year, interest was calculated at a rate of 8% per year on the actual value of the assets.

Returns from cocoon production

The respondents' earnings from the sale of cocoons at the cocoon market and other by-products that contributed to the returns were valued at the average price for the market.

Net income realized from cocoon production

The value of by-products and the value of cocoons transferred at current market rates are included in the gross income. By deducting the total cost of producing cocoons from the gross income, the net return on cocoon production was established.





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Benefit Cost Ratio (BCR)

It indicates the returns generated for every rupee of investment, both in mulberry leaf production and silkworm cocoon production. It was calculated by adopting the following formula.

$$\text{BCR} = \frac{\text{Gross returns}}{\text{Total Cost}}$$

RESULTS AND DISCUSSION

The results of the current investigations are tabulated in Tables 1 to 4 and Explained along with the works of previous research in the following paragraphs.

Cost of mulberry leaf production

It was observed from table 01 that the total cost incurred by mulberry cultivation growers during the period per year was Rs. 53,500. Out of this, variable costs and fixed costs accounted for about 87.85 and 12.15 per cent respectively. The major item of variable cost was on shoot harvest Rs.9,500 which formed 17.76 per cent of total cost followed by manure and application charges Rs.7,100 had 13.27 per cent, intercultural operation in mulberry garden Rs.6,500 had 12.15 per cent, imputed value of family labour Rs.6,400 had 11.96 per cent, farm yard manure Rs.5,500 had 10.28 per cent and irrigation charges, irrigation water, farm yard manure and others are 7.48, 4.67, 4.67 and 3.74 per cent of the total cost respectively. The fixed cost accounted for Rs.6,500 of which major item was the imputed apportioned cost of mulberry garden 6.54 per cent followed by depreciation 4.77 per cent and Land revenue 0.84 per cent, respectively.

Returns from mulberry leaf production

It was observed from the table 02 that the average mulberry leaf yield per acre was 25,072 kilogram's which formed of Rs.53,500 to the total cost. An average cost of the mulberry leaves sold at Rs.2.13 per kilogram. However, the price of mulberry leaf was Rs. 4 per kilogram. Hence, this price was used to arrive at the gross return, net return and benefit cost ratio from mulberry leaves which amounted to (Rs. 1,00,288, Rs. 47,788 and 1.89:1/acre/year) respectively. On an average, the quantity of the by-product obtained from an acre was Rs. 1,000 per year, added to the gross return.

Cost of Cocoon Production

It was observed from the table 03 that the total cost of production of cocoons for the silkworm rearing of 100 DFLs of among the total costs (variable and fixed) was Rs. 17,950 (Rs. 16,200 and Rs. 1,750) respectively. The major item of variable cost was on leaf Rs. 6,000 which formed 33.43 per cent of the total cost followed by labour Rs. 4,800 had 26.74 per cent, chawki worms Rs. 2500 had 13.93 per cent and other transportation and marketing, disinfectants and other costs are 8.37, 4.19 and 3.62 per cent of the total establishment cost respectively. The fixed cost accounted for Rs. 1,750, of which the item was depreciation on buildings and equipment 9.75 per cent respectively.

Returns from Cocoon Production

It was observed from table 04 that the results on per acre gross and net returns from silk cocoon production for each rearing 100 DFLs. It could be seen from the results that the silkworm rearing of 100 DFLs on an average cocoon yield 74.93 per kilogram, followed by the cost for one kilogram of cocoon production was Rs. 260.41 and the average market price of cocoons was Rs. 505.94 per kilogram respectively. However, an average includes the value of by-products obtained from 100 DFLs at 5 per cent of gross returns added. On the other hand, returns generated for cocoons for the gross returns, net returns and benefit-cost ratio amounted was Rs. 39,805.58 per 100 DFLs, Rs. 21,855.58 per 100 DFLs and 2.22:1 per 100 DFLs, respectively.





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CONCLUSION

The production of cocoons is profitable in the study region. The analysis of indicated that the average leaf yield per acre was 25,072 kilogram's which formed of Rs. 53,500 to the total cost. On the other hand, returns generated for cocoons for the gross returns, net returns and benefit-cost ratio amounted was Rs. 39,805.58 per 100 DFLs, Rs. 21,855.58 per 100 DFLs and 2.22:1 per 100 DFLs, respectively. It is a farm-based, labor-intensive, and commercially attractive economic activity falling under the cottage and small scale sector. If this industry flourishes on a large scale in rural areas, the chronic unemployment problem in the Krishnagiri district will be solved to a considerable extent.

Abbreviations: FYM - Farm Yard Manure, DFLs – Disease Free Layings, BCR – Benefit Cost Ratio, MT – Metric Tonnes.

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Table.01. Cost of Mulberry leaf production (Unit: Rs/acre/year)

| S. No | Particulars | Value | Percentage |
|----------|--|---------------|--------------|
| A | Variable cost | | |
| 1 | Intercultural operation in mulberry Garden | 6,500 | 12.15 |
| 2 | Manure and application charges | 7,100 | 13.27 |
| 3 | FYM | 2,500 | 4.67 |
| 4 | Fertilizer and application charges | 5,500 | 10.28 |
| 5 | Irrigation Water | 2,500 | 4.67 |
| 6 | Irrigation Charges | 4,000 | 7.48 |
| 7 | Shoot harvest | 9,500 | 17.76 |
| 8 | Pruning and cleaning of plants | 1,000 | 1.87 |
| 9 | Miscellaneous | 2,000 | 3.74 |
| 10 | Imputed Value of Family labour | 6,400 | 11.96 |
| | Total variable cost | 47,000 | 87.85 |
| B | Fixed Cost | | |
| 1 | Land revenue | 450 | 0.84 |
| 2 | Depreciation | 2,550 | 4.77 |
| 3 | Apportioned cost of Mulberry Garden | 3,500 | 6.54 |
| | Total Fixed Cost | 6,500 | 12.15 |
| | Total Cost (A+B) | 53,500 | 100 |

Table.02. Returns from Mulberry leaf production (Unit: Rs/acre/year)

*Price of leaf @ Rs. 4/kg; value of by-products = Rs. 1000/-

| S. No | Particulars | Value |
|-------|----------------------|----------|
| 1 | Leaf Yield (kg) | 25,072 |
| 2 | Total cost (Rs.) | 53,500 |
| 3 | Cost of Leaf (Rs/kg) | 2.134 |
| 4 | Gross return (Rs.) * | 1,01,288 |
| 5 | Net return (Rs.) | 47,788 |
| 6 | BCR | 1.89 |



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Table.03. Cost of Cocoon Production (Unit: Rs/100DFLs)

| S. No | Particulars | Value | Percentage to the total |
|----------|--|---------------|-------------------------|
| A | Variable Cost | | |
| 1 | Chawki worms | 2,500 | 13.93 |
| 2 | Leaf | 6,000 | 33.43 |
| 3 | Disinfectants | 750 | 04.19 |
| 4 | Labour | 4,800 | 26.74 |
| 5 | Transportation and Marketing | 1,500 | 08.37 |
| 6 | Other costs | 650 | 03.62 |
| | Total Variable cost | 16,200 | 90.25 |
| B | Fixed cost | | |
| 1 | Depreciation on building and equipment | 1,750 | 09.75 |
| | Total Fixed cost | 1,750 | 09.75 |
| | Total cost (A+B) | 17,950 | 100.00 |

Table.04. Returns from Cocoon Production (Unit: Rs/100 DFLs)

| S. No | Particulars | Value |
|-------|--------------------------|-----------|
| 1 | Cocoon yield (kg) | 73.93 |
| 2 | Total cost (Rs.) | 17,950 |
| 3 | Cost/kg of Cocoons (Rs.) | 260.41 |
| 4 | Price of cocoons/kg | 505.94 |
| 5 | Gross return (Rs.) * | 39,805.58 |
| 6 | Net returns (Rs.) | 21,855.58 |
| 7 | BCR | 2.22 |

*Includes value of by-products @ 5%





***In silico* Molecular Docking Analysis of Anti-Viral Drugs against Structural and Non-Structural Proteins of SARS-CoV-2**

K.Sakthishabarish^{1*}, S. Nivetha² and S. Karthik Sundaram³

¹Research Scholar, Marine Biology Lab, School of Bio Sciences and Technology, Vellore Institute of Technology (VIT), Vellore- 632 014, Tamil Nadu, India

²Production Executive, Tropical Bio Sciences, Pollachi Main Road, Coimbatore – 641 031, Tamil Nadu, India

³Assistant Professor, PG and Research Department of Microbiology, Dr. N. G. P. Arts and Science College, Coimbatore - 641 048, Tamil Nadu, India.

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***Address for Correspondence**

K.Sakthishabarish

Research Scholar,
Marine Biology Lab,
School of Bio Sciences and Technology,
Vellore Institute of Technology (VIT),
Vellore- 632 014, Tamil Nadu, India
E.Mail: sakthishabarish.k@gmail.com



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ABSTRACT

Numerous people lost their valuable life and many of them are still facing difficulties because of the Covid-19 pandemic over the past few years. Still, there was no permanent solution for the Covid-19 infection. Even though various types of vaccines are available, the virus gets mutated and developed into a more virulent form with a high rate of transmission. Still, researchers are giving their best to find a potential antiviral drug to save the lives of people all around the world. Even with the availability of many commercial antiviral drugs, many deaths were reported all over the world. In this study, we have chosen several commercially available antiviral drugs as a ligand for the molecular docking against major Structural proteins (Sp) (E484K, K417T, N501Y, S13I) and Non- structural proteins (Nsp) (7K7P, 2IDY, 6WXD, 7BQ7, 6NUR) as a drug target for the molecular interaction studies. Based on the protein-ligand interactions, binding affinity and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) analysis, REM, AZI, DEX, and IVE shown better interactions with the target proteins. Based on this study, these drugs were identified as potent drugs against SARS-CoV-2. These drugs could be effective in inhibition of viral replication and transcription of SARS-CoV-19 viral infection.

Keywords: SARS CoV-2; Molecular docking; antiviral drugs; Structural proteins (Sp); Non-Structural proteins (Nsp); ADMET analysis; Covid-19 virus.



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INTRODUCTION

Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), has resulted in a pandemic around the world known as COVID-19 [1]. On 31st December 2019, a mysterious pneumonia was detected in Wuhan City in China. On 7th January 2020, the new coronavirus (2019-nCoV) was identified as the causative agent, and WHO later named it Covid-19 [2]. Coronavirus is very small in size (65–125 nm in diameter) with single-stranded positive RNA virus. This belongs to the Family – Coronaviridae, Subfamily – coronaviridae, Order – nidovirales [3]. The coronaviruses have spike proteins like viral glycoprotein. They are club-shaped projections from the viral envelope resembling a crown that was named spikes glycoprotein(s) [4]. According to the serotype and genomic characteristics, Coronaviruses are categorized into four important genera, they are alpha (α), beta (β), delta (γ), and gamma (δ) coronaviruses. The α and β -corona viruses are a zoonotic group found in bats and the γ and δ -corona viruses are a group of rodents found in avian species [5]. There are seven types of coronavirus strains that infect humans (229E-CoV, NL63-CoV, OC43-CoV, HKU1-CoV, SARS-CoV, MERS-CoV, and Covid-19). Coronavirus possesses the large genomes (26.4-31.7 kb) with the G+C content varying from 32-43% [6, 7]. In general, Coronaviruses cause widespread respiratory, gastrointestinal, and central nervous system diseases in humans and other animals [8, 9].

For the SARS CoV-2 viral infection and transmission, Structural proteins (Sp) and non-structural proteins (Nsp) play a vital role in viral entry into the host cell and viral replication inside the host cell. 16 non-structural and 4 structural proteins were reported in the SARS-CoV-2 structure. So we have chosen several structural (E484K, K417T, N501Y, S13I) and non-structural (7K7P, 2IDY, 6WXD, 7BQ7, 6NUR) proteins as the drug target for the molecular interaction analysis using molecular docking [10]. In this study, we are going to analyze the molecular interaction of selected drugs Lopinavir (LPV), Ritonavir (RTV), Tamiflu (TAM), Chloroquine (CQ), Hydroxychloroquine (HCQ), Ribavirin (RIB), Remdesivir (REM), Azithromycin (AZI), Ivermectin (IVE), Favipiravir (FAV), Dexamethasone (DEX) and Isoniazid (INH) with the structural and non-structural proteins by using molecular docking method. Molecular docking studies were carried out to predict the best binding antiviral drugs against the structural and non-structural proteins of SARS-CoV-2 [11]. Based on this study we got a preliminary idea about the binding affinity of various antiviral drugs against the target protein.

Virulence mechanism of structural proteins

There are four structural proteins in SARS CoV-2, they are Spike S, Envelope E, Membrane M, and Nucleocapsid N protein [12]. The S, M, and E proteins together form the envelope of virus. E is the smallest and M is the largest structural protein responsible for the shape of the virus. The transmembrane proteins S and M are involved in virus assembly. N protein remains associated with the viral replication cycle by RNA forming a nucleocapsid inside the envelope [13]. The Centers for Disease Control and Prevention has highlighted that mutation in the spike protein of SARS-CoV-2 might have a possible impact on protection from acquired immunity. Mutation in the substitution of lysine for glutamic acid at the 484th position is called E484K. Mutations occur in the spike protein Receptor Binding Domain (RBD), a key antigenic target and it evolved independently in multiple virus lineages [14].

N501Y & K417T mutations found in P.1/ B1351 strains. P1 has three changes in the Receptor Binding Domain (K417T, E484K, and N501Y). These changes have the potential to modulate ACE2/RBD, which potentially leads to increased transmissibility these mutated residues also have the potential to modulate the neutralization of SARS-CoV-2 by natural or vaccine-induced antibody response [15]. In December 2020, new variants of SARS-CoV-2 carrying some mutations in the spike protein were documented in the UK and South Africa. Which showed increased receptor binding and infectivity by mutation of residue 501 in the RBD from Asparagine to Tyrosine (N501Y) [16]. Strain B.1.427/B.1.429 was detected in California at the beginning of 2021. It carries spike glycoprotein mutations in the signal peptide S13I mutations resulting in total loss of neutralization for 10 out of 10 N-Terminal domains [17].





Virulence Mechanism of Non-structural Proteins (Nsp)

ORF1a and ORF1b are the two-open reading fragments that encode Nsp1 to Nsp11 and Nsp1 to Nsp16 and they are further translated into polyproteins. The “C” terminus of Nsp1 binds and blocks the mRNA entry channel of the host cell to translate and promotes cellular mRNA degradation [18]. Nsp3 proteins are responsible for viral replication [19]. Nsp9 plays a major role in viral genome RNA replication and gives protection from nucleases [20, 21]. Negatively charged Nsp12 has minimal RNA-dependent RNA polymerase activity. Other non-structural protein cofactors like Nsp7 and Nsp8 stimulate Nsp12 for region stabilization and polymerase activity. Depending on the RNA polymerase active sites, the Nsp12, Nsp8, and Nsp7 complex are capable of initiation of the De nova pathway. Nsp12 interacts with Nsp5, Nsp9, and Nsp13. While Nsp8 interacts with Nsp7, Nsp9, Nsp10, Nsp13, and Nsp14. Within the viral replication complex, these two Nsp forms a hub for many protein-protein interactions [22]. Nsp16 is a positively charged active site for RNA cap binding. Nsp10 controls the methylase activity in both Nsp14 and Nsp16 [23].

MATERIALS AND METHODOLOGY

Proteins and Ligands

Various structural and non-structural proteins of SARS-CoV-2 responsible for viral replication and the evasion of host immune responses were selected. 3D crystalline structure for structural proteins N501Y (PDB ID:7MJL) [16], S13I (PDB ID:7N8I) [24], K417T (PDB ID: 7NX8) [15], D614G (PDB ID: 6XS6) [25], E484K (PDB ID: 7E8F) [26], and Non-Structural proteins such as NSP1 (PDB ID: 7K7) [18], NSP3 (PDB ID: 2IDY) [27], NSP9 (PDB ID: 6WXD) [21], NSP16 (PDB ID: 7BQ7) [28], NSP7,8,12 complex (PDB ID: 6NUR) [22], were retrieved from PDB database. Various antiviral drugs such as Lopinavir (LPV), Ritonavir (RTV), Tamiflu (TAM), chloroquine (CQ), Hydroxychloroquine (HCQ), Ribavirin (RIB), Remdesivir (REM), Azithromycin (AZI), Ivermectin (IVE), Favipiravir (FAV), Dexamethasone (DEX) and Isoniazid (INH) were chosen as a ligand for docking against the structural and non-structural proteins.

Docking and Analysis

Structures of the receptor and ligands were retrieved from the PDB [29] and PubChem [30] databases. Molecular docking was performed using AutoDock Vina v1.1.2 [31] and UCSF Chimera version 1.15 [32]. Further protein and ligand interactions and binding affinity were analyzed using PyMOL 2.4.1 [33] and Discovery Studio Visualizer v21.1.0.20298 [34, 35]. Also, we carried out ADMET analysis for the screened antiviral drugs using Swiss-ADME [36, 37] and admet SAR software [38].

Ligand and protein preparation

A one-dimensional representation (Canonical Smiles) was retrieved from PubChem and constructs into a 3D structure using UCSF Chimera version 1.15. By minimizing the energy, addition of hydrogen atoms, and addition of charges by Gasteiger methods, the ligand was prepared for molecular docking. Similarly, 3D structure of SP and NSP were retrieved from Protein Data Bank in PDB format. Before proceeding to docking analysis, the protein was subjected to a refinement and energy optimization process to prepare the protein. The formal atomic charges were fixed for the amino acid residues and energy minimization was carried out. Also, the crystal structure of the protein was prepared by removing all the water molecules and adding hydrogen atoms into their structure.

RESULT

Structural protein K484K docked with all the selected antiviral drugs. REM showed the highest binding potential followed by DEX and IVE with the docking score of -7.1, -7.0, and -6.4 kcal/mol respectively. The lowest binding potential was shown by RIB with a -5.1 kcal/mol docking score (Table 1). The K484K -REM complex contains two amino acid residues Leu R:335 and Glu A:169 three hydrogen bonds (Table 2). A minimum bond distance of 2.31 Å of Ser R:359 showed a C-H bond (Figure 2).



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Structural protein K417T docked with all the selected antiviral drugs. REM showed the highest binding potential followed by TAM and IVE with the docking score of -7.0, -6.8, and -6.8 kcal/mol respectively. The lowest binding potential was shown by LPV and RTV with a -5.0 kcal/mol docking score (Table 1). The K417T -REM complex contains two amino acid residues Ser E:469 and Gln E:474 with three hydrogen bonds (Table 3). A minimum bond distance of 2.02 Å of Gln E:474 showed an H- bond (Figure 3). Structural protein N501Y docked with all the selected antiviral drugs. REM showed the highest binding potential followed by AZI and FAV with the docking score of -6.8, -6.7, and -6.5 kcal/mol respectively. The lowest binding potential was shown by RTV with a -4.7 kcal/mol docking score (Table 1). The N501Y -REM complex contains three amino acid residues Gly A:238, Leu A:517, and Ser A:514 with three hydrogen bonds (Table 4). A minimum bond distance of 2.09 Å of Leu A:517 showed an H- bond (Figure 4).

Structural protein SI3I docked with all the selected antiviral drugs. IVE showed the highest binding potential followed by DEX and TAM with the docking score of -6.6, -6.5, and -6.5 kcal/mol respectively. The lowest binding potential was shown by LPV with a -4.5 kcal/mol docking score (Table 1). The SI3I -IVE complex contains no H bond. Instead the C-H bond is present thrice with three amino acid residues, such as Leu A:226, Asp A:228, and Phe A:43 (Table 5). The minimum bond distance of 1.97 Å of Asp A:228 showed an H- bond (Figure 5). Non-structural protein Nsp1 docked with all the selected antiviral drugs. REM showed the highest binding potential followed by AZI and RTV with the docking score of -6.6, -6.3, and -5.9 kcal/mol respectively. The lowest binding potential was shown by INH with a -4.3 kcal/mol docking score (Table 1). Nsp1-REM complex contains three amino acid residues such as Lys B:38, Arg B:34, and Asn B:117 has a total of eight hydrogen bonds (Table 6). A minimum bond distance of 2.07 Å of Arg B:34 showed an H- bond (Figure 6). Non-structural protein Nsp3 docked with all the selected antiviral drugs. AZI showed the highest binding potential followed by DEX and LPV with the docking score of -7.9, -7.0, and -6.7 kcal/mol respectively. The lowest binding potential was shown by INH with a -4.9 kcal/mol docking score (Table 1). The Nsp3-AZI complex contains a single amino acid residue Gly A:77 with two hydrogen bonds (Table 7). A minimum bond distance of 1.87 Å of Gly A:77 showed H- bond (Figure 7).

Non-structural protein Nsp9 protein docked with all the selected antiviral drugs. DEX showed the highest binding potential followed by IVE and RTV with the docking score of -7.9, -7.8, and -7.7 kcal/mol respectively. The lowest binding potential was shown by INH with a -5.5 kcal/mol docking score (Table 1). The Nsp9-DEX complex contains single amino acid residue Leu A:9 (Table 8) with a C-H bond in a 2.58 Å bond distance (Figure 8). Non-structural protein Nsp 7, 8, 12 docked with all the selected antiviral drugs. LPV showed the highest binding potential followed by REM, AZI, and IVE with the docking score of -8.8, -7.2, -7.0, and -7.0 kcal/mol respectively. The lowest binding potential was shown by INH with a -5.6 kcal/mol docking score (Table 1). Nsp 7, 8, 12-LPVcomplex contains two amino acid residue Trp A:216, and Gln A:117 with two hydrogen bonds (Table 9). A Minimum bond distance of 1.76 Å of Trp A:216 showed an H- bond (Figure 9). Non-structural protein Nsp16 docked with all the selected antiviral drugs. LPV showed the highest binding potential followed by REM and IVE with the docking score of -7.6, -6.9, and -6.7 kcal/mol respectively. The lowest binding potential was shown by INH with a -5.1 kcal/mol docking score (Table 1). Nsp16 -LPVcomplex contains five amino acid residues Asn A:198, Lys A:46, Try A:132, Lys A: 170, and Asp A:130 with six hydrogen bonds (Table 10). A minimum bond distance of 2.34 Å of Lys A: 170 showed an H- bond (Figure 10).

DISCUSSION

The highest negative binding energy and the least bond distance are the two major factors determining the strength of the interaction. Less bond distance gives good stability and better interaction. High negative binding energy with the highest number of hydrogen bonds denotes the effective and strong interaction between the protein and the ligand. REM, IVE, and TAM are the antiviral drugs that show the highest binding affinity against structural proteins. FAV, AZI, and DEX show the average binding score. But LPV, RTV, CQ, HCQ, RIB, and INH show the least binding affinity with structural proteins. In non-structural proteins, DEX, REM, LPV, and IVE show the good binding



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affinity. RTV and AZI show average binding affinity. But the other antiviral drugs show the least binding affinity with molecular docking of non-structural proteins. The highest binding score of -8.8 kcal/mol is for LPV drug against the Nsp7, 8, 12 complexes. A least binding score of -4.3 kcal/mol with the INH drug against the Nsp1 protein. In structural proteins, remdesivir has the highest binding affinity of -7.1 (kcal/mol). Liponavir showed the least binding affinity of -4.5 (kcal/mol) with the structural protein.

Based on the docking results, antiviral drugs REM, AZI, DEX, and IVE show the highest binding affinity in both structural and non-structural proteins. While the drugs CQ, HCQ, RIB, FAV, and INH show the least binding score in the molecular docking using auto dock vina. But Chloroquinone and Hydroxychloroquinone drugs showed the highest binding affinity against three major structural proteins (main-protease (M_{pro}), papain-like protease (PL_{pro}) and RNA-dependent RNA polymerase (RdRp)) were reported in early on studies [37]. Other drugs like TAM, LPV, and RTV show average binding affinity with the non-structural proteins. Similarly, various potent antiviral drugs (Ivermectin, Remdesivir, Chloroquine, Hydroxychloroquine, Fapinavir) were docked against ACE-2 and TMPRSS2 receptors of SARS-CoV-2 virus. Based on this study, ivermectin and remdesivir were identified as a promising drug for the treatment of SARS-CoV-2 infection was reported in previous study [5, 39].

REM shows the highest binding score of -7.1, -7.0, and -6.8 with structural proteins (E484K, K417T, and N501Y). Because REM forms more hydrogen bonds with the least bond distance. This makes REM to form strong and stable interaction with the structural proteins. AZI forms the highest binding affinity of -7.9 with Nsp3 (2IDY). It forms 2 hydrogen bonds with the least bond distance of 1.87 Å. DEX forms the highest binding affinity of -7.9 with Nsp9 (6WXD). Similarly, Remdesivir and azithromycin showed better inhibition against Nsp3 and Nsp9 virulent enzymes were reported in previous study [40]. LPV forms the highest binding affinity of -8.8 and -7.6 with the Nsp16 (7BQ7) and Nsp7, 8, 12 (6NUR). It forms 2 hydrogen bonds with the least bond distance 1.76 Å. These H bond and bond distance makes better and stronger interaction. IVE forms the highest binding affinity of -6.6 with the structural protein S13I. Based on the previous study, among remdesivir (RMD) and glycyrrhizin (GA), GA showed highest binding affinity (-8.21 Kcal/mol), though RMD showed stable and better interaction with the main protease enzyme (M_{pro}) of Covid-19 virus [41]. Also Glycyrrhizic acid showed highest binding affinity and good ADMET properties [42].

Based on the SWISS-ADME analysis of drugs, REM, AZI and IVE are violates twice the major Lipinski's rules. Drugs such as REM, AZI and IVE violates the Lipinski's rule high molecular weight (>500 Dalton) and high number of H bond acceptor (>10). These three drugs also have low GI absorption and they are not crosses the BBB (Blood Brain Barrier). AZI also has a high number of H bond donors (5). LPV and RTV are the other major drugs which also violate Lipinski's rules (Table 11). Apart from that CQ and HCQ show the high GI absorption and they also cross the BBB. Moreover, they don't violate any of Lipinski's rules. But CQ and HCQ show low binding affinity with the molecular docking with structural and non-structural proteins. It indicates CQ and HCQ having the higher permeability and drug absorption, compared to other drugs (Table 11).

CONCLUSION

In this study we identified REM, AZI, DEX, and IVE as the antiviral drugs that showed effective binding at the target sites in both structural and non-structural proteins to inhibit the viral replication and transcription. More number of hydrogen bonds and least bond distance are the two major criteria in the anti-viral drugs such as REM, AZI, DEX and IVE for the effective binding and strong interaction in the target sites. In ADMET analysis, compared to other drugs, dexamethasone (DEX) has high GI absorption and falls under Lipinski's rule of drugs. Still this is an in silico study, further research on development of combined drugs using CADD with high efficiency and more inhibition against the Covid-19 viral infection. In future studies, this in silico approaches will be playing a key role in the better prediction of new drug discovery against the Covid-19 infection.





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DECLARATION

Conflict of interest:

The author declares that they have no conflict of interest in this article.

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Table 1: Molecular docking result of antiviral drugs against Structural Proteins (SP) and Non-Structural Proteins (NSP).

| Proteins and Drugs | Binding Energy ΔG (kcal/mol) | | | | | | | | | |
|--------------------|--------------------------------------|-------|-------|-------|------------------------|-------|-------|-------|-----------|--|
| | Structural Protein | | | | Non-Structural Protein | | | | | |
| | E484K | K417T | N501Y | S13I | Nsp1 | Nsp3 | Nsp9 | Nsp16 | Nsp7,8,12 | |
| | (Unit : kcal/mol) | | | | (Unit : kcal/mol) | | | | | |
| LPV | -5.2 | -5.0* | -4.8 | -4.5* | -5.3 | -6.7 | -7.2 | -7.6* | -8.8* | |
| RTV | -5.2 | -5.0* | -4.7* | -5.1 | -5.9 | -6.0 | -7.7 | -6.2 | -6.9 | |
| TAM | -6.2 | -6.8 | -6.3 | -6.5 | -4.6 | -5.3 | -5.6 | -5.2 | -5.7 | |
| CQ | -5.3 | -5.3 | -5.6 | -4.8 | -4.4 | -5.6 | -5.9 | -5.5 | -6.1 | |
| HCQ | -5.3 | -5.7 | -5.6 | -5.4 | -4.9 | -6.0 | -5.7 | -5.9 | -6.2 | |
| RIB | -5.1* | -5.6 | -5.1 | -5.2 | -5.6 | -5.3 | -6.2 | -5.4 | -6.4 | |
| REM | -7.1* | -7.0* | -6.8* | -6.3 | -6.6* | -6.6 | -7.6 | -6.9 | -7.2 | |
| AZI | -5.8 | -6.4 | -6.7 | -5.7 | -6.3 | -7.9* | -5.7 | -5.7 | -7.0 | |
| IVE | -6.4 | -6.8 | -6.3 | -6.6* | -4.5 | -6.1 | -7.8 | -6.7 | -7.0 | |
| FAV | -6.3 | -6.1 | -6.5 | -5.6 | -4.8 | -5.1 | -5.6 | -4.7* | -5.7 | |
| DEX | -7.0 | -5.9 | -6.1 | -6.5 | -5.8 | -7.0 | -7.9* | -6.0 | -7.3 | |
| INH | -5.4 | -5.1 | -4.9 | -5.3 | -4.3* | -4.9* | -5.5* | -5.1* | -5.6* | |

* Highest and lowest binding score.

Table 2: Molecular docking analysis of E484K structural protein with Remdesivir (REM) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Type of interaction |
|----------|--------|--------------------|------------------|-------------------------|
| E484K | REM | CYS R:361 | 4.69 | Pi-Alkyl |
| | | LEU R:335 | 2.39 | Conventional H bond |
| | | PRO R:337 | 4.53, 4.05 | Pi-Alkyl |
| | | PHE A:168 | 5.04 | Pi-Alkyl |
| | | LEU A:229 | 4.45 | Alkyl |
| | | TYR A:170 | 4.16 | Pi-Alkyl |
| | | PRO A:230 | 5.23, 4.33 | Alkyl |
| | | GLU A:169 | 2.81, 2.65 | Conventional H bond |
| | | ASN R:360 | 2.57 | Unfavorable donor-donor |
| | | SER R:359 | 3.56, 2.31 | C-H bond |
| | | ARG R:357 | 2.68, 2.39, 2.06 | Unfavorable donor-donor |



Sakthishabarish *et al.*,**Table 3: Molecular docking analysis of K417T structural protein with Remdesivir (REM) drug.**

| Receptor | Ligand | Amino acid residue | Bond distance | Type of interaction |
|----------|--------|--------------------|---------------|---------------------|
| K417T | REM | ARG E:457 | 4.39 | Pi –cation |
| | | SER E:469 | 2.60 | Conventional H bond |
| | | TYR E:473 | 5.27 | Pi-Pi T shaped |
| | | LYS E:458 | 5.20 | Pi-Alkyl |
| | | GLN E:474 | 2.17 | Conventional H bond |
| | | GLN E:474 | 3.93 | Pi-Sigma |
| | | GLN E:474 | 5.27 | Pi-Pi T shaped |
| | | GLN E:474 | 2.02 | Conventional H bond |
| | | GLN E:474 | 3.09 | C-H bond |
| | | CYS E:480 | 2.15 | C-H bond |

Table 4: Molecular docking analysis of N501Y structural protein with Remdesivir (REM) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Type of interaction |
|----------|--------|--------------------|---------------|---------------------|
| N501Y | REM | GLY A:238 | 2.21 | Conventional H bond |
| | | THR A:430 | 3.75 | Pi-Sigma |
| | | LEU A:517 | 2.09 | Conventional H bond |
| | | ASPA:428 | 2.84 | C-H bond |
| | | SER A:514 | 2.54 | Conventional H bond |
| | | TYR A:396 | 5.36 | Pi-Pi stacked |
| | | GLU A:516 | 4.93 | Pi- Anion |
| | | PHL A:464 | 5.24 | Pi-Pi stacked |
| | | PHL A:464 | 5.08 | Pi-Alkyl |

Table 5: Molecular docking analysis of S13I structural protein with Ivermectin (IVE) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Type of interaction |
|----------|--------|--------------------|---------------|--------------------------------|
| S13I | IVE | LEU A:226 | 2.72 | C-H bond |
| | | TYR A:170 | 4.43 | Pi-Alkyl |
| | | PRO A:230 | 5.25 | Alkyl |
| | | PRO A:225 | 4.09 | Alkyl |
| | | ASP A:228 | 1.97 | C-H bond |
| | | ASP A:228 | 2.88 | Unfavorable Acceptor- Acceptor |
| | | PHE A:43 | 3.72 | C-H bond |
| | | PHE A:43 | 5.29 | Pi-Alkyl |



Sakthishabarish *et al.*,**Table 6: Molecular docking analysis of (Nsp1) Non- structural protein with Remdesivir (REM) drug.**

| Receptor | Ligand | Amino acid residue | Bond distance | Types of interaction |
|----------|--------|--------------------|------------------------------|----------------------|
| Nsp1 | REM | LEU B:7 | 5.47 | Pi –Alkyl |
| | | VAL B: 5 | 3.35, 3.88 | Pi-sigma |
| | | LYS B: 38 | 3.92 | Pi-sigma |
| | | LYS B: 38 | 3.98 | Pi-alkyl |
| | | LYS B: 38 | 2.10, 2.61, 2.44, 2.91, 2.50 | Conventional H bond |
| | | ARG B:34 | 2.07, 2.53 | Conventional H bond |
| | | ARG B:34 | 3.94 | Alkyl |
| | | ASN B:117 | 2.32 | Conventional H bond |
| | | LYS B:116 | 4.16 | Pi- alkyl |
| | | LYS B:116 | 4.85 | Pi –cation |
| | | LYS B:2 | 2.82, 2.85 | C-H bond |

Table 7: Molecular docking analysis of (Nsp3) Non- structural protein with Azithromycin (AZI) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Types of interaction |
|----------|--------|--------------------|---------------|----------------------|
| Nsp3 | AZI | PHE A:107 | 4.69 | Pi- Alkyl |
| | | PHE A:88 | 5.14 | Pi- Alkyl |
| | | TRP A:83 | 4.12 | Pi- Alkyl |
| | | ALA A:86 | 3.78, 4.03 | Alkyl |
| | | LEU A:28 | 3.55 | C-H bond |
| | | GLU A:82 | 5.38 | Attractive Charge |
| | | GLY A:77 | 1.87, 2.47 | Conventional H bond |

Table 8: Molecular docking analysis of (Nsp9) Non- structural protein with Remdesivir (REM) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Types of interaction |
|----------|--------|--------------------|---------------|----------------------|
| Nsp9 | DEX | LEU A:9 | 2.58 | C-H bond |

Table 9: Molecular docking analysis of (Nsp7,8,12) Non- structural protein with Lopinavir (LPV) drug.

| Receptor | Ligand | Amino acid residue | Bond distance | Types of interaction |
|-----------|--------|--------------------|---------------|----------------------|
| Nsp7,8,12 | LVP | PHE A:192 | 4.41 | Pi – alkyl |
| | | MET A:196 | 4.87 | Alkyl |
| | | ILE A:201 | 4.08 | Pi-alkyl |
| | | ILE A:201 | 4.24 | Alkyl |
| | | PHE A:222 | 5.56 | Pi-Pi stacked |
| | | ALA A:195 | 4.12 | Pi – alkyl |
| | | ALA A:195 | 4.21 | Alkyl |
| | | TRP A:216 | 1.76 | Conventional H bond |
| | | TRP A:216 | 2.51 | C-H bond |
| | | GLN A:117 | 2.72 | Conventional H bond |
| | | GLN A:117 | 2.84 | C-H bond |
| | | | | LYS A:188 |



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Table 10: Molecular docking analysis of (Nsp16) Non- structural protein with Lopinavir (LPV) drug.

| Receptor | Ligand | Aminoacid residue | Bond distance | Types of interaction |
|----------|--------|-------------------|---------------|----------------------|
| Nsp16 | LPV | ASN A:198 | 2.40 | Conventional H bond |
| | | LYS A:46 | 2.36, 2.47 | Conventional H bond |
| | | SER A:201 | 1.08 | Donar- Donar |
| | | GLU A:203 | 2.87 | C-H bond |
| | | TRY A:132 | 2.96 | Conventional H bond |
| | | TRY A:132 | 4.22 | Pi - alkyl |
| | | LYS A:170 | 2.34 | Conventional H bond |
| | | MET A:131 | 4.20, 5.22 | alkyl |
| | | LEU A:100 | 3.99, 5.12 | Alkyl |
| | | ASP A:99 | 2.62 | C-H bond |
| | | PRO A:134 | 3.90 | Pi - alkyl |
| | | ASP A:130 | 2.70 | Conventional H bond |
| | | ASP A:130 | 2.82 | C-H bond |
| | | ASP A:75 | 4.32 | Pi-anion |

Table 11: SWISS-ADME and admetSAR analysis of the chosen antiviral drugs

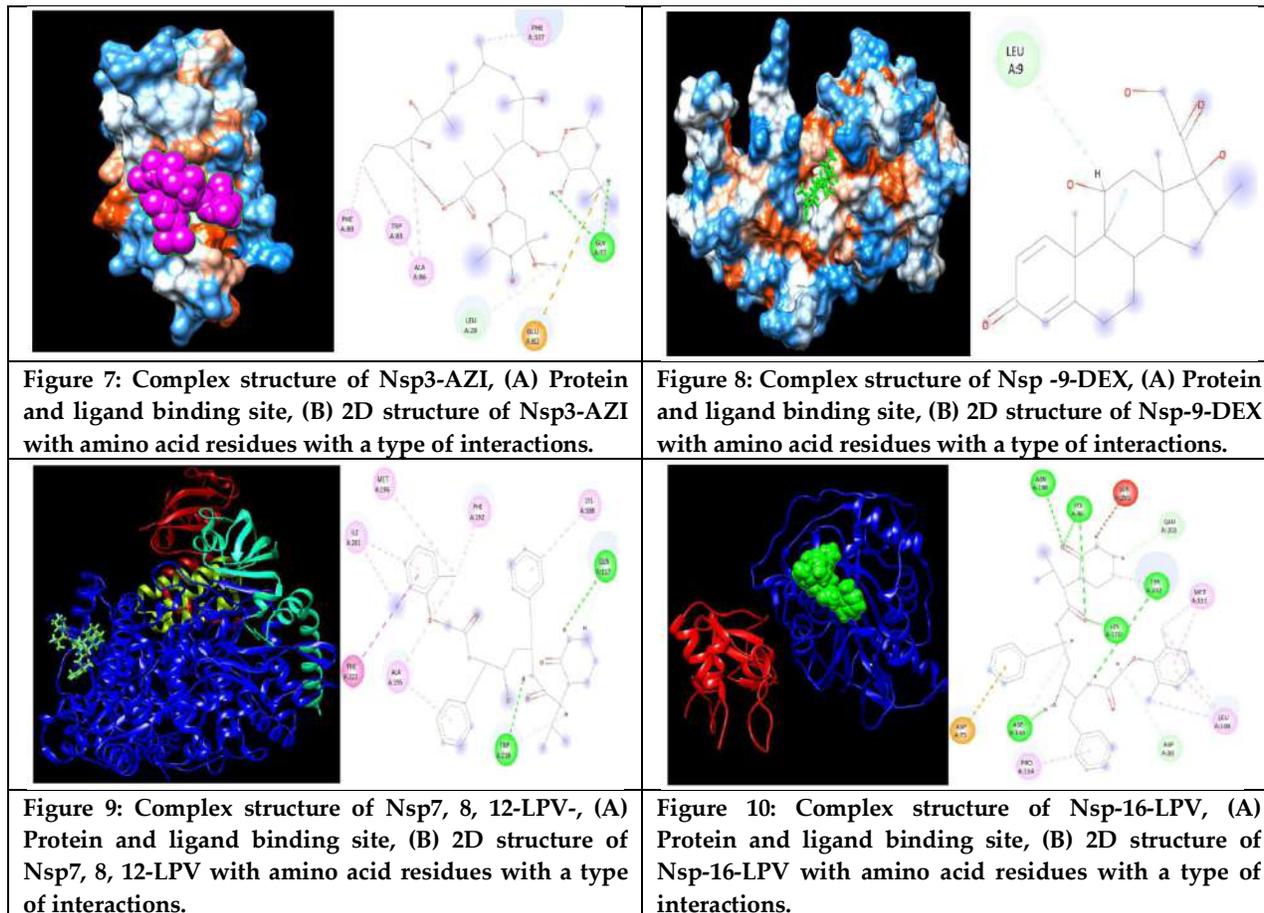
| Antiviral Drug | Molecular Weight (≤ 500 Da) | No. of rotatable bonds (≤ 10) | No. of H bond acceptor (≤ 10) | No. of H bond donor (≤ 5) | LogP (≤ 5) | GI Absorption | BBB Permeant | Bio availability | Synthetic Accessibility |
|----------------|-----------------------------------|--------------------------------------|--------------------------------------|----------------------------------|-------------------|---------------|--------------|------------------|-------------------------|
| LPV | 628.8* | 17* | 5 | 4 | 4.4 | High | No | 0.55 | 5.67 |
| RTV | 720.94* | 22* | 7 | 4 | 5.04 | Low | No | 0.17 | 6.45 |
| TAM | 312.4 | 9 | 5 | 2 | 1.43 | High | No | 0.55 | 4.44 |
| CQ | 319.87 | 8 | 2 | 1 | 4.15 | High | Yes | 0.55 | 2.76 |
| HCQ | 335.87 | 9 | 3 | 2 | 3.37 | High | Yes | 0.55 | 2.82 |
| RIB | 244.2 | 3 | 7 | 4 | -2.18 | Low | No | 0.55 | 3.89 |
| REM | 602.58* | 14* | 12* | 4 | 1.5 | Low | No | 0.17 | 6.33 |
| AZI | 748.98* | 7 | 14* | 5 | 2.02 | Low | No | 0.17 | 8.91 |
| IVE | 875.09* | 8 | 14* | 3 | 4.37 | Low | No | 0.17 | 10 |
| FAV | 157.1 | 1 | 4 | 2 | -0.27 | High | No | 0.55 | 2.08 |
| DEX | 392.46 | 2 | 6 | 3 | 2.14 | High | No | 0.55 | 5.47 |
| INH | 137.14 | 2 | 3 | 2 | -0.35 | High | No | 0.55 | 1.24 |

*Violation of Lipinski's rule; BBB –Blood Brain Barrier; GI- Gastro Intestinal.





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Clinical Evaluation of Siddha Herbal Formulation Nelli Mulli Ilagam in the Treatment of Pitha Paandu (Iron Deficiency Anaemia)

M.G.Chithra^{1*}, T.Lakshmi Kantham² and Meena Kumari R³

¹PG Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai-47, Tamil Nadu, India.

²Associate Professor, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai 47, Tamil Nadu, India.

³Director, National Institute of Siddha, Tambaram Sanatorium, Chennai, Tamil Nadu, India.

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*Address for Correspondence

Chithra M G,

PG Scholar,

Department of Maruthuvam,

National Institute of Siddha,

Tambaram Sanatorium,

Chennai- 47, Tamil Nadu, India.

E.Mail: chithramanibsms@gmail.com



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ABSTRACT

The siddha system of medicine is a established medical system that uses a scientific and holistic approach to provide preventive, promotive and curative. As per Siddha tradition the term “Paandu” is obtained from the character of veluppunoi. The etiology and symptoms of Paandunoi is corresponded to the iron deficiency anaemia. Around 30% of the total world population is anaemic and half of these, some 600 million people have iron deficiency. To evaluate the therapeutic effect of Nelli Mulli Ilagam, to study the improvement of clinical symptoms and assessment of haemoglobin level in Pitha Paandu patients before and after treatment. Nelli Mulli Ilagam was prepared according to sastric literature Agathiyar Vaithya Vallathi 600. Then clinical trial was conducted in Out-Patients Department of NIS. 30 Patients were selected based on the selection criteria and informed Consent was acquired from all the patients. Complete clinical history, complaints and duration, examination findings and laboratory findings was recorded in the prescribed proforma. Among 30 patients, 18 (60%) patients estimated as good clinical improvement, 9 (30%) patients estimated as moderate clinical improvement, and 3 (10%) patients estimated as mild clinical improvement and 13 (43.33%) patients showed increase of 1 – 2.5grams in the haemoglobin level. I conclude the trial drug is found to be safety, therapeutically efficacious and the research can be expanded with the same medication in more numbers of patients in the effective treatment of Pitha Paandunoi because of its clinical and laboratory results.





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Keywords: Siddha herbal formulation, Nelli Mulli Ilagam, Pitha Paandu

INTRODUCTION

Dietary inadequacy is most common issue in developing countries. As per the World Health Organization (WHO) approximately 1.62 billion peoples suffer from iron deficiency anaemia worldwide which compose 47% in preschool – age children, 25% in school children, 30% in non-pregnant women and 24% in people older than 60 years of age with 12% in adult men which is the least. (1) The commonest source of anaemia, particularly among the most vulnerable groups are nutritional disorders and infections. (2) Anaemia is a global public health problem affecting both developing and developed countries with major consequences for human health as well as social and economical development (3). World Health Organisation defines “ Anaemia is a condition in which the number of red blood cells or their oxygen - carrying capacity is insufficient to meet physiological needs, which vary by age, sex, attitude, smoking and pregnancy status” (4). The most important etiology of Iron Deficiency Anaemia (IDA) are increased blood loss, increased requirements, inadequate dietary intake and decreased absorption (5). IDA occurs at all age and involves both male and female, adolescent girls are more susceptible to get (6). As per National Family Health Survey (NFHS) nearly 50 – 80% of Indian mothers suffer from anaemia due to Iron deficiency (7). As per Siddha tradition the term “Paandu” is derived from the character of veluppunoi. Paandunoi is a disease characterized by changes in the body’s natural colour and pallor found in the skin, nails and conjunctiva. The etiology of Paandunoi are poor dietary intake, excessive intake of salty, sour food items, toxic foods, excessive chewing of betel nut and tobacco, pica, malabsorption conditions like worm infestation, liver disease, chronic blood loss such as haemorrhoids, dysentery and menorrhagia (8). These are correlated with the etiology of Anaemia in Biomedicine. Number of medicine, herbs, herbomineral preparations available in Siddha system of medicine in treating anaemia. So, I have gone through various siddha literatures to find a suitable, cost effective, safely haematinic for anaemic patients. And I have decided to take “NELLI MULLI ILAGAM” which is suggested PithaPaandu (Iron Deficiency Anaemia) specifically in the sastric literature AgathiyarVaithyaVallathi 600 (9)

MATERIALS AND METHODS

Subject Selection

The clinical trial was conducted in Out-Patients Department of National Institute of Siddha, Chennai. Before beginning the clinical study, the study was registered in clinical trial registry – India (**Reg No: CTRI/2021/08/035488**). When patients reported at OPD NO 1, Department of Maruthuvam, Ayothidoss Pandithar Hospital, NIS with symptoms of inclusion criteria were subjected to screening test and documentation was done by using screening proforma. Among 70 patients screened, 30 patients who fulfilled the inclusion criteria were recruited for the trial. Informed Consent was obtained from all the patients before starting the clinical trial. The qualitative study for the trial drug was performed in Biochemistry lab, National Institute of Siddha, Chennai - 47. Phytochemical analysis, Physiochemical evaluation, analysis of aflatoxin, TLC and HPTLC, Heavy metal analysis were conducted in Nobel Research Solution Laboratory, Perambur, Chennai.

Selection Criteria

Inclusion Criteria

- Age : 18-60 years
- Sex : both sex and transgender.
- Abnormal peripheral smear study – Microcytic Hypochromic Anaemia
- Hb level less than normal range, For male: 9-12 gms/dl, For female: 8-11 gms /dl
- Patient who are willing to undergo blood investigation.
- Patients who are willing to participate in study and signing in informed consent form.





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Exclusion Criteria

- Presence of any associated severe systemic illness (e.g. Cancer, AIDS)
- Chronic Kidney Disease
- Diabetes mellitus
- Family history of inherited defects (Known case of Sickle Cell Anaemia, Thalassemia, Aplastic Anaemia)
- Pregnant women
- Patients who are not willing to give blood sample.

The symptoms of PithaPaandu like pallor of skin, blurred vision, excessive thirst, chesttightness, breathlessness, giddiness, pungent or bitter taste. In this open clinical trial, patients reporting at the OPD with above clinical symptoms were examined clinically for enrolling in the study based on the inclusion and exclusion criteria. The patients who got enrolled were informed about the study, study drug, possible objectives and outcomes of the study in their vernacular language. After ascertaining the patient's willingness, informed consent was obtained from consent form. Complete clinical history, complaints and duration, examination findings and laboratory findings was recorded in the prescribed proforma. Patients were advised to take the study drug and appropriate dietary advice.

Required Raw Drugs

- | | |
|---|--------------------|
| 1. Nellikulli(<i>Phyllanthusemblica</i> ,Linn) | - 20 palam (700 g) |
| 2. Athimadhuram (<i>Glycyrrhizaglabra</i> ,Linn) | - 2 palam (70 g) |
| 3. KoogaiNeeru(<i>Marantaarundinacea</i> ,linn) | - 2 palam (70 g) |
| 4. Thiratchai (<i>Vitisvinifera</i> ,linn) | - 2 palam (70 g) |
| 5. Thippili(<i>Piper longum</i> ,Linn) | - 3 palam (105 g) |
| 6. Perinthu (<i>Phonexdactilifera</i> ,Linn) | - 3 palam (105 g) |
| 7. Sarkarai | - 25 palam (875) |
| 8. Honey | - ½ padi (750 ml) |

**Nelli Mulli Ilagam**

The above drugs were purified and make anilagam with correct consistency. The prepared Ilagam gave in a pure ziplock cover. On 1st day, the patients were advised to take oil bath with **Arakkuthylam** before starting treatment with trial drug to regulate the azhalkutram. On 2nd day patients were advised to take purgative medication i.e. **Agasthiyarkuzhambu** (Milagalavu) mixed with **Milaguthool** (Pepper powder) at early morning in empty stomach. Patients were advised to take rest on next day.

Then the trail drug "NELLI MULLI ILAGAM" 8 gram after food twice a day along with milk was given continuously for 45 days, patients should visit hospital once in 15 days. Specific diet restrictions such as tobacco, betel chewing, tea, coffee and alcohol, sour taste food were advised to avoid, during the trial period. The trial drug was provided for every 15 days. Laboratory investigations were done on 0th and 46th day for the assessment of safety



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of the patients and efficacy of the drug. After completion of the trial, the patients were followed up for the next 3 months in OPD.

OBSERVATIONS AND RESULTS

For this clinical study totally 70 patients were screened of which 40 patients were excluded and 30 patients were selected and treated in the Out – patient department of Ayothidoss Pandithar Hospital, National Institute of Siddha, Chennai – 47. Adverse reactions of the trial drug were not observed during the course of the study. Results of the clinical trial were observed with respect to the following criteria.

In Age Distribution Among 30 patients treated, 10 (33.33%) patients were reported from the age between 18 – 30 years, 10 (33.33%) patients were reported from the age between 31 – 40 years, 8 (26.7%) patients were reported from the age between 41 – 50 years, 2 (6.7%) patients were reported from the age between 51 – 60 years (Table 1).

In Gender Distribution 29 (96.67 %) patients were female and 1 (3.33 %) patient were male (Table 2).

In Menstrual History 20 (66.67 %) patients had regular menstrual history, 8 (26.7 %) patients had irregular menstrual history, 1(3.33 %) patient attained menopause (Table 3).

In Obstetrics History 15 (50%) patients had normal delivery, 4 (13.3%) patients had caesarean delivery, 2 (6.67%) patients had postpartum haemorrhage (Table 4). Out of 30 patients, 10 (33.33%) patients had family history of anaemia, 20 (66.67%) patients did not have family history of anaemia (Table 5).

In Socio Economic Status 2 (6.66%) patients were Upper economic status, 17 (56.67%) patients were Middle class people and 11 (36.67%) patients were poor economic status. The percentage is more in Middle economic group (Table 6).

In Causative Factor 7(23.33%) patients had hypothyroidism, 9 (30%) patients had menorrhagia, 5 (16.67%) patients had haemorrhoids, 3 (10%) patients had fibroid and 6 (20%) patients not having any causes (Table 7).

In history of surgery:15 (50%) patients had past history of surgery and 15 (50%) patients did not have past history of surgery (Table 8). In EzhuUdalkattugal, before treatment, **Saaram** (noted as fatigue) and **Senneer**(noted as pallor, reduction of haemoglobin level) were affected in all 30 (100%) patients. **Oon** was affected in 21 (70%) patients noted as pain paedaledema, **Kozhuppu** was affected in 6 (20 %) patients, **Enbu** was affected in 2 (6.67%) patients, **Suronitham** was affected as 9 (30%) patients noted as Oligomenorrhoea and Menorrhagea. After treatment, **Saaram** was affected in 6 (20 %) patients, **Senneer** was affected in 9 (30 %) patients, **Oon** was affected in 4 (13.33 %) patients, **Kozhuppu** was affected in 2 (6.67 %) patients, **Enbu** was affected in 1(3.33%) patients, **Suronitham** was affected in 2 (6.7 %) patients (Table 9).

In EnnVagaitervu, before treatment **Sparisam** (noted as dryness, cold sensation, excessive sweat) was affected in 17 (56.67%) patients, **Naa** (pallor, coated, glossitis, angular stomatitis, baldness, fissure, dryness, pungent or bitter taste) was affected in 21 (70%) patients. **Niram**(pallor) and **vizhi** (pallor) was affected in 30 (100 %) all patients. **Malam** (noted as constipation and diarrhoea) was affected in 6 (20 %) patients. **Mozhi** and **Moothiram** were normal in all patients. After treatment **Sparisam** was affected in 7 (23.33 %) patients, **Naa** was affected in 10 (33.3 %) patients, **Niram** (pallor) and **Vizhi** (pallor) were affected in 8 (26.67 %) patients, **Malam** was normal in all patients (Table 10). Among 30 patients of which 13 (43.33%) patients showed increase of 1 – 2.5 grams in the haemoglobin level, 14 (46.67%) patients showed increase of 0.1 – 0.9 grams in the haemoglobin level, 3 (10 %) patients showed decrease the haemoglobin level from its base level, and none of the patients were observed without improvement in haemoglobin level(Table 11).Among 30 patients, 18 (60 %) patients assessed as good improvement, 9 (30 %) patients assessed as



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moderate improvement and 3 (10 %) patients assessed as mild improvement (Table 12). In clinical features, all patients were improved after treatment (Table 13).

DISCUSSION

The inference obtained from the study showed, the vulnerability of the female population towards the disease Pitha Paandu. Heavy menstrual blood loss, abnormal uterine bleeding and pregnancy put women at risk of developing iron deficiency anaemia. The percentage of IDA is more in Middle economic group. Developing countries reported more Iron Deficiency Anaemia patients because of higher middle and poor economic status people whom in general consume low nutritional food.

The percentage is more in house wife as well as working women. Self – care was not taken into consideration like nutritious and balanced diet by this group of people which leads to Iron depletion. The percentage was more in rural area people. Due to the over consumption of fast foods and unhealthy diet in the urban areas leads to iron deficiency. There is a relationship between iron deficiency anaemia and irregular menstruation. Anaemia triggered by an iron deficiency can cause irregular menstruation, and likewise, women with irregular menstruation can suffer from iron deficiency anaemia from excessive blood loss. Iron deficiency anaemia at delivery is associated with an increased risk for caesarean section and adverse maternal and neonatal outcomes in otherwise healthy women. The prevalence of anaemia increases with age and frequently identified in older surgical patients. Anaemia is associated with increased postoperative morbidity and mortality (10).

Among 30 patients, 7(23.33%) patients had hypothyroidism. There is a relationship between anaemia and hypothyroidism. Anaemia in hypothyroidism might effect from bone marrow depression, reduced erythropoietin production, comorbid diseases or associated iron, vitamin B12 or folate deficiency. Modified iron metabolism and oxidative stress may contribute to anaemia in hypothyroidism (11). Among 30 patients, 9 (30%) patients had menorrhagia. In developing countries like India where the nutritional iron deficiency is very much frequent in the perimenopausal age group. Menstrual disorder accounts for 5% - 10% of the women presenting with iron deficiency anaemia (IDA) in the perimenopausal age group (12). Among 30 patients, 5 (16.67%) patients had haemorrhoids, Rectal bleeding due to piles can leads to large amounts of blood loss from the body. Health conditions such as ulcers, polyps or haemorrhoids can cause iron deficiency anaemia (13).

Among 30 patients, 3 (10%) patients had fibroid which causing heavier menstrual bleeding from the endometrium. Heavy blood loss from uterine fibroids can also denote an excess loss of haemoglobin (14). Among 30 patients, 6 (20%) patients were not having any causes. But their dietary habit was poor. So, it can under a type of nutritional deficiency anaemia. Preliminary phytochemical analysis of the extracts of Nelli Mull illagam showed presence of **Alkaloids, Flavanoids, Steroids, Triterpenoids, Phenols, Tannins and Protein** were detected. These are the responsible for the therapeutic action of the trial drug. Heavy metal analysis, specific pathogen test, Pesticide residual analysis, Aflatoxin test were negative.

No Adverse reaction of the drug was observed during the course of the study. Among 30 patients, 18 (60%) patients assessed as good clinical improvement, 9 (30%) patients assessed as moderate clinical improvement, and 3 (10%) patients assessed as mild clinical improvement. All laboratory investigations were done on 46th day. Among 30 patients, 13 (43.33%) patients showed increase of 1 – 2.5 grams in the haemoglobin level, 14 (46.67%) patients showed increase of 0.1 – 0.9 grams in the haemoglobin level. The statistical analysis of Haematological parameters was statistically highly significant. (P value <0.01).





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Statistical Analysis**The Statistical report states that the Mean \pm Standard deviation for,**Haemoglobin before treatment is 9.74 ± 1.03 and after treatment is 10.66 ± 1.17 which is statistically significant ($p < 0.00$)RBC before treatment is and after treatment is 4.05 ± 0.57 which is 54.42 ± 0.76 statistically significant ($p < 0.00$)PCV before treatment is 32.34 ± 2.79 and after treatment is 35.02 ± 2.57 which is statistically significant ($p < 0.00$)MCV before treatment is 74.13 ± 7.75 and after treatment is 75.57 ± 7.84 which is statistically significant ($p < 0.00$)MCH before treatment is 22.61 ± 2.76 and after treatment is 23.59 ± 2.62 which is statistically significant ($p < 0.00$)MCHC before treatment is 30.18 ± 1.39 and after treatment is 31.55 ± 1.87 which is statistically significant ($p < 0.00$)**CONCLUSION**

An open clinical trial was conducted to evaluate the therapeutic potential of Nelli Mull illagam in the treatment of Pitha Paandu. Among 30 patients, 18 (60%) patients assessed as good clinical improvement, 9 (30%) patients assessed as moderate clinical improvement, and 3 (10%) patients assessed as mild clinical improvement. Regarding Haemoglobin level, among 30 patients, 13 (43.33%) patients showed increase of 1 – 2.5 grams in the haemoglobin level, 14 (46.67%) patients showed increase of 0.1 – 0.9 grams in the haemoglobin level. From the statistical studies, the Mean Standard deviation for haemoglobin before treatment was 9.74 ± 1.03 and after treatment was which was 10.66 ± 1.17 statistically highly significant ($P < 0.01$). Thus, the Siddha herbal formulation Nelli Mull illagam had been proved clinically to be haematonic and effective in treating PithaPaandunoi. Hence, I conclude the trial drug is found to be safety, therapeutically efficacious and the research can be expanded with the same medication in more numbers of patients in the effective treatment of Pitha Paandunoi because of its clinical and laboratory results. Poor economic status, chronic blood loss like haemorrhoids, menorrhagia, fibroid in uterus, anal fissure were associated to IDA. If we will treating the causes of anaemia, we will definitely get good prognosis in IDA.

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| <p>AGE DISTRIBUTION</p> <table border="1"> <thead> <tr> <th>AGE GROUPS IN YEARS</th> <th>PERCENT AGE</th> </tr> </thead> <tbody> <tr> <td>18 – 30</td> <td>33.33 %</td> </tr> <tr> <td>31 – 40</td> <td>33.33 %</td> </tr> <tr> <td>41 – 50</td> <td>26.66 %</td> </tr> <tr> <td>51 - 60</td> <td>6.66 %</td> </tr> </tbody> </table> | AGE GROUPS IN YEARS | PERCENT AGE | 18 – 30 | 33.33 % | 31 – 40 | 33.33 % | 41 – 50 | 26.66 % | 51 - 60 | 6.66 % | <p>GENDER DISTRIBUTION</p> <table border="1"> <thead> <tr> <th>Gender</th> <th>Percentage</th> </tr> </thead> <tbody> <tr> <td>MALE</td> <td>3.33 %</td> </tr> <tr> <td>FEMALE</td> <td>96.67 %</td> </tr> </tbody> </table> | Gender | Percentage | MALE | 3.33 % | FEMALE | 96.67 % | | | | |
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| Table : 1 Age Distribution | Table : 2 Gender Distribution | | | | | | | | | | | | | | | | | | | | |
| <p>MENSTRUAL DISTRIBUTION</p> <table border="1"> <thead> <tr> <th>Category</th> <th>Percentage</th> </tr> </thead> <tbody> <tr> <td>REGULAR</td> <td>66.67 %</td> </tr> <tr> <td>IRREGULAR</td> <td>26.67 %</td> </tr> <tr> <td>MENOPAUSE</td> <td>3.33 %</td> </tr> <tr> <td>NOT APPLICABLE (MALE)</td> <td>3.33 %</td> </tr> </tbody> </table> | Category | Percentage | REGULAR | 66.67 % | IRREGULAR | 26.67 % | MENOPAUSE | 3.33 % | NOT APPLICABLE (MALE) | 3.33 % | <p>OBSTETRICS HISTORY DISTRIBUTION</p> <table border="1"> <thead> <tr> <th>Category</th> <th>Percentage</th> </tr> </thead> <tbody> <tr> <td>NORMAL...</td> <td>50 %</td> </tr> <tr> <td>CESAREAN...</td> <td>13.33 %</td> </tr> <tr> <td>POST PARTUM...</td> <td>6.67 %</td> </tr> <tr> <td>NOT APPLICABLE...</td> <td>30 %</td> </tr> </tbody> </table> | Category | Percentage | NORMAL... | 50 % | CESAREAN... | 13.33 % | POST PARTUM... | 6.67 % | NOT APPLICABLE... | 30 % |
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| IRREGULAR | 26.67 % | | | | | | | | | | | | | | | | | | | | |
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| Category | Percentage | | | | | | | | | | | | | | | | | | | | |
| NORMAL... | 50 % | | | | | | | | | | | | | | | | | | | | |
| CESAREAN... | 13.33 % | | | | | | | | | | | | | | | | | | | | |
| POST PARTUM... | 6.67 % | | | | | | | | | | | | | | | | | | | | |
| NOT APPLICABLE... | 30 % | | | | | | | | | | | | | | | | | | | | |
| Table : 3 Menstrual Distribution | Table : 4 Obstetrics History Distribution | | | | | | | | | | | | | | | | | | | | |





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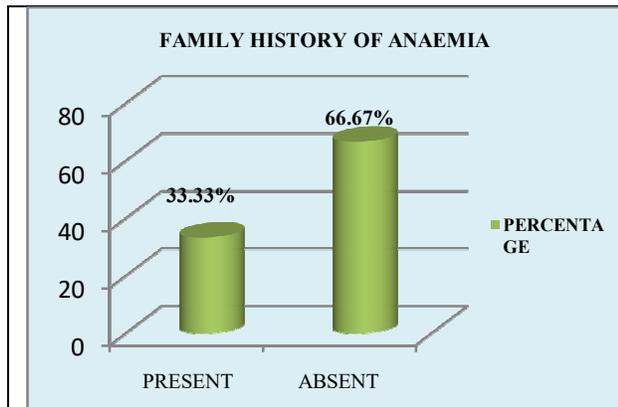


Table : 5 Family History of Anaemia

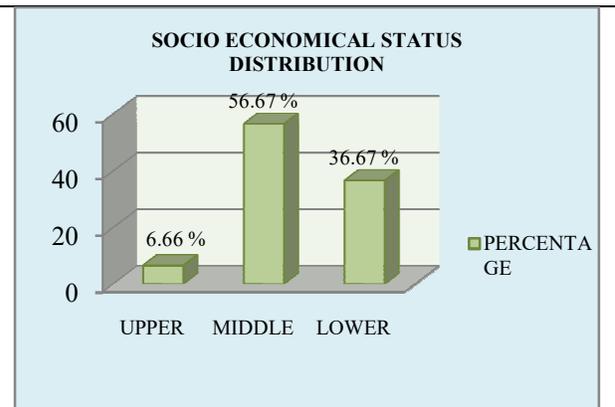


Table : 6 Socio Economical Status Distribution

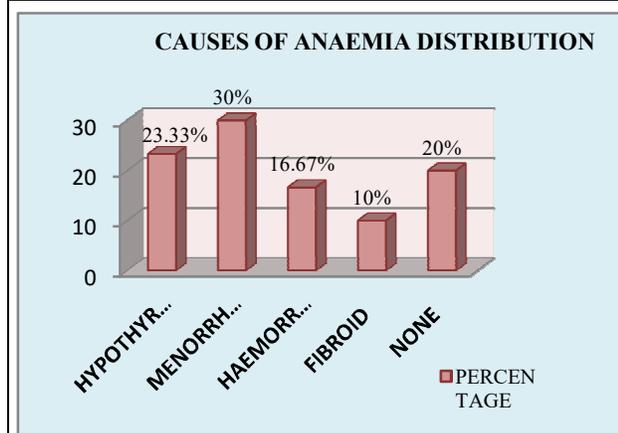


Table : 7 Causes of Anaemia Distribution

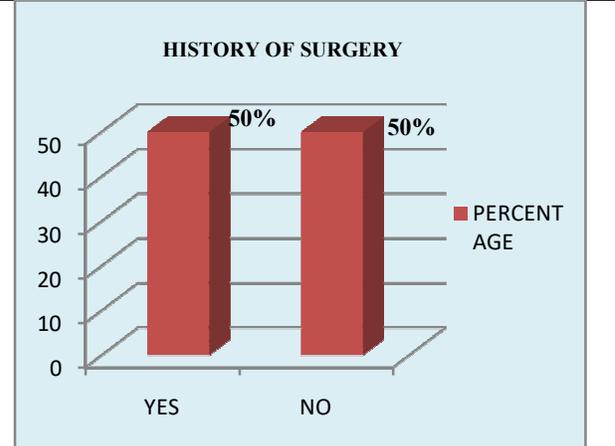


Table : 8 History of Surgery

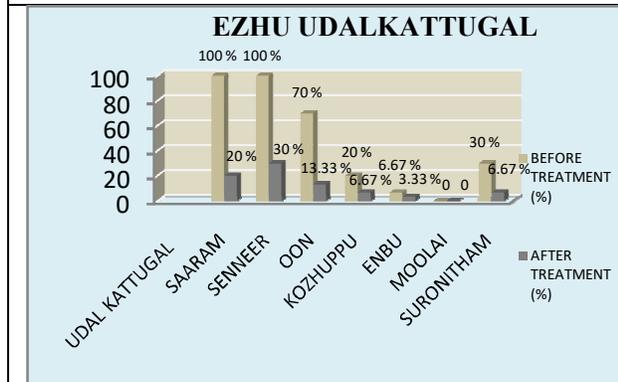


Table :10(A)Ennvagai Thervugal

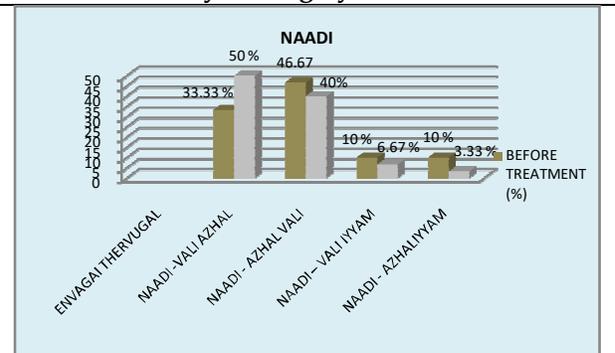


Table 10 (b) Naadi





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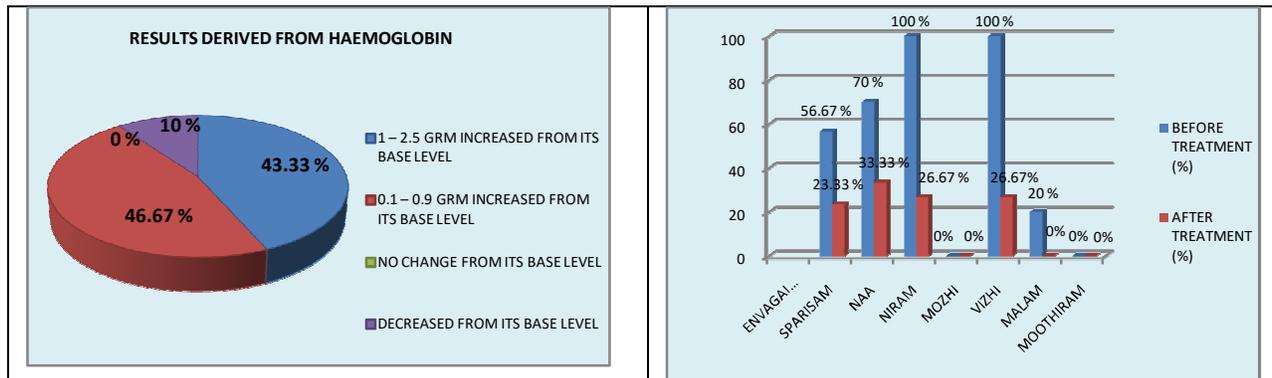


Table : 10 Results Derived From Haemoglobin

Table : 11

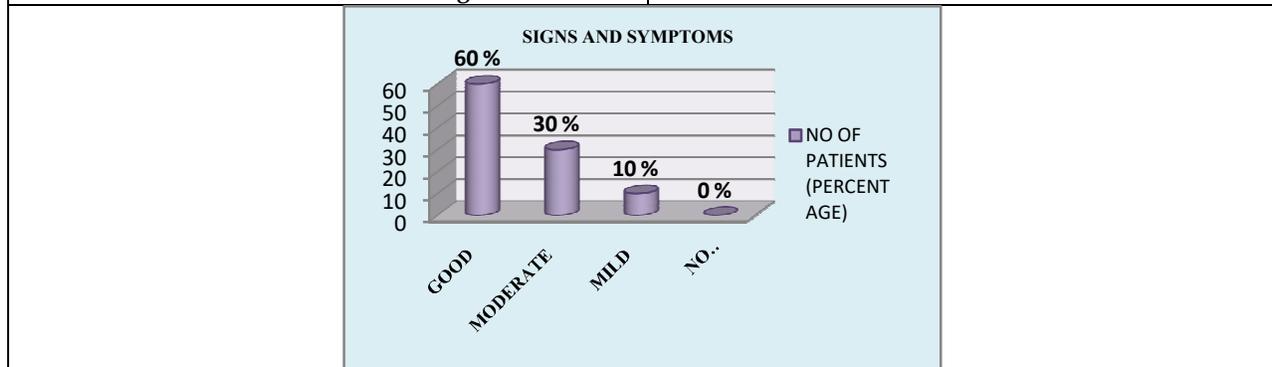


Table : 12 Signs and Symptoms

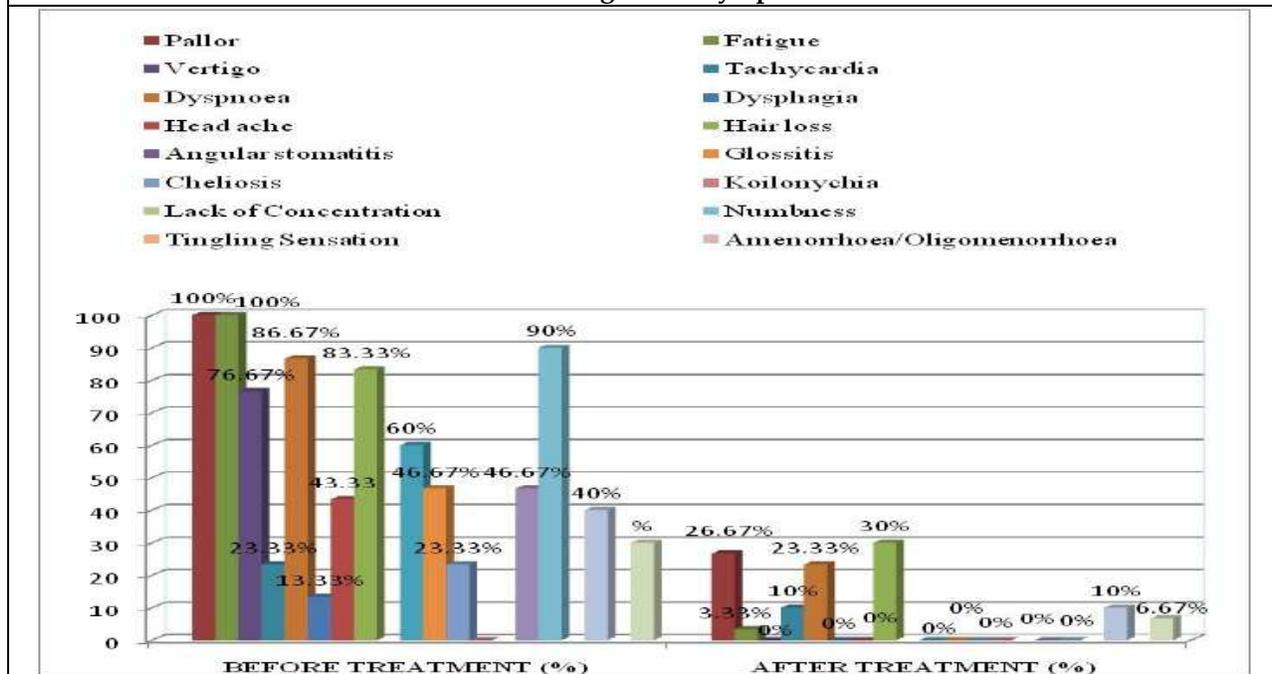


Table :13 Clinical Features





Anticancer Potential of *Acalypha indica* Linn. - A Review

Supriya Reddy.G^{1*} and Kavitha N Chilaka²

¹Research Scholar, Department of Pharmacology, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh -530045, India.

²Assistant Professor, GITAM Institute of Pharmacy, GITAM (Deemed to be University), Rushikonda, Visakhapatnam, Andhra Pradesh -530045, India.

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*Address for Correspondence

Supriya Reddy.G

Research Scholar,

Department of Pharmacology,

GITAM Institute of Pharmacy,

GITAM (Deemed to be University),

Rushikonda, Visakhapatnam,

Andhra Pradesh -530045, India.

E.Mail: 121965201507@gitam.in



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ABSTRACT

With more people looking for health and wellness practices free from side effects brought on by artificial chemicals, medicinal plants are transitioning from fringe to mainstream use. Over 3500 plants are officially recognized in India for their therapeutic use. Over 6000 plants are reportedly used in India for traditional, alternative, and natural medicines. Since ancient times, people have employed plants and their mixtures as medicines. There is evidence that plants can be used to treat a variety of illnesses in the Ayurveda and other ancient Indian literatures. The annual herb *Acalypha indica* L is upright and covered in silky hairs on its many long branches. The Siddha claims that *Acalypha* treats disorders of the teeth and gums, burns, toxins of plant and mixed origin, stomach ache, Pitha-related illnesses, bleeding piles, irritations, stabbing pain, wheezing, sinusitis, and neutralizes the Kabha factor's predominance. To treat a variety of illnesses and problems, whole-plant medicines have been employed in traditional medicine. Therefore, the current review aims to provide a thorough overview of the literature on its pharmacological, traditional, and pharmacological uses. It has been reported that this herb, which is widely used in traditional Indian medicine and many other countries, has anti-cancer properties.

Keywords: *Acalypha indica* linn, anticancer, Pharmacognosy, Phytochemistry





Supriya Reddy and Kavitha N Chilaka

INTRODUCTION

In 2018, there were 9.6 million cancer-related deaths and 18.1 million new cases. Cancer has 36 different forms, with men and women being the primary populations affected by colorectal, liver, lung, stomach, and prostate cancers, respectively [1]. The study of cancer treatment has expanded significantly. Both traditional and extremely contemporary methods are used to treat cancer. Chemotherapy, radiation therapy, or surgery are just a few of the methods utilized to treat cancer. All of them, however, have some drawbacks [2]. The use of conventional chemicals is accompanied by toxicities and adverse effects [3]. But because conventional chemotherapeutic techniques have failed, new strategies are required for the control of diseases as the issue endures. Therefore, in order to reduce the number of deaths caused by cancer, innovative cancer preventive and treatment methods are required. A very safe, non-toxic, and widely accessible source of cancer-fighting chemicals is now herbal medicine. Because of a variety of qualities they exhibit, herbs are thought to neutralize the effects of diseases in the body [4].

The usage of herbs was long neglected due to the development of the industrial sector and industrial medicine [11]. The development of new procedures has decreased obstacles relating to natural chemicals, and interest has grown in using such natural constituents in the pharmaceutical business [12, 13]. According to the World Health Organization, traditional therapy approaches are used in 80% of countries worldwide [14]. With the aid of contemporary biomolecular science, which recognizes several significant properties, such as anticancer, anti-inflammatory, and anti-virus, we can better understand the impacts or actions of herbs on diverse targets. As the effects of such herbal medicine are becoming better understood, their effectiveness in treating various malignancies has also been established. For instance, hepatocellular carcinomas (HCC) are the fifth most prevalent cancer in the world, and their frequency is rising [15, 16]. It has been demonstrated in numerous research on the treatment and prevention of HCC with herbal medicine that all phases of HCC, including start, development, and progression, may be impacted by herbal constituents [17, 18].

However, there is a misconception that there are no safety or side effect concerns with herbal components when they are used as pharmaceuticals. There are numerous plant species that are harmful to human health. Similar to this, some substances found in otherwise beneficial plants can be harmful to cells. It has been demonstrated through testing that even anticancer herbs have lethal effects [19]. In the United States of America, the "dietary supplement health and education act" regulates herbs as dietary supplements. This study emphasizes the mechanisms of certain very significant anticancer plants, the research into those mechanisms, the components that make up those active ingredients, and the laws that are in place for those plants.

Development of cancer

Cancer is an uncontrolled cell development that can be fatal in our body. It causes an imbalance in the body and damages healthy cells. It is among the most serious health issues in both industrialized and developing nations. It is quite challenging to identify the precise an etiology of cancer. Tobacco usage, alcohol use, environmental pollution, infectious agents, cultural practices, and way of life are some of the more well-known causes of this illness, though. Tobacco, obesity, radiation, infection, genetics, stress, environmental contaminants, and a lack of physical exercise (lifestyle, economic, and behavioral variables) are environmental factors that contribute to cancer fatalities. Lung cancer is 90% a result of smoking [20]. Larynx, bladder, kidney, stomach, pancreatic, and other cancers are also caused by it [21]. About one in five cancer deaths worldwide are caused by tobacco [21]. 30–35% of cancer-related fatalities are attributed to physical inactivity, obesity, and diet [22]. The chance of developing cancer is thought to increase with physical inactivity. Over nutrition is responsible for more than half of the effects of diet. Aflatoxin B1 causes liver cancer, betel nut chewing causes mouth cancer, and some specific foods are linked to certain types of malignancies [23]. For example, a high-salt diet can cause stomach cancer. Up to 10% of people who are exposed to both ionizing and non-ionizing UV radiation get cancer. Ionizing radiation comes from radon gas and medical imaging; however it isn't a particularly potent mutagen. As with radon and tobacco smoke, radiation becomes more effective when combined with other cancer-causing substances [24]. Skin cancer is caused by long-term exposure to



**Supriya Reddy and Kavitha N Chilaka**

UV rays from sunlight. Less than 0.3% of the population possesses genetic mutations that cause cancer, therefore hereditary causes of cancer can occasionally be present. Hereditary non-polyposis colorectal cancer and inherited mutations in the BRCA1 and BRCA2 genes are two examples of hereditary cancers. By encouraging cell proliferation, several hormones can have a significant impact on the growth of cancer [25].

The concept of "staging" is crucial in assessing the severity of cancer. Patients may receive medication recommendations that are appropriate for their stage of cancer. Cancer has four phases, each with unique characteristics and symptoms.

These are listed as follows:

STAGE 1: The early stages of cancer which show no symptoms externally and the tumour are still undergoing development. A routine physical examination can detect cancer in its early stages. If cancer was found at this stage, it would be easier to treat.

STAGE 2: Scans in this example show the tumour to be clearly evident. There are a couple of obvious indications.

STAGE 3: The benign tumour has fully developed and is showing symptoms.

STAGE 4: This is the final stage of cancer, and there is no chance of recovery. The tumour has metastasized, or spread, to distant sections of the body. Skin cancer patches and cachexia's obvious indications of abrupt, considerable weight loss are both present.

Preventive methods for cancer

Changes in diet, quitting tobacco use, managing inflammatory illnesses efficiently, and taking nutritional supplements that support immune functioning are all significant preventive measures for the majority of malignancies. The malignancy is currently being treated with radiotherapy, chemotherapy, and medications made of chemicals. Anti-metabolites, alkylating agents, platinum analogues, and anti-tumor antibiotics are some of the most frequently utilized cancer chemotherapy drugs. Chemotherapy and radiotherapy, however, put patients under a lot of stress and further harm their health. Thus, research is currently being done to find new anticancer drugs in nature, particularly plants. Plants have always been the foundation of ancient medical practices, and for thousands of years, they have continuously given mankind medicines. Based on the results of thousands of years of use, plants have the potential to be therapeutic. The clay tablets contain the first documented descriptions of hundreds of medicinal plants, including opium and myrrh [26]. For millennia, people have utilized herbal medicines to cure a variety of illnesses. Plant extracts are used in herbal therapy to cure illness and advance the patient's health. The goal of herbal medicine is to reestablish the body's capacity for defense, control, and healing. The medicinal plants are used to make a number of contemporary medications. It is possible to consume herbal goods as powder, paste, tablets, liquid, or raw material (extract). When produced improperly, consumed excessively, or used for the wrong purposes, this can be toxic [27]. To avoid negative effects, it is crucial to investigate the efficacy of herbal medicines. Thus, methods for identifying possible applications of plant extracts for the treatment of cancer have been created through research. The many types of cancer have already been treated with a variety of herbs. In addition to containing a variety of bioactive substances, medicinal plants also exhibit a wide spectrum of biological activity, including anti-tumor, anti-viral, anti-inflammatory, and anti-malarial action. For the creation of numerous medications, knowledge of medicinal plants has been crucial. Anticancer properties of several medicinal plants are used to find a lead compound that can block the development of cancer. Medicinal plant has various secondary metabolites such as terpenoids, flavanoids, alkaloid and steroids that have different pharmacological properties [28]. Medicinal plants such as *Acalypha indica* Linn, *Allium sativum*, *Annona muricata*, *Berberies aristata*, *Catharanthus roseus*, *Linum usitatissimum*, *Podophyllum hexeandrum*, *Rubia cordifolia*, *Withania somnifera*, etc., show potential role in the inhibition of cancer cell proliferation. Therefore this chapter provides an overview of *Acalypha indica* Linn medicinal plant and their major bioactive compounds utilized for treatment of cancer.



**Supriya Reddy and Kavitha N Chilaka*****Acalypha indica* Linn****PLANT PROFILE**

Scientific name: *Acalypha indica*

Acalypha indica Linn. is a little annual shrub that commonly grows as a troublesome plant on Indian plains, along roadsides, and in gardens. It is established in tropical Africa, Asia, and Polynesia after then. Tannins, the pyranoquinolinone alkaloid flindersin, kaempferol glycosides, mauritianin, clitorin, nicotiflorin, biorobin, the cyanogenicglucosideacalphyin, the acalphyamides, the aurantiamide, and succinimide were some of the chemical components isolated from *A. indica* [29].

The World Health Organization describes most malignancies as a method of unchecked mobile growth and spread that might appear almost wherever inside the frame [30]. Nine, 958,133 deaths from this disease were caused by the 19,292,789 cases of most cancers reported worldwide in 2020 [31]. Other studies have examined the *Acalypha* genus' anticancer hobby, namely *A. indica*'s methanolic (ME), hexane (HE), and chloroform extracts. The Vero (non-tumor kidney) cells were no longer cytotoxic to these extracts, while the NCIH187 (lung carcinoma) cells showed anticancer activity. Similar to how L-quebrachitol was extracted from the extract, it was then characterized using NMR [32].

A slight cytotoxic impact of *A. fruticosa* AE turned into tested, which inhibited the proliferation of MDA-MB-435S (cancer mobileular line) and Hep3B (hepatocellular carcinoma) cells similarly to the safety of DNA towards oxidative harm precipitated with H₂O₂ [33]. wilkesiana well-known shows an anti-proliferative impact on U87MG (probable glioblastoma cells), A549 (lung carcinoma cells), and MCR5 (non-tumor lung) cells with their ethyl acetate extract, and a morphological take a look at showed apoptosis and DNA harm [6]. The increase inhibition impact on MDA-MB-468 and MCR5 cells have been evaluated the use of the EE of wilkesiana [34]. The National Cancer Institute reports 232,340 cases of breast cancer in females. A 69-year-old Caucasian woman with breast cancer was diagnosed with MCF-7 in the year 1970 [35]. The name MCF-7 refers to the Michigan Cancer Foundation-7, a facility in Detroit where Herbert Soule and a colleague installed the mobile phone line in 1973.

Acalypha indica is certainly considered one among weed plant life that includes vital medicinal values for human fitness applications. It may be determined normally in India, Sri Lanka Thailand and Pakistan. The extracts of diverse elements of the plant, leaves, roots and stem elements are used for medicinal functions to deal with diverse sicknesses including the attention infections, breathing troubles, rheumatism, and pores and skin troubles and to lower blood sugar level. Different extraction strategies are used for acquiring lively additives from *Acalypha indicia* generally; Soxhlet extraction has a excessive performance and accuracy however the thermal pressure would possibly degrade goal photochemical additives. Herbal drugs were gambling a crucial position in remedy and therapy for diverse sicknesses and physiological situations in conventional strategies practiced including Ayurveda, Unani and Siddha. This form of remedy, additionally referred to as traditional remedy, turned into the principle supply of scientific remedy for the duration of this time [36]. However, civilization has modified and with it has come the advent of extra superior strategies and strategies, main the subsequent generations to generally tend to pick out contemporary-day remedy over traditional remedies.

The facts associated to traditional remedies are regularly vanishing for the reason that preceding generations have become older and demise without successors. This understanding is handed directly to the subsequent generation, via experiments and statement and oral teaching [37]. Therefore, it's far essential to have right documentation from the extant practitioners considering traditional remedies are an opportunity course to treating diverse forms of human sicknesses [38]. Traditional or traditional medicinal practices primarily based totally on herbal plant life were identified with the aid of using the World Health Organization (WHO, 2002) as dependable medicinal reassets for healing sports. The medicinal plant life are to be had round backyards, settlements, spreading alongside roadsides, and residence compounds Medicinal plant life are identified as cappotential supply of bioactive compounds. More than 80%of contemporary-day tablets are derived without delay from reasserts of plant life and microbes. Natural merchandise derived from medicinal plant life has huge variety of pharmacological significance.



**Supriya Reddy and Kavitha N Chilaka****Botanical Description of *Acalypha indica***

Older generations in many countries, particularly in Africa and Asia, are well-known for using the traditional medicinal plant *Acalypha indica*. It thrives in the majority of the western and southern sections of the African northeast, as well as in Somalia, Ethiopia, and other places. The plant can also be found in the wettest tropical and temperate regions of Asia, Europe, and both North and South America. It spreads like a weed in backyards, shrubs, along roads, and other places, including household and agricultural properties [39]. Since this plant has a close connection to Ayurveda, Sidda, and Unani medical practices carried out with the aid of elder Indian generations, many *Acalypha indica* manuscripts have been published globally and come from Indian locations. [40].

Taxonomy

The leaves have sub obtuse crenate-serrate and base cuneate and glabrous thin. Their petiolate is normally longer than the slender, blade, and stipulate minute [41]. The leaves of the *Acalypha indica* are easy and organized spirally; 0.02, 12.00 cm petiole lengthy; blade widely ovate to ovate-lanceolate; 2 – nine cm × 1 – five cm; base cuneate; apex acute; margins toothed; membranous; sparingly quick hairs to nearly glabrous is nature on each surfaces; extra furry alongside the midrib; five-veined at base and with four to five pairs of lateral veins. One month after germination, the stem begins off evolved to show woody because it matures. The stem of the *Acalypha indica* is sparing to densely furry. The branches are numerous, ascending, lengthy and finely pubescent.

Distribution

Acalypha indica grows evidently in wet, temperate, and tropical regions alongside the equator cross-continental of Asian, Africa, Europe, Australia and South and North America. The Indian humans have the documented statistics of plant usage for his or her conventional drugs in addition to traditional drugs [42]. Many Australians identified this plant of their location however is much less willing to devour it [43]. *Acalypha indica* additionally may be determined with inside the Arabia Gulf location primarily based totally at the document that they ate up this plant as a food [44]. *Acalypha indica* is likewise a not unusual place weed determined in West Africa and south Nigeria [45].

Ethnomedicinal Practices

Most of the practices come from humans within side the Asian and African regions. Some humans in India are normal purchasers of this plant leave considering its far part of the Ayurveda exercise. Meanwhile, different international locations use this plant as a part of their remedy however utilization is minimal.

Phytochemical Study

Phytochemical and Nutrient Constituent of *Acalypha indica* the sparkling *Acalypha indica* plant has a huge form of vitamins including carbohydrates, proteins, vitamins, and lipids. They determined to put together its documentation with unique statement of important and non-important heavy metals content material as part of the natural standardization preparation. *Acalypha indica* has excessive iron content material, accompanied with the aid of using copper, nickel zinc, and chromium which can be beneficial for sufferers with mineral deficiencies troubles.

Chemical constituents

The arial portions include flavonoids such the kaempferol glycosides mauritianin, clitorin, nicotiflorin, and biorobin as well as the cyanogenic glycoside acalyphin (a 3-cyanopyridone derivative). Additionally identified substances include tannins, -sitosterol, acalyphamide, aurantiamide, succinimide, and flindersin (a pyranoquinolinone alkaloid). The iridoid substances isodihydronepetalactone and isoiridomyrmecin are those that draw cats.

Anti- cancer activity

Acalypha indica plant extract additionally has the capacity to grow to be an anticancer plant as suggested with the aid of using [41]. Three forms of most cancers mobileular strains were examined with *Acalypha indica* leaf extracts along with KB-Oral hollow space most cancers, MCF7-breast most cancers and PC3 human prostate mobileular most cancers. The anti-most cancers pastime turned into decided via MTT assay method. The ethanolic extract of *Acalypha indica* inhibited MCF7-breast most cancers with an inhibition attention (IC 50) cost of 35µg/ml. Two requirements



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were used within side the assay for assessment which turned into doxorubicin and ellipticine. The IC₅₀ cost for doxorubicin and ellipticine have been 8.8µg/ml and 0.5µg/ml, respectively. The MCF7- breast most cancers and the KB-Oral hollow space most cancers have been taken into consideration nonreactive with the methanolic extract for the reason that inhibition attention handed 50µg/ml [42]. In addition, a current take a look at has discovered that the quebrachitol ought to take part in numerous vital mechanisms as cappotential anti-most cancers tablets both thru arrest or opposite pathways.

CONCLUSION

The review conducted on *Acalypha indica* indicate that the plant has many potential compounds useful for treatment of wounds, malaria inflammation specially cancer. It also exhibit antioxidant, antimicrobial, anti-inflammatory, wound healing and anti-diabetic activity. Various phytochemicals isolated from the plant have been investigated in laboratory animals for their therapeutic benefits. Many pharmacological studies prove to establish *in vitro* anticancer studies of the plant *Acalypha indica*. Further research will enhance the analysis of therapeutic efficacy of the plant. Efforts should be made to study various products of plants for their therapeutic benefit. The gift assessment suggests the pharmacological take a look at of the *Acalypha indica* and diverse phytochemical compounds answerable for it that have been suggested. The complete plant of *Acalypha indica* were utilized in traditional medicinal drug and conventional medicinal drug for many years and the research finished but has authenticated the scientific practices.

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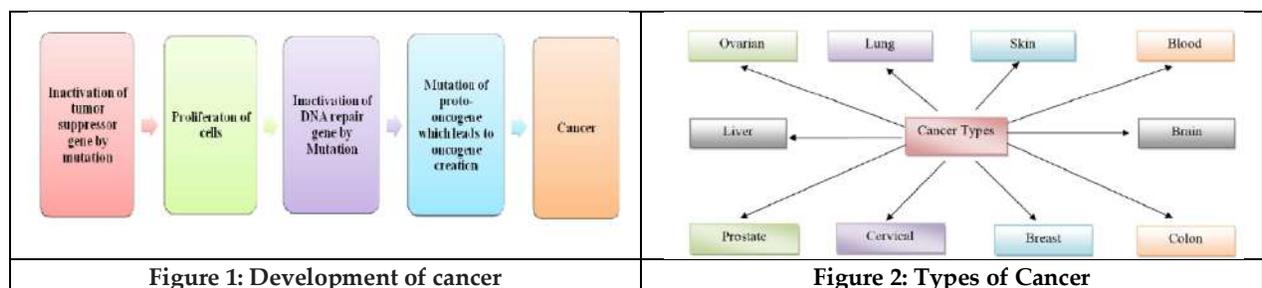


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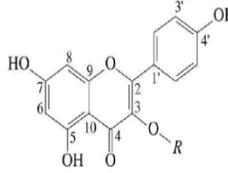
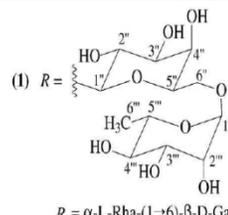
Table 1: Scientific classification

| | |
|----------|------------------|
| Kingdom | Plantae |
| Clade | Tracheophytes |
| Clade | Angiosperms |
| Clade | Eudicots |
| Clade | Rosids |
| Order | Malpighiales |
| Family | Euphorbiaceae |
| Subtribe | Acalyphinae |
| Genus | <i>Acalypha</i> |
| Species | <i>A. indica</i> |





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| | |
|---|---|
|  | <div style="display: flex; justify-content: space-around;"> <div data-bbox="876 289 1104 462">  </div> <div data-bbox="1136 289 1364 504">  <p>(1) $R = \alpha\text{-L-Rha-(1\rightarrow6)-}\beta\text{-D-Gal}$</p> </div> </div> <div style="margin-top: 10px;"> <p>(2) $R = \alpha\text{-L-Rha-(1\rightarrow6)-}\beta\text{-D-Glc}$ (3) $R = \alpha\text{-L-Rha-(1\rightarrow2)-}\alpha\text{-L-Rha-(1\rightarrow6)-}\beta\text{-D-Glc}$ (4) $R = \alpha\text{-L-Rha-(1\rightarrow2)-}\alpha\text{-L-Rha-(1\rightarrow6)-}\beta\text{-D-Gal}$</p> </div> |
| <p>Figure 3: <i>Acalypha indica</i></p> | <p>Figure 4: Isolated Compounds [Biorobin (1), Kaempferol Derivatives Nicotiflorin (2), Clitorin (3), Mauritianin (4)]</p> |





Buying Behaviour and Purchasing Habit of Consumer towards Branded and Unbranded Milk in Chidambaram Town Cuddalore District of Tamil Nadu

R. Selvakumar^{1*}, R. Azhagesan², M. Vignesh² and T. Lingavarayan²

¹Assistant Professor, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University, Annamalai Nagar- 608002, Tamil Nadu, India

²Research Scholar, Department of Agricultural Economics, Faculty of Agriculture, Annamalai University, Annamalai Nagar- 608002, Tamil Nadu, India

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*Address for Correspondence

R. Selvakumar,

Assistant Professor,

Department of Agricultural economics,

Faculty of Agriculture, Annamalai University,

Annamalai Nagar,

Tamil Nadu, India – 608002

E. Mail: selvakumaragri83@gmail.com



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ABSTRACT

Mammary glands in mammals produce milk, a transparent, nutrient-rich liquid meal. Infant mammals (including people who are breastfed) rely on it as their main source of nutrition until they can digest other types of food. The main animal industry product, milk, has long been acknowledged as a complete natural food. It promotes development and health maintenance. In terms of global milk production, India comes in ranked 1, followed by Germany, China, and the United States of America. India has led the world in milk production for the previous 15 years. Production is crucial since the majority of India's economy is based on agricultural and animal husbandry. India continues to be the largest milk producer in the world. Because of the several measures the government has made to promote animal output, milk production has significantly grown. 198.44 million tonnes and 209.96 million tonnes, respectively, will be produced in 2019–20 and 2020–21 of milk will be produced, representing yearly growth of 5.81%. Around 427 grams of milk are available per person daily in 2020–21. In the year of 2019–20, the total milk production of Tamil Nadu was around 88 lakh tonnes. Based on this data the Salem district in the 1st position of milk production, followed by Villupuram, Thiruvannamalai district respectively. The objective of the research is to understand the milk purchasing habit of the different consumers in Chidambaram town and to find the consumer preference for purchasing branded and unbranded milk. According to the study's findings, about 77% of the respondents were swayed by neighbors' recommendations for unbranded milk. However, in the case of branded milk, advertisements had the

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greatest impact on responses and it is around 85 per cent. The forms of milk products used around 75 per cent of consumer had preferred to the branded panner, followed by 64 per cent of consumer were butter, 54 per cent had preferred ghee. In case of unbranded milk 67 per cent of consumers had preferred to the curd, followed by 46 per cent of consumers were ghee, and 36 per cent had preferred for butter. Around 42 per cent of consumers preferring branded milk were found to have monthly expenses more than 3 thousand rupees, followed by 1000-2000 monthly expenses (26 per cent). In case of unbranded milk consumers 50 percent fall below 2 thousand rupees monthly expenses category, followed by 30 per cent in 2-3 thousand rupees monthly expenses.

Keywords: Milk, Milk Production, Purchasing Habit, Consumer Preference, Branded and Unbranded Milk, Likert Scaling Technique.

INTRODUCTION

Mammary glands in animals produce milk, a transparent, nutrient-rich liquid meal. It is the primary source of nutrition for young animals, including breastfeeding humans, before they can digest other forms of food. Milk, the primary output of the livestock business, has long been recognized as a complete natural food. It encourages growth and the preservation of health. India leads the world in terms of milk production, followed by the United States of America, China, and Germany. India has led the world in milk production for the previous 15 years. Production is crucial since the majority of India's economy is based on agricultural and animal husbandry. India surpassed the United States as the world's top milk producer in 2010 with an output of 121.8 million tonnes (Hemmeet *et al.*, 2003). India produced around 165.4 million tonnes of milk in 2016–17. In 2019–20, the country produced 198.4 million tons of milk, an increase of 5.7% from 187.7 million tons in 2018–19. According to the National Dairy Development Board, there are now 374 grams of milk available per person each day. India is the world's largest provider of skimmed milk powder while exporting few other milk products. India may soon start importing more dairy products than it exports because of expanding domestic demand and a huge supply-demand disparity.

Global Scenario

The top four countries that export milk and milk products worldwide are Brazil, India, China, and the United States. Prior to becoming self-sufficient in 2016, the two major milk and milk product importers worldwide, China and Russia, contributed to the global milk surplus. Worldwide, more than six billion people consume milk and dairy products. Dairy farming households number around 750 million people. By 2016, there was an excess of milk globally due to ongoing advancements in the efficiency of milk production. Russia and China ceased importing milk and became self-sufficient. Theoretically, in 2015, the European Union stopped providing subsidies to the dairy industry. Since "environmental incentives" have taken the place of direct subsidies, the government now purchases milk when the cost drops to Rs. 15677 per 1,000 litters. Under a voluntary insurance policy in the United States, producers get compensated according to the price of milk and the cost of feed. India's milk production increased by 20.38 percent, from 467.2 million tonnes in 2014–17 to 562.4 million tonnes in 2017–20.

Indian Scenario

India continues to be the largest milk producer in the world. Because of the several measures the government has made to promote animal output, milk production has significantly grown. 198.44 million tonnes and 209.96 million tonnes of milk will be produced in 2019–20 and 2020–21, respectively, indicating annual increase of 5.81%. Each person will have access to 427 grams of milk per day in 2020–21. Milk is processed and marketed by 170 Milk Producers' Cooperative Unions, which are federated into 15 State Cooperative Milk Marketing Federations. Numerous brands have been created throughout the years by cooperatives such as Amul (Gujarat), Vijaya (AP),



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Verka (Punjab), Saras (Rajasthan), Nandini (Karnataka), Milma (Kerala), and Gokul (Kolhapur). 10% of the milk eaten in India, the second-largest producer of milk in the world, is produced in Punjab. Punjab has the highest national average for milk availability per person. Typically, milk is sold in bottles, unpackaged, or in polythene sachets. When selling loose milk or milk derivatives like ghee, butter, paneer, etc., there is a possibility of contamination and the problem of adulteration. These problems can be handled by offering branded milk and milk-related items. However, the cooperative and private milk facilities in Punjab only sell 14% of the milk that is produced there. With a daily processing capacity of 57 lakh liters, of which about 59% is utilised, the state has 62 milk processing facilities in the private and cooperative sectors (Taneja, 2007).

Due to differences in factors like income levels, family size and composition, educational attainment and resulting nutrient awareness, availability of substitutes and prices, consumer tastes and preferences, and many other factors, consumption of milk and milk products varies across the various socioeconomic groups in urban areas. The consumer's choice to buy or pass on a product represents the marketing professional's ultimate test. More important than just creating a quality product, should be given consideration are consumer preferences in terms of pricing, ease of availability, etc. Species-to-species variations in milk content are significant. There are a number of variables that can change, including the kind of protein, the ratio of protein to fat and sugar, the concentration of different vitamins and minerals, the size of the butterfat globules, and the firmness of the curd. For instance: On average, 100 grams of human milk contain 72 kcal of calories are included in 1.1% protein, 4.2% fat, and 7.0% lactose. Cow's milk typically contains 3.4% protein, 3.6% fat, 4.6% lactose, 0.7% minerals, and 66 kcal per 100 grams. While donkey and horse milk have the lowest quantities of fat per unit of nutrients, seal and whale milk may have more than 50% fat. In the year of 2019-20, the total milk production of Tamil Nadu was around 88 lakh tonnes. Based on this data the Salem district in the 1st position of milk production, followed by Villupuram, Thiruvannamalai district respectively.

Problem Focus

One of the healthiest foods, milk is typically consumed by people of all ages, regardless of their socioeconomic status. About 35% of the milk produced in India is processed (of which 13% is accounted for by the organized dairy industry), with the remaining milk either being consumed on farms or being marketed as fresh, unpasteurized milk through unorganized channels. Due to its affordability and freshness, unbranded milk is become more preferred by consumers. In light of this context, the current investigation was conducted.

Objectives

1. To understand the Milk purchasing habit of the different consumers in Chidambaram Town.
2. To find the consumer preference for purchasing Branded and Unbranded Milk.

DATA AND METHODOLOGY

Sample Selection

The study was taken up in Chidambaram municipality of Cuddalore district. It is purposively selected for the study. There are 111 unbranded milk shops in Chidambaram town. Out of it 60 no. of shops were selected. Chidambaram municipal area comprises of 1,415 households. The list of households was prepared using the records maintained by municipality office. A sample of 120 households were selected randomly from this list and contacted directly to collect necessary information.

Data Collection

With the aid of a thorough and previously tested interview schedule, the primary data were obtained via personal interviews from 60 milk consumers who purchased either branded or unbranded milk. Secondary information was gathered from a variety of published sources. The consumer's interview covered topics such basic consumer traits,





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preferences for unbranded milk, issues consumers had while buying unbranded milk, and variables that might have influenced their decision to buy unbranded milk. To get relevant findings, the collected data were collated and subjected to statistical analysis.

Tools of Analysis

To achieve the goals, the various parts of the study were analyzed using the following analytical techniques.

Percentage Analysis

To make straightforward comparisons, percentages were calculated to analyze sample characteristics like age, education, family size, etc.

$$\text{Percentage} = \frac{\text{Number of respondents}}{\text{Total sample size}} \times 100$$

Likert Scaling Technique

On a five-point scale, the sample respondents were asked to rate their preference for unbranded milk with respect to the various features as excellent, very good, good, bad, or very bad. To calculate the mean score toward the sample's degree of satisfaction, the replies were recorded and the scores were added. The scores for each factor are listed as shown in Table 1 below. Thus, the likert scaling algorithm gives each of the five replies a scale value. Every statement in the instrument receives the same treatment. The instrument generates a total score for each responder as a result, which would indicate how favorable the respondent is to the stated point of view. The score values would be revealing if the instrument had 30 statements;

30 × 5 = 150 most favourable response possible

30 × 3 = 90 a neutral attitude

30 × 1 = 30 most unfavourable attitude

Any individual's score would range from 30 to 150. A score of 90 or higher indicates a favorable impression from the customer, while a score of 90 or less indicates an unfavorable opinion and 90 or exactly 90 indicates a neutral attitude.

RESULT AND DISCUSSION

Milk Purchasing Habit among Branded and Unbranded Milk and milk product Consumers in the Study Area Influencing factors to Buy Branded and Unbranded Milk

The views of respondents on what causes them to purchase branded and unbranded milk were gathered, and the results are shown in Table 2 and Fig 1. As can be seen from Table 2, 77% of respondents who consumed unbranded milk were influenced by neighbors who gave their word-of-mouth recommendations for the product. However, in the case of branded milk, commercials had the greatest impact on responders, accounting for almost 85% of them.

Forms of Milk Products

The details on type of products purchased were collected from respondents and results are given in Table - 3 and Fig - 2. It could be seen from the Table - 3 that 75 per cent of consumer had preferred to the branded panner, followed by 64 per cent of consumer were butter, 54 per cent had preferred ghee. In case of unbranded milk 67 per cent of consumers had preferred to the curd, followed by 46 per cent of consumers were ghee, and 36 per cent had preferred for butter.

Monthly Expenses

The details for monthly expenses for the products were collected from the respondents for branded and unbranded milk and results are furnished in Table - 4 and Fig - 3. It could be seen from the Table - 4, that 42 per cent of consumers preferring branded milk were found to have monthly expenses more than 3 thousand rupees, followed





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by 1000-2000 monthly expenses (26 per cent) In case of unbranded milk consumers 50 percent fall below 2 thousand rupees monthly expenses category, followed by 30 per cent in 2-3 thousand rupees monthly expenses.

CONCLUSION

According to the study's findings, about 77% of the respondents were swayed by neighbors' recommendations for unbranded milk. However, in the case of branded milk, commercials had the greatest impact on responders, accounting for almost 85% of them. The forms of milk products used around 75 per cent of consumer had preferred to the brandedpanner, followed by 64 per cent of consumer were butter, 54 per cent had preferred ghee. In case of unbranded milk 67 per cent of consumers had preferred to the curd, followed by 46 per cent of consumers were ghee, and 36 per cent had preferred for butter. Around 42 per cent of consumers preferring branded milk were found to have monthly expenses more than 3 thousand rupees, followed by 1000-2000 monthly expenses (26 per cent) In case of unbranded milk consumers 50 percent fall below 2 thousand rupees monthly expenses category, followed by 30 per cent in 2-3 thousand rupees monthly expenses.

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Table 1: Likert Five Point Scale used for the Satisfaction Level

| Particulars | Excellent | Very good | Good | Bad | Very bad |
|-------------|-----------|-----------|------|-----|----------|
| Scale | 5 | 4 | 3 | 2 | 1 |

Table 2: Factors Influencing to Buy Branded and Unbranded Milk(n=120)

| S. No. | Influencer | Branded Milk | | Unbranded Milk | |
|--------|------------|-----------------------|----------------|-----------------------|----------------|
| | | Number of respondents | Percentage (%) | Number of respondents | Percentage (%) |





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| | | | | | |
|----|-------------------|----|-----|----|-----|
| 1. | Neighbours | 5 | 8 | 46 | 77 |
| 2. | Friends/relatives | 4 | 7 | 10 | 17 |
| 3. | Advertisement | 51 | 85 | 4 | 6 |
| | Total | 60 | 100 | 60 | 100 |

Table 3: Forms of Milk Products Used (n= 120)

| S. No. | Products | Branded Milk | | Unbranded Milk | | Total |
|--------|----------|-----------------------|----------------|-----------------------|----------------|----------|
| | | Number of respondents | Percentage (%) | Number of respondents | Percentage (%) | |
| 1. | Butter | 77 | 64 | 43 | 36 | 120(100) |
| 2. | Curd | 40 | 33 | 80 | 67 | 120(100) |
| 3. | Paneer | 105 | 88 | 15 | 12 | 120(100) |
| 4. | Ghee | 65 | 54 | 55 | 46 | 120(100) |

Table 4: Monthly Expenses(n=120)

| Si.No. | Monthly expenses | Branded milk | | Unbranded milk | |
|--------|------------------|-----------------------|----------------|-----------------------|----------------|
| | | Number of respondents | Percentage (%) | Number of respondents | Percentage (%) |
| 1 | >1000 | 10 | 16 | 2 | 3 |
| 2 | 1000-2000 | 15 | 26 | 30 | 50 |
| 3 | 2000-3000 | 10 | 16 | 18 | 30 |
| 4 | <3000 | 25 | 42 | 10 | 17 |
| | Total | 60 | 100 | 60 | 100 |

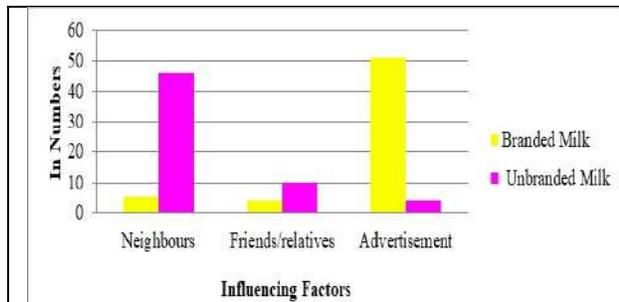


Fig 1: Factors Influencing to Buy Branded and Unbranded Milk

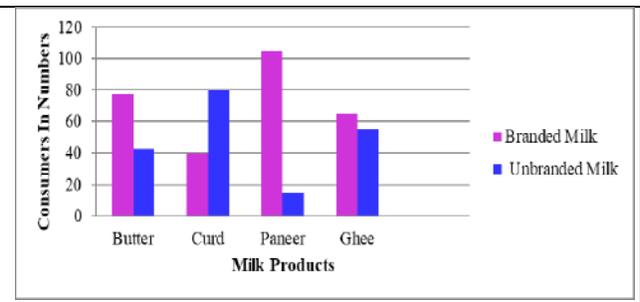


Fig 2: Forms of Milk Products Used

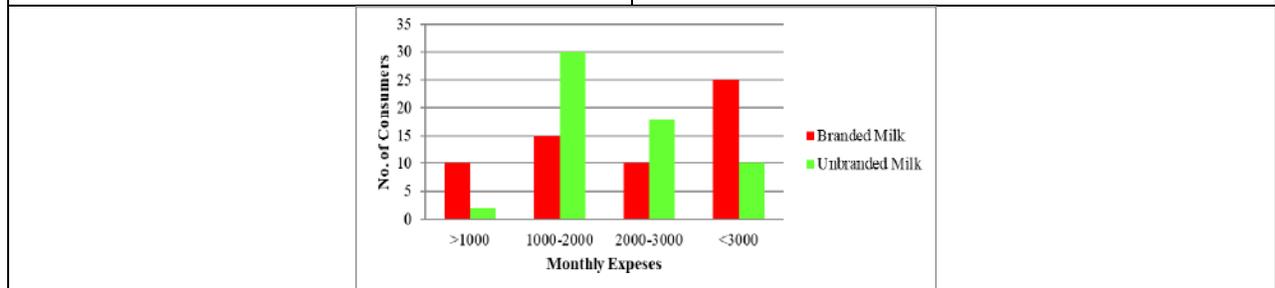


Fig 3: Monthly Expenses





Scientometric Analysis of Cyber Security Research and its Outcomes at the Global Level

Jobin Sebastian^{1*}, Prakash Abraham² and P. Sakthivel³

¹Ph.D Scholar, Department of Political Science and Public Administration, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

²College Librarian, Mary Matha Arts and Science College, Mananthavady, Kerala, India.

³Professor, Department of Political Science and Public Administration, Annamalai University, Annamalai Nagar-608002, Tamil Nadu, India

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*Address for Correspondence

Jobin Sebastian

Ph.D Scholar,

Department of Political Science and Public Administration,

Annamalai University,

Annamalai Nagar-608002, Tamil Nadu, India

E. Mail: frkuriakosev@gmail.com



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ABSTRACT

A life without cyberspace is out of the question at present. It becomes one of the necessities of human life. The recent technological developments and inclination of humans toward them provide immeasurable advantages and threats even. This study develops a scientometric overview, a quantitative analysis of scholarly publications to identify research trends in the area of cyber security from 2012 to 2021. From the 10364 research outputs of the Web of Science database, the researchers aim to list the annual growth rate, publication categories, prolific authors, countries, institutions, and prominent research areas. The results of the study reveal that cyber security has multidisciplinary nature and there is a gradual growth in the number of publications. The study identifies Das A. K., United States, Chinese Academy of Sciences, and IEEE Access as the most prolific author, country, institution, and source respectively. More secure and efficient networking systems along with awareness of cyber security would minimize the number of crimes in cyberspace.

Keywords: Cyber security, Information security, internet security, computer security, web security, Scientometrics.

INTRODUCTION

We reach at the high time to update the traditional list of human needs. Along with the need for food, water, shelter, and air; the internet becomes one of the necessities. Globally, the life of everyone is centered upon the internet;





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without which day-to-day life would be standstill. While listing the immeasurable advantages of the internet in life, the insecurity that it creates has to be noted. The dependency on information communication and technology (ICT) is at high level and the number of cyber threats increases simultaneously. In this situation, the thought of cyber security becomes a high topic to be discussed and practiced. The deconstruction of the concept of cyber security gives two domains 'cyber' and 'security'. The term 'cyber' is related to cyberspace, electronic communication networks, virtual reality, etc. And the term 'security' has a general sense; the central idea is to be free from danger or attack. Thus, cyber security is the process to secure cyberspace. As the number and nature of cybercrimes vary, the concept of cyber security gets changed from information technology issues to strategic management issues and techno-legal-management issues[1]. The perception of cyber security can certainly be changed in the coming days.

There are a number of definitions of cyber security. "Cyber security consists largely of defensive methods used to detect and thwart would be intruders" [2]. "Cyber security entails the safeguarding of computer networks and the information they contain from penetration and from malicious damage or disruption" [3]. The term cyber security is used broad sense and its definitions are subjective, context-based and certain times make confusion; in such a situation, Craigen *et al.* define it as "the organization and collection of resources, processes, and structures used to protect cyberspace and cyberspace-enabled systems from occurrences that misalign de jure from de facto property rights" [4]. In this definition, the terms de jure and de facto mean perceived and actual respectively. The procedures to safeguard data, information, networks, and computer programs from unauthorized access and use as well as intended or unintended change or destruction.

The term "scientometrics" was coined by Vasily Nalimov and Mulchenko in 1960. Scientometrics, the science of measurement and analysis of science would help to evaluate emerging concepts and identify the trends of research and publications in a specific area of study through quantifications of works of literature so that the possibility of further research and policy creations are opened. Thus, this paper analyses the annual growth rate and relative growth rate, publication categories, prolific countries, authors, institutions, and prominent research areas connected to the research outputs of the Web of Science (WoS) database in cyber security from 2012 to 2021.

LITERATURE REVIEW

Loan *et al.* [5] conducted a scientometric study on the research productivity of cyber security from the articles accessed from the WoS database. The study aimed to identify prominent research areas, collaborative trends, and relative growth rate and doubling time of publication output from 2011 to 2020. The results showed that cyber security had a connection with computer and information security; the field of cyber security has an increasing trend in research outputs across the globe. The bibliometric analysis of Ho and Luong [6] on the research trends in cybercrime victimization during 2010 - 2020 from the WoS database was to find out productive authors, prominent sources, active institutions, and leading countries through statistical evidence and visual findings. The findings of the study specified the upward trend of publication in the area of research and the USA and its institutions and authors had the top position in the area of cybercrime victimization.

The scientometric analysis of Dhawan *et al.*[7] was on the global research output in cyber security. They had analyzed the publications and citations on cyber security from 1998 to 2019 from the Scopus database. From the analysis of 10607 publications, the cyber security research areas had registered 46.41% of growth with an average citation impact of 5.05 per paper. International collaboration was considered as the driving force behind the growth of research in the area of cyber security. The bibliometric analysis of the journals indexed in the WoS database in connection with computer science research was done by Zurita *et al.* [8]. The research area of computer science was delimited by the categories namely, artificial intelligence, cybernetics, hardware and architecture, information systems, interdisciplinary applications, software engineering, and theory and methods. The purpose of the study was to list the name of leading institutions from 1991 to 2015 and among 78 institutions, the institutions from the USA were the most influential. Rai *et al.*[9] made a quantitative analysis of the research trends in cyber security from





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2001 to 2018 based on the Scopus database. They examined 2720 publications on cyber security to quantify the growth pattern, collaborations, citations, authorship, and country-wise contribution. Mandani and Ramirez [10] analyzed 2202 articles published on digital governance and cyber security from 1999 to 2018 in the WoS database to understand how the private information of the firms was secured. Firms cooperated with the latest technologies related to the internet for their upgradation; they should be vigilant enough to secure their intellectual properties. The researchers declared that cyber security was a growing problem from the perspective to protect the information regarding a firm and the proper understanding of cyber security governance and its relation to technologies would be the proper answer to the existing issues. Jalali *et al.* [11] conducted a bibliometric analysis of 472 English language articles on the cyber security of healthcare information systems from the databases of PubMed and WoS. The technological advancements and digitalization process in the healthcare area necessitated the need for security for safe, reliable, and effective healthcare delivery. The study identified that cyber security had to play a central role in healthcare delivery in the days to come as it was dependent more on latest technologies.

Objectives

The basic objective of this study is to analyze the global productivity of research in the area of cyber security from 2012 to 2021. It is carried out by the following objectives.

1. To analyze the year-wise distribution and growth of literature on cyber security during 2012-2021.
2. To know the annual growth rate (AGR), relative growth rate (RGR), and doubling time (DT) of cyber security research.
3. To find out the publication types, language-wise distribution, and multidisciplinary nature of publications related to cyber security research output at the global.
4. To identify the most twenty prolific authors, countries, institutions, and journals in the area of cyber security research.
5. To identify the major observations of prominent research outputs in the area of cyber security.

METHODOLOGY

To analyze the productivity in the area of cyber security, the data for 10 years (2012 – 2021) were collected from the WoS database. The bibliographic information on the publication was downloaded from WoS in plain text format by giving the key terms cybersecurity, cyber security, computer security, network security, and internet security from 2012 to 2021. The bibliographical details retrieved were about 10364 research papers. The downloaded text format was converted into HistCite and BibExcel and analyzed with Microsoft Excel to connect to desired objectives. \

Analysis and Interpretation

Year-wise Distribution and Growth of Publications in Cyber Security

The formula to find out the Annual Growth Rate is,

$$\text{Annual Growth Rate} = \frac{\text{Final Value} - \text{Start Value}}{\text{Start Value}} \times 100$$

The data in Table 1 shows that there are 10364 publications in the areas of cyber security research from 2012 to 2021. During the period of study, it can be analyzed that there is a gradual growth in the number of publications. The highest percentage of publication is in 2021 with 25.42%. Annual Growth Rate is one of the measures to find out the growth rate of publications in any field. The study on the percentage of growth in these years concludes that the highest growth of publication is in 2018 with 49.53 and the lowest growth is noted in 2014 with 4.79 when the number of publications of each year is compared to that of previous years. It is evident that there is continuous growth in the publication output from 2012 to 2021.





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The formula developed by Mahapatra [12] is used to find out the Relative Growth Rate (RGR) and Doubling Time (DT). The formula is to find out the increase in the number of articles based on a certain period of time. The formula is the following,

$$\text{RGR} = \frac{W2 - W1}{T2 - T1}$$

In which,

RGR = Relative Growth Rate over the specific period of the interval

W1 = Log_e (natural log of the initial number of contributions)

W2 = Log_e (natural log of the final number of contributions)

T1 = The unit of initial time

T2 = The unit of final time

$$\text{Doubling Time (DT)} = \frac{0.693}{R}$$

In which

R = Growth Rate

Table 2 and Figure 1 show the chronological distribution of publications in the area of cyber security. The relationship between RGR and DT is of an inverse nature. RGR is one of the measures to find out the growth rate of publications in any field. There is a gradual decrease from 0.75 to 0.30 in the number of publications when RGR is considered, but in 2018 and 2019 the rate is constant. But in the case of DT, there is a gradual growth from 0.92 to 2.31. The average RGR and DT of cyber security research output for 10 years are 0.35 and 1.73 respectively.

Analysis of Publication Types in Cyber Security Research Outputs

Publications in the areas of cyber security come in different document formats like articles, reviews, editorial materials, conference papers, and new items. Table 3 shows the details of the publication types of research output during 2012 - 2021. A total of 10364 documents can be divided into sixteen categories in which 9028 documents with 87.11% are published as articles and review with 527 (5.09%) documents and editorial materials with 391 (3.77%) documents come in the second and third rank. It is clear that the articles are the most produced form of documents. The analysis of publication category based on the number of citations confirm that articles (273518), review (47378), and article early access (6329) have the highest number of citations. ACP of review early access (126) and editorial material early access (92) have the highest amount compared to articles (30.30) and review (89.90).

Analysis of Language-wise Distribution of Research on Cyber Security

The language-wise analysis of 10364 documents in the cyber security research area during 2012 - 2021 is listed in Table 4. The total number of 10364 documents are published in 14 different languages. English language with 10306 (99.44%) documents and 172251 citations has the highest position and shows its wider acceptance. The English language is highly preferred for publishing documents on cyber security. Spanish, German, and Norwegian have the second, third, and fourth ranks with 14, 13, and 7 documents respectively.

Author-wise contribution of publication in the area of cyber security from 2012 to 2021 is tabulated in Table 5. During the period of study, there are 10364 publications and these 10364 publications are created by 22630 authors. The average number of authors per publication would be 2.18. The details of 20 prolific authors based on the number of publications are placed in Table 5. Das AK has secured first place with 95 publications. Liu Y, Zhang J, and Park Y have the second, third, and fourth place with 68, 61, and 51 publications respectively. The analysis of the productivity of authors based on the number of citations shows that Das AK (4636) has the highest number of citations; Kumar N with 2439 citations has secured second place. It is apparent that both of these authors have the highest h-index score. Das AK (41), and Kumar N (27) have the highest h-index score. The meaning of citation sum within h-core is how many citations an author has received to get the particular h-index. Das AK has received 3652 citations for his publications to score his h-index 41. Kumar N has received 2261 citations to claim his h-index 27.





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Analysis of Country-wise Distribution of Research on Cyber Security

The research outputs of cyber security from 2012 to 2021 are from 113 countries. Table 6 displays the top 20 countries which have the highest number of publications. The highest share of publications is from the United States with 28.90% followed by China with 24.30%. They are followed distantly by England (8.70%), India (7.30%), and Australia (6.70%). Among 113 countries, only the United States (2989), and China (2518) have publications more than a thousand. The United States, China, and England have the highest total citations of 62472, 44600, and 20849 respectively. However, the assessment of the average citation per paper reveals that Canada (26.17), Singapore (25.81), and Australia (25.55) have the highest average citations.

Analysis of Institution-wise Contribution of Documents on Cyber Security

The analysis of the total publication of 10364 documents published from 2012 to 2021 on the cyber security research area shows that they belong to 6593 different institutions. Among 6593 institutions, the top 20 institutions are listed in Table 7 based on the greatest number of publications. Chinese Academy of Sciences, Xidian University, and Nanyang Technological University Singapore come in the first three positions having 156, 126, and 110 documents with 1.51%, 1.22%, and 1.06% respectively. Table 7 gives the details of the citations that each institution received for their publications. Though the International Institute of Information Technology Hyderabad has published 88 (0.88%) documents, it comes in first place with 4324 citations having 49.14 as the average citation per paper. Chinese Academy of Sciences comes the next having 3843 citations with a 24.64 average, but the average citations of Carnegie Mellon University (31.31) and Nanyang Technological University Singapore (31.22) are higher than the Chinese Academy of Sciences.

Analysis of Journal-wise Distribution of Research on Cyber Security

The total number of 10364 publications in cyber security research appeared in 1483 different sources. The top 20 sources in which the highest number of documents were published are listed in Table 8. As per the records displayed in Table 6, IEEE Access gets the first rank with 781 documents and the same source has the highest number of citations (11920). The sources named Computer & Security, Security & Communication Network, and Sensors have the second, third and fourth rank respectively with 346, 249, and 221 documents. Though IEEE Communications Surveys and Tutorials has the 15th rank with 80 papers based on the number of papers, it has received 9445 citations, and 118.06 is the average which is the highest.

Prominent Research Areas Connected to Cyber Security Research Outputs

All the publications in the areas of cyber security have multidisciplinary nature. The total of 10364 publications from 2012 to 2021 belongs to 18676 research areas. Table 9 exhibits the first 20 prominent research areas based on the number of publications. The analysis of the table confirms that the prominent areas of the research outputs of cyber security are computer science (6049), engineering (4216), and telecommunications (2728). This is the indication that the discussion on cyber security is mostly related to these areas. The study on citations also proves the same. The average citation per publication suggests that automation and control systems (28.08) and transportation (26.38) have the highest average.

Cyber Security Crimes and its Nature

It becomes unimaginable a world without the internet. The report on the worldwide digital population published by Statistic Research Department on 20th September 2022 reports that more than five billion internet users are in the world [13]. The Internet, being the core pillar to connect billions of people, creates impacts in all aspects of human life. The gradual growth in the process of digitalization simultaneously results in the increase of cybercrime in both number and complexity. It becomes strenuous to identify and detect the very nature of cybercrimes due to their newness in the form and it is the same with handling and avoiding the emerging threats in cyberspace. For “it is pervasive, violent, ubiquitous and increasingly sophisticated” [14].

In the initial stage, cybercrime was associated with computer crime alone; and Parker [15] defined it into four categories where computer is used as the object, environment, instrument, and symbolic of crime. The thorough





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analysis on the nature of cybercrime discloses its transition from individual personal data to the matters of organizational and national integrity like extortion, identity theft, data breach, and phishing attacks [16]. A comprehensive understanding of the cybercrime is possible when it is approached from a multidisciplinary way where the legal and criminal aspects are discussed by combining the application of computer network systems in various spheres of life.

FINDINGS

1. It is reported the gradual growth in the number of publications from 2012 to 2021. When the year 2021 reports 2634 publications which is the highest, the year 2018 marks the highest annual growth rate with 49.53.
2. Among the sixteen publication types, articles have secured the majority of research output with 87.11% (9028).
3. The inverse nature of RGR and DT is visible in the analysis; RGR decreases from 0.75 to 0.30 with an average of 0.35 whereas DT increases from 0.92 to 2.31 with an average of 1.73.
4. The author-wise distribution of publications has identified that Das AK has the first rank in the number of publications (95), the number of citations (4636), and h-index (41).
5. IEEE Access has the highest share of cyber security related publications (7.54) and its total citation is 11920. The journal named IEEE Communications Surveys and Tutorials has acquired 9445 citations from 80 publications and its average citation per paper is 118.60 which is the highest.
6. The preferred language for publishing cyber security related issues is English and its global share is 99.44%.
7. Chinese Academy of Sciences secures the first rank in the institutional wise analysis of the total publications(156) and the total number of citations (3843).
8. The highest share of publications in the area of cyber security is from the United States with 28.90% and the calculation of the average citation per paper reveals that Canada comes at the top with 26.17.
9. The multidisciplinary nature of cyber security is visible in the analysis of prominent research areas. Cyber security is related to 18676 research areas; an indication that it is a high topic to be discussed.

CONCLUSION

Cyber security becomes a growing problem for business and other fields as the implementation of technologies and applications in various processes becomes one of the prerequisites [17]. The exponential growth in the number of cyber threats speaks of the enforcement of security measures in cyberspace. At present, the virtual world becomes the deciding factor in the daily activities of human beings than ever before. The steady rise in cybercrimes insists to discuss on cyber security. The quantitative analysis of cyber security publication output data in the WoS database shows the progressive nature of publication from three digits in 2012 to four digits in 2021; the gradual growth of research works in this area is to analyze its impact in the society. The period of study reports 10364 documents in cyber security research. The primary indication from the number of publications is that cyber security is one of the serious topics of discussion among academicians and scientists. The progressive growth rate of cyber security research compels us to have periodical analysis in this field and qualitative analysis in the publication is also necessary for the future. On the one side more secure and efficient networking systems are to be implemented to minimize the number of cybercrimes and the awareness on the other side [18].

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Table 6 : Year-wise Distribution and Growth of Publications in Cyber Security

| Year | No. of Publication | Percentage | Cumulative Publication | Cumulative Percentage | Annual Growth Rate |
|------|--------------------|------------|------------------------|-----------------------|--------------------|
| 2012 | 313 | 3.02 | 313 | 3.02 | |
| 2013 | 355 | 3.43 | 668 | 6.45 | 13.42 |
| 2014 | 372 | 3.58 | 1040 | 10.04 | 4.79 |
| 2015 | 491 | 4.74 | 1531 | 14.77 | 31.99 |
| 2016 | 634 | 6.12 | 2165 | 20.89 | 29.12 |
| 2017 | 739 | 7.13 | 2904 | 28.02 | 16.56 |
| 2018 | 1105 | 10.66 | 4009 | 38.68 | 49.53 |
| 2019 | 1590 | 15.34 | 5599 | 54.02 | 43.89 |





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|------|-------|-------|-------|-------|-------|
| 2020 | 2131 | 20.56 | 7730 | 74.59 | 34.03 |
| 2021 | 2634 | 25.42 | 10364 | 100 | 23.60 |
| | 10364 | 100 | | | |

Table 7 : Relative Growth Rate and Doubling Time of Publications on Cyber Security

| Year | No. of Publication | Cumulative No. of Publication | W1 | W2 | RGR | DT |
|------------------|--------------------|-------------------------------|------|------|-------------|-------------|
| 2012 | 313 | 313 | | 5.75 | | |
| 2013 | 355 | 668 | 5.75 | 6.50 | 0.75 | 0.92 |
| 2014 | 372 | 1040 | 6.50 | 6.95 | 0.45 | 1.54 |
| 2015 | 491 | 1531 | 6.95 | 7.33 | 0.38 | 1.82 |
| 2016 | 634 | 2165 | 7.33 | 7.68 | 0.35 | 1.98 |
| 2017 | 739 | 2904 | 7.68 | 7.97 | 0.29 | 2.39 |
| 2018 | 1105 | 4009 | 7.97 | 8.30 | 0.33 | 2.10 |
| 2019 | 1590 | 5599 | 8.30 | 8.63 | 0.33 | 2.10 |
| 2020 | 2131 | 7730 | 8.63 | 8.95 | 0.32 | 2.17 |
| 2021 | 2634 | 10364 | 8.95 | 9.25 | 0.30 | 2.31 |
| 2012-2021 | 10364 | Mean | | | 0.35 | 1.73 |

Table 3: Analysis of Publication Types in Cyber Security Research Outputs

| No | Publication Type | No. of Publication | Percentage | Total Citation | Average Citation per Paper |
|----|----------------------------------|--------------------|------------|----------------|----------------------------|
| 1 | Article | 9028 | 87.11 | 273518 | 30.30 |
| 2 | Review | 527 | 5.09 | 47378 | 89.90 |
| 3 | Editorial Material | 391 | 3.77 | 2619 | 6.70 |
| 4 | Article; Proceedings Paper | 165 | 1.59 | 6010 | 36.42 |
| 5 | Article; Early Access | 117 | 1.13 | 6329 | 54.09 |
| 6 | News Item | 36 | 0.35 | 8 | 0.22 |
| 7 | Meeting Abstract | 34 | 0.32 | 7 | 0.21 |
| 8 | Book Review | 27 | 0.26 | 36 | 1.33 |
| 9 | Letter | 11 | 0.11 | 129 | 11.73 |
| 10 | Correction | 10 | 0.09 | 10 | 1 |
| 11 | Article; Book Chapter | 6 | 0.06 | 492 | 82 |
| 12 | Review; Early Access | 5 | 0.05 | 630 | 126 |
| 13 | Article; Retracted Publication | 4 | 0.04 | 252 | 63 |
| 14 | Retraction | 1 | 0.01 | 1 | 1 |
| 15 | Editorial Material; Early Access | 1 | 0.01 | 92 | 92 |
| 16 | Article; Data Paper | 1 | 0.01 | 17 | 17 |
| | | 10364 | 100 | 337528 | |

Table 4 : Analysis of Language-wise Distribution of Research on Cyber Security

| Rank | Language | No. of Publication | Percentage | Total Citations |
|------|------------|--------------------|------------|-----------------|
| 1 | English | 10306 | 99.44 | 172251 |
| 2 | Spanish | 14 | 0.13 | 65 |
| 3 | German | 13 | 0.12 | 6 |
| 4 | Norwegian | 7 | 0.07 | 8 |
| 5 | Hungarian | 5 | 0.05 | 4 |
| 6 | Portuguese | 4 | 0.04 | 33 |





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|----|--------------|--------------|------------|---|
| 7 | Turkish | 4 | 0.04 | 1 |
| 8 | Chinese | 2 | 0.02 | 4 |
| 9 | Czech | 2 | 0.02 | 2 |
| 10 | French | 2 | 0.02 | 5 |
| 11 | Slovene | 2 | 0.02 | 4 |
| 12 | Danish | 1 | 0.01 | 2 |
| 13 | Polish | 1 | 0.01 | 0 |
| 14 | Russian | 1 | 0.01 | 4 |
| | Total | 10364 | 100 | |

Table : 5 Analysis of Prolific Authors in Cyber Security Research Outputs

| Rank | Name of the Author | No. of Publication | Total Citation | Average Citation per Paper | h-index | Citation sum within h-core |
|------|--------------------|--------------------|----------------|----------------------------|---------|----------------------------|
| 1 | Das AK | 95 | 4636 | 48.80 | 41 | 3652 |
| 2 | Liu Y | 68 | 732 | 10.77 | 13 | 514 |
| 3 | Zhang J | 61 | 1661 | 27.23 | 21 | 1397 |
| 4 | Park Y | 51 | 986 | 19.33 | 21 | 763 |
| 5 | Choo KKR | 48 | 1368 | 28.50 | 19 | 1221 |
| 6 | Xiang Y | 47 | 1638 | 34.85 | 20 | 1453 |
| 7 | Zhang Y | 47 | 984 | 20.94 | 16 | 842 |
| 8 | Zheng WX | 44 | 1014 | 23.05 | 18 | 828 |
| 9 | Wang J | 43 | 808 | 18.79 | 12 | 683 |
| 10 | Kumar N | 42 | 2439 | 58.07 | 27 | 2261 |
| 11 | Liu J | 41 | 777 | 18.95 | 13 | 672 |
| 12 | Weng J | 40 | 1346 | 33.65 | 18 | 1238 |
| 13 | Li X | 38 | 1055 | 27.76 | 17 | 972 |
| 14 | Havlin S | 37 | 892 | 24.11 | 14 | 740 |
| 15 | Wang Y | 37 | 644 | 17.41 | 10 | 548 |
| 16 | Kim J | 36 | 660 | 18.33 | 11 | 570 |
| 17 | Liu X | 34 | 999 | 29.38 | 17 | 892 |
| 18 | Yan Z | 33 | 730 | 22.12 | 16 | 636 |
| 19 | Li J | 32 | 1026 | 32.06 | 11 | 951 |
| 20 | Karri R | 32 | 597 | 18.65 | 11 | 524 |

Table 6 : Analysis of Country-wise Distribution of Research on Cyber Security

| Rank | Country | No. of Publication | Percentage | Total Citation | Average Citation per Paper |
|------|--------------|--------------------|------------|----------------|----------------------------|
| 1 | USA | 2989 | 28.90 | 62472 | 20.90 |
| 2 | China | 2518 | 24.30 | 44600 | 17.71 |
| 3 | England | 904 | 8.70 | 20849 | 23.06 |
| 4 | India | 755 | 7.30 | 15747 | 20.86 |
| 5 | Australia | 692 | 6.70 | 17679 | 25.55 |
| 6 | South Korea | 584 | 5.60 | 9579 | 16.40 |
| 7 | Canada | 440 | 4.20 | 11516 | 26.17 |
| 8 | Spain | 434 | 4.20 | 6378 | 14.70 |
| 9 | Italy | 409 | 3.90 | 8203 | 20.06 |
| 10 | Saudi Arabia | 354 | 3.40 | 5247 | 14.82 |
| 11 | Germany | 321 | 3.10 | 4449 | 13.86 |
| 12 | Israel | 276 | 2.70 | 3098 | 11.23 |





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|----|-----------|-----|------|------|-------|
| 13 | Pakistan | 276 | 2.70 | 4687 | 16.98 |
| 14 | Singapore | 273 | 2.60 | 7045 | 25.81 |
| 15 | Japan | 269 | 2.60 | 3663 | 13.62 |
| 16 | Taiwan | 213 | 2.10 | 2473 | 11.61 |
| 17 | France | 202 | 1.90 | 3457 | 17.11 |
| 18 | Iran | 160 | 1.50 | 2975 | 18.59 |
| 19 | Poland | 159 | 1.50 | 1360 | 8.55 |
| 20 | Brazil | 157 | 1.50 | 2931 | 18.67 |

Table 7 : Analysis of Institution-wise Contribution of Documents on Cyber Security

| Rank | Name of the Institution | No. of Publication | Percentage | Total Citation | Average Citation Per Pater |
|------|---|--------------------|------------|----------------|----------------------------|
| 1 | Chinese Academy of Sciences | 156 | 1.51 | 3843 | 24.64 |
| 2 | Xidian University | 126 | 1.22 | 2369 | 18.8 |
| 3 | Nanyang Technological University, Singapore | 110 | 1.06 | 3435 | 31.22 |
| 4 | King Saud University | 108 | 1.04 | 2138 | 19.8 |
| 5 | Beijing University of Posts & Telecommunications | 104 | 1 | 1895 | 18.22 |
| 6 | Deakin University | 101 | 0.98 | 2822 | 27.94 |
| 7 | Tsinghua University | 99 | 0.96 | 2577 | 26.03 |
| 8 | Ben Gurion University Negev | 90 | 0.87 | 1035 | 11.5 |
| 9 | International Institute of Information Technology Hyderabad | 88 | 0.85 | 4324 | 49.14 |
| 10 | New York University | 88 | 0.85 | 1588 | 18.05 |
| 11 | University Texas San Antonio | 88 | 0.85 | 1921 | 21.83 |
| 12 | Carnegie Mellon University | 80 | 0.77 | 2505 | 31.31 |
| 13 | Korea University | 76 | 0.73 | 1234 | 16.24 |
| 14 | Purdue University | 76 | 0.73 | 1087 | 14.3 |
| 15 | Shanghai Jiao Tong University | 75 | 0.72 | 1185 | 15.8 |
| 16 | Southeast University | 73 | 0.7 | 1466 | 20.08 |
| 17 | University of Electronic Science Technology of China | 71 | 0.69 | 1903 | 26.8 |
| 18 | Bar Ilan University | 69 | 0.67 | 1136 | 16.46 |
| 19 | Massachusetts Institute of Technology MIT | 68 | 0.66 | 1582 | 23.27 |
| 20 | National University of Singapore | 66 | 0.64 | 1492 | 22.61 |





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Table 8 : Analysis of Journal-wise Distribution of Research on Cyber Security

| Rank | Name of the Journal | No. of Publication | Percentage | Total Citation | Average Citation per Paper |
|------|---|--------------------|------------|----------------|----------------------------|
| 1 | IEEE Access | 781 | 7.54 | 11920 | 15.26 |
| 2 | Computers & Security | 346 | 3.34 | 7201 | 20.81 |
| 3 | Security and Communication Networks | 249 | 2.4 | 1887 | 7.58 |
| 4 | Sensors | 221 | 2.13 | 2253 | 10.19 |
| 5 | IEEE Transactions on Information Forensics and Security | 179 | 1.73 | 5345 | 29.86 |
| 6 | IEEE Security & Privacy | 140 | 1.35 | 785 | 5.61 |
| 7 | Applied Sciences-Basel | 138 | 1.33 | 1122 | 8.13 |
| 8 | IEEE Transactions on Smart Grid | 124 | 1.19 | 5278 | 42.57 |
| 9 | Electronics | 120 | 1.16 | 1545 | 12.88 |
| 10 | Computer Networks | 118 | 1.14 | 3555 | 30.13 |
| 11 | IEEE Internet Of Things Journal | 114 | 1.09 | 4888 | 42.88 |
| 12 | Future Generation Computer Systems-the International Journal of Esience | 109 | 1.05 | 4411 | 40.47 |
| 13 | Wireless Personal Communications | 106 | 1.02 | 866 | 8.17 |
| 14 | Journal of Network and Computer Applications | 81 | 0.78 | 2861 | 35.32 |
| 15 | IEEE Communications Surveys and Tutorials | 80 | 0.77 | 9445 | 118.06 |
| 16 | Computer | 80 | 0.77 | 493 | 6.16 |
| 17 | IEEE Transactions on Industrial Informatics | 75 | 0.72 | 287 | 3.83 |
| 18 | Journal of Intelligent & Fuzzy Systems | 73 | 0.71 | 289 | 3.96 |
| 19 | IEEE Transactions on Dependable and Secure Computing | 70 | 0.67 | 1778 | 25.4 |
| 20 | ENERGIES | 69 | 0.67 | 721 | 10.45 |

Table 9 : Prominent Research Areas Connected to Cyber Security Research Outputs

| No | Research Areas | No. of Publication | Total Citation | Average Citation per Paper |
|----|--|--------------------|----------------|----------------------------|
| 1 | Computer Science | 6049 | 109138 | 18.04 |
| 2 | Engineering | 4216 | 81545 | 19.34 |
| 3 | Telecommunications | 2728 | 49872 | 18.28 |
| 4 | Physics | 443 | 4474 | 10.09 |
| 5 | Science & Technology | 422 | 6767 | 16.04 |
| 6 | Chemistry | 407 | 3854 | 9.47 |
| 7 | Mathematics | 333 | 2827 | 8.49 |
| 8 | Automation & Control Systems | 327 | 9183 | 28.08 |
| 9 | Business & Economics | 324 | 5034 | 15.54 |
| 10 | Instruments & Instrumentation | 297 | 3235 | 10.89 |
| 11 | Government & Law | 275 | 2784 | 10.12 |
| 12 | Operations Research & Management Science | 245 | 5570 | 22.74 |





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|----|---------------------------------------|-----|------|-------|
| 13 | Materials Science | 244 | 2725 | 11.17 |
| 14 | Information Science & Library Science | 189 | 4118 | 21.79 |
| 15 | Energy & Fuels | 178 | 3370 | 18.93 |
| 16 | Psychology | 139 | 1874 | 13.48 |
| 17 | International Relations | 133 | 1741 | 13.09 |
| 18 | Transportation | 127 | 3350 | 26.38 |
| 19 | Medical Informatics | 116 | 2356 | 20.31 |
| 20 | Environmental Sciences & Ecology | 110 | 1155 | 10.50 |

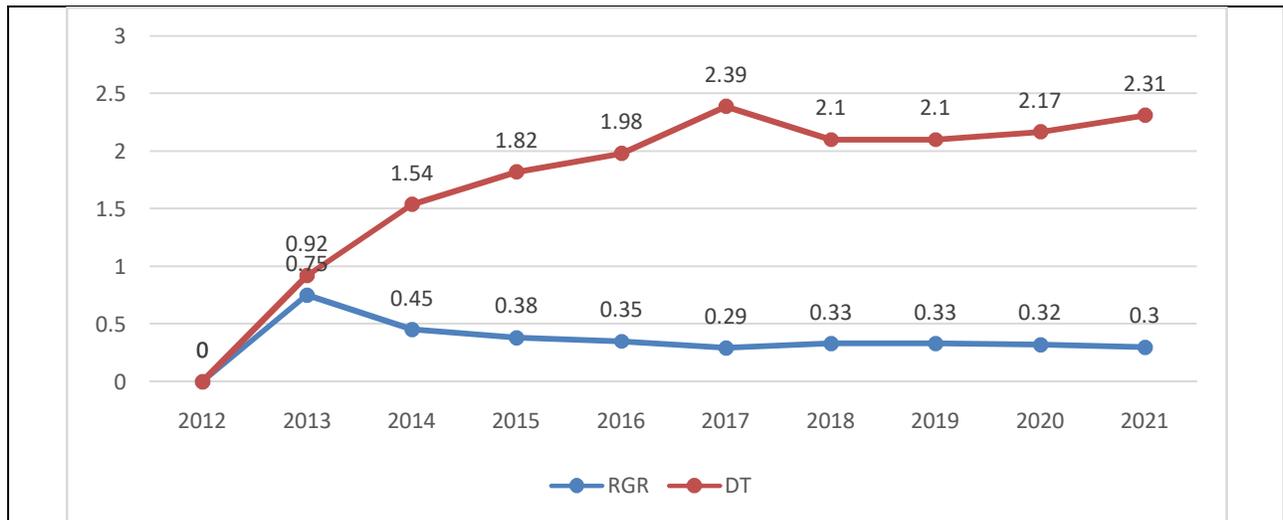


Figure 1 : Relative Growth Rate and Doubling Time of Publications on Cyber Security





Psychobiological Underpinnings of Cyberchondria Behaviour

Geeta Khanwani¹ and Suyesha Singh^{2*}

¹Research Scholar, Department of Psychology, Manipal University Jaipur, Jaipur, Rajasthan, India.

²Assistant Professor, Department of Psychology, Manipal University Jaipur, Jaipur, Rajasthan, India.

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*Address for Correspondence

Suyesha Singh

Assistant Professor,

Department of Psychology,

Manipal University Jaipur,

Jaipur, Rajasthan, India.

suyesha.singh@jaipur.manipal.edu



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ABSTRACT

Intense fear and anxiety due to sudden unprecedented events such as covid-19 pandemic has been one of the serious health concerns. Population around the globe experience a turmoil of physiological and psychological arousal leading to biochemical changes which may impact the overall health and wellbeing. A constant threat and anxiousness during the uncertainty leads to long-term health hazards such as fluctuations in biological materials like magnesium and calcium salts, neuronal circuitry, and neurotransmitters like Gama-aminobutyric acid (GABA). To combat the threatening situation, one tends to engage in defending behaviours as a coping mechanism. In the era of digitalisation, digital platforms have been widely adopted as a route for communication and sharing of information especially during the surge of covid-19 pandemic. However, ambiguous nature of digital media inflamed the fear and anxiety related to covid-19 pandemic leading to cyberchondria behaviour. Studies have highlighted the role of digital media in influencing the health information seeking behaviour but there is limited understanding of underlying amplifying biopsychosocial factors responsible for predicting cyberchondria behaviour. The current study aims to understand the bio-psychological underpinnings predicting cyberchondria behaviour. Mechanism of neural circuitry, chemical messengers, and psychological theoretical models i.e., the theory of motivated information management (TMIM) [1] and risk information seeking and processing model (RISP) [2] has been adopted under the present study to understand cyberchondria behaviour. The study will contribute towards gaining insight about the chemical messengers and biochemical changes in the human biopsychological nature leading to cyberchondria behaviour. Physiological interventions such as oral administration of magnesium salts, carbamazepine (an antiepileptic drug responsible for raising intracellular magnesium), calcium channel blockers such as verapamil, diltiazem, or nimodipine can help in reducing the symptoms related to anxiety. Behavioural



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modification therapeutic interventions can also be promoted as an early intervention therapy especially during the uncertainty.

Keywords: *Cyberchondria, Psychobiology, Chemical messengers, Neuronal Circuitry*

INTRODUCTION

The dualistic nature of digital media has raised the concerns of massive spread of digital mis-infodemic which may negatively impact the biopsychological implications especially during the health emergencies such as covid-19 pandemic [3,4]. Raised concerns of stress and anxiety due to uncertainty cause significant physiological alterations. Biological materials such as magnesium and calcium salts and bio-messengers such as hormones, saliva, plasma, neurotransmitters, and cerebral-spinal fluid (CSF), lead to activation of hypothalamic-pituitary-adrenal (HPA) axis and autonomic nervous system (ANS). Biomarkers are the traits that can be measured and assessed as an indicator of regular biological or pathological processes to therapeutic responses. It plays a crucial role to identify and manage fear and anxiety especially during uncertain times leading to engagement in online searches of health-related information[5]. Excessive, compulsive, and repeated digital exposure to seek health-related information elicited the maladaptive health-related behaviour widely recognised as “cyberchondria behaviour”[6]. Health anxiety i.e., irrational, and obsessive worry about one’s health, is one of the major underlying predictors of cyberchondria especially during the unprecedented events. The vicious cycle of health anxiety is modulated and regulated by the individual’s control, beliefs, and attitude towards the unprecedented event [7,8]. Henceforth, it is crucial to understand the underlying biopsychological factors contributing towards cyberchondria behaviour during the unprecedented events such as covid-19 pandemic. Therefore, current study has proposed a neural circuitry model to understand cyberchondria behaviour under the context of covid-19 pandemic. Furthermore, present study has also adopted two information management models i.e., the theory of motivated information management (TMIM) [1] and risk information seeking and processing model (RISP) [2] for predictive analysis of cyberchondria behaviour.

Neural Circuitry Model Explaining Cyberchondria Behaviour

Physiological response to threat especially during the unprecedented times like health pandemic exaggerated the fear reactivity response. Fear has been identified as an underlying response to stress and anxiety during the unpredictable or threatening times. There has been involvement of several mechanisms to generate and mediate fear reactivity response to conditioned threat signal. Thalamus consolidates sensory signals from the primary sensory cortices (PSC) and sends signals to amygdala. Dorsal anterior cingulate cortex (DACC) and amygdala process aversive signals and pass on the output to basal ganglia, hypothalamus, and brain stem to generate defensive behaviour. For instance, to deal with fear and health anxiety elevated during the uncertain times like covid-19 pandemic engagement in online searches of health-related information worked as a defensive behavioural response. Hippocampus is responsible for encoding information associated with fear and threat cues. Further hippocampus plays a crucial role in regulating amygdala implicated extinction of fear by achieving safer conditions such as updating and validating about health-related information. The medial prefrontal cortex mediates and regulates fear response inputs coming from hippocampus and thalamus and projecting towards amygdala to mediate and control fear behaviour under the complex environmental situations.

Physiological mechanisms and Neurotransmitters

The expression of fear and anxiety circuitry under the unprecedented times involves complex and synchronised activity of brain neuronal circuitry with coordinated actions of biological material such as magnesium and calcium and chemical messengers such as salivary extracts, hormones, plasma and neurotransmitters. Magnesium plays a crucial role in all essential biochemical mechanisms of the human physiology. Deficit in levels of magnesium may contribute to various nonspecific neuropsychological changes like agitation, fear, anxiety, stress and neuronal



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damages. Similarly, calcium channel blockers chemicals also help to reduce the anxiety arousal[9,10]. Anxiety also shows association with cortisol level, lysozyme, immunoglobulin A, melatonin, chromogranin A, fibroblast growth factor 2 and alpha amylase in saliva[11]. Cortisol i.e., common stress biomarker activates other stress hormones such as adrenaline and noradrenaline. Peripheral plasma assessment indicated that abnormal levels of serotonin, 5-hydroxytryptamine (5-HT), brain-derived serum neurotrophic factor (BDNF) and neuropeptide S (NPS) showed significant variance among the individual with anxious traits compared to control group [12]. 5-Hydroxyindoleacetic acid (5-HIAA) and neuropeptides are common biomarkers in CSF[13]. Gama-aminobutyric acid (GABA) is one of the major inhibitory neurotransmitters responsible for modulation and regulation of fear and anxiety spectrum present in inter-neuronal structure of amygdala region. Low levels of GABA elevate anxious traits in an individual leading to physiological and behavioural response to uncertainty. Constant uncertainty and threat to health leads to deficit of amino acids like GABA leading to elevated health anxiety. The vicious cycle of anxious traits and constant uncertainty raised the concerns of negative emotional regulation and excessive endorsement of health-related information[14,15,16]. To demonstrate the biological mechanism of neural circuitry and biomaterials involved in modulating and regulating cyberchondria behaviour under the physiological context, a diagrammatic representative physiological model (Figure 1) has been proposed under the present study.

Psychological Mechanisms in Cyberchondria Behaviour

The physiological framework demonstrates the biological response and adaptivity of fear and threat by the modulation and regulation of neural circuitry and neurotransmitters. Psychological factors i.e., one's thought, beliefs, perception, and attitude towards an event also play a significant role in predicting their response and control over the unprecedented situation. Hence, psychological, and cognitive patterns affect one's perception and control over the situation which further influence how one assesses and interprets the environment as threatening or non-threatening [17].

Theory of Motivated Information Management (TMIM)

TMIM model depicts that an individual passes through three phase processes while deciding whether they want to seek or avoid information related to any event especially during the uncertainty. It is followed by the two-stage process of mediating role of information seeker and information provider. Information seeker involves in *interpretation phase* wherein assessment of uncertainty has been processed. Uncertainty refers to an experience where one cannot predict the situation. Uncertainty discrepancy i.e., difference between the amount of uncertainty one has and the amount one desires to have, serves as a mediating factor for information seeking process. Inclined uncertainty discrepancy raises the concerns of health anxiety during the unprecedented times such as COVID-19 pandemic. To deal with the elevated emotional turmoil i.e., health anxiety, an individual mediates into evaluation phase. *Evaluation phase* relies on two major conditions i.e., outcome expectancy (i.e., pros and cons of the health-related information to deal with the uncertainty) and efficacy assessments (i.e., whether health-related information is enough to subside the uncertainty discrepancy). Furthermore, evaluation phase relies on information provider i.e., communication efficacy (relying on health-related websites for seeking health-related information), coping efficacy (individual's perception that gathered health-related information can help to cope with the uncertainty) and target efficacy (individual is able to cope or manage the uncertainty with the health-related information). Finally, one decides to either seek or avoid health-related information under *decision phase* wherein they seek relevant information (i.e., adopting relevant sources such as internet for seeking health-related information about the pandemic) or avoid relevant information (i.e., avoiding seeking information if one considers it as risky or damaging). It is mediated by individual cognitive reappraisal i.e., individual's perception and attitude towards uncertainty.

Risk Information Seeking and Processing (RISP) Model

RISP model demonstrates that health risk-seeking information and processing is mediated by the subjective analysis of an individual i.e., discrepancy factor between what one knows about the event and what one should be knowing about the event. Information gap judgement is regulated by several factors i.e., personal characteristics such as attitude and beliefs, perception of hazard posed by the risk, worry associated with risk and perceived social norms available to learn about the unprecedented event. Lastly, beliefs about the information available and perception of





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individual's ability to gather the information act as a link between the perceived information gap and information seeking and processing intentions [18]. Based on the outline structure demonstrated by TMIM and RISP model, we hypothesised that when people experience uncertain situations such as covid-19 pandemic which they perceive as a threat to their health, they would indulge more in compulsive internet search of health-related information. Drawing on the hybrid model of cyberchondria behaviour, we postulated that health anxiety, intolerance to uncertainty and pandemic attitude can be the significant predictors of cyberchondria behaviour. Therefore, a proposed model of cyberchondria behaviour represented (Figure: 2) has been designed under the present study.

The present study can contribute to developing better health and wellness intervention plans to foster the health and wellbeing of the human population especially during the unprecedented events which has the potential to aggravate health anxiety, intolerance towards uncertainty and negative attitude leading to massive engagement in cyberchondria behaviour. Oral administration of magnesium salts, carbamazepine (an antiepileptic drug responsible for raising intracellular magnesium), calcium channel blockers such as verapamil, diltiazem, or nimodipine shows a significant role in reducing anxiety symptoms[9,10]. Behavioural modification therapeutic interventions can also be useful as an early intervention therapy during the uncertainty. Therefore, formulation of cyberchondria behaviour monitoring and management intervention plan can safeguard the population at risk.

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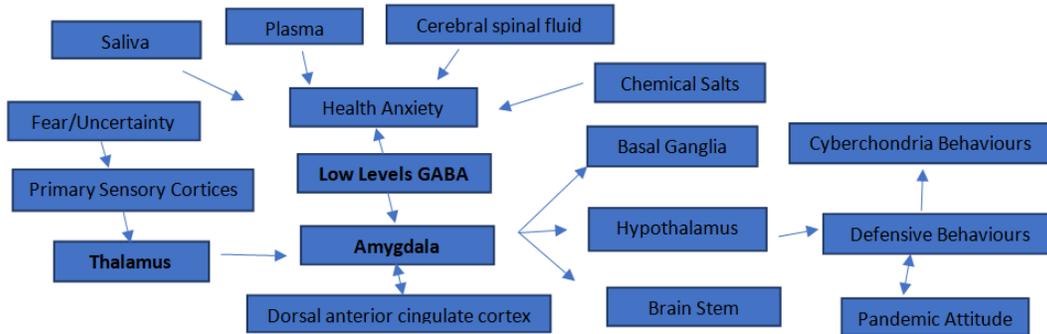


Figure 1: Representation of Neural Circuitry and Biological Markers in Cyberchondria

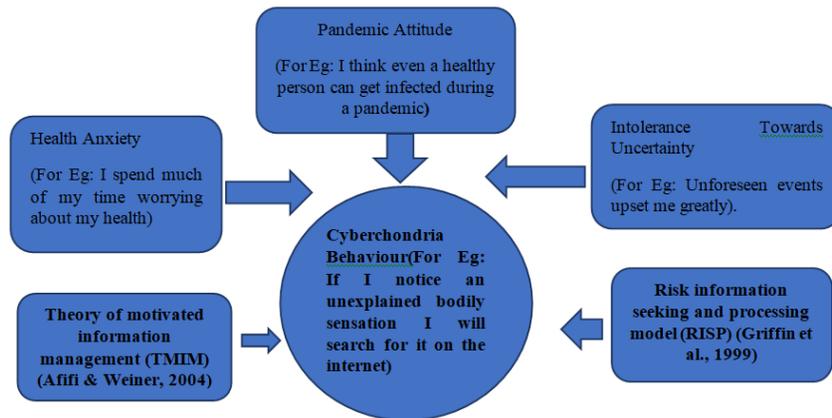


Figure 2: Proposed Psychological Model for Cyberchondria Behaviour





A Comparative Study of Contrast Enhancement and Brightness Preservation of Ancient Inscription Image using Different Histogram Equalization Algorithms

Bapu D. Chendage^{1*} and Rajivkumar S. Mente²

¹Research Scholar, School of Computational Sciences Punyashlok Ahilyadevi Holkar Solapur University, Solapur 413255, Maharashtra, India

²Assistant Professor, School of Computational Sciences Punyashlok Ahilyadevi Holkar Solapur University, Solapur 413255, Maharashtra, India

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*Address for Correspondence

Bapu D. Chendage

Research Scholar,
School of Computational Sciences,
Punyashlok Ahilyadevi Holkar Solapur University,
Solapur 413255, Maharashtra, India



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ABSTRACT

Image enhancement techniques in digital image processing bring out the details that are hidden in the image. Image contrast enhancement without changing other parameters of image is very difficult task. This paper focused on several contrast enhancement techniques using histogram equalization. Histogram equalization is most used method for the contrast enhancement. However, the histogram equalization is not the best method for the enhancing the brightness of image because it produces different brightness in the output image with respect to input image. There are several histogram equalization algorithms are available to overcome the brightness preservation problem. The Dualistic sub image histogram equalization (DSIHE) and brightness preservation bi histogram equalization (BBHE) methods are used to divide the image histogram into two parts based on statistical features then equalize each part independently. The Contrast Limited Adaptive Histogram Equalization (CLAHE) is used to divide image into different regions, called tiles and then neighboring tiles are combined using bilinear interpolation to remove artificial boundaries. It is used to solve over amplification of the contrast. The conventional histogram equalization (HE) is used to stretch the dynamic range of pixel values to enhance the contrast. The experimental study is based on Peak Signal to Noise Ratio (PSNR), Mean Square Error (MSE), Mean Absolute Error (MAE) and Enhancement Measurement Error (EME).

Keywords : Histogram Equalization, Brightness Preservation, Contrast Enhancement, Peak Signal to Noise Ratio, Mean Absolute Error, Enhancement Measure Error, DSIHE, BBHE, CLAHE, RMSHE.





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INTRODUCTION

Contrast enhancement is an important area in digital image processing for human visual perception. Contrast enhancement is one of the preprocessing steps and it is used for enhancement of ancient inscriptions and other image processing applications (Patel O., Maravi Y. P. Et al,2013),(Scott E Umbaugh, 1998). There are many methods are available for image contrast enhancement (Patel O., Maravi Y. P. Et al, 2013)-(Kumar, P. 2014).In this paper, author implemented some contrast enhancement methods. Histogram Equalization is one of the popular methods used for contrast enhancement (Scott E Umbaugh, 1998). Histogram equalization is spread the pixel intensity over the full intensity range. But HE has “Mean Shift “problem (Kim, Y. T.1997), it shift the mean intensity value to center gray level of intensity range. So this technique is not useful in the case of brightness preservation. So overcome this problem new technique is proposed by Yeong Taeg Kim called as BBHE (Brightness Bi Preservation using Histogram Equalization) (Kim, Y. T.1997). This technique divides image into two parts based on mean value. Later on histogram equalization is applied on two part separately, first minimum gray level to mean and secondly mean to maximum gray level (Lu L., Zhou, Y., Et al,2010).

Later, Yu Wan, Qian Chen, and Bio Min Zang invented new method called as Dualistic Sun-image Histogram Equalization (DSIHE)(Chen, S. D., Ramli, A. R.,2003) They claimed that this method is effective than BBHE in terms of brightness preservation and entropy. The DISHE method work similar to BBHE, it divides input image into two parts based of Cumulative Density Function (CDF) rather than mean value. DSIHE divides image equal size blocks i.e. number of pixel in block 1 is equal to number of pixel in block 2. The Contrast Limited Adaptive Histogram Equalization (CLAHE) is the part of Adaptive Histogram Equalization (AHE). It operates on small regions of image rather than entire image. These small regions are called as tiles. The neighbor tiles are combined using bilinear interpolation to remove artificial boundaries. The CLAHE is used to solve oversampling problem. Digital image processing is the process that takes image as input and produce image or characteristics of image as output. It has many applications such as medical imaging, face recognition, pattern recognition and many more. Captured inscription image may be degraded by noise and less contrast. So this will leads to poor quality of image. So there is requirement to enhance image using enhancement techniques.

In the spatial domain, enhancement is done by directly modification of pixel values. Many researchers are used gray level image enhancement on spatial domain. Frequency domain enhancement techniques are based on modification of Fourier Transform of image)-(Kumar, P. 2014). Image is improved by using different frequency range. Image enhancement deals with quality enhancement of a digital image. Here authors discussed some enhancement methods based histogram equalization for the quality enhancement of inscription images.

Histogram Equalization

Histogram equalization is used to enhance the appearance of the image. This method divides image into different sections and equalize sections independently. Suppose the image ‘x’ has number of occurrences of gray level ‘i’. The probability of an occurrence of pixel at gray level ‘i’ is given by

$$p_x(i) = \frac{n_i}{n}, 0 < i < L \quad (1)$$

where $p_{x(i)}$ is the image histogram that is normalized to [0,1], n is number of pixel of image, n_i is value of pixel at certain gray level and L is maximum gray level. The corresponding Cumulative Density Function (CDF) is given by

$$CDF(i)x = \sum_{j=0}^i p_x(j) \quad (2)$$

where x is an image, i is occurrences of gray level.

Histogram equalization techniques map input image into dynamic range $[X_0, X_{L-1}]$ using CDF as transformation function. The transformation function $f(x, y)$ using CDF is given by

$$f(x, y) = X_0 + (X_{L-1} - X_0) * cdf(xt) \quad (3)$$





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Then the output image $Y=y(i, j)$ can be expressed as

$$Y = \{f(x(i, j)) \mid \forall x(i, j) \in X\} \tag{4}$$

This method describes histogram equalization on gray scale image (Michael Chan, 2022). This method is also applicable for color image by separating three colors independently i.e. Red, Green and Blue (Wang, Q., & Ward, R. K.2007).The main problem of histogram equalization is that this method normally changes the brightness of entire image significantly. And that makes output image very bright or dark. So the brightness preservation is unsatisfactory (url, 2023). The brightness is very important characteristics in enhancement of digital image (Chen, S. D., & Ramli, A. R. 2003). So there is need to use brightness preservation techniques. In the above table maximum PSNR is 19.5254 for image SI1 and minimum PSNR for image SI10 i.e. 11.4239. Similarly EME is maximum is for images SI1, SI2, SI3 and SI7. The minimum value of EME is for image SI10. The maximum MAE is for the image SI7 and minimum for SI10. The highest value of the statistical measure shows better contrast enhancement of an image.

Brightness Preservation Bi Histogram Equalization (BBHE)

In order to overcome the problem of histogram equalization many brightness preservation methods were developed. One of the brightness preservation methods is BBHE. This method is introduced by kim (Kim, Y. T.1997). The BBHE method divides image into two sub histograms based on brightness of input image. One image contains the pixel values less than mean and other contains larger pixel value than mean. The image is having gray levels 0 to L-1(255). These gray levels are divided into two parts. The first part ranges from 0 to X_T and second from X_{T+1} to L-1. The original input image $f(x,y)$ is divided into f_L and f_U is given by

$$f = f_L \cup f_U$$

$$f_L = \{f(i, j) \mid f(i, j) \leq f_m \forall f(i, j) \in f\} \tag{5}$$

And

$$f_U = \{f(i, j) \mid f(i, j) > f_m \forall f(i, j) \in f\} \tag{6}$$

where f_m is mean of the image f and consider $f_m \in \{0, L-1\}$ the image f_L is composed of $\{f_0, f_1, \dots, f_m\}$ and f_U is composed of $\{f_{m+1}, f_{m+2}, \dots, f_{L-1}\}$. After dividing an image there is need to apply CDF as transformation function. The transformation function is given by

$$TL(f_k) = f_0 + (f_m - f_0)CL(f_k) \tag{7}$$

And

$$TU(f_k) = f_m + 1 + (f_L - 1 - f_m + 1)CU(f_k) \tag{8}$$

Based on the above transformation images are equalized independently. The combined result of equalized image using above transform will produce output image.

Dualistic Sub image Histogram Equalization (DSIHE)

The idea behind BBHE is to decompose image into two parts and equalize each part separately. The DSIHE was invented by Later, Yu Wan, Qian Chen, and Bio Min Zang [4].This method aims to decomposing image using mean gray level and produce high entropy image. The input image is decomposed into equal sub image (number of pixels are same) in two part, one is dark and one is bright.

Contrast Limited Adaptive Histogram Equalization (CLAHE)

Adaptive Histogram Equalization (AHE) is a digital image processing technique used to enhance the contrast of an image. It computes several histograms; each represents different regions of the image. So it is suitable for enhance the local contrast of the image and edge of different objects of an image. Contrast Limited Adaptive Histogram Equalization is the type of the AHE. The CLAHE removes the over amplification problem. The CLAHE has three major parts: tiles generation, histogram equalization and bilinear interpolation. The input image is divided into small regions called as tiles. Histogram equalization is applied on each tile independently. Then CDF is calculated for





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histogram values. CDF values of each tile are scaled and mapped with input image pixel values. The resulting tiles are combined using bilinear interpolation, to generate output image with better contrast.

Recursive Mean Separate Histogram Equalization (RMSHE)

This method is used to deal with limitation of Brightness Preservation Bi Histogram Equalization (BBHE) method. This method provides not only better but scalable brightness. BBHE separates the input histogram into two separate images based on mean value and equalize separately. The RMSHE can make histogram recursively of separate image based on mean value for the enhancement of image. The recursive histogram nature of RMSHE method produces scalable brightness.

RESULTS AND DISCUSSION

Enhancement Measures

Author used 175 images to shows the performance of above histogram equalization techniques. The author selected some standard images to evaluate the performance of HE, BBHE, DSIHE and CLAHE. The performance is evaluated on the basis of Peak Signal to Noise Ratio (PSNR), Mean Square Error (MSE), Mean Absolute Error (MAE), and Enhancement Measurement Error (EME).

Peak Signal to Noise Ratio

For the Peak Signal to Noise Ratio assume input image is $f(x, y)$ which contains $M \times N$ pixels, and output image is $G(x, y)$.

First calculate the Mean Square Error (MSE).

$$MSE = \frac{\sum_{x=1}^M \sum_{y=1}^N |f(x, y) - G(x, y)|^2}{M \times N} \quad (9)$$

Now Peak Signal to Noise Ratio (PSNR) is

$$PSNR = 10 \log_{10} \frac{(L - 1)^2}{MSE} \quad (10)$$

PSNR is statistical measure used to calculate noise ratio between two images (Sim, K. S., Tso, C. P Et al 2007). The high value of PSNR indicate good quality image.

Mean Absolute Error

The Mean Absolute Error is the statistical measure of error between paired observations. The MAE is calculated as the sum of absolute error divide by sample size. The MAE is given by

$$MAE = \frac{\sum_{i=1}^n |y_i - x_i|}{n} \quad (11)$$

where y_i is prediction and x_i is measurement . x_i is true value.

Enhancement Measurement Error:

EME represent contrast scoring and provide clear information. High EME value indicates high contrast image. The image having K_1 , K_2 and K_3 blocks. EME is defined by





$$EMSE = \frac{1}{\sum_{k_1} \sum_{k_2} \sum_{k_3} \left[\frac{I_{ijk}^{\max}}{I_{ijk}^{\min}} \right]}$$

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where I_{\max} and I_{\min} represents the maximum and minimum intensity inside the block denoted by superscript ijk . The ratio between maximum and minimum is contrast ratio.

CONCLUSION

This paper presents comparative study of different histogram equalization methods for the enhancement of ancient inscriptions. The methods like HE, BBHE, DSIHE, CALHE and RMSHE are studied. HE, BBHE and DSIHE methods are not able to handle the brightness preservation. The RMSHE method handles brightness preservation very effectively. The error rate in RMSHE method is very low this leads to better PSNR value. The result is in table1 to table 5 shows the quality metrics value using the different methods. The RMSHE method has maximum PSNR values so this method is best suitable for the contrast enhancement of the ancient inscriptions. In future author use discrete wavelets transform to divide image into four parts. Later use RMSHE technique to enhance contrast.

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<https://in.mathworks.com/matlabcentral/fileexchange/74234-image-contrast-enhancement-by-he-techniques>

Table 1: Sample values of quality metrics using HE Method





| Image | PSNR_HE | EME_HE | MAE_HE |
|-------|---------|---------|--------|
| SI1 | 19.5254 | 156.028 | 6.4924 |

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| | | | |
|------|---------|----------|---------|
| SI3 | 15.4199 | 156.6846 | 20.5049 |
| SI4 | 16.9442 | 144.4252 | 14.0373 |
| SI5 | 16.7812 | 152.9778 | 13.0535 |
| SI6 | 16.7065 | 155.5535 | 30.0901 |
| SI7 | 13.7752 | 156.8848 | 31.5178 |
| SI8 | 14.7044 | 155.7243 | 23.4658 |
| SI9 | 15.0032 | 154.5589 | 28.9943 |
| SI10 | 11.4239 | 89.8159 | 0.0051 |

Table 2: Sample values of quality metrics using BBHE method

| Image | PSNR_BBHE | EME_BBHE | MAE_BBHE |
|-------|-----------|----------|----------|
| SI1 | 21.1442 | 151.3905 | 7.816 |
| SI2 | 13.922 | 156.1355 | 19.7592 |
| SI3 | 13.4364 | 153.1381 | 22.8468 |
| SI4 | 16.3157 | 114.8269 | 9.5379 |
| SI5 | 15.9794 | 138.985 | 9.6239 |
| SI6 | 18.7595 | 127.5247 | 19.5153 |
| SI7 | 14.4687 | 149.7366 | 22.189 |
| SI8 | 14.8806 | 124.427 | 19.3498 |
| SI9 | 16.0036 | 128.6799 | 19.3436 |
| SI10 | 15.6145 | 77.6156 | 0 |

Table 3: Sample values of quality metrics using DSIHE method

| Image | PSNR_DSIHE | EME_DSIHE | MAE_DSIHE |
|-------|------------|-----------|-----------|
| SI1 | 18.563 | 150.1262 | 4.1372 |
| SI2 | 13.9579 | 156.1355 | 24.0751 |
| SI3 | 13.4327 | 153.1381 | 23.9086 |
| SI4 | 16.8059 | 117.5583 | 14.398 |
| SI5 | 15.9794 | 142.098 | 15.1965 |
| SI6 | 16.9541 | 129.271 | 29.3472 |
| SI7 | 14.2495 | 150.688 | 25.9491 |
| SI8 | 14.8556 | 124.427 | 21.5884 |
| SI9 | 15.081 | 135.316 | 29.8233 |
| SI10 | 11.4597 | 72.2128 | 0 |

Table 4: Sample values of quality metrics using CLAHE method

| Image | PSNR_CLAHE | EME_CLAHE | MAE_CLAHE |
|-------|------------|-----------|-----------|
| SI1 | 16.6509 | 132.2789 | 12.7015 |
| SI2 | 15.3678 | 96.0838 | 18.6588 |
| SI3 | 15.9586 | 76.0214 | 18.4113 |
| SI4 | 15.2931 | 145.1176 | 15.7234 |
| SI5 | 15.5867 | 150.6843 | 14.3387 |





| | | | |
|---|---------|----------|---------|
| SI6 | 16.4384 | 130.3393 | 25.4611 |
| SI7 | 15.057 | 94.2035 | 26.3462 |
| SI8 | 15.4771 | 106.3641 | 20.3275 |
| Bapu D. Chendage and Rajivkumar S. Mente | | | |
| SI10 | 12.8619 | 110.4769 | 3.1396 |

Table 5. RMSHE method image with high contrast and respective histogram

| Image | PSNR_RMSHE | EME_RMSHE | MAE_RMSHE |
|-------|------------|-----------|-----------|
| SI1 | 19.3704 | 50.241 | 13.9991 |
| SI2 | 18.9063 | 44.5534 | 15.4573 |
| SI3 | 15.753 | 38.0222 | 12.9169 |
| SI4 | 17.6578 | 55.9919 | 30.2803 |
| SI5 | 15.0619 | 51.0813 | 10.2235 |
| SI6 | 18.0752 | 61.9133 | 12.1661 |
| SI7 | 18.2712 | 39.3379 | 14.9953 |
| SI8 | 19.0517 | 40.0693 | 9.1041 |
| SI9 | 18.4335 | 49.1906 | 14.1646 |
| SI10 | 10.4634 | 93.8479 | 0 |

Table 6. Advantages and Limitations of HE methods

| Sr. No. | Name of HE Method | Advantages | Limitations |
|---------|--|---|--|
| 1 | Histogram Equalization (HE) | Best enhancement technique based on Cumulative Density Function (CDF) | Dose not preserve the brightness, creates artifacts and less sharpness |
| 2 | Brightness Preservation Bi Histogram Equalization (BBHE) | It preserves the brightness of the image | The appearance of original image may change and produce different brightness respect to original image |
| 3 | Dualistic Sub Image Histogram Equalization(DSIHE) | The luminance of an image is effectively maintained | It totally change appearance of original image |
| 4 | Contrast Adaptive Limited Histogram Equalization (CALHE) | It is used to solve over amplification problem | Change the total contrast of image |
| 5 | Recursive Mean Separate Histogram Equalization (RMSHE) | It enhances all parts of the image by recursively constructing histogram. | The number of sub histogram increases rapidly(in power of two) |

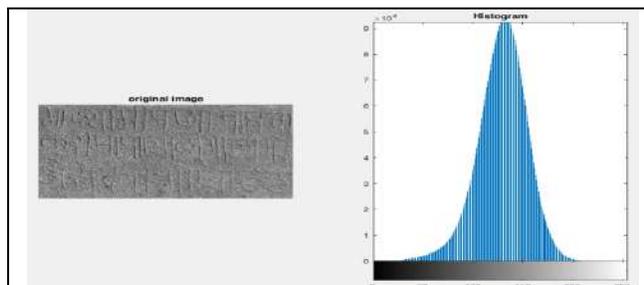


Figure 1: Input image with low contrast and respective histogram

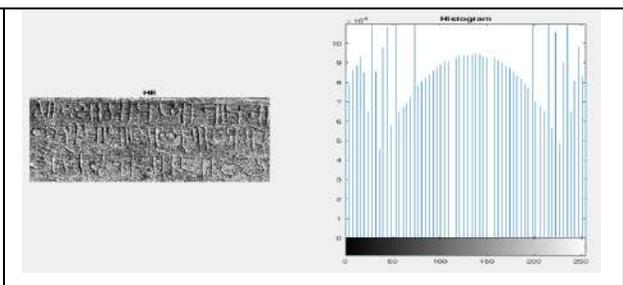


Figure 2: Result of HE method image with high contrast and respective histogram





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| | |
|--|--|
| | |
| <p style="text-align: center;">Figure 3: Process of BBHE</p> | <p style="text-align: center;">Figure 4: Result of BBHE method image with high contrast and respective histogram</p> |
| | |
| <p style="text-align: center;">Figure 5: Process of DSIHE</p> | <p style="text-align: center;">Figure 6: Result of DSIHE method image with high contrast and respective histogram</p> |
| | |
| <p style="text-align: center;">Figure 7: Result of CLAHE method image with high contrast and respective histogram</p> | <p style="text-align: center;">Figure 8: Result of RMSHE method image with high contrast and respective histogram</p> |





X-Linked Adrenoleukodystrophy (ALD): an Overview of Rare Neurological Disorder

Nithya Raju^{1*}, Aishwarya Vijay², Amrutha B Raja², Ashwini Murugan², Aswini Suresh²

¹Assistant Professor, Swamy Vivekanandha College of Pharmacy, Elayampalayam-637205, Namakkal District, Tamil Nādu, India.

²Vth Pharm.D Students, Swamy Vivekanandha College of Pharmacy, Elayampalayam - 637205, Namakkal District, Tamil Nadu, India.

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*Address for Correspondence

Nithya Raju

Assistant Professor,

Swamy Vivekanandha College of Pharmacy,

Elayampalayam-637205, Namakkal District,

Tamil Nādu, India.

E. Mail: nithyapharma14@gmail.com



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ABSTRACT

An uncommon type of irreversible degenerative neurological disorder called X-Linked Adrenoleukodystrophy (ALD) has an extensive spectrum of clinical presentations. Its distinguishing characteristics include gradual cerebral degeneration of the brain spinal cord axonal deterioration, and adrenal and testicular inadequacy. ALD evolved from a gene that encodes for the Adrenoleukodystrophy protein (ABCD1), which is related to the regulation of fatty acid metabolism. When the ABCD1 protein deteriorates, higher concentrations of very long-chain fatty acids can be detected in bloodstreams and tissues. Medical signs and laboratory evaluations, such as monitoring blood levels of very long chain fatty acids (VLCFAs) and anomalous magnetic resonance imaging (MRI), are used to assess ALD. Allogeneic Hematopoietic stem cell transplantation enables a reversal of disease progression for people with the chronic, cerebral form of ALD. This review's objective is to offer a succinct summary of the most recent data on ALD and to provide an overview of recent developments in both diagnosis and treatment.

Keywords: Adrenoleukodystrophy, ABCD1 Gene, Very long chain fatty acid, cerebral adrenoleukodystrophy, adrenomyeloneuropathy.





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INTRODUCTION

Testicles, the cortex of the adrenal gland, nerves in the peripheral regions, and the brain's nervous system are all impacted by the inherited X-linked illness called adrenoleukodystrophy.[1] The terms “adreno” denote the suprarenal gland, “leuko” means the substantia alba of the brain and “dystrophy” indicates irregular growth. This is the most prominent peroxisomal disease associated with the accumulation of unusually extended-chain fatty acids, that leads to destruction of tissues and white matter demyelination. [2,3,4] The first known instance of this condition was probably reported and coined by Michael Blaw in 1970[1,5,6]. ALD is a severe neurological condition, brought on by polymorphism in the ABCD1 gene, which codes for the X-chromosomal adrenoleukodystrophy (ALD). The human adrenoleukodystrophy protein represents a 745-amino-acid long ATP-binding sequence of protein. [7] The prevalence of adrenal leukodystrophy is estimated to be 1 in 10,000–17,000 people worldwide. Since there exists only one X chromosome in men, X-linked disorders are more prevalent in men and females have two X chromosomes, hence they are less likely to be affected. ALD can be passed to male offspring by female carriers of the disease who possess the faulty gene on one of their X chromosomes [2]. VLCFA plasma levels are measured for the diagnosis, allowing for the recognition of 100% of afflicted men and 80% to 95% of heterozygous women [8].

Based on the organ affected and the age of onset, four major subgroups of ALD are identified. They are;

- The Zellweger syndrome is linked to the recessive autosomal disorder known as the neonatal subtype.
- Childhood cerebral (cALD) form is brought on by demyelination of the brain's white matter. Patients often start showing symptoms from 4 to 10 years old, and their prognosis is bleak. Typically, patients pass away 6 to 24 months after diagnosis.
- Men in their mid-20s and older are most commonly affected by adrenomyeloneuropathy (AMN). Compared to cALD, it advances more slowly. Only 10–20% of guys have the variant that progresses quickly.
- Addison's disease, also known as adrenal insufficiency, appears between adolescence and adulthood. This portion of the spectrum is frequent in middle age and typically advances to AMN [2].

Epidemiology

All parts of the world experience it. ALD was once thought to be an X-linked recessive disease. Males exhibit with ALD in its most severe forms, although by the age of 60, 80% of females experiencing ALD, the manifestations of myelopathy are lower. [9,11] There is significant phenotypic variation, and male carriers are more severely affected than female carriers. Males and females are affected by X-ALD at a rate of 1:21,000 and 1:14,000, respectively. (10,11) According to published epidemiological reports, there are relatively more women than men. ALD is a relatively widespread genetic illness that impacts the peroxisomes. It is thought to affect 1 in 20,000 people. Patients who are Latino or African in descent have a greater illness incidence. [12] One in 50,000 people are affected by N-ALD. [13]

Etiology

The ABCD1 gene alteration is connected to the aetiology of X-ALD illness. (15,4) The very-long-chain fatty acids (VLCFAs) transmit pathway in the peroxisomes, where VLCFAs could be involved in further metabolic processes, is significantly influenced by the ABCD1 gene. Any mutation in the PTS1 receptor, PEX10, or PEX13 genes results in the neonatal type. (14,4) Depending on the afflicted organs and the patient's age during appearance, four primary subtypes of ALD have been identified:

- Neonatal
- Child brain development
- Adrenomyeloneuropathy
- Insufficiency of the adrenals.

Unknown is the mechanism by which an abnormality in the metabolism of VLCFAs results in a deadly neuroinflammatory response in the brain. According to one idea, VLCFAs cause demyelination by weakening the axonal myelin sheath. (4)





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Clinical Manifestations

The signs of adrenal leukodystrophy vary from person to person since it has varying expressivity. The milder form, adrenomyeloneuropathy (AMN), most often causes a person to become increasingly stiff and weak in their legs as they age, as well as sphincter problems, sexual dysfunction, and frequently impaired adrenocortical function. All of these symptoms worsen over time. Mania and psychosis can develop, despite the fact that higher motor neuron involvement and irregularities of gait are the most typical presenting signs. [2]

Based on the four main varieties of adrenoleukodystrophy, specifically:

Children’s cerebral type (cALD): The children in this category grow normally for the first few years, but in the early years of school, neurological symptoms like learning disabilities, seizures, newly developed behavioural issues, loss of speech, vision loss, deafness, and difficulty coordinating movement start to appear. [2,16,17]

Adrenomyeloneuropathy (AMN): The most typical variety is called adrenomyeloneuropathy (AMN). There are issues with the nervous system and the adrenals. It commonly manifests in early adulthood and includes clumsiness in the limbs, stiffness, weakness, discomfort in the hands and feet, muscular spasms, urinary issues, and erectile dysfunction. [2,18]

Adulthood cerebral type: Behavioural changes, memory problems, slurred speech, and cognitive impairments are all signs of AMN in men. [2,19]

Adrenal insufficiency type: Adrenal insufficiency is only adrenal deficit but no neurological issues. Between childhood and adulthood, symptoms appear diminished appetite, darker skin, low blood pressure, weakened muscles, and vomiting. Males can acquire any type of the disease, while roughly half of females with the ABCD1 mutation experience middle-aged onset of AMN symptoms. Females rarely develop cerebral types or adrenal insufficiency. [2,20] When it comes to psychiatric symptomatology, it can be challenging to pinpoint when personality or mood changes first appear; if they do, they are frequently attributed to other reasons. [2]

Clinical Aspects Of X-ALD Pathogenesis

The two primary clinical manifestations of X-ALD are inflammatory cerebral ALD and adrenomyeloneuropathy (AMN). [22,23,24] It is a slowly progressing axonopathy affecting the sensory ascending and motor descending spinal cord pathways with 100% penetrance in men and 65% in heterozygous women by the age of 60 years is one of the main clinical symptoms seen by X-ALD patients as they age.[21]

Cerebral Demyelinating Variant of X-ALD

The most severe manifestation of X-ALD is cerebral ALD. Between the ages of 5 and 12 years, there is a 35–40% chance that a newborn male with an ABCD1 gene mutation would get cerebral ALD. As it was already established, 20% of adult AMN males are also susceptible to cerebral ALD. The commencement of cerebral ALD and the rate at which the disease advances are correlated with age: the earlier cerebral demyelination begins, the faster the disease advances. Patients do not experience neurological symptoms until they exhibit cerebral demyelination on a brain MRI.[22,23,25,26]

Adrenomyeloneuropathy In Males And Heterozygous Female

The most prevalent ALD phenotype represents AMN. [22,23,24] The initial signs of AMN typically show in male patients between the ages of 20 and 30 but typically before the fifth decade, in newborn males with X-ALD, as determined by the alteration in the ABCD1 gene. By the age of 60, the actual prevalence of AMN in heterozygous female will likely be near to 65%. [27,28]

Biochemical Aspects Of X- ALD

Unbranched very long-chain fatty acids are degraded as a result of the consecutive reactions of the catalysts of the beta-oxidation pathway inside the peroxisomal matrix. Patients with XALD have accumulated levels of saturated





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VLCFA, particularly C26:0, which can be used as a diagnostic marker for the condition [23,31] The peroxisomal ATP-binding cassette is encoded by the ABCD1 gene. CoA-activated fatty acids, are valid substrates encoding the human ABCD1 protein, and cultured X-ALD fibroblasts effectively limit the peroxisomal beta-oxidation of these fatty acids [30,31,32] The peroxisomal membrane also harbors ABCD2 and ABCD3 in addition to ABCD1. [35]

Genetic Aspects of X-ALDs

Methods for directional sequencing were employed to identify the ABCD1 gene. [33] The peroxisomal transmembrane protein, which has 745 amino acid sequences and an overall ABC transporter framework, has been generated by the 19.9 kb long gene, which also includes 10 exons [34]. Three key studies highlight the lack of a widespread genotype-phenotype connection in X-ALD:

- All clinical manifestations within a single family can result from the same ABCD1 mutation.
- Complete deletion of the ABCD1 polypeptide.
- Patients with the full range of X-ALD symptoms have been found to have the often-occurring mutation (a deletion involving two bases in exon 5).

The ABCD1 gene has mutations, this shows that in every instance, this gene causes X-ALD to be hereditarily transmitted. This condition was passed down by an X-linked gene. [21,35,36]

Pathophysiology of X-ALD

The gene for ATP-binding channel subgroup D (ABCD 1), which is responsible for expressing the ABCD 1 transporter, resides on the peroxisomes. Such fatty acids are transported within the peroxisome using this protein from where they are further processed into smaller fatty acids. [2,3,37] Due to the accumulation of such fatty acids, the neurons in the central nervous system get demyelinated, the spinal cord develops axonopathy, and the adrenal glands are prone to become underactive. Additionally, it has been demonstrated that VLCFA sets off an inflammatory cascade that causes demyelination and neurodegeneration. [2,38]

Mechanism of free radical damage and inflammation of adrenoleukodystrophy

Genetic modifications in the transmembrane protein, which resides on the peroxisome will prevent the transport of these fatty acids to the peroxisome for oxidation, cause VLCFA to accumulate *in vivo* (Fig 1). In addition to increasing the amount of Radicals and reactive oxygen species in the mitochondria, cumulative VLCFA and malfunctioning peroxisomes also interfere with the GSK3- antioxidant pathway, leading to redox dysfunction and activating the pathway associated with NF- κ B to cause aggravation. [39]

Childhood cerebral (cALD)

Childhood X-linked ALD (cALD) is characterised by accelerated neurodegeneration and early mortality. VLCFA levels have been reported to be higher in cALD than in AMN, which causes oxidative stress and fast axon degeneration (Fig 2). The blood-brain barrier may be impacted by these VLCFAs, allowing macrophages to pass through more easily and release cytokines and chemokines to prolong the inflammatory cascade. These substances have been connected to the peroxisome's malfunction. In cALD, the demyelination extends from the central corpus callosum to the parietal occipital white matter. Thus, gene polymorphism in the pathway leads to VLCFA accumulation. [2,3,21]

Diagnostic Findings

To diagnose X-ALD in the laboratory, elevated VLCFA levels and a gene changes examination are necessary. [40]

Blood Test

Determining the amount of an extremely long-chain fatty acid: A plasma test is conducted to determine the concentration of VLCFAs, which are typically higher in males with ALD and three VLCFA parameters are used in the assay. [2,41,42]





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Genetic Testing

To identify the ABCD1 mutations: Genomic screening must be carried out to validate the detection of ALD in cases of increased VLCFA levels or VLCFA ratios that are below normal. Large removals (3%), frame alterations (24%), amino acid sequence insertions/deletions (6%), and accidental mutations (12%) results in truncated peptides, which prevent ABCD1 from being detectable. [41]

Newborn Screening

To do this, a Tandem mass spectrometer is used to gauge the amount of VLCFA present in the dried blood spots. [2]

MRI Scan (Magnetic Resonance Imaging)

The most effective imaging technology is MRI approach for testing people with X-linked Adrenoleukodystrophy. Several intrinsic features, including proton density, T1 and T2 tranquilly, macromolecular form, and freed fluid transport properties, and metabolic composition, can distinguish between normal and aberrant brain in ALD. [24]

Regarding anatomical sites, MRI progression, five MRI patterns of ALD were described they are;

1. In depth of white matter can be found in the corpora callose's splenium and parieto-occipital regions. (mostly in children).
2. Genus of the colossal commissure found in adolescents.
3. Corticospinal projection fibres are 12% found in adulthood.
4. Arbor vitae is 1% seen in adults.
5. Combined subcortical 2.5% noticed in youngsters. [2,43]

Treatment

A multidisciplinary team including experts in eye disease, hearing science, genealogy, hormonal disorders, and neuroscience, psychiatry, reinsertion, and Hospice care is required for the management of ALD. There is no causative therapy for X-ALD. Nevertheless, a common therapeutic approach has been evolved over the past ten years and, depending on the clinical condition, a number of analyses endure unreliable results about ALD therapy. Currently, treatments for X-ALD include dietary supplements utilising Lorenzo's oil (LO), hormone substitution therapy, as well as additional methods. Prior to the emergence of neurological problems, adrenal insufficiency frequently shows early signs. It is a frequent cause of morbidity and occurs frequently in otherwise asymptomatic males with ALD. By carefully monitoring these boys and acting quickly, this condition can be avoided. While mineralocorticoid replacement is not always required, the daily needs for glucocorticoids, varying from 10 to 40 mg, are identical as those for other forms of adrenal insufficiency. The dose needs to be modified to match the greater demand when illness strike. (47,56,57)

Cerebral Adrenoleukodystrophy

Patients with early ALD should have HSCT as their preferred treatment. If carried out during the disease's initial phases, allogenic HSCT provides numerous advantages with decreased transplant-related mortality. [63] Males without cerebral involvement on brain MRIs are not advised to undergo HSCT considering that 50% of them are anticipated to remain without illness. [44] However, such therapy fails to cure myeloneuropathy, avoid adrenal involvement, or reverse neurological abnormalities. [45] A diminished conditioning strategy that utilised fludarabine, melphalan, and minimal dose entire-body radiation produced the best outcomes. [47] Advanced cerebral adrenoleukodystrophy (cALD) patients are managed with supportive care because HSCT has not been proven to be beneficial

Adrenomyelopathy

The current approach to treating AMN is mostly supportive and focuses on treating urinary disarray, incontinence, stiffness, and discomfort (pregabalin, gabapentin), as well as restoration treatment. According to one study, functional electrical stimulation can help myeloneuropathy patients walk more easily. (44,46) The benefits of HSCT in AMN alone have not been demonstrated, and It might worsen myeloneuropathy manifestations. (45,47) While



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age-matched untreated mice experienced severe neurological decline, intravenous injection of the gene encoded in an adeno-associated virus (AV) genotype 9 vector resulted in a close to average motor function over a period of twenty-four months. Intrathecal injection of rAAV9 vector in the spinal cord of primates to express ALDP in large amounts. (46,47)

Adrenal Insufficiency Type

ALD patients receive the same treatment for primary adrenal insufficiency as those with other causes. (48,54,55) The chosen glucocorticoid is hydrocortisone since it is converted to cortisol and has the least impact on the epiphyses during childhood. (51,54) Three doses of 8–12 mg/m²/day of hydrocortisone, with the morning dose being the most potent, create the median beginning treatment. (48,49,50,54) Mineralocorticoid deficit in glucocorticoid-deficient patients should be checked every six months, and as clinically necessary, fludrocortisone and salt supplements should be given. (54) Patients and caretakers need to be informed on what to do in the event of sickness or other severe physical strain are verbally and in writing on how to provide hydrocortisone doses for stress. If oral hydrocortisone cannot be taken in an emergency, administering a subcutaneous injection of hydrocortisone sucrose 50-100 mg/m²/dose is recommended. Additionally, patients must be told to put on identifying that states they have adrenal insufficiency and are steroid-dependent. (49,54) Adrenal crises can be avoided by stress-dosing protocols during sedation and general anaesthesia. (52,53,54)

Gene Therapy

X-linked Adrenoleukodystrophy CD34+ immune cells with movement in blood from the peripheral area were transduced, which showed the correction of VLCFA levels in the cells. Extended production of adrenoleukodystrophy amino acid in monoblast and mast cells produced from embryonic stem cells were produced after ALD cells expressing CD34 were transduced. Stem cells engrafted and producing monocytes and macrophages. (56,47) It has also been demonstrated that after being infused into immunodeficient mice Mammalian CD34+ cells travel into the central nervous system from blood from the peripheral arteries, where they mature into perivascular, ramified microglia. Since lentiviral transfer of genes doesn't impact the maturation of human CD34+ lymphocytes into microglia, which persons CD34+-derived microglial would likely be driven to the sites of brain damage. (57,47) Future treatments for X-ALD and other inherited CNS illnesses may involve transplanting these transduced cells. (47)

Supportive Care

A Cross-dimensional system tailored to the extent of the condition is required for treatment after the diagnosis of X-ALD. Neurologists and neuropediatric keep an eye on their patients and administer medications. Physical therapists are frequently needed, while dieticians and speech therapists can offer assistance with employment and loss of bulbar muscle function. The effectiveness of sildenafil in treating individuals with spinal cord injuries has been demonstrated, and it may be an option for people with AMN. (62) Due to the high frequency of behavioural abnormalities in AMN patients with cerebral involvement, psychiatric consultation is frequently necessary. (58,63)

Dietary Therapy Of Adrenoleukodystrophy

In individuals with adrenoleukodystrophy, food constraint of C26:0 have not proven successful in reducing serum concentrations of these fatty acids. (58,59) Contrarily, restricting phytanic acid may have demonstrated to help alleviate Refuse sickness by lowering plasma phytanate concentration. It was proposed that dietary oleic acid supplementation and C26:0 restriction could reduce plasma levels of very long chain fatty acids. (58,60) Currently being studied is how combined nutrition therapy affects neurological function. However, it was recently discovered that after 355 days on the diet, a 21-year-old patient with adrenomyeloneuropathy and a 46-year-old lady heterozygous for adrenoleukodystrophy both showed improved peripheral nerve function testing. (58,61)



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DISCUSSION

Adrenoleukodystrophy was characterized according to ongoing necrosis of the central and the peripheral nervous systems and insufficient adrenal function, in response to the accumulation of extremely lengthy chain fatty acids (VLCFA) in the adrenal. The determination regarding VLCFA plasma levels serves as the basis for the diagnosis. Fatty acid transporter ALDP is dysfunctional, which impairs VLCFA oxidation and causes excessive VLCFA build-up in tissues.

CONCLUSION

Leukodystrophy, a rare class of diseases, including a condition known as adrenoleukodystrophy (ALD). It's a genetic condition connected to ABCD1. DNA mutations in both men and women. Adrenoleukodystrophy is largely a white matter neurodegenerative. Because it is difficult and unambiguous to make a clinical diagnosis of this uncommon condition, blood testing and imaging studies continue to be the most practical methods for doing so. Although there is no cure, supportive care is nevertheless important while the condition is present.

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Conflict of Interest

The authors declare that there is no conflict of interest

Abbreviation

X-ALD: X-Linked Adrenoleukodystrophy, VLCFAs: Very Long Chain Fatty Acids, MRI: Magnetic Resonance Imaging, ALDP: Adrenoleukodystrophy Protein, AMN: Adrenomyeloneuropathy, cALD: Childhood cerebral Adrenoleukodystrophy, N-ALD: Neonatal Adrenoleukodystrophy, GSK3: Glycogen synthase kinase 3, NRF2: Nuclear factor erythroid 2-related factor 2, NF- κ B: Nuclear factor kappa B, HSCT: Haematopoietic Stem Cell Transplant, rAAV9: Adeno-Associated Virus Serotype 9, LO: Lorenzo's oil.

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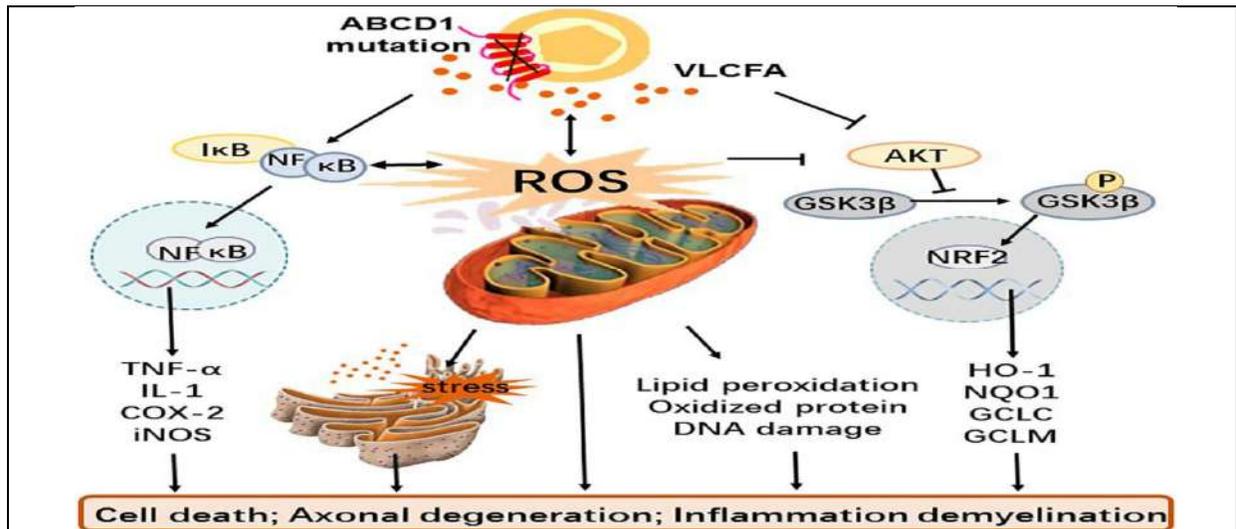
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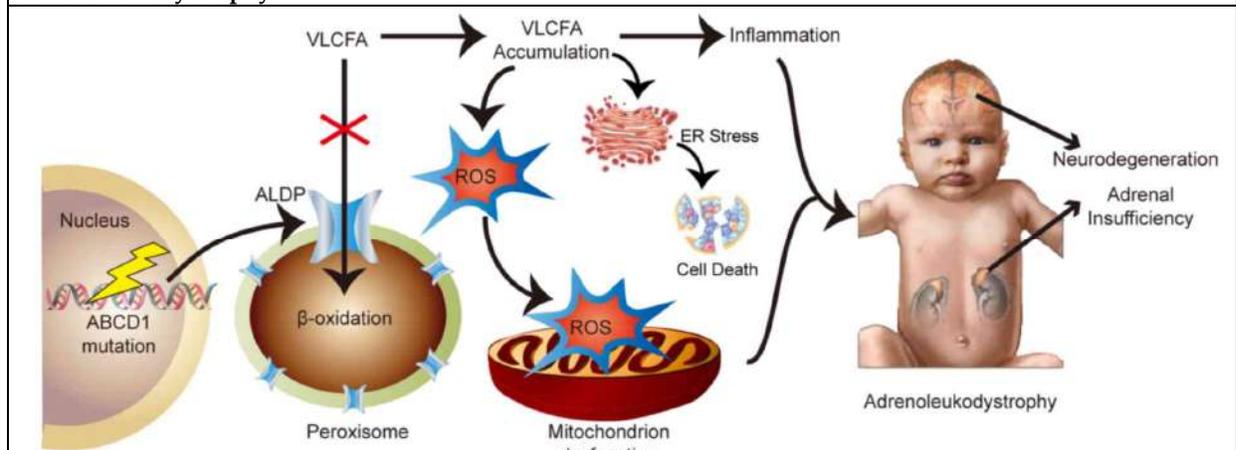


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Source: Yu J, Chen T, Guo X, Zafar MI, Li H, Wang Z, Zheng J. The role of oxidative stress and inflammation in X-link adrenoleukodystrophy. *Frontiers in Nutrition*. 2022;9.

Fig 1: Schematic representation of mechanism of oxidative stress and inflammation of x-linked adrenoleukodystrophy.



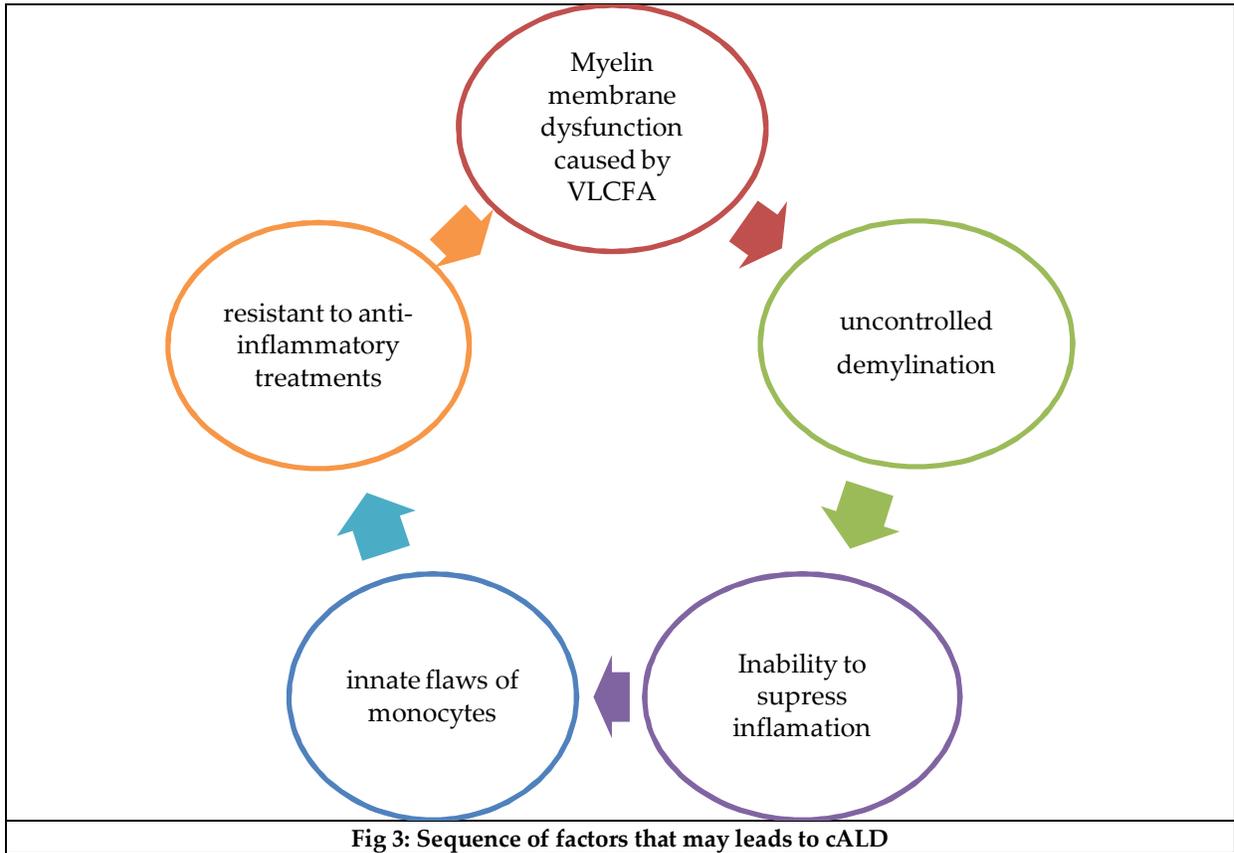
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Fig 2: Schematic representation of pathogenesis of cALD





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Mitigation of Voltage Sag and Swell in Smart Grid by using Ant-Lion Intelligent Controller

S. Bharathi^{1*}, D. Chandra Sekhar¹, M. Dilip Kumar², V Usha Rani³ and P. Sarala⁴

¹Assistant Professor, Department of EEE, Malla Reddy Engineering College (Autonomous), Secunderabad, Telangana, India.

²Associate Professor, Department of EEE, St. Peters Engineering College (Autonomous), Secunderabad, Telangana, India.

³Assistant Professor, Department of EEE, Nadimpalli Satyanarayana Raju Institute of Technology (Autonomous), Visakhapatnam, Andhra Pradesh, India

⁴Associate Professor, Department of EEE, Malla Reddy Engineering College (Autonomous), Secunderabad, Telangana, India.

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*Address for Correspondence

S. Bharathi¹

Assistant Professor, Department of EEE,
Malla Reddy Engineering College (Autonomous),
Secunderabad, Telangana, India.

E.Mail: dcsekhar@mrec.ac.in



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ABSTRACT

In the operational condition of an electrical power system, the need for proper utilization with quality of utilization is primal. Where different types of quality measures are deployed such as the linear filters and adaptive filters to condition the current quality, a power flow controller is deployed to compensate for the dissipation losses or fault tolerance. Where efforts are made in enhancing power quality, efforts are also made in the utilization of it. With the rapid and ever-increasing demand for power supply and rapid increases in industrial and urbanization, the demand has exceeded the supply capacity of all generation systems. To compensate for the demanded power requirement, in addition to the existing power generation units, additional subunits are added to the power system to compensate for the demanded supply. Smart grids are designed as a cluster of various generation units and consumption units. The demanded power is processed in these smart grids and using a processing algorithm, these grids play a crucial part in adjusting the power supply allocation to compensate for it. Here, the grid systems are either designed for a concentric parameter or multi-objective monitoring in making a decision. The issue is with the complexity in the parameter validation, where multi-objective monitoring gives the benefit of accurate scheduling, the complexity in parameter monitoring is higher. The main objective of the proposed is weight-defined parameter monitoring of power scheduling in multi-parameter monitoring, where the past approach of a preference-based scheduler is to be developed with different intelligent controller techniques like UNITED POWER FLOW CONTROLLER (UPFC) with ANT-LION



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optimization (ALO) algorithm is proposed and compared with ANFIS, Adaptive FLC, Reduced-order FL, FOPI, and FOFL. The PQ issue in the system is helped by the UPFC device. A shunt active power filter is used in series with an Artificial Neural Network (ANN) with an ALO-based controller to improve UPFC performance by allaying current and voltage power quality (PQ) concerns. It proved that our proposed system is best in smart grid applications using MATLAB/Simulink.

Keywords: UPFC, ALO, ANFIS, Adaptive FLC, Reduced-order FL, FOPI and FOFL Smart Grid, MATLAB/Simulink.

INTRODUCTION

There are no dispensing devices, nonlinear loads, or utilities employed as compensation in the electrical power system. This complicated structure guides the power system toward an unsteady energy source [1]. Complicated energy systems known as "smart grids" (SGs) utilized two-way communication between distributed generation (DG), control systems, and loads to improve voltage stability, create the good utilization of RES, self-repair systems when a problem arises, and provide customers with the chance to control their electricity usage and save funds on maintenance cost [2]. Regardless of the complexity of most attacks, the Energy station represents a unique confrontational goal in the smart grid. [3]. Series and shunt compensators are combined in UPQC. Upgraded maximum frequencies, dynamic reactive and active energy control, voltage sag, voltage disbalance, voltage flicker, and voltage are all outcomes of its use [4]. Even from the end perspective, the user's PV technology has become the most enticing. Due to its dependability and simplicity, applications. However, scheme intricacies like load variations and parameter modifications may prevent PI/PID control from functioning normally even when it is perfectly aligned [6]. As an outcome of the power system's unsteady or disruption of synchronization, Due to a lack of dissipative torque, these vibrations become more intense. The major scheduling approach is defined as time-based operational, or demand based. In these systems, the power scheduling is operated based on the measurement of demanded power and the available generation [8-10]. Where advanced algorithms were deployed for proper decision-making, all the existing approaches were based on discrete parameter monitoring. Here, the characteristic of the demand variation is not been considered. Longer periodic observation of the demand may lead to a more accurate switching performance of the generation units.

The Foremost Contribution of the Proposed Methodology

- UPFC with ANT-LION optimization (ALO) algorithm is proposed and compared with ANFIS, Adaptive FLC, Reduced-order FL, FOPI, and FOFL to develop a weight-specified parameter tracking of power planning in multi-parametric monitoring, where the past method of priority scheduler is to be established.
- UPFC performance can be improved by easing concerns about current and voltage PQ by using an ANN with an ALO-based controller.
- To use and schedule the virtual generation plants in demand-supply, a hybrid system of virtual power units must be integrated and scheduled, from which a balance relation between generation and demand must be derived. Utilizing the MATLAB/Simulink environment, every parameter must be verified.

LITERATURE SURVEY

In this section, a few relevant works on intelligent controllers for smart grid performance are examined. Rahbari, O, et.al., have offered a flexible control system that makes the smartest grids, electric cars with plugs, and renewable energy sources. This study suggests a workable solution to the issues associated with integrating renewable energies & into the electrical grid for electric vehicles, considering source generation unpredictability & uneven energy use, by using a new adaptive intelligent controller. It is suggested to use an innovative hybrid control





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strategy based on the optimum power flow issue and just a neuro-fuzzy adaptive inference system with particle swarm optimization resulting in a rise in the voltage's total harmonic distortion rather regularly. Irfan, M. et.al., have proposed, Possibilities and difficulties in managing smart grids from a Pakistani perspective. This paper provides a thorough analysis of developments in smart grid control. The methodologies are highlighted by the smart grid. Pakistan must make use of its natural resources, including wind, hydropower, and solar energy. To reduce future energy shortages, urgent action is required. Bayindir, R. et.al., have suggested Applications for smart grid technology. This paper analyzed data transmission methods, smart grid applications, and technological energy efficiency in smart grids. Scientists and engineers investigating the smart grid are expected to find this study to be a key source of direction. Derakhshan, G. et.al., have discussed improving demand response programs in smart grids. The following four types of residential consumers were surveyed for this study, which was conducted on some residential houses in Tehran, Iran, during the summer: When payment costs for various periods are reduced, our optimization model employs the Shuffled Frog Leaping (SFL) algorithms & Teaching & Learning Based Optimization (TLBO) to plan smart grid utilization.

He, Y., et.al., have proposed, using an intelligent mechanism based on deep learning, the smart grid can identify fake information injection attacks in real-time. In this study, FDI assaults' behavioral characteristics are detected using deep learning methods. historical measurement data is employed, and the behavioral features are then used for real-time FDI attack identification. Keshtkar, A. et.al, have proposed wireless sensors, smart grids, and fuzzy logic for smart home load reduction. In order to reduce the load on HVAC systems in houses, this study offers a Fuzzy Logic Technique (FLA) that provides wireless sensors and smart grid incentives. A PCT that can handle TOU & RTP is being constructed in MATLAB/GUI to mimic a real thermostat. It functions as a "simulator engine" to assess FLA's effectiveness in various contexts. The main objective of energy systems and smart grids is the integration of household HVAC technologies in the context.

PROPOSED METHODOLOGY

The system's load demand increases as a result of the increased utility use brought on by urbanization and industrialization. Regarding protection and energy reliability and performance troubles, integrated approach sources are unable to satisfy the required energy needs in today's modern power grid. To resolve these issues, dispersed energy resources and RES have been utilized. The most cutting-edge technology currently in use, the RES system, can boost system steadiness and effectiveness. Among the most practical remedies for PQ problems like voltage sag, sale, and fluctuation is the FACT system. Therefore, UPFC is employed in the RES in this paper to address these power quality issues. A PV system that is linked to the smart grid makes up the suggested RES. The RES is linked to a smart grid, which results in issues with power quality due to nonlinear loads, critical loads, and unexpected loads. This results in a problem with reactive power mismatch and more voltage instability. The UPFC may be the best tool for dealing with power quality issues and enhancing voltage regulation in RES linked to the smart grid. The modeling approach is depicted in Figure. 1 and includes batteries and photovoltaics linked to a smart grid. To meet the needs of the customer, RES is used. The battery energy storage system is utilized to distribute essential electricity and store excess power generated by solar panels. Power quality issues in RES systems connected to the smart grid are the primary concern in maintaining stability and dependability conditions. To handle PQ difficulties including sag, swell, and fluctuation, a UPFC is constructed using a smart grid-connected RES system.

Method for storing energy in batteries

Systems that store energy in batteries are utilized to meet load demands when the RES power supply is insufficient.

$$b^*_p = p^*_{PV}(t) + p^*_{WT}(t) - \frac{P^*_I(t)}{\epsilon^*_i} \text{----- (1)}$$

$P^*_I(t)$ = Demand for system load,

b^*_p = Battery power.



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An important battery feature known as the State of Charge (SOC) is linked to RES's inability to produce enough energy and excessive power production as shown in equation 1.

PV cell analogous circuit:

The quantity of solar panels interconnected in a Photovoltaic system in series and parallel determines the voltage, current, open-circuit voltage & short-circuit current.

DC-DC converter analogous circuit:

Figure 3 shows a circuit equivalent to a DC-DC boost converter. Switch 1 is initially closed, while Switch 2 is initially in the open position. Inductor L's (I_L) current is now starting to rise from zero. The inductor current flows to the load at this point while the capacitor stores the charges, & switch-two is closed and switch one is opened. When the output voltage is stable, the ON-OFF states of switches 1 and 2 fairly represent the contingent excessive value of the output voltage.current.

Load(smart grid, AC load):

The three-phase electric power system is the industry standard for generating, distributing, and transmitting alternating current. It is a type of polyphase system, and in most cases, the most widely used way to transfer power in the world's electrical grids. It is also possible to use to power large motors and other heavy loads. Results and discussions

Case 1: Mitigation of Sag Signal

The voltage sag is to be corrected in order to ensure linear and consistent system operation. By providing the necessary voltage with the help of UPFC, to meet the needs of the load, the voltage is rectified. The voltage from the source, injection voltage, and load voltage are all shown in a sag analysis in Figure 4.

ANFIS is contrasted with the modified sag signal produced by the proposed method. Signals that are already in use include Adaptive FLC, Reduced order FL, FOPI, and FOFL. The suggested method corrects the sag signal in a time window of 0.15 to 0.3 seconds. Regarding sag correction, the suggested method performs better than convolutional alternatives.

Case 2: Mitigation of Swell Signal

The performance of the suggested method is estimated using the swell condition. By combining the flaw with the source, the swell condition is introduced into the system. Examining the swell state, varying the sources, and rating the performances. Additionally, the injected voltage for the swell signal is compensated using the ANTLIN built on the UPFC, and the compensated load voltage is ultimately reached at 440 V. The swell signal is decreased in the suggested method over a time interval of 0.15 to 0.3 sec. In comparison to other techniques, the effectiveness of voltage swell alleviation as can be observed from this graph, the proposed method is better than ANFIS, Adaptive FLC, Reduced order FL, FOPI, and FOFL. In comparison to reduced order FL, Adaptive FLC, and ANFIS, the swell signal is greatly diminished.

CONCLUSIONS

This paper described the improvement of PQ carried out by the proper design of control techniques in a grid-connected RES with the load. The RES's irregularity causes PQ problems, which the ANT-LION optimization algorithm methodology attempts to address. The simulation results are used to analyze the various PQ troubles, such as voltage sag and harmonics, to show how superior the proposed work is. The proposed work's mitigation level is greater than that of the current algorithms. The percentage of THD, which is 2.07%, is also examined and contrasted with the THDs of traditional algorithms. In comparison to traditional algorithms, the suggested technique's UPFC with ANT-LION optimization algorithm is more effective.

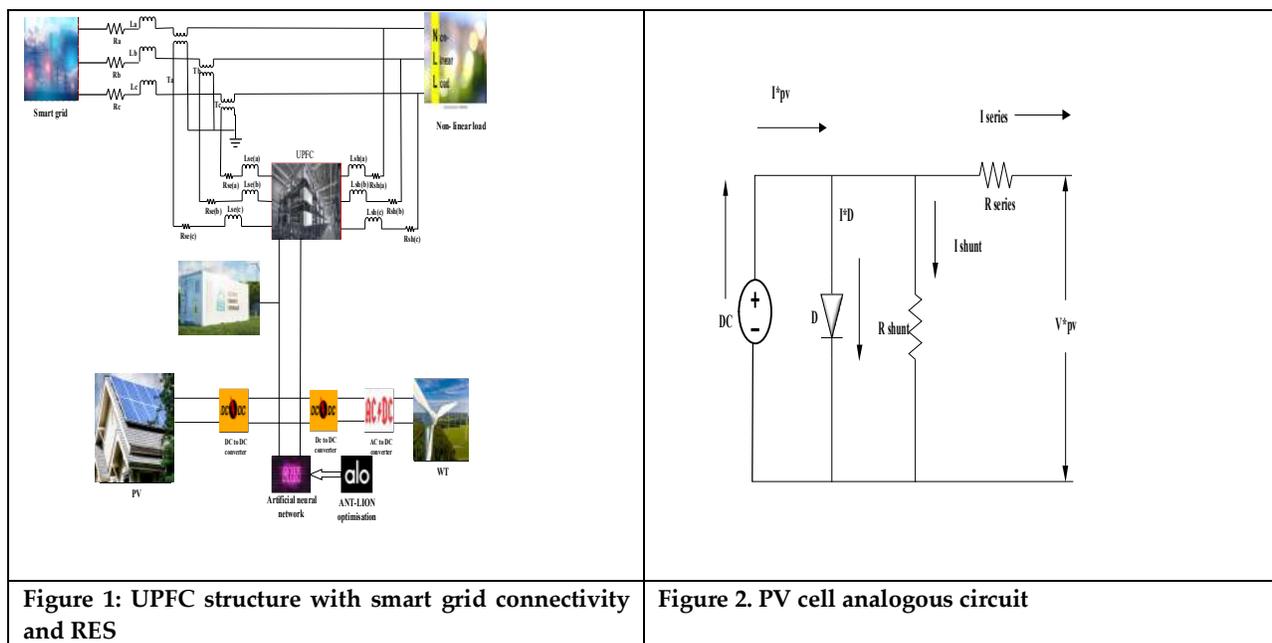




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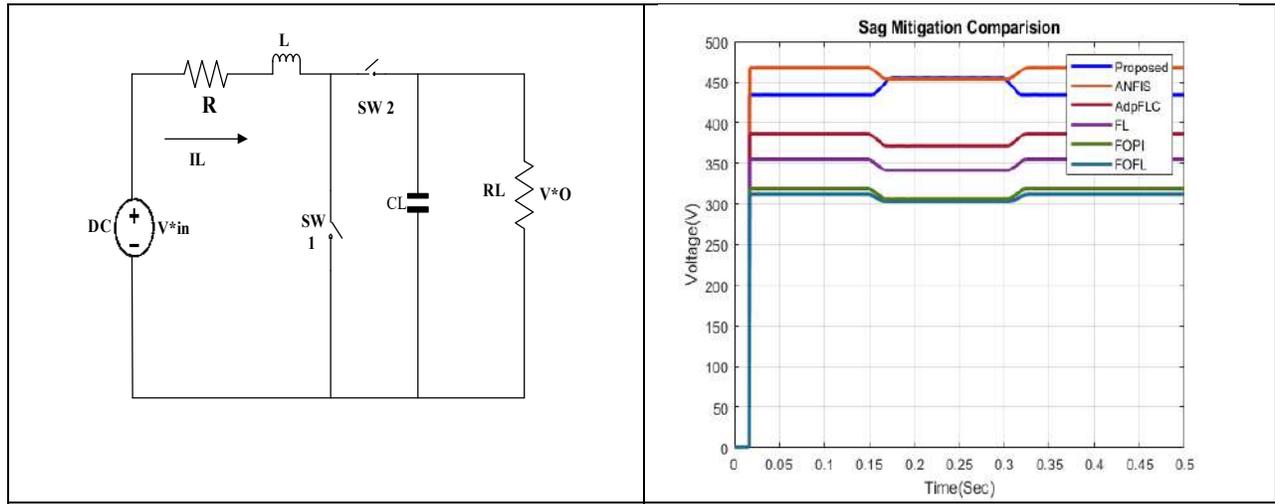


Figure 3: DC-DC converter analogous circuit

Figure 4: Comparison of sag mitigation

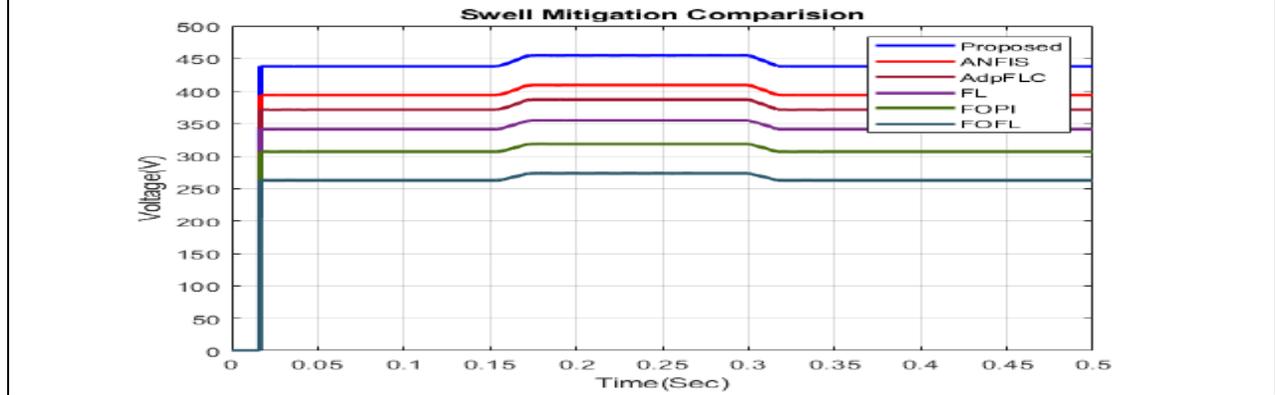


Figure 5: Comparison of Swell mitigation





New Generalized \hat{g} -Closed Functions in an Intuitionistic Fuzzy Topological Space

P.Deepika¹ and M. Rameshpandi^{2*}

¹Research Scholar, Department of Mathematics, P.M.T. College, Usilampatti, Madurai District, Tamil Nadu, India .

²Associate Profeseor, Department of Mathematics, P.M.T. College, Usilampatti, Madurai District, Tamil Nadu, India.

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*Address for Correspondence

M. Rameshpandi

Associate Profeseor,

Department of Mathematics,

P.M.T. College, Usilampatti,

Madurai District,

Tamil Nadu, India.

E.Mail: proframesh9@gmail.com



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ABSTRACT

Main aim of this paper, we introduce the new notions of intuitionistic fuzzy generalized closed functions called an intuitionistic fuzzy \hat{g} -continuous functions and intuitionistic fuzzy $i-\hat{g}$ closed functions in intuitionistic fuzzy topological spaces. Further more, we discuss some of their properties and characterizations. Several examples are given to illustrate the statements.

2010 Mathematics Subject Classification, 54A40, 03F55.

Key words and phrases : Intuitionistic fuzzy functions, Intuitionistic fuzzy \hat{g} -continuous functions and Intuitionistic fuzzy $i-\hat{g}$ closed functions

INTRODUCTION

Concept of fuzzy set by Zadeh [21] and fuzzy topology by Chang [4] , several researchers were conducted on the generalizations of the notions of fuzzy sets and fuzzy topology. The concept of fuzzy sets was introduced by Atanassov [1] as a generalization of fuzzy sets. Coker [3] introduced intuitionistic fuzzy topological spaces using the notion of intuitionistic fuzzy sets. Intuitionistic fuzzy closed function was introduced by Jeon, Jun and Park [9]. Rajarajeswari and Krishna Moorthy [10] introduced the notion of intuitionistic fuzzy weakly generalized closed





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functions and studied its properties. In [5], we introduced intuitionistic fuzzy \hat{g} -closed sets. In this paper, we introduce the new notions of intuitionistic fuzzy generalized closed functions called an intuitionistic fuzzy \hat{g} -continuous functions and intuitionistic fuzzy $i\hat{g}$ closed functions in intuitionistic fuzzy topological spaces. Further more, we discuss some of their properties. Several examples are given to illustrate the statements.

Preliminaries

We given some definitions and note some fundamental results necessary for our present study in an intuitionistic fuzzy topological space.

Definition 2.1 [1] Let X be a non empty set. An intuitionistic fuzzy set (IFS in short) A in X can be described in the form

$$A = \{(x, \mu_A(x), \nu_A(x)) \mid x \in X\}$$

where the function $\mu_A : X \rightarrow [0, 1]$ is called the membership function and $\mu_A(x)$ denotes the degree to which $x \in A$ and the function $\nu_A : X \rightarrow [0, 1]$ is called the non-membership function and $\nu_A(x)$ denotes the degree to which $x \notin A$ and $0 \leq \mu_A(x) + \nu_A(x) \leq 1$ for each $x \in X$.

Denote IFS(X), the set of all intuitionistic fuzzy sets in X

Definition 2.2 [1] The intuitionistic fuzzy sets $0 \sim = \{(x, 0, 1) \mid x \in X\}$ and $1 \sim = \{(x, 1, 0) \mid x \in X\}$ are called the empty set and the whole set of X respectively.

Definition 2.3 [3] An intuitionistic fuzzy topology (IFT in short) on X is a family τ of IFSs in X satisfying the following axioms :

1. $0 \sim, 1 \sim \in \tau$,
2. $G_1 \cap G_2 \in \tau$ for any $G_1, G_2 \in \tau$,
3. $\cup G_i \in \tau$ for any family $\{G_i \mid i \in J\} \subseteq \tau$.

In this case the pair (X, τ) is called an intuitionistic fuzzy topological space (IFTS in short) and any IFS in τ is known as an intuitionistic fuzzy open set (IFOS in short) in X .

The complement A^c of an IFOS A in an IFTS (X, τ) is called an intuitionistic fuzzy closed set (IFCS in short) in X . Denote $\text{IFO}(X)$, the set of all intuitionistic fuzzy open sets of X .

Definition 2.4 An IFS A in an IFTS (X, τ) is said to be an

1. intuitionistic fuzzy regular closed set (IFRCS in short) if $A = \text{cl}(\text{int}(A))$, [7]
2. intuitionistic fuzzy semi closed set (IFSCS in short) if $\text{int}(\text{cl}(A)) \subseteq A$, [2]
3. intuitionistic fuzzy α -closed set (IF α CS in short) if $\text{cl}(\text{int}(\text{cl}(A))) \subseteq A$, [8]
4. intuitionistic fuzzy semi pre closed set (IFSPCS in short) if $\text{int}(\text{cl}(\text{int}(A))) \subseteq A$. [20]

Definition 2.5 An IFS A in an IFTS (X, τ) is said to be an

1. intuitionistic fuzzy regular open set (IFROS in short) if $A = \text{int}(\text{cl}(A))$, [7]
2. intuitionistic fuzzy semi open set (IFSOS in short) if $A \subseteq \text{cl}(\text{int}(A))$, [7]
3. intuitionistic fuzzy α -open set (IF α OS in short) if $A \subseteq \text{int}(\text{cl}(\text{int}(A)))$, [8]
4. intuitionistic fuzzy semi pre open set (IFSPPOS in short) if $A \subseteq \text{cl}(\text{int}(\text{cl}(A)))$. [20]

Definition 2.6 [15] Let A be an IFS in an IFTS (X, τ) . Then the α -interior of A ($\alpha\text{int}(A)$ in short) and the α -closure of A ($\alpha\text{cl}(A)$ in short) are defined as $\alpha\text{int}(A) = \cup \{G \mid G \text{ is an IF}\alpha\text{OS in } (X, \tau) \text{ and } G \subseteq A\}$, $\alpha\text{cl}(A) = \cap \{K \mid K \text{ is an IF}\alpha\text{CS in } (X, \tau) \text{ and } A \subseteq K\}$. $\text{sint}(A)$, $\text{scl}(A)$, $\text{spint}(A)$ and $\text{spcl}(A)$ are similarly defined. For any IFS A in (X, τ) , we have $\alpha\text{cl}(A^c) = (\alpha\text{int}(A))^c$ and $\alpha\text{int}(A^c) = (\alpha\text{cl}(A))^c$.





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Definition 2.7 An IFS A in (X, τ) is said to be an

1. intuitionistic fuzzy generalized closed set (IFGCS in short) if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is an IFOS in (X, τ) , [18]
2. intuitionistic fuzzy generalized semi closed set (IFGSCS in short) if $\text{scl}(A) \subseteq U$ whenever $A \subseteq U$ and U is an IFOS in (X, τ) , [14]
3. intuitionistic fuzzy semi generalized closed set (IFSGCS in short) if $\text{scl}(A) \subseteq U$ whenever $A \subseteq U$ and U is an IFOS in (X, τ) , [17]
4. intuitionistic fuzzy generalized semi pre closed set (IFGSPCS in short) if $\text{spcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is an IFOS in (X, τ) , [13]
5. intuitionistic fuzzy α generalized closed set (IF α GCS in short) if $\alpha\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is an IFOS in (X, τ) . [15]

The complements of the above mentioned intuitionistic fuzzy closed sets are called their respective intuitionistic fuzzy open sets.

Denote $\text{IFSGO}(X)$, the set of all intuitionistic semi-generalized open sets of X .

Definition 2.8 Let f be a function from an IFTS (X, τ) into an IFTS (Y, σ) . Then f is said to be an

1. intuitionistic fuzzy closed function (IF closed function in short) if $f(A)$ is an IFCS in (Y, σ) for every IFCS A of (X, τ) [9],
2. intuitionistic fuzzy α -closed function (IF α closed function in short) if $f(A)$ is an IF α CS in (Y, σ) for every IFCS A of (X, τ) [9],
3. intuitionistic fuzzy semi closed function (IFS closed function in short) if $f(A)$ is an IFSCS in (Y, σ) for every IFCS A of (X, τ) [9],
4. intuitionistic fuzzy generalized closed function (IFG closed function in short) if $f(A)$ is an IFGCS in (Y, σ) for every IFCS A of (X, τ) [19],
5. intuitionistic fuzzy generalized semi-closed function (IFGS closed function in short) if $f(A)$ is an IFGSC in (Y, σ) for every IFCS A of (X, τ) [19],
6. intuitionistic fuzzy α generalized closed function (IF α G closed function in short) if $f(A)$ is an IF α GCS in (Y, σ) for every IFCS A of (X, τ) [16],
7. intuitionistic fuzzy α generalized semi closed function (IF α GS closed function in short) if $f(A)$ is an IF α GSCS in (Y, σ) for every IFCS A of (X, τ) [12],
8. intuitionistic fuzzy ω closed function (IF ω closed function in short) if $f(A)$ is an IF ω CS in (Y, σ) for every IFCS A of (X, τ) [17],
9. intuitionistic fuzzy generalized semi pre closed function (IFGSP closed function in short) if $f(A)$ is an IFGSPC in (Y, σ) for every IFCS A of (X, τ) [13],
10. intuitionistic fuzzy generalized irresolute (IFG irresolute in short) if $f^{-1}(B)$ is IFGCS in X for every IFGCS B in Y [12].

Definition 2.9 [11] Let be an IFTS (X, τ) is called

1. an intuitionistic fuzzy \hat{g} - $T_{\frac{1}{2}}$ -space (briefly, IF \hat{g} - $T_{\frac{1}{2}}$ space) if every IF \hat{g} CS in (X, τ) is an IFCS in (X, τ) ,
2. an intuitionistic fuzzy $\hat{g}\alpha$ - $T_{\frac{1}{2}}$ -space (briefly, IF $\hat{g}\alpha$ - $T_{\frac{1}{2}}$ space) if every IFGCS in (X, τ) is an IF \hat{g} CS in (X, τ) .

IF \hat{g} -closed functions

Definition 3.1 A function $f: (X, \tau) \rightarrow (Y, \sigma)$ is called an intuitionistic fuzzy \hat{g} -closed (briefly, IF \hat{g} closed) if $f(H)$ is an IF \hat{g} CS in (Y, σ) for every IFCS H of (X, τ) .





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Example 3.2 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then $f(H)$ is an IF \hat{G} CS in (Y, σ) for every IFCS H of (X, τ) . This verifies that f is an IF \hat{G} closed function.

Example 3.3 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.6, 0.5), (0.3, 0.4) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for IFCS $H = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ of (X, τ) , $f(H)$ is not an IF \hat{G} CS in (Y, σ) . This verifies that f is not an IF \hat{G} closed function.

Definition 3.4 Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be a function. f is said to be an intuitionistic fuzzy \hat{G} -open (briefly, IF \hat{G} open) if for every IFOS H of (X, τ) , $f(H)$ is an IF \hat{G} OS in (Y, σ) .

Example 3.5 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then $f(H)$ is an IF \hat{G} OS in (Y, σ) for every IFOS H of (X, τ) . Therefore f is an IF \hat{G} open function.

Example 3.6 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.6, 0.5), (0.3, 0.4) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFOS $H = \langle \alpha, (0.6, 0.5), (0.3, 0.4) \rangle$ of (X, τ) , $f(H)$ is not an IF \hat{G} OS in (Y, σ) . Therefore f is not an IF \hat{G} open function.

Proposition 3.7 Every IF closed function is an IF \hat{G} closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an IF closed function. Let H be an IFCS in X . Since f is an IF closed function, $f(H)$ is an IFCS in Y . Since every IFCS is an IF \hat{G} CS [5], $f(H)$ is an IF \hat{G} CS in Y . Hence f is an IF \hat{G} closed function.

Remark 3.8 The converse of Proposition 3.7 is not true in general as shown in the following example.

Example 3.9 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.7, 0.8), (0.2, 0.1) \rangle$ and $H_2 = \langle \beta, (0.6, 0.7), (0.3, 0.2) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for IFCS $H = \langle \alpha, (0.2, 0.1), (0.7, 0.8) \rangle$ of (X, τ) , $f(H)$ is not an IFCS in (Y, σ) . Therefore f is not an IF closed function. But f is an IF \hat{G} closed function.

Proposition 3.10 Every IF \hat{G} closed function is an IFGSP closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an IF \hat{G} closed function. Let H be an IFCS in X . Since f is an IF \hat{G} closed function, $f(H)$ is an IF \hat{G} CS in Y . Since every IF \hat{G} CS is an IFGSPCS [5], $f(H)$ is an IFGSPCS in Y . Hence f is an IFGSP closed function.

Remark 3.11 The converse of Proposition 3.10 is not true in general as shown in the following example.

Example 3.12 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.5, 0.4), (0.4, 0.5) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for IFCS $H = \langle \alpha, (0.4, 0.5), (0.5, 0.4) \rangle$ of (X, τ) , $f(H)$ is not an IF \hat{G} CS in (Y, σ) . Therefore f is not an IF \hat{G} closed function. But f is an IFGSP closed function.

Proposition 3.13 Every IF \hat{G} closed function is an IF ω closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an IF \hat{G} closed function. Let H be an IFCS in X . Since f is an IF \hat{G} closed function, $f(H)$ is an IF \hat{G} CS in Y . Since every IF \hat{G} CS is an IF ω CS [5], $f(H)$ is an IF ω CS in Y . This verifies that f is an IF ω closed function.

Remark 3.14 The converse of Proposition 3.13 is not true in general as shown in the following example.





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Example 3.15 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.2), (0.6, 0.7) \rangle$ and $H_2 = \langle \beta, (0.4, 0.3), (0.5, 0.6) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \alpha, (0.6, 0.7), (0.3, 0.2) \rangle$ of (X, τ) , $f(H)$ is not an $IF\hat{g}CS$ in (Y, σ) . Therefore f is not an $IF\hat{g}$ closed function. But f is an $IF\omega$ closed function.

Proposition 3.16 Every $IF\hat{g}$ closed function is an IFG closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $IF\hat{g}$ closed function. Let H be an IFCS in X . Since f is an $IF\hat{g}$ closed function, $f(H)$ is an $IF\hat{g}CS$ in Y . Since every $IF\hat{g}CS$ is an IFGCS [5], $f(H)$ is an IFGCS in Y . This proves that f is an IFG closed function.

Remark 3.17 The converse of Proposition 3.16 is not true in general as shown in the following example.

Example 3.18 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.2, 0.3), (0.7, 0.6) \rangle$ and $H_2 = \langle \beta, (0.6, 0.5), (0.3, 0.4) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \alpha, (0.7, 0.6), (0.2, 0.3) \rangle$ of (X, τ) , $f(H)$ is not an $IF\hat{g}CS$ in (Y, σ) . Therefore f is not an $IF\hat{g}$ closed function. But f is an IFG closed function.

Proposition 3.19 Every $IF\hat{g}$ closed function is an $IF\alpha G$ closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $IF\hat{g}$ closed function. Let H be an IFCS in X . Since f is an $IF\hat{g}$ closed function, $f(H)$ is an $IF\hat{g}CS$ in Y . Since every $IF\hat{g}CS$ is an $IF\alpha GCS$ [5], $f(H)$ is an $IF\alpha GCS$ in Y . This verifies that f is an $IF\alpha G$ closed function.

Remark 3.20 The converse of Proposition 3.19 is not true in general as shown in the following example.

Example 3.21 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.2), (0.6, 0.7) \rangle$ and $H_2 = \langle \beta, (0.5, 0.6), (0.4, 0.3) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \alpha, (0.6, 0.7), (0.3, 0.2) \rangle$ of (X, τ) , $f(H)$ is not an $IF\hat{g}CS$ in (Y, σ) . Therefore f is not an $IF\hat{g}$ closed function. But f is an $IF\alpha G$ closed function.

Proposition 3.22 Every $IF\hat{g}$ closed function is an IFGS closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $IF\hat{g}$ closed function. Let H be an IFCS in X . Since f is an $IF\hat{g}$ closed function, $f(H)$ is an $IF\hat{g}CS$ in Y . Since every $IF\hat{g}CS$ is an IFGSCS [5], $f(H)$ is an IFGSCS in Y . Which proves that f is an IFGS closed function.

Remark 3.23 The converse of Proposition 3.22 is not true in general as shown in the following example.

Example 3.24 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.2), (0.6, 0.7) \rangle$ and $H_2 = \langle \beta, (0.2, 0.4), (0.7, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \alpha, (0.6, 0.7), (0.3, 0.2) \rangle$ of (X, τ) , $f(H)$ is not an $IF\hat{g}CS$ in (Y, σ) . Therefore f is not an $IF\hat{g}$ closed function. But f is an IFGS closed function.

Definition 3.25 Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be a function is called an intuitionistic fuzzy ζ -closed (briefly, $IF\zeta$ closed) if $f(H)$ is an $IF\zeta CS$ in (Y, σ) for every IFCS H of (X, τ) .

Proposition 3.26 Every $IF\hat{g}$ closed function is an $IF\zeta$ closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $IF\hat{g}$ closed function. Let H be an IFCS in X . Since f is an $IF\hat{g}$ closed function, $f(H)$ is an $IF\hat{g}CS$ in Y . Since every $IF\hat{g}CS$ is an $IF\zeta CS$ [5], $f(H)$ is an $IF\zeta CS$ in Y . This proves that f is an $IF\zeta$ closed function.

Remark 3.27 The converse of Proposition 3.26 is not true in general as shown in the following example.





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Example 3.28 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.5, 0.6), (0.4, 0.3) \rangle$ and $H_2 = \langle \beta, (0.3, 0.2), (0.6, 0.7) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_2) = n_2$. Then for an IFCS $H = \langle \alpha, (0.4, 0.3), (0.5, 0.6) \rangle$ of (X, τ) , $f(H)$ is not an $\widehat{IF\hat{G}CS}$ in (Y, σ) . Therefore f is not an $\widehat{IF\hat{G}}$ closed function. But f is an $\widehat{IF\zeta}$ closed function.

Proposition 3.29 Every $\widehat{IF\hat{G}}$ closed function is an $\widehat{IF\alpha GS}$ closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $\widehat{IF\hat{G}}$ closed function. Let H be an IFCS in X . Since f is an $\widehat{IF\hat{G}}$ closed function, $f(H)$ is an $\widehat{IF\hat{G}CS}$ in Y . Since every $\widehat{IF\hat{G}CS}$ is an $\widehat{IF\alpha GSCS}$ [5], $f(H)$ is an $\widehat{IF\alpha GSCS}$ in Y . This verifies that f is an $\widehat{IF\alpha GS}$ closed function.

Remark 3.30 The converse of Proposition 3.29 is not true in general as shown in the following example.

Example 3.31 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.2, 0.3), (0.7, 0.6) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_2) = n_2$. Then for an IFCS $H = \langle \alpha, (0.7, 0.6), (0.2, 0.3) \rangle$ of (X, τ) , $f(H)$ is not an $\widehat{IF\hat{G}CS}$ in (Y, σ) . Therefore f is not an $\widehat{IF\hat{G}}$ closed function. But f is an $\widehat{IF\alpha GS}$ closed function.

Definition 3.32 Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be a function is said to be an intuitionistic fuzzy $\widehat{g\alpha}$ -closed (briefly, $\widehat{IF\hat{G}\alpha}$ closed) if $f(H)$ is an $\widehat{IF\hat{G}\alpha CS}$ in (Y, σ) for every IFCS H of (X, τ) .

Proposition 3.33 Every $\widehat{IF\hat{G}}$ closed function is an $\widehat{IF\hat{G}\alpha}$ closed function.

Proof. Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $\widehat{IF\hat{G}}$ closed function. Let H be an IFCS in X . Since f is an $\widehat{IF\hat{G}}$ closed function, $f(H)$ is an $\widehat{IF\hat{G}CS}$ in Y . Since every $\widehat{IF\hat{G}CS}$ is an $\widehat{IF\hat{G}\alpha CS}$ [5], $f(H)$ is an $\widehat{IF\hat{G}\alpha CS}$ in Y . This verifies that f is an $\widehat{IF\hat{G}\alpha}$ closed function.

Remark 3.34 The converse of Proposition 3.33 is not true in general as shown in the following example.

Example 3.35 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.6, 0.7), (0.3, 0.2) \rangle$ and $H_2 = \langle \beta, (0.3, 0.7), (0.6, 0.2) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_2) = n_2$. Then for IFCS $H = \langle \alpha, (0.3, 0.2), (0.6, 0.7) \rangle$ of (X, τ) , $f(H)$ is not an $\widehat{IF\hat{G}CS}$ in (Y, σ) . Therefore f is not an $\widehat{IF\hat{G}}$ closed function. But f is an $\widehat{IF\hat{G}\alpha}$ closed function.

Remark 3.36 The following example shows that an $\widehat{IF\alpha}$ closed function and $\widehat{IF\hat{G}}$ closed function are independent of each other.

Example 3.37 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.8, 0.6), (0.2, 0.4) \rangle$ and $H_2 = \langle \beta, (0.3, 0.2), (0.7, 0.8) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_2) = n_2$. Then for an IFCS $H = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ of (X, τ) , $f(H)$ is not an $\widehat{IF\hat{G}CS}$ in (Y, σ) . Therefore f is not an $\widehat{IF\hat{G}}$ closed function. But f is an $\widehat{IF\alpha}$ closed function.

Example 3.38 Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.15, 0.25), (0.75, 0.65) \rangle$, $H_2 = \langle \beta, (0.2, 0.3), (0.7, 0.6) \rangle$ and $H_3 = \langle \gamma, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, H_3, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_2) = n_2$. Then for an IFCS $H = \langle \alpha, (0.75, 0.65), (0.15, 0.25) \rangle$ of (X, τ) , $f(H)$ is not an $\widehat{IF\alpha CS}$ in (Y, σ) . This verifies that f is not an $\widehat{IF\alpha}$ closed function. But f is an $\widehat{IF\hat{G}}$ closed function.

Remark 3.39 The following example shows that an IFS closed function and $\widehat{IF\hat{G}}$ closed function are independent of each other.





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Example 3.40

1. Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.6, 0.5), (0.3, 0.4) \rangle$ and $H_2 = \langle \beta, (0.2, 0.3), (0.7, 0.6) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ of (X, τ) , $f(H)$ is not an $IF\hat{G}CS$ in (Y, σ) . Hence f is not an $IF\hat{G}$ closed function. But f is an IFS closed function.

2. Let $X = \{m_1, n_1\}$ and $Y = \{m_2, n_2\}$. Let $H_1 = \langle \alpha, (0.1, 0.2), (0.8, 0.7) \rangle$, $H_2 = \langle \beta, (0.2, 0.3), (0.7, 0.6) \rangle$ and $H_3 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, H_3, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$. Then for an IFCS $H = \langle \beta, (0.8, 0.7), (0.1, 0.2) \rangle$ of (X, τ) , $f(H)$ is not an IFSCS in (Y, σ) . Thus f is not an IFS closed function. But f is an $IF\hat{G}$ closed function.

Theorem 3.41 Let $f: X \rightarrow Y$ be an $IF\hat{G}$ closed $\Leftarrow f(H)$ is an $IF\hat{G}OS$ in Y for every IFOS in X .

Proof. H is an IFOS in X . This implies H^c is an IFCS in X . Since f is an $IF\hat{G}$ closed, $f(A^c)$ is an $IF\hat{G}CS$ in Y . Since $f(H^c) = (f(H))^c$, $f(H)$ is an $IF\hat{G}OS$ in Y . Thus $f(H)$ is an $IF\hat{G}OS$ in Y for every IFOS in X .

Conversely, H is an IFCS in X . Then H^c is an IFOS in X . By hypothesis, $f(H^c)$ is an $IF\hat{G}OS$ in Y . Since $f(H^c) = (f(H))^c$, $f(H)$ is an $IF\hat{G}CS$ in Y . This proves that f is an $IF\hat{G}$ closed function.

Theorem 3.42 A function $f: (X, \tau) \rightarrow (Y, \sigma)$ be an $IF\hat{G}$ closed function. Then f is an IF closed function if Y is an $IF\hat{G}T_{\frac{1}{2}}$ space.

Proof. Let H be an IFCS in X . By hypothesis, $f(H)$ is an $IF\hat{G}CS$ in Y . Since Y is an $IF\hat{G}T_{\frac{1}{2}}$ space, $f(H)$ is an IFCS in Y . This proves that f is an IF closed function.

Theorem 3.43 A function $f: (X, \tau) \rightarrow (Y, \sigma)$ is an $IF\hat{G}$ closed function. Then f is an IFG closed function if Y is an $IF\hat{G}aT_{\frac{1}{2}}$ space.

Proof. Let H be an IFCS in X . By hypothesis, $f(H)$ is an $IF\hat{G}CS$ and hence IFGCS in Y . Since Y is an $IF\hat{G}aT_{\frac{1}{2}}$ space, $f(H)$ is an $IF\hat{G}CS$ and hence IFGCS in Y . This proves that f is an IFG closed function.

Remark 3.44 The following example shows that composition of two $IF\hat{G}$ closed functions but not an $IF\hat{G}$ closed function.

Example 3.45 Let $X = \{m_1, n_1\}, Y = \{m_2, n_2\}$ and $Z = \{m_3, n_3\}$. Let $H_1 = \langle \alpha, (0.7, 0.6), (0.2, 0.3) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.6, 0.5) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$, $\sigma = \{0_{\sim}, 1_{\sim}\}$ and $\delta = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X, Y and Z respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = m_2$ and $f(n_1) = n_2$, and define a function $g: (Y, \sigma) \rightarrow (Z, \delta)$ by $g(m_2) = m_3$ and $g(n_2) = n_3$. Then f and g are $IF\hat{G}$ closed functions. Consider an IFCS $H = \langle \beta, (0.2, 0.3), (0.7, 0.6) \rangle$. Then H is an IFCS in β . But $(f \circ g)(H)$ is not an $IF\hat{G}CS$ in X . This verifies that the composition of two $IF\hat{G}$ closed functions but not an $IF\hat{G}$ closed function.

Theorem 3.46 A $f: (X, \tau) \rightarrow (Y, \sigma)$ is an IF closed function. Let $g: (Y, \sigma) \rightarrow (Z, \delta)$ be an $IF\hat{G}$ closed function. Then $g \circ f: (X, \tau) \rightarrow (Z, \delta)$ is an $IF\hat{G}$ closed function.

Proof. Let H be an IFCS in X . By hypothesis, $f(H)$ is an IFCS in Y . Since g is an $IF\hat{G}$ closed function, $g(f(H))$ is an $IF\hat{G}CS$ in Z . Hence $g \circ f$ is an $IF\hat{G}$ closed function.

IF $i-\hat{G}$ closed functions

In this section, we introduce intuitionistic fuzzy $i-\hat{G}$ closed functions and study its properties.





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Definition 4.1 Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be a function. f is said to be an intuitionistic fuzzy irresolute \hat{g} closed (briefly, IFi- \hat{g} closed) if $f(H)$ is an IF \hat{g} CS in (Y, σ) for every IF \hat{g} CS H in (X, τ) .

Example 4.2 Let $X = \{m_1, m_2\}$ and $Y = \{n_1, n_2\}$. Let $H_1 = \langle \alpha, (0.6, 0.7), (0.3, 0.2) \rangle$ and $H_2 = \langle \beta, (0.5, 0.6), (0.4, 0.3) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = n_1$ and $f(m_2) = n_2$. This verifies that f is an IFi- \hat{g} closed function.

Example 4.3 Let $X = \{m_1, m_2\}$ and $Y = \{n_1, n_2\}$. Let $H_1 = \langle \alpha, (0.3, 0.4), (0.6, 0.5) \rangle$ and $H_2 = \langle \beta, (0.2, 0.3), (0.7, 0.6) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = n_1$ and $f(m_2) = n_2$. Consider an IFS $H = \langle \beta, (0.6, 0.5), (0.3, 0.4) \rangle$. Then H is an IFCS in X . This implies H is an IF \hat{g} CS in X . But $f(H)$ is not an IF \hat{g} CS in Y . Therefore f is not an IFi- \hat{g} closed function.

Proposition 4.4 If $f: (X, \tau) \rightarrow (Y, \sigma)$ is an IFi- \hat{g} closed function, then f is an IF \hat{g} closed function.

Proof. Let f be an IFi- \hat{g} closed function. Let H be any IFCS in X . Since every IFCS is an IF \hat{g} CS [5], H is an IF \hat{g} CS in X . By hypothesis, $f(H)$ is an IF \hat{g} CS in Y . This proves that f is an IF \hat{g} closed function.

Remark 4.5 The converse of Proposition 4.4 is not true in general as shown in the following example.

Example 4.6 Let $X = \{m_1, m_2\}$ and $Y = \{n_1, n_2\}$. Let $H_1 = \langle \alpha, (0.7, 0.6), (0.3, 0.4) \rangle$ and $H_2 = \langle \beta, (0.3, 0.4), (0.7, 0.6) \rangle$. Then $\tau = \{0_{\sim}, H_1, 1_{\sim}\}$ and $\sigma = \{0_{\sim}, H_2, 1_{\sim}\}$ are IFTs on X and Y respectively. Define a function $f: (X, \tau) \rightarrow (Y, \sigma)$ by $f(m_1) = 1 - n_1$ and $f(m_2) = 1 - n_2$. Then f is an IF \hat{g} closed function. But f is not an IFi- \hat{g} closed function. Since the IFS $H = \langle \alpha, (0.2, 0.3), (0.8, 0.7) \rangle$ is an IF \hat{g} CS in X , but $f(H) = \langle \beta, (0.8, 0.7), (0.2, 0.3) \rangle$ is not an IF \hat{g} CS in Y . Hence f is an IF \hat{g} closed function, but not an IFi- \hat{g} closed function.

Theorem 4.7 Let $f: (X, \tau) \rightarrow (Y, \sigma)$ be an IFi- \hat{g} closed function. Then f is an IF closed function if Y is an IF $\hat{g}T_{\frac{1}{2}}$ space.

Proof. Let H be an IFCS in X . Then H is an IF \hat{g} CS in X . By hypothesis, $f(H)$ is an IF \hat{g} CS in Y . Since Y is an IF $\hat{g}T_{\frac{1}{2}}$ space $f(H)$ is an IFCS in Y . This verifies that f is an IF closed function.

Theorem 4.8 If $f: (X, \tau) \rightarrow (Y, \sigma)$ and $g: (Y, \sigma) \rightarrow (Z, \delta)$ be IFi- \hat{g} closed functions. Then $g \circ f: (X, \tau) \rightarrow (Z, \delta)$ is an IFi- \hat{g} closed function.

Proof. Let H be an IF \hat{g} CS in X . Then $f(H)$ is an IF \hat{g} CS in Y . Since g is an IFi- \hat{g} closed function, $g(f(H))$ is an IF \hat{g} CS in Z . This proves that $g \circ f$ is an IFi- \hat{g} closed function.

Theorem 4.9 If $f: (X, \tau) \rightarrow (Y, \sigma)$ is an IF \hat{g} closed function and $g: (Y, \sigma) \rightarrow (Z, \delta)$ is an IFi- \hat{g} closed function, then $g \circ f: (X, \tau) \rightarrow (Z, \delta)$ is an IF \hat{g} closed function.

Proof. Let H be an IFCS in X . Since f is an IF \hat{g} closed function, $f(H)$ is an IF \hat{g} CS in Y . Since g is an IFi- \hat{g} closed function, $g(f(H))$ is an IF \hat{g} CS in Z . This proves that $g \circ f$ is an IF \hat{g} closed function.

Theorem 4.10 A function $f: (X, \tau) \rightarrow (Y, \sigma)$ is an IFi- \hat{g} closed function. Then f is an IFG irresolute function if Y is an IF $\hat{g}aT_{\frac{1}{2}}$ space.

Proof. Let H be an IFCS in X . Then H is an IF \hat{g} CS in X . By hypothesis, $f(H)$ is an IF \hat{g} CS and hence IFGCS in Y . Since Y is an IF $\hat{g}aT_{\frac{1}{2}}$ space, $f(H)$ is an IF \hat{g} CS in Y . This proves that f is an IFG irresolute function.





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Analysis of Contextual Word Similarity using Clustering Technique

K.Ananthi^{1*} and M.Balakrishnan²

¹Assistant Professor, Department of Computer Science and Business Systems, Sri Eshwar College of Engineering, Kinathukadavu-641 202, Coimbatore, Tamil Nadu, India.

²Department of Artificial Intelligence and Data Science, Dr.Mahalingam College of Engineering and Technology, Pollachi- 642003, Coimbatore, Tamil Nadu, India.

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*Address for Correspondence

K.Ananthi

Assistant Professor,
Department of Computer Science and Business Systems,
Sri Eshwar College of Engineering,
Kinathukadavu-641 202,
Coimbatore, Tamil Nadu, India.
E.Mail: ananthikss5@gmail.com



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ABSTRACT

An unsupervised learning technique called clustering groups a set of items into clusters with the goal of making the clusters as similar as feasible to one another while making the clusters as different from one another as possible. A similarity function is frequently used in clustering techniques to compare multiple data pieces. In this paper, a novel word clustering method for statistical language modelling is presented. Our method uses a condensed clustering algorithm and contextual word similarity as its classification criterion. The INRS speech recognizer was put to the test using the ATIS spontaneous English corpus. The rate of word correctness rises thanks to automatic word classification.

Keywords: negative data clustering, pattern-based similarity, similarity measurements

INTRODUCTION

The term "distance-based clustering," sometimes known as "distance-based object clustering," is widely used. There is a minimum distance between any two data objects that are in the same cluster, and there is a maximum distance between any two data objects that are in different clusters. The level of association between two data patterns or objects is based on how similar they are to one another. The clustering method groups related data elements based on a similarity score. These distance metrics, including the Euclidean, Manhattan, Murkowski, and Cosine similarity, are often employed to group items together based on similarities. Any two data objects within a cluster are closest to one another, while the distance between any two data objects at their largest distance.





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The following categories can be used to categorise clustering algorithms:

1. One-dimensional clustering
2. Clustering based on density
3. Using a hierarchy of clusters

A single dataset can be divided or combined into several layered divisions using hierarchical clustering techniques. These nested partitions may have agglomerative, top-down, divisive, or bottom-up hierarchies. Some claim that partitioned clustering is the most typical kind of clustering technology. In the partition clustering approach, each of the "k" partitions is a cluster made up of data points. A specific objective function is used to build the partition. Even if they are "different" from the data items of other clusters, data objects within a cluster are "similar" to one another. When the amount of clusters required is constant, partitioned clustering techniques are advantageous. A few strategies for partitioning clustering are KMeans, PAM (Partition around Methods), and CLARA. Clusters of any shape can be produced using density-based clustering techniques.

A review of the distance metrics used for clustering

Distance measurements can be used to identify how similar the data objects are to one another. Obtaining a suitable distance/similarity function for a certain situation is a crucial requirement for metric calculation. Metric functions, also referred to as distance functions, calculate the separation between entries in a collection. Metric space is the name for a set with a metric. This paper illustrates the k-means clustering method using the Euclidean distance metric. Create a function called Similarity as part of the process is standard (X,Y). When two X and Y, objects or sets belonging to the same class, are compared, they are said to be "similar" and this is what the function's value indicates.

Identical Measures

An similar metric is the distance between two different points of data. Instead of measuring the degree of connectedness between two data items, similarity evaluates the degree of divergence between two data items [9]. The effectiveness of many algorithms depends on the choice of an adequate distance function across the input data set in practise.

Similarity Measurement

The following sections provide a comprehensive overview of the similarity measure functions frequently used for clustering in literature.

Euclidean separation

The accepted industrial standard metric for geometric issues is Euclidean distance. Simply described, it is the typical distance between two places. In clustering issues, especially text clustering, Euclidean distance is frequently used. Another standard distance metric employed by the K-means algorithm is the Euclidean distance. The following equation (1) shows how to use the Euclidean distance to calculate the square root of the coordinate difference between two objects. [7].

$$Dist_{XY} = \max_k |X_{ik} - X_{jk}| \quad (1)$$

Cosine distance

The formula that follows [36] yields the cosine of the angle between two vectors is how the cosine distance metric for clustering is calculated. The angle between the n-dimensional vectors A and B is shown in this instance.

$$\theta = \arccos(A \cdot B / \|A\| \|B\|) \quad (2)$$



**Ananthi and Balakrishnan****Jaccard distance**

The Jaccard distance quantifies the similarity between the two pieces of data by dividing the intersection of the two by their union, as indicated in equation (3) below. In addition, the classification of ecological species was done using the Jaccard similarity measure.

Manhattan distance

According to equation (4) below, the Manhattan distance is a unit of measurement that pinpoints the precise discrepancies in coordinates between two data objects. [7]: $Dist_{XY} = |X_{ik} - X_{jk}|$ (3)

Distance metrics' limitations in clustering

There are some situations when it is possible to use distance measurements to capture correlations between the data elements. Even if a group of data objects are spatially separated from one another, there is a good chance that they will share data patterns[2] and have different distance measurements from one another. Distance-based metrics are inappropriate for representing the behaviour of the data series because they only compare the actual distance between two data points. One way to understand how data series behave is to look at how patterns of data points are linked and disassociated[2]. This may draw attention to how they are related. [2,3]

Based on similar data patterns, clustering is necessary

Figures 2 and 3 display examples of data patterns. The bottom two data objects in Figure 2 are relatively close to one another in terms of distance, and they all create a changing pattern. The data patterns in Figure 3 create a scaling connection.

As a result, the objective is to group data objects with similar patterns, as well as those that are physically adjacent to one another, as shown in Figs. 2 and 3. The focus of this study is in this direction.

A Flexible Clustering Technique Is Required

Aside from the problem with utilising distance-based measurements for grouping, several other problems exist with clustering techniques. The user must define the number of clusters at the beginning of the process, which adds to the overhead of the process, and many clustering approaches, for instance, struggle with noisy data or outliers.

Therefore, in addition to grouping data based on similar patterns, it is crucial to cluster data properly in order to avoid the issues mentioned above. One of the directions for the research is this.

Area of the Data

Terminology used in a document can help give text information context or meaning. In the application, "context" is defined by many academics. Context can also be ascertained by looking at the relevant terms that are associated with a document [2]. By utilising the characteristics of the data to create the context, this idea of discovering the "context" of data may also be used to non-textual data[2]. These contexts can be produced once the data has been sorted according to behaviour rather than proximity to a location. Each recently created cluster has a context[2] attached to it. These can be created utilising radar data, such as ionosphere data from the University of California, Irvine (UCI) Repository, as well as medical diagnosis data, such as Pima Indian Diabetes data from the UCI Repository.

Assessment of Positive Data

It could be difficult to get unfavorable data. On this topic, not much research has been done. The bulk of clustering theories and distance functions fail to cluster negative data effectively. They are limited to using only persuasive evidence, such as the Pima Indian Diabetes statistics. A wide range of unfavorable radar readings make up the ionosphere data. It depends on the ionosphere structures these radar measurements produce whether they are beneficial or harmful. Information from radar, such as ionosphere, is one example of unfavorable information. We discovered that a scale that may be applied to observed data, such as readings for diabetes, can function with negative and positive weights in medical diagnostic software. The advice of medical professionals assisted in the acquisition of this knowledge. Negative term weighting in text mining techniques offer.



**Ananthi and Balakrishnan****Distance measurement of Clustering Techniques**

Negative data clustering could be difficult. Little research has been done on this subject. Negative data cannot be clustered efficiently using the majority of distance functions or clustering theories. Only favourable evidence—like the Pima Indian Diabetes statistics—can be used by them.

The ionosphere data are a collection of unfavourable radar measurements. The ionosphere structures these radar measurements create determine whether they are advantageous or detrimental. Data from the ionosphere is one example of negative information from radar. We found that applications for medical diagnostics can work with negative and positive weights on a scale that can be applied to observed data like readings for diabetes with the help of doctors' suggestions. Text mining techniques offering negative term weighting

Limitations and Results from Current Systems

The following problems and conclusions have been established following an examination and analysis of the current systems: In clustering, distance-based similarity variables are most frequently utilised. The relationships between the data pieces may not always be revealed by distance measures. Even if a group of data objects were spatially separated from one another[2] based on measures of distance, there is a good possibility that they would have similar data patterns.

CONCLUSION

We propose to address the aforementioned constraints in our system after a thorough analysis of existing systems.. The advantages of the systems under discussion are suggested to be combined by this system. Because distance-based metrics fail to adequately capture the behaviour of data series, our system uses the context similarity coefficient, an efficient data association technique, in conjunction with pattern-based similarity grouping. Making this method applicable to negative data will enable the clustering of radar data for the ionosphere. This clustered ionosphere data can be used as the background for similarity matrix input based clustering, a potent threshold-generating technique that will help keep outliers out of clusters.

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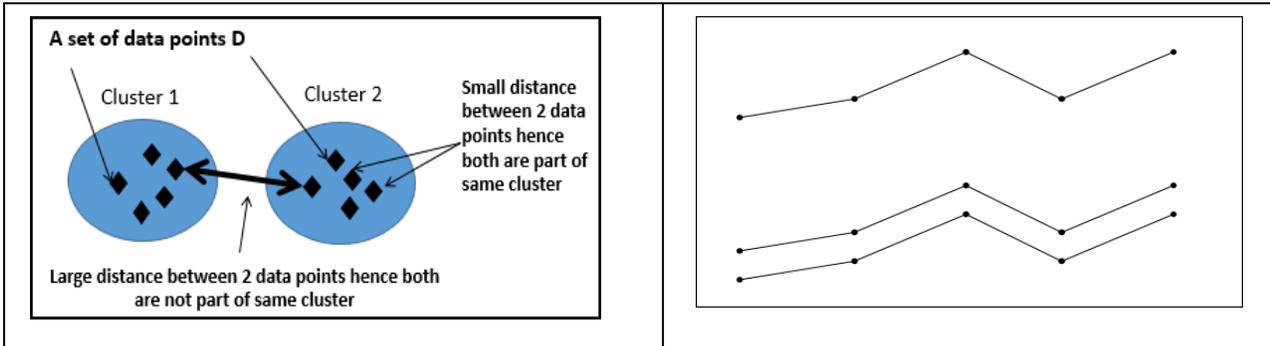


Fig1: The generalized clustering procedure employing distance metrics, as an example

Figure 2: Data items create a shifting pattern

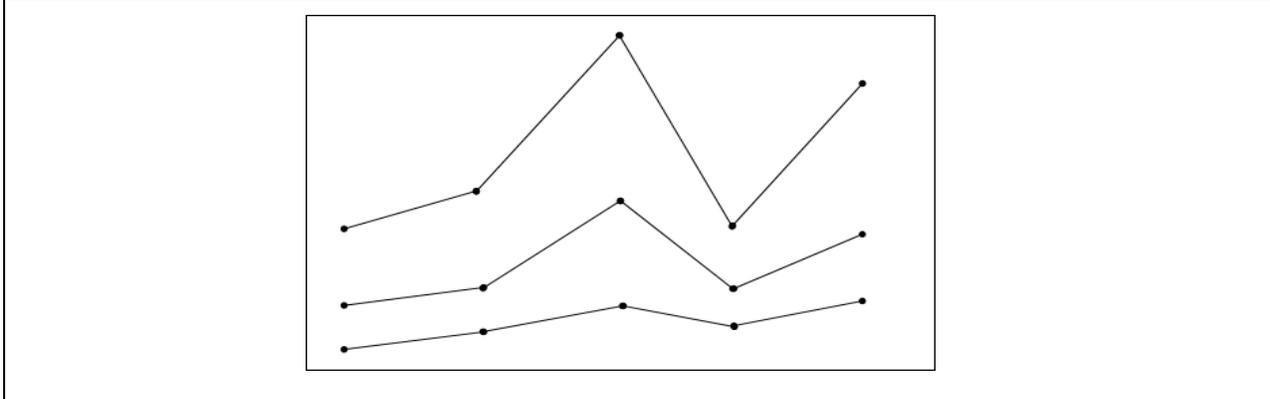


Figure 3: Data items create a scaling pattern





Age-Dependent Effect of Covid-19 Infection on the Cognitive Functioning of Patient's Post-Hospital Discharge

Ashwani Kumar¹, Amber Aanad², Amardeep Kaur Saini¹ and Manju Devi^{3*}

¹MPT Student, School of Allied Medical Sciences, Lovely Professional University, Phagwara, Punjab - 144402, India.

²Ph.D. Scholar, School of Allied Medical Sciences, Lovely Professional University, Phagwara, Punjab - 144402, India.

³Assistant Professor, School of Allied Medical Sciences, Lovely Professional University, Phagwara, Punjab -144402, India.

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*Address for Correspondence

Manju Devi

Assistant Professor,
School of Allied Medical Sciences,
Lovely Professional University,
Phagwara, Punjab -144402, India.
E.Mail: manju.23207@lpu.co.in



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ABSTRACT

The coronavirus is still evolving and there is growing concern of cognitive impairment after Covid-19 infection. The after-effects of Covid-19 on cognitive ability is underrated and less explored. The objective of this study is to identify the cognitive impairment in Covid-19 patients discharged from hospital and its effect on age. The study included 250 subjects (125 each in Covid-19 survivors and control group). Covid-19 patient data were collected from different local hospitals and multicentres located in Jalandhar and Kapurthala district between September 2022 to January 2023. Participants were evaluated for their cognitive functions using Montreal Cognitive Assessment (MoCA) tool. The overall prevalence of cognitive impairment was 76% and 29.60% in Covid-19 survivors and control group, respectively and 41-50 age group were mostly affected with 14.40%. Males (45.60%) were more affected than females (30.40%). Significant difference was observed between two groups in overall and all sub-components of MoCA score ($p < 0.05$). ANOVA shows a significant interaction effect between the three different age groups on total MoCA score ($P < 0.001$). The total MoCA score positively correlated with abstraction ($r = .281, p = .002$), naming positively correlated with attention ($r = .377, p < .001$) and visuospatial ($r = .370, p < .001$) whereas gender correlated negatively with attention ($r = -.203, p = .023$). Cognitive impairment was prevalent among Covid-19 survivors specifically in the early 40s. Males were more affected than females. The abstraction, naming, attention, and visuospatial were the most affected domains.





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Keywords: Covid-19, cognitive impairment, Montreal cognitive assessment, MoCA, Covid-19 survivors

INTRODUCTION

Coronavirus disease (Covid-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2). In the last two decades, SARS-CoV-2 is the third Corona-virus epidemic after Severe Acute Respiratory Syndrome Coronavirus-1 (SARS-CoV-1) [1,2] and Middle East Syndrome Coronavirus (MERS-CoV) [3-5]. The Covid-19 infection started spreading globally from December 2019 [6] then rapidly became a global pandemic with 670 million cases has been reported globally and 45 million cases in India [7] and it is still a threat for public health system. Study suggests that Covid-19 damages different organs with different impacts, it causes damage to respiratory system, central nervous system (CNS) and viscera through infection and blood flow constraints [4,8,9]. However, complications on central nervous system such as anosmia, ageusia, headache, confusion, encephalopathy, impairment of consciousness, stroke, cerebral venous sinus thrombosis (CVST), intracerebral haemorrhage, coma and seizure in acute phases have been reported in many studies [10-13]. Moreover, underlying mechanism leading to cerebrovascular damage could be endothelitis, stasis, disseminated intravascular coagulation, capillary thrombosis, pericyte damage, impaired neurovascular coupling, blood brain barrier leakage and inflammation [1,4,14].

Other factors might be the cause of change in function and structure of the CNS are neurotrophic changes, particularly in hippocampus; cytokine storm which will cause brain tissue damage and increase the probability of cognitive impairment (CI) [10]. Additionally, several studies have shown that patients recovered from Covid-19 have severe cerebral white matter abnormalities which leads to cognitive decline [13,15,16]. The decline in cognitive ability has been reported by various researchers as one of the complication of Covid-19 infection and it ranges between 40-60% of CI on Montreal Cognitive Assessment (MoCA) [5,14,17-20]. Domains of the cognitive function like visuospatial function, attention, language, and delayed recall were the most affected as reported by some studies [10,18,21]. Cognitive decline impacted the quality of life of Covid-19 survivors which lead to depression and anxiety also. Despite the fact that a large amount of comprehensive data on acute symptoms and clinical management has been collected and analyzed, there are currently few evidences available for the long-term impact of Covid-19 on patients' cognitive functioning. Post-acute care for cognitive and mental health disabilities may go unnoticed, particularly in low-resource settings where all efforts were focused on containing Covid-19 transmission and providing appropriate care for critically ill patients [22]. Therefore, there is an urgent need to understand the ongoing impacts of Covid-19 on the cognitive functioning of patients among all ages to plan and administer necessary treatment effectively as very few studies has been conducted in India and specifically in the northern state of India. Following this reason, we intend to conduct the investigation using MoCA scale to identify the prevalence of CI and specify the more likely affected age group after the Covid-19 infection.

METHODOLOGY

The present research project was accepted by the Clinical Research Ethical Committee of Applied Medical Sciences, Lovely Professional University (LPU/IEC/2022/01/07) (Appendix-V). This cross sectional study was conducted from September 2022 to January 2023 among recovered Covid-19 survivors of Jalandhar and Kapurthala districts. The Participants were identified through the Covid-19 patient database from local hospitals and multi-centres located in Jalandhar and Kapurthala district and were contacted for participation in face-to-face interviews. Control group was chosen from the first-degree relatives of Covid-19 survivor group to eliminate the impact of variable environmental and genetical factors. Participants within the age of 20-50 years who currently resides in Jalandhar and Kapurthala districts of Punjab were eligible to participate in the survey. Different strategies were employed to reach various participants as possible who fit into the inclusion criteria. The study included Covid-19 survivor of both genders with a confirmed history of Covid-19 infection based on PCR test report and no recurrence of the same in the past 6



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months. However, Participants were excluded if they had any other diagnosed medical, neurological or psychiatric condition and who were non-cooperative and failed to provide the informed consent. On the basis of selection criteria, 250 participants were recruited and categorised into Covid-19 survivor group and control group with 125 participants each.

On the first page of general assessment form, the researchers informed the participants about the objectives of the study. Additionally, they were informed that their identities would be kept confidential. Participants informed consent was the preliminary section of the survey. Participants had to respond to close ended questions (yes/no) to confirm their willingness to proceed with the survey voluntarily. Later, participants were provided with single page MoCA assessment screening tool and were scored on a maximum of 30 points.

Data Analysis

The data was analyzed descriptively and statistically using SPSS version 26. Descriptive data included continuous and categorical variable, continuous variables were presented as mean, standard deviation (SD), and standard error (SE), and categorical variables were shown as frequency and percentages. Kolmogorov-Smirnov test was used to check normality of participants in both Covid-19 survivors and control group. Independent t-test was applied to evaluate whether there is a significant difference in the MoCA score or not among the survivor and control group. Additionally, ANOVA was used to evaluate the impact of cognitive performance within the survivor and control group. Correlation between the different cognitive scores and demographic data were analyzed using the Pearson correlation for the continuous variable.

Descriptive Analysis

In Covid-19 survivors group, 61 Participants were aged between 20-30, 31 Participants were aged between 31-40, 31 Participants were aged between 41-50 with total male (61%) and female (39%). Whereas in control group, 57 Participants were aged between 20-30, 31 Participants were aged between 31-40, 41 Participants were aged between 41-50 with total male (59.2%) and female (65.8%). The mean MOCA score of Participants in 20-30, 31-40, 41-50 age groups in Covid-19 survivor (Figure 3) and control groups were 22.70, 19.45, 16.16 respectively and 25.82, 25.95, 23.96 respectively (Table 1). The normality of the sample was tested using Kolmogorov-Smirnov test with significance of $p < .001$.

Prevalence of CI

The overall prevalence of CI was 76% and 29.60% in Covid-19 survivors and control groups respectively. The age group of 20-30 was having 15.20% of mild CI whereas 41-50 age group was affected mostly with 14.40% of moderate CI (Table 2).

Comparison between Covid-19 survivors and control group

The CI was compared between the groups using independent t-test. Significant difference was observed between the two groups in terms of total MoCA score, visuospatial, abstraction, recall, orientation, language and attention with $p < 0.01$ and naming $p < 0.05$ (Table 3).

Comparison within Covid-19 survivors and control group

The linear mixed repeated measure analysis (ANOVA) within the Covid-19 survivor group revealed that there was a significant interaction effect between the three different age groups (20-30, 31-40 and 41-50) on total MoCA score ($P < .001$), whereas control group also shown significant differences between the age group on total MoCA score ($p = .002$), however there were no significant differences between male and female ($p = .730$) (Table 4).

MANOVA between age groups of the Covid-19 survivors and control group suggested that there was no significant difference on sub components of MoCA (Table 5,6).





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Correlation between age, gender and MoCA scores

The Pearson correlation shows significant correlation between age, gender and sub component of MoCA in Covid-19 survivor group. Age negatively correlated with total MoCA score ($r = -.474, p < .001$) as the cognitive performance decreases with increase in age. Gender and attention were negatively correlated ($r = -.203, p = .023$) as the female in our study had higher attention deficit than male. Total MoCA score positively correlated with abstraction ($r = .281, p = .002$) as total MoCA increases with increase in abstraction. Further naming was positively correlated with attention ($r = .377, p < .001$) and visuospatial ($r = .370, p < .001$) which suggest that attention and visuospatial increases with increase in naming (Figure 5).

Further the age, gender, total MoCA and sub component of MoCA shows significant correlation in control group as well. Total MoCA score and age negatively correlated ($r = -.285, p = .001$) as cognition decline with age. Visuospatial were positively correlated among naming ($r = .449, p < .001$), attention ($r = .647, p < .001$), language ($r = .344, p < .001$), abstraction ($r = .359, p < .001$), recall ($r = .539, p < .001$), orientation ($r = .247, p = .005$) as visuospatial inputs positively impact attention, language, abstraction, recall and orientation. Naming positively correlated with recall ($r = .194, p = .03$), language ($r = .214, p = .017$) and attention ($r = .480, p < .001$) as naming component positively impact language and attention (Figure 6).

On the other hand, attention positively correlated with orientation ($r = .203, p = .024$), recall ($r = .483, p < .001$), abstraction ($r = .297, p = .001$) and language ($r = .185, p = .039$). This suggest that attention increases with increase in abstraction, recall and language. Language positively correlated with orientation ($r = .182, p = .042$), and abstraction ($r = .218, p = .015$) which implies that language ability improves with orientation and abstraction. Lastly, recall positively correlated with abstraction ($r = .325, p < .001$) and orientation ($r = .196, p = .028$) which suggest that participants with better recall ability have good abstraction and orientation ability and vice-versa(Figure 6).

DISCUSSION

The Covid-19 since its inception, has claimed 6.8 million lives so far globally and has not stopped yet [7]. The impact of virus infection varies from country to country based on ethnicity, climate and geographical location [35]. After Covid-19 infection, survivors who got discharged from hospital were assumed to have recovered in terms of respiratory complication which was their primary concern problem at the time of hospitalization regardless of the CIs [10]. Many studies have reported the CI among Covid-19 survivors globally [4,18,21,24,28,36]. Specifically, in India, the impact of Covid-19 infection on cognitive ability is significant as evidenced by the findings of prior studies [19,32,37-39]. However, the impact of Covid-19 infection on cognitive ability in different age groups of Covid-19 survivors and its comparison with first-degree relatives of Covid-19 survivors for eliminating the impact of variable environmental and genetic factors has not been established.

Prevalence of cognitive impairment in Covid-19 survivors

The findings of this study revealed that the Covid-19 survivors have higher prevalence of CI (76%) than their first-degree relatives, which suggest that Covid infection increases the likelihood of developing CI and it in line with the study of Italy in which they reported that 79% prevalence of CI were in Covid-19 survivors after 1 month of hospital discharge and it decreased to 75% in 3 and 6 months follow up [15]. Whereas the findings of a French study reported 66% prevalence of CI in Institutionalised Covid-19 patient upon discharge which is lower than this study's finding. Another study from Egypt reported the prevalence of CI to be 51.8%[10]. US based study which was conducted on non-critical, mild to moderate Covid-19 infection reported frequency of objective CI of 40%[18]. Recent study conducted in the hilly region of northern India reported 28% prevalence of CI[19]. whereas similar study conducted in hot climatic region of Rajasthan, India reported 34% prevalence of CI among institutionalized Covid-19 survivors [32].





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Gender and cognitive impairment

The prevalence of CI among male and female Covid-19 survivors was 40.60% and 35.40% respectively reported in this study. Previous study of Rajasthan reported that males were more affected than female (42.3% vs 29.1%) [32]. Whereas study from Egypt reported higher prevalence of CI among female (43.5%) than male (8.2%) as very few studies have specified the prevalence of CI based on gender [18].

Age and Cognitive performance

The majority of Covid-19 survivors in 41-50 age group had lower MoCA score as compare to those in 20-40 age group which suggest that CI progresses from mild to moderate in late 30s or early 40s. Study conducted in Egypt of Covid-19 survivors with mean age of 56.8 years suggested that older population who recovered from Covid-19 are more vulnerable for CI with having significance of $p < .01$ [20]. Another study of Haifa, North Israel on Covid-19 infection aged 19-72 years with mean age of 49.5 years were assessed on total MoCA score, findings suggested that older group are more likely to have higher cognitive deficit after Covid-19 infection [21]. These studies are in line with this study and suggests that older group of Covid-19 survivors had higher CI however they haven't compared the effect of Covid-19 based on age.

Correlation between age, gender and MoCA

This study finding suggested that the cognitive performance decreases with increase in age among Covid-19 survivors which stands true in general population as white matter volume and tract integrity declines with age [26]. Prior studies have not reported the effect of Covid-19 on CI based on age, but have reported domains specific CI using MoCA or similar cognitive assessment tool. Domains of MoCA including abstraction, attention, visuospatial, and naming was mostly affected in our study. Previous study of US reported that visuospatial function of Covid-19 survivors was affected most [18]. Another study of Haifa, Northern Israel identified abnormalities in visuospatial, attention and language in their study [21]. The visuospatial, attention, language, and delayed recall were the most affected domains in Covid-19 survivors reported by the cross sectional study of Egypt [10]. The study of Covid-19 survivors from hilly region of Northern India reported visuospatial function and attention to be most affected [19]. The result of study conducted in Ragolu district of India reported visuospatial, naming and language to be the most commonly affected domains [37].

Strengths and Limitations

The findings of this study acknowledge cognitive performance in the Covid-19 survivors group and had sufficient sample size to show significant result for CI using MoCA scale. Additionally, the study population comprised of patients with diverse background, and disease severity and who have been discharged from the hospital upon recovery. Only those patients who have recovered from the Covid-19 infection between 6 months to 1 year were included. Cognitive ability genetically varies from individual to individual, to ensure the unbiased population distribution we included a family member of the Covid-19 survivors in the control group. Despite the fact that our results showed mild and moderate CI, we accept certain limitations by employing the MoCA scale, which is less sensitive for minor CI. Results were interpreted based on the total MoCA score, but since each person has a unique IQ, they may vary person to person⁵.

Clinical Implications

Clinical evaluation of patients with Covid-19 patients' cognitive performance is important, both during hospitalisation and after discharge. Any cognitive impairment can be identified and treated effectively with the findings of a thorough neurological examination. In addition to assessing cognitive functioning, healthcare providers should also monitor patients for the development of long-term cognitive impairment. This may involve regular cognitive assessments over an extended period of time, as well as ongoing communication with patients to monitor for any changes in cognitive function.





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Recommendations

Medical professionals must to inform patients and their families about any possible Covid-19 cognitive side effects and how to handle them. This may involve providing information on cognitive rehabilitation techniques, strategies for improving memory and attention, and ways to manage the emotional and psychological impact of cognitive impairment. In general, healthcare professionals should take Covid-19 patients' ongoing effects on cognitive functioning into account when treating and managing them. Regular evaluations, good communication, and the right interventions may reduce the disease's long-term cognitive impacts and enhance patients' overall quality of life.

CONCLUSION

CI was prevalent among Covid-19 survivors specifically in late 30s or early 40s. Male were more affected than female as male score was less on MoCA in comparison to female. The abstraction, naming, attention, and visuospatial were the most affected domains of MoCA in Covid-19 survivors group. Future study should focus on using biomarkers to find out the structural changes in brain after Covid-19 infection and may use the performance-based outcome measures as an assessment tool.

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DECLARATION

The authors declare that they have no any conflict of interest either financial or personal.

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Author Contribution

All authors participated in the literature search, manuscript preparation, and feedback on final manuscript.

Ethical Approval

This study was approved by Institutional Ethical Committee of Lovely Professional University vide approval number LPU/IEC/2022/01/07.

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Table 8. Descriptive analysis of the data

| Covid-19 survivors | N | Mean | Std. Deviation |
|--------------------|-----|--------|----------------|
| 20-30 | 63 | 22.698 | 4.390 |
| 31-40 | 31 | 19.451 | 3.758 |
| 41-50 | 31 | 16.612 | 3.852 |
| Total | 125 | 20.384 | 4.810 |
| Control group | | | |
| 20-30 | 57 | 25.824 | 2.323 |
| 31-40 | 41 | 25.951 | 2.438 |
| 41-50 | 27 | 23.963 | 2.821 |
| Total | 125 | 25.464 | 2.579 |
| Gender | | | |
| Male | 77 | 20.350 | 4.857 |
| Female | 48 | 20.437 | 4.784 |





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Table 9. Prevalence of cognitive impairment based on age and gender

| | Mild CI | Moderate CI | Total CI |
|--|---------|-------------|----------|
| Age | | | |
| 20-30 years | 15.20% | 6.40% | 21.60% |
| 31-40 years | 22.40% | 7.20% | 29.60% |
| 41-50 years | 10.40% | 14.40% | 24.80% |
| Overall (21-50 years) | 48% | 28% | 76% |
| Gender | | | |
| Male | 29.60% | 16.00% | 45.60% |
| Female | 18.40% | 12% | 30.40% |
| CI-Cognitive impairment. Note- None of the Participants were having severe CI | | | |

Table 10. Independent T-test between Covid-19 survivors and control group

| | F | Sig. | t | df | Sig. (2-tailed) |
|------------------|--------|-------|---------|-----|-----------------|
| MoCA total score | 55.568 | 0 | -10.405 | 248 | .000 |
| Naming | 4.107 | 0.044 | 2.059 | 248 | 0.041 |
| Visuospatial | 39.76 | 0 | 7.809 | 248 | .000 |
| Abstraction | 6.578 | 0.011 | 4.162 | 248 | .000 |
| Recall | 9.504 | 0.002 | 7.995 | 248 | .000 |
| Orientation | 78.45 | 0 | 3.981 | 248 | .000 |
| Language | 31.185 | 0 | 7.865 | 248 | .000 |
| Attention | 1.931 | 0.166 | 5.731 | 248 | .000 |

Table 11. ANOVA in Covid-19 survivors and control group

| | Sum of Squares | df | Mean Square | F | Sig. |
|---------------------------|----------------|-----|-------------|--------|-------|
| Covid-19 survivors | | | | | |
| Between Groups | 792.012 | 2 | 396.006 | 23.255 | 0.000 |
| Within Groups | 2077.556 | 122 | 17.029 | | |
| Control | | | | | |
| Between Groups | 77.977 | 2 | 38.988 | 6.367 | 0.002 |
| Within Groups | 747.111 | 122 | 6.124 | | |

Table 12. MANOVA within Covid-19 Survivors group

| | | Sum of Squares | df | Mean Square | F | Sig. |
|-------------|----------------|----------------|-----|-------------|-------|-------|
| Orientation | Between Groups | 0.062 | 2 | 0.031 | 0.506 | 0.604 |
| | Within Groups | 7.426 | 122 | 0.061 | | |





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| | | | | | | |
|--------------|----------------|---------|-----|-------|-------|-------|
| | Total | 7.488 | 124 | | | |
| Abstraction | Between Groups | 1.549 | 2 | 0.774 | 2.751 | 0.068 |
| | Within Groups | 34.339 | 122 | 0.281 | | |
| | Total | 35.888 | 124 | | | |
| Recall | Between Groups | 0.192 | 2 | 0.096 | 0.073 | 0.930 |
| | Within Groups | 161.008 | 122 | 1.320 | | |
| | Total | 161.200 | 124 | | | |
| Language | Between Groups | 0.403 | 2 | 0.202 | 0.496 | 0.610 |
| | Within Groups | 49.597 | 122 | 0.407 | | |
| | Total | 50.000 | 124 | | | |
| Naming | Between Groups | 2.091 | 2 | 1.046 | 1.582 | 0.210 |
| | Within Groups | 80.661 | 122 | 0.661 | | |
| | Total | 82.752 | 124 | | | |
| Visuospatial | Between Groups | 1.812 | 2 | 0.906 | 0.695 | 0.501 |
| | Within Groups | 158.988 | 122 | 1.303 | | |
| | Total | 160.800 | 124 | | | |
| Attention | Between Groups | 1.636 | 2 | 0.818 | 0.729 | 0.485 |
| | Within Groups | 136.956 | 122 | 1.123 | | |
| | Total | 138.592 | 124 | | | |

Table 13. MANOVA within the control group

| | | Sum of Squares | df | Mean Square | F | Sig. |
|--------------|----------------|----------------|-----|-------------|-------|-------|
| Visuospatial | Between Groups | 1.262 | 2 | 0.631 | 0.208 | 0.813 |
| | Within Groups | 370.930 | 122 | 3.040 | | |
| | Total | 372.192 | 124 | | | |
| Naming | Between Groups | 1.049 | 2 | 0.524 | 0.860 | 0.426 |
| | Within Groups | 74.439 | 122 | 0.610 | | |
| | Total | 75.488 | 124 | | | |
| Attention | Between Groups | 0.741 | 2 | 0.371 | 0.250 | 0.779 |
| | Within Groups | 181.131 | 122 | 1.485 | | |
| | Total | 181.872 | 124 | | | |
| Language | Between Groups | 2.041 | 2 | 1.021 | 0.998 | 0.372 |
| | Within Groups | 124.759 | 122 | 1.023 | | |
| | Total | 126.800 | 124 | | | |
| Abstraction | Between Groups | 2.219 | 2 | 1.109 | 3.036 | 0.052 |
| | Within Groups | 44.581 | 122 | 0.365 | | |
| | Total | 46.800 | 124 | | | |
| Orientation | Between Groups | 0.302 | 2 | 0.151 | 0.819 | 0.443 |
| | Within Groups | 22.498 | 122 | 0.184 | | |
| | Total | 22.800 | 124 | | | |
| Recall | Between Groups | 4.249 | 2 | 2.124 | 1.072 | 0.346 |
| | Within Groups | 241.879 | 122 | 1.983 | | |
| | Total | 246.128 | 124 | | | |





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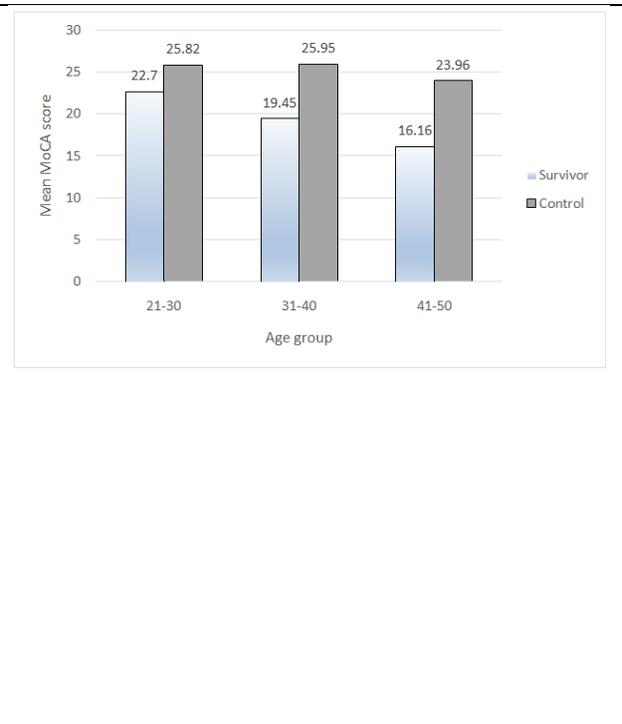
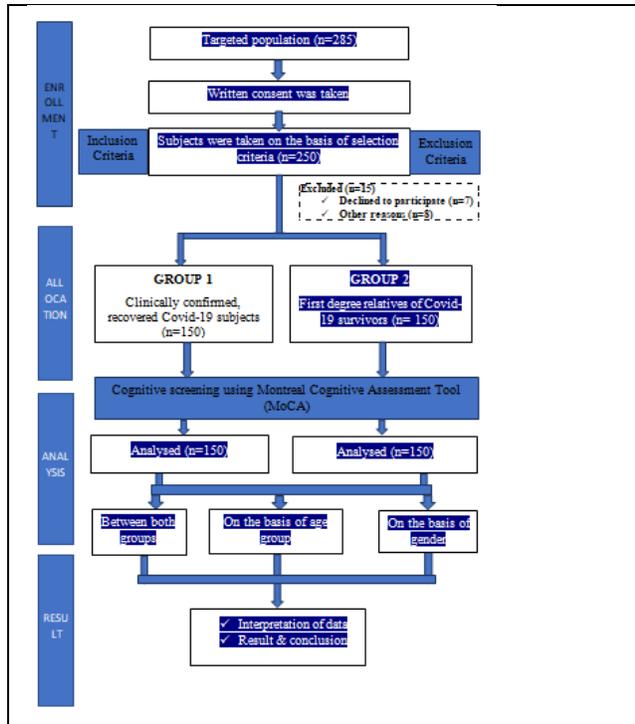


Figure 1 STROBE flow chart

Figure 2 Mean of total MoCA score in Covid-19 survivors and Control group

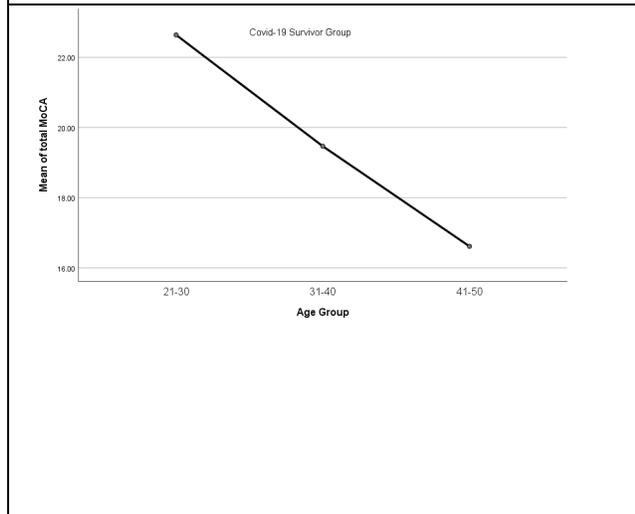


Figure 3 Comparison of total MoCA score in different age group of Covid-19 survivors

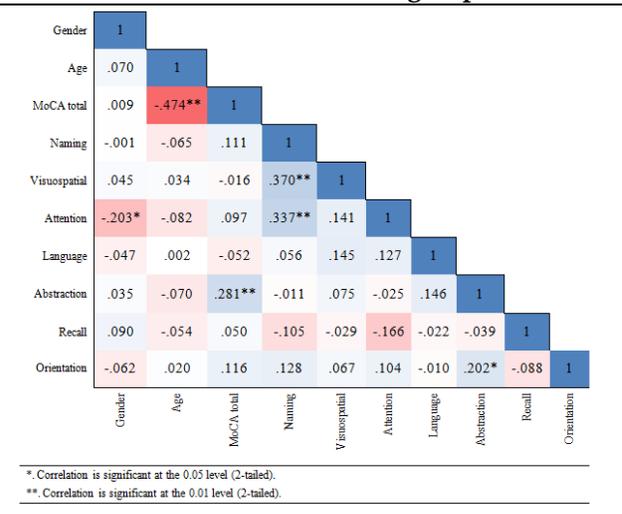


Figure 4 Correlation among total MoCA score and its component along with age and gender in Covid-19 survivor group





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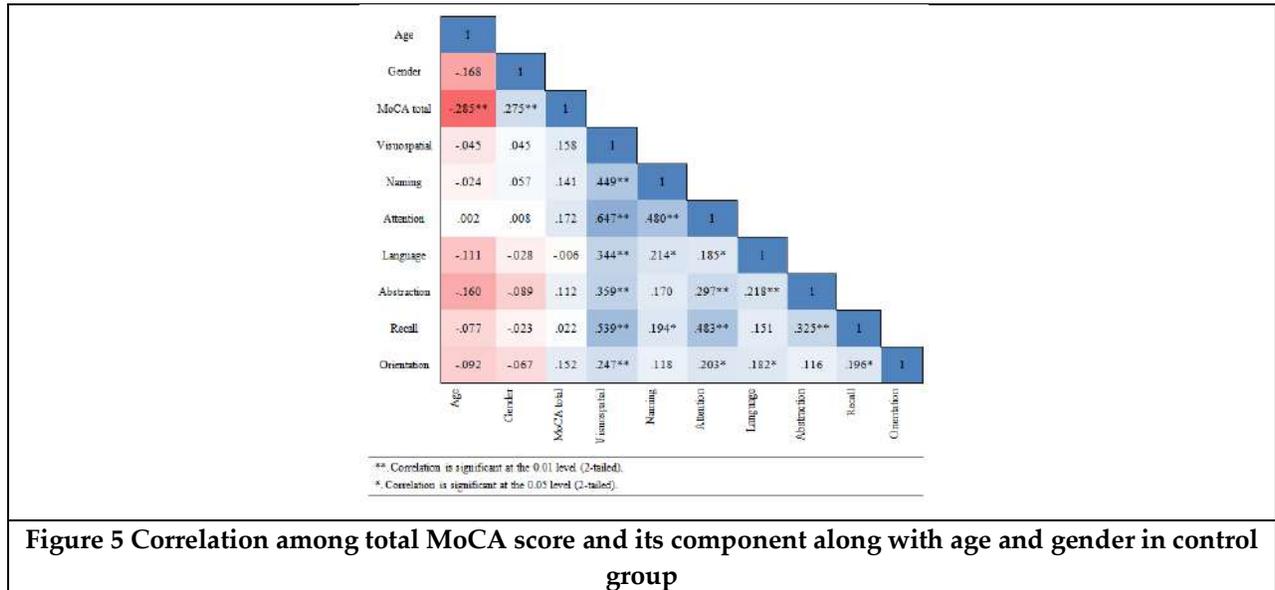


Figure 5 Correlation among total MoCA score and its component along with age and gender in control group





Effect of Core Stability Exercise on Pulmonary Function in Postmenopausal Women – an Interventional Study

Devki Krishnakumar Mehta^{1*}, Pragna Gondaliya² and Krishna Sheth¹

¹MPT Scholar, Shree K.K.Sheth Physiotherapy College, Rajkot, Gujarat, India.

²Associate Professor and Ph.D. Scholar, Shri K.K. Sheth Physiotherapy College and Marwadi University, Rajkot, Gujarat, India.

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*Address for Correspondence

Devki Krishnakumar Mehta

MPT Scholar,

Shree K.K.Sheth Physiotherapy College,

Rajkot, Gujarat, India.

E.Mail: mehtadevki14@gmail.com



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ABSTRACT

Menopause is a significant yet natural biological process. Respiratory problems have risen throughout menopause. Significant hormonal changes brought on by menopause may have an impact on inflammation and immunology, which may compromise lung function. This study aimed to find out the effect of core stability exercise on pulmonary function in postmenopausal women. A total of 42 subjects aging from 50-65 years were allocated and divided into two groups. Group A received core stability exercise and group B received conventional exercise for 5 days/week for 6 weeks. Pulmonary function test to evaluate FVC, FEV1, and FEV1/FVC was performed pre and post of intervention. Results indicated that following an intervention, participants' lung function significantly improved in both groups but more in group A ($p < 0.05$). In conclusion, postmenopausal women who engage in core stability exercises can enhance their pulmonary function.

Keywords: Core stability exercises, PFT, Postmenopausal Women.

INTRODUCTION

Menopause is the permanent cessation of ovarian follicular activity or menstruation. Menopause causes decreased estrogen and progesterone activity. Reproductive hormones fluctuate significantly throughout the menopausal transition, and this fluctuation may be to blame for some particular symptoms like hot flashes and mood swings [1]. Women over 45 are regarded as being in menopause when they have gone 12 months without a period, have consistently low levels of circulating estradiol, and have high levels of gonadotropins. The hypothalamo-pituitary-





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gonadal axis undergoes substantial alterations during menopause, including an increase in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels as 17-estradiol generation in the ovaries slows [2]. Increased systemic inflammation and pulmonary inflammation are linked to low levels of 17-estradiol. Forced vital capacity (FVC) and forced expiratory volume in one second (FEV1) are adversely correlated with the inflammatory markers C-reactive protein and interleukin-6 [3]. These results suggest a probable link between greater lung function loss after menopause. Knowing how to preserve excellent health and quality of life in later years is crucial given that life expectancy now is well beyond menopause is significant [4]. Yet, it is still unclear how aging in women's reproductive systems may affect their respiratory health. An extensive population-based survey's recent longitudinal study revealed a link between menopause and an increase in respiratory symptoms, including a greater prevalence of new-onset asthma [4]. The "core," also known as the lumbopelvic-hip complex, is a three-dimensional region with muscular limits that include the diaphragm (superior), abdominal and oblique muscles, paraspinal and gluteal muscles, pelvic floor, and hip girdle (inferior) [6]. The capacity to stabilize the position and movement of the trunk above the pelvis is the primary role of the diaphragm in core stability. Diaphragmatic control may improve inspiration while easing dyspnea and the effort required to breathe [7]. Here the purpose of the study was to find out the effectiveness of core stability exercise on pulmonary function in postmenopausal women. Pulmonary function was measured by FEV1, FVC, and FEV1/FVC ratio with the help of a spirometer.

Aim of the study

To compare the effectiveness of core stability exercise and conventional exercise on pulmonary functions in postmenopausal women.

Objectives of the study

- 1) To determine the effect of core stability exercise on pulmonary function in postmenopausal women.
- 2) To determine the effect of conventional exercise on pulmonary function in postmenopausal women.
- 3) To determine the effect of core stability exercise and conventional exercise on pulmonary function in postmenopausal women.

METHOD

Sampling: Purposive sampling

Study design: Experimental comparative study

Sampling technique: Simple random sampling

Duration of study: 5 days/ week for 6 weeks

Sample size: 42 subjects were randomly selected from k.k. sheth physiotherapy college, Rajkot.

42 subjects were randomly selected from Shri K. K. Sheth Physiotherapy College, Rajkot. The study was conducted on 42 subjects between the age group of 50-65 years. Subjects were taken from clinics in and around Rajkot based on random sampling. Before participation aims and objectives of the study were explained. All subjects were screened and detailed medical history was taken to exclude any serious illness. Informed consent was signed by the subjects for their voluntary participation. Then the following information was recorded for each patient: name, age, sex, address, height, weight, BMI, heart rate, and systolic and diastolic blood pressure. Subjects were requested to continue their normal activities and avoid any other form of exercise for the duration of the study. 21 subjects in each group were divided randomly. Group A received core stability exercises in form of curl-ups, bilateral SLR, unilateral SLR, hip extension in prone, alternate arm and leg lift in a quadruped position, and bridging [7]. While group B received conventional exercise in form of mild to moderate intensity exercise according to ACSM'S guidelines, including warm-up, aerobic exercise (12 repetitions 2 sets), and cool down [10].





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Inclusion Criteria

- Age: 50-65 Years
- Individuals who are willing to participate in the study

Exclusion criteria

- Subject with a known case of any neurological condition
- Subject with a known case of any other cardiovascular conditions
- Subject with any history of upper or lower limb fracture and other musculoskeletal disorders or disease that would restrict the performance of the exercise.
- Athletes
- Those who are performing regular physical exercise
- Smokers and alcohol drinkers

RESULT

Data analysis was done by using SPSS version 21. Normality was measured by Shapiro wilk test which showed data was not following normal distribution. Within the group, Wilcoxon signed the rank test and between the group, Mann Whitney U test was applied. Both within the group and between the group showed a significant difference within the group and between the group FEV1, FVC ($P > 0.05$) but no significant difference between the group of FEV1/FVC measures. ($P < 0.05$)

DISCUSSION

The baseline data of the demographic and outcome variable did show a significant difference between the subjects in the two groups. By performing core stability exercises, intraabdominal pressure increases, and eventually, intrathoracic pressure becomes negative, which helps to improve the inspiratory capacity of the lungs [7]. Diaphragm is the chief inspiratory muscle as well as core muscle. By directly working on diaphragm strengthening there will be increased inspiratory capacity. Working on abdominals that are responsible for forceful expiration will improve expiratory capacity mainly FEV1 [8]. Strongoli et al. reported in the literature that performing core workouts led to a rise in trans diaphragmatic pressure; this index, which measures the diaphragm's maximum force at functional residual capacity, may be used to measure the effectiveness of a workout. Muscular weakness—compared to rest, some of which may be sufficient to give the respiratory muscles a training stimulus. In a different research, Brilla et al. found that a 6-week regimen of core workouts and inspiratory muscle training enhanced core function in healthy volunteers.

By performing aerobic exercise, the body needs more oxygen and creates more carbon dioxide while exercising because muscles are working harder. Breathing must rise from around 15 times per minute (12 liters of air) at rest to between 40 and 60 times per minute (100 liters of air) during activity to meet this additional demand. Moreover, circulation quickens to ensure that the muscles have enough oxygen to continue working. The chronic effect of aerobic exercise on pulmonary function causes lower respiratory rate, increase lung compliance, decreases work of breathing, increase chest expansion, and increase lung volumes and capacities [11]. Hence alternate hypothesis was accepted that core stability exercise, as well as aerobic exercise, can improve pulmonary functions in postmenopausal women.

CONCLUSION

The study concluded that women who engaged in core stability exercises and conventional exercise programs improved their declined pulmonary functions but core stability exercise showed more significant differences in





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comparison to conventional therapy. Core muscle stability exercise, as well as aerobic exercise, had significant effects on improving pulmonary functions in postmenopausal women.

Clinical implication

The result suggests that in postmenopausal women with deteriorating function of the pulmonary system, regular physical activity along with core stability exercise can improve pulmonary function.

Limitation of the study

Small sample size

Core muscles endurance was not measured

Further recommendations

Core stability exercise on a swiss ball

Large sample size

Abbreviations:

BMI: BODY MASS INDEX

FEV1: FORCED EXPIRATORY VOLUME

FVC: FORCED VITAL CAPACITY

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10. ACSM's Guidelines for Exercise Testing and Prescription
11. Your lungs and exercise,

Table 1: Mean and SD values for data

| | GROUP A | GROUP B |
|---------------|------------------|------------------|
| AGE | 44.91 ± 4.741 | 39.22 ± 6.514 |
| PRE FEV1 | 1.163 ± 0.293 | 1.65 ± 0.68 |
| PRE FVC | 1.20 ± 0.3517 | 1.7740 ± 0.6854 |
| PRE FEV1/FVC | 93.91 ± 9.538 | 85.0248 ± 13.86 |
| POST FEV1 | 1.650 ± 0.293 | 1.9328 ± 0.624 |
| POST FVC | 2.820 ± 0.3704 | 2.0985 ± 0.6456 |
| POST FEV1/FVC | 94.6443 ± 4.2430 | 97.6365 ± 4.1985 |





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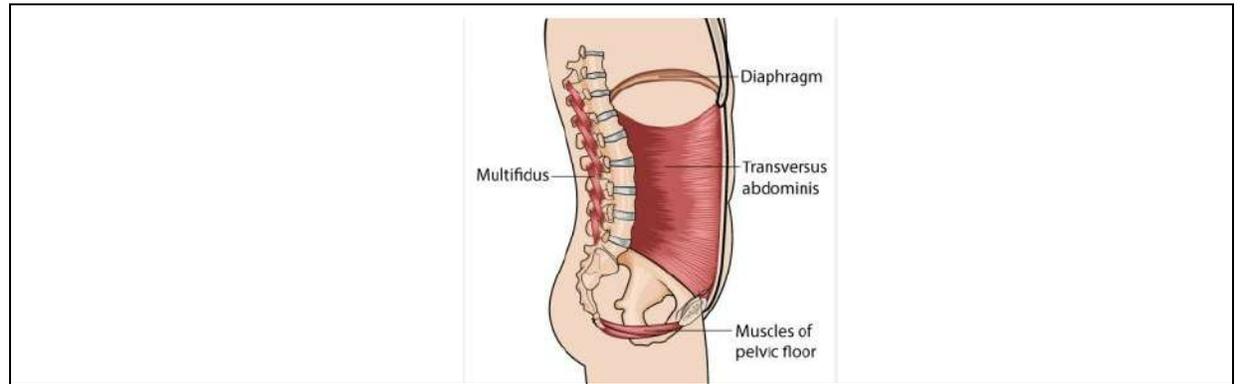


Figure 1: Core Muscles

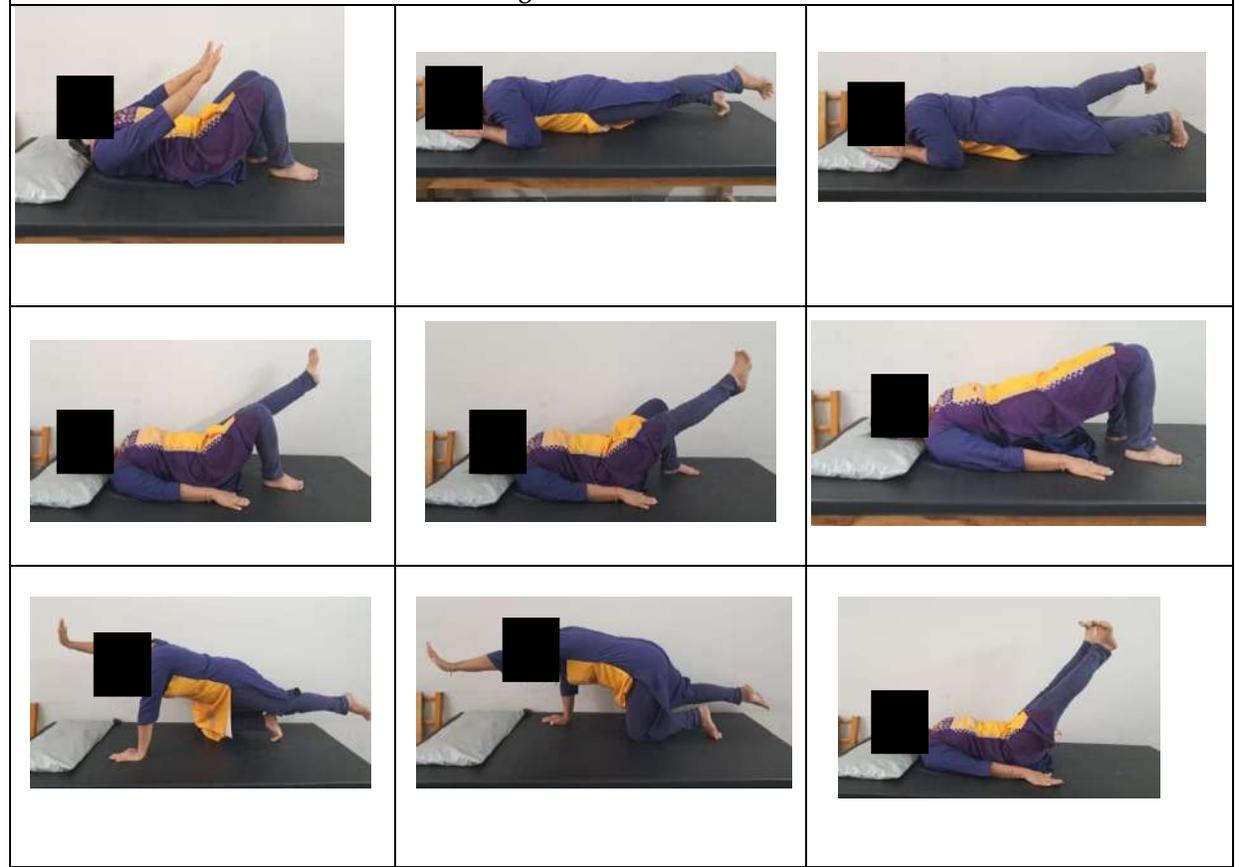


Fig. 2. Exercise





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| | | |
|---|---|--|
| <p>comparison of mean value of FVC in group A</p> | <p>COMPARISON OF MEAN VALUE OF FEV1 IN GROUP A</p> | <p>COMPARISON OF FEV1/FVC RATION IN GROUP A</p> |
| <p>Fig. 3. Comparison of mean value of FVC in group A</p> | <p>Fig. 4. Comparison of mean value of fev1 in group a</p> | <p>Fig. 5. Comparison of FEV1/FVC ration in group a</p> |
| <p>COMPARISON OF MEAN VALUE OF FVC IN GROUP B</p> | <p>COMPARISON OF MEAN VALUE OF FEV1 IN GROUP B</p> | <p>COMPARISON OF MEAN VALUE OF FEV1/FVC IN...</p> |
| <p>Fig. 6. Comparison of mean value of FVC in group b</p> | <p>Fig. 7. Comparison of mean value of FEV1 in group b</p> | <p>Fig. 8. Comparison of mean value of FEV1/FVC in group</p> |
| <p>COMPARISON OF MEAN VALUE OF FVC BETWEEN GROUP A AND GROUP B</p> | <p>COMPARISON OF MEAN VALUE OF FEV1 BETWEEN GROUP AND GROUP B</p> | <p>COMPARISON OF MEAN VALUE OF FEV1/FVC IN GROUP A AND GROUP B</p> |
| <p>Fig. 9. Comparison of mean value of FVC between group a and group b</p> | <p>Fig. 10. Comparison of mean value of FEV1 between group and group b</p> | <p>Fig. 11. Comparison of mean value of FEV1/FVC in group a and group b</p> |





Feeding Habits and Habitat use of Indian Peafowl (*Pavo cristatus*) in Specific Sites in Bharatpur Rajasthan

Bhagat Singh^{1*} and Manju Lata²

¹Assistant Professor, Department of Zoology, M.S.J. Govt. College, Bharatpur Rajasthan, India

²Professor, Department of Zoology, M.S.J. Govt. College, Bharatpur, Rajasthan, India

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*Address for Correspondence

Bhagat Singh

Assistant Professor,

Department of Zoology,

M.S.J. Govt. College,

Bharatpur Rajasthan, India

E.Mail: bpenghore@gmail.com.



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ABSTRACT

This paper discusses habitat utilization, food and feeding habits, and roosting preference of Indian Peafowl *Pavocristatus* in different areas of Bharatpur District. These are Village Kandholi in Tehsil Rupbas (location I) and Village Saman-Penghore in Tehsil Kumher (location II) From February 2022 to March 2023. Indian Peafowl showed a preference for flowers, leaves, and fruits of wide-ranging cultivated crops and indigenous trees. It was further recorded that the preference for food items depended upon the different crops, drop foliage, and uncultivated areas. Roosting was noted on indigenous trees at location I as compared to buildings/ roofs at location II. Indian Peafowl substantially used tree species such as Eucalyptus, Pear, Poplar, Neem, Amaltas, Peepal, Jamun, Mango, Banyan, Ber, and Sheesham for roosting. Although the diet of peafowl constituted both animal and vegetable matter, vegetable matter comprised 90%. Analysis of feeding and roosting habits will be useful for the conservation of Indian Peafowl and to minimize the conflict with humans.

Keywords: Feeding, Indian Peafowl, roosting, tree species

INTRODUCTION

The term peafowl is used for two species of birds (*Pavocristatus* (Indian peafowl) and *Pavomuticus* (Green peafowl) the genera *Pavo* of the pheasant family, Phasianidae. The Indian Peafowl, *Pavocristatus* Linnaeus 1758 is an occupant breeder of South Asia and set up across India and also in Sri Lanka. It was declared the National Bird of India in 1963 due to its flagship value found in its glorious position in mythology (Ali & Ripley, 1987).



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The physical appearance and behavior of peafowl have always created interest and intrigued naturalists for a long back. The distribution of peafowl is almost widespread and quite common in northern India, but due to the high demand for its train feathers, it is presently under threat. It is a bird of scrub jungles and forest edges, showing affinity to moist, dry deciduous, and semiarid biomes. It is also found in agricultural fields, along streams with good vegetation, and close to human habitations in a semi-feral condition (Johnsgard, 1986). The species is virtually an omnivorous and opportunistic feeder on a wide variety of insects, plants, seeds, tender shoots, amphibians, reptiles, and worms (Baker & Inglis, 1930).

Indian Peafowl preferably roost on high, open trees to protect themselves from tree-climbing, night predators. It roosts on trees and also uses tall buildings where trees are scarce (Gadgil, 1972). Roosting is the most common process of birds, which lowers the loss of body heat and decreases the risks associated with predators. The roosts of Indian Peafowl give us a lot of management strategies to safeguard the trees in the relevant habitats (Trivedi and John Singh, 1996).

MATERIALS AND METHODS**Study area**

The present study was carried out from February 2022 to March 2023 in different areas of Bharatpur District. These are Village Kandholi in Tehsil Rupbas (location I) and Village Saman-Penghore in Tehsil Kumher (location II). The location has a large stretch of the hilly area with rich biodiversity and agricultural fields. Location II consists of a large area of agricultural fields with dense vegetation. It is a medium size village.

The field area is distributed under different categories of crops such as wheat, pearl millet, vegetables, fodder, and orchards. In addition, there is a rich diversity of trees in the study areas. The roosting habits of Indian Peafowl were observed in both areas. The tree species used by Indian Peafowl for roosting in orchards, scrubby and dense regions of both the study areas were recorded and identified. The point count method was used to record the feeding habits of Indian Peafowl (Blondel *et al.*, 1981). Monthly and seasonal records of tree species utilized for roosting activity by Indian Peafowl were maintained.

RESEARCH METHODOLOGY

Peafowl was directly observed under natural conditions during three time periods: 0600–1000 hrs, 1000–1400 hrs, and 1400–1800 hrs respectively. Observations were recorded in a field notebook and the duration of each observation was noted with a stopwatch. When a bird was sighted, it was followed to the extent possible. Observations were taken with the help of binoculars, photography, and videography done by Nikon D3500 DSLR Camera. To understand the roosting behavior of peafowl, observations were made in the early morning and late evening. Because peafowl regularly roosts during dusk, and congregates till dawn, (Sharma, 1979) suggested that counting peafowl at roosting sites at dusk and dawn could determine their abundance. Whenever a roost tree was located, it was marked with paint, and various parameters such as tree height, site location and elevation, distance from a water body, distance from human path/road, canopy cover, and roosting height were recorded. Whenever roosting behavior was observed, time of arrival, time of settling, and flock size were noted.

RESULTS AND DISCUSSION

The Indian Peafowl are omnivorous and feed on seeds, insects, fruits, small mammals, and reptiles. The present study revealed that Indian Peafowl spends more time feeding on different types of herb matter than animal matter. Similar observations were recorded (Navaneethakannan, 1981). They were observed to spend maximum time in feeding activity as compared to other activities. They were found to forage in crop fields in groups or individually during early morning and evening. It was observed that Indian Peafowl preferred the leaves of certain plant species



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but was infrequently set up to feed on the flowers of the same plant species (Table 1). At Location, I, Indian Peafowl was set up to feed on the no identical cereal and fodder crops like wheat, paddy, pearl millet, and berseem, and also consume flowers and leaves of pear. Indian Peafowl was observed to feed on flowers/leaves/fruits of *Brassica campestris* (flowers, leaves), *Trifolium alexandarium* (leaves), *Triticum aestivum* (leaves), *Oryza sativa* (leaves), *Chenopodium album* (Flowers, leaves), *Parthenium hysterophoresus*(flowers, leaves), *Pisum sativum* (flowers, leaves), *Pyrus communis* (leaves, fruit), *Ficus benghalensis*(flowers, fruit), *Ficus rumphii*(flowers, fruit) at the location I. At location II, they eat up a variety of cereal and fodder crops like maize, pearl millet, wheat, paddy, and vegetables viz: brinjal, tomato, chilies, carrot, potato, and radish. At location II, they foraged on the crops like *Brassicacampestris* (flowers, leaves), *Trifolium Alexandria*(leaves), *Triticum aestivum*(leaves), *Oryza sativa* (leaves), *Chenopodium album* (Flowers, leaves), *Ficus rumphii*(flowers, fruit) (Table 1)

(Chopra and Kumar,2014) also recorded the same kinds of observations.(Yasmin & Yahya,1996) also recorded that Indian Peafowl fed on seeds, leaves, and wild herbs. At location, I, females were reflected to spending further time in feeding activities than males. Males spent much more time feeding than females at location II. They were sighted feeding on small insects at both locations. Flocks of Indian Peafowl were observed to consume different types of invertebrates (mostly insects) and reptiles (Table 2).

Johnsingh& Murali (1980) reported that Indian Peafowl fed on a wide range of crops such as groundnut, tomato, paddy, chilly, and bananas in the farmed areas. Berries and drupes of plants such as *Carissa*, *Lantana*, *Zizyphus*, and wild figs (*Ficus*) were favored foods of Indian Peafowl. At the location, I, the sites preferred by Indian Peafowl were surveyed where they spent maximum time of day and night roosting. There was a rich diversity of tree types i.e. why they preferred tall trees with dense foliage or thorny trees for roosting. (Veeramani,1999)reported the same observations that Indian Peafowl preferred thick foliaceous species for roosting and voluminous birds roost on altitudinous trees and small birds need small trees for roosting.(Johansgard,1986) mentioned analogous results that Indian Peafowl (*Pavocristatus*) roosted on tall trees.

During our observations, Indian Peafowl was set up reposing in the sunshine found during the morning and resting in shadowed areas during the hot afternoon. At night, they roosted on top of trees. It might be related to their safety from predators or the paucity of buildings. Indian Peafowl was noticed resting under the shades during noon observations, and its behavior differed in the evening observations. It seemed to have a preference for open areas in the evening hours. They were found to prefer trees like Eucalyptus, Poplar, Lasura, Amaltas, Peepal, Jamun, Mango, Banyan, Pilkan, and Dhek for roosting. All these were stout branched trees that could afford moving space, accommodating their long train feathers as well as could withstand their weight. The roost tree height ranged from 8-25m and the roost height varied between 7-20m. Similar observations were given by (Dodia,2011), that Indian Peafowl roosted mainly on *Azadiracta indica*, *Ficus bengalensis*, *Eucalyptus*, and *Cocos nucifera* in Gujarat state.

CONCLUSION

It could be concluded that the wide feeding range and diversity in its roosting preferences of Indian Peafowl might have enabled it to live in different sub-habitat types within the agrarian geography in both locations. Further ecological and ethological studies should be conducted on Indian Peafowl to better understand its ecological niche in formulating its conservation strategies in different niches of the agro ecosystem. From this study, it is gathered that the peafowl is an omnivore that relies substantially on vegetable matter; prefers large trees with thick foliage, or spiny trees for roosting; and prefers open scrub vegetation for foraging. Stray dogs seem to be the major predator for Peafowl in these locations.





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Table 1: Different types of plants and vegetation consumed by Indian Peafowl

| | Common Name | Scientific name | Location I | Location II |
|------------|-------------|-------------------------------|-----------------|-----------------|
| Crops | Mustard | <i>Brassica campestris</i> | Flowers, leaves | Flowers, leaves |
| | Wheat | <i>Triticum aestivum</i> | Leaves | Leaves |
| | Berseem | <i>Trifolium alexandrinum</i> | Leaves | Leaves |
| | Matar | <i>Pisum sativum</i> | Flowers, Leaves | ----- |
| Wild plant | Bathua | <i>Chenopodium album</i> | Flowers, leaves | Flowers, leaves |
| Trees | Bargad | <i>Ficus benghalensis</i> | Flowers, Fruits | Flowers, Fruits |
| | Mango | <i>Mangifera indica</i> | Fruits | ----- |

Table 2: List of animals consumed by Indian Peafowl

| Common name | Order/family |
|---------------|--------------|
| Beetles | Coleoptera |
| Grasshopper | Orthoptera |
| Bugs | Hemiptera |
| Termite | Isoptera |
| Ant | Hymenoptera |
| Earthworm | Oligocheata |
| Garden Lizard | Lacertilia |
| Small Snake | Ophidia |





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Figure 1- Flock of Indian peahens foraging and feeding on *Cynodon dactylon*.



Figure 2- Indian Peafowl Foraging and Roosting



Figure 3- Indian peahen feeding on Household Waste



Figure 5- Indian Peafowl roosting on Eucalyptus trees.





Digital Pharma: How Software Solutions are Shaping the Pharmaceutical Industry

T.Naresh Narayanan¹, M.Arjun², Sangita Mishra² and M.P. Venkatesh^{3*}

¹M. Pharm Student, Pharmaceutical Regulatory Affairs Group, Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagar, Mysuru-570015, Karnataka, India

²Ph.D. Scholar, Pharmaceutical Regulatory Affairs Group, Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagar, Mysuru-570015, Karnataka, India

³Associate Professor, Department of Pharmaceutics, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Sri Shivarathreeshwara Nagar, Mysuru-570015, Karnataka, India and Guest Assistant Professor, Faculty of Pharmaceutical Sciences, UCSI University, Malaysia.

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*Address for Correspondence

M.P. Venkatesh

Associate Professor,

Department of Pharmaceutics,

JSS College of Pharmacy, JSS Academy of Higher Education and Research,

Sri Shivarathreeshwara Nagar, Mysuru-570015, Karnataka, India and

Guest Assistant Professor,

Faculty of Pharmaceutical Sciences,

UCSI University, Malaysia.

E.Mail: venkateshmpv@jssuni.edu.in



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ABSTRACT

The pharmaceutical sector relies extensively on advanced software for optimizing drug discovery, development, manufacturing, and regulatory compliance. This study delves into the pivotal role of software in this industry. Computational tools aid in identifying potential drug candidates through virtual screening and molecular modeling. Data management software enhances clinical trial efficiency, facilitating data handling and collaboration. Clinical Data Management Systems streamline data collection, validation, and analysis, integrating information from various sources for evidence-based decision-making. Process simulation software ensures quality and regulatory adherence in manufacturing. Manufacturing Execution Systems elevate production processes by seamlessly integrating data and processes. Quality control and regulatory submission software maintain production standards and expedite approvals. Regulatory Information Management Systems enable precise regulatory compliance through unified data management. Pharmacovigilance software monitors adverse





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events post-market, aiding safety and compliance. Supply chain management software optimizes distribution efficiency. In sum, software solutions drive research, operational efficiency, and compliance in pharmaceuticals, contributing to safe and effective global healthcare solutions.

Keywords: Pharmaceutical Software, RIMS, Clinical Data Management System, Electronic Document Management System.

INTRODUCTION

Pharmaceutical Regulatory Affairs is a critical function within the pharmaceutical industry that involves ensuring that pharmaceutical products are developed, manufactured, and marketed in compliance with regulatory requirements. To accomplish this task, various software tools are used to manage the regulatory information, documentation, and compliance activities. The software tools used in Pharmaceutical Regulatory Affairs help in ensuring regulatory compliance, maintaining quality standards, and producing safe and effective pharmaceutical products.[1]Pharmaceutical and biopharmaceutical industries historically have not been the forerunners of groundbreaking engineering innovations and contemporary chemical engineering approaches. A regulatory framework that protected the final product's quality and examined the characteristics of batch-based operations, raw materials and end products set process conditions, and in-process materials has been used to monitor the production of pharmaceutical goods for many years.[2]Testing methodologies for both small molecule and biopharmaceutical products have generally acknowledged limitations relating to this uniformity. On both sides of the manufacturing spectrum, cutting-edge techniques have been successfully applied to improve our understanding of processes and products.[3]

Software is employed extensively in pharmaceutical regulatory affairs in a number of crucial areas, including:

- Electronic Document Management Systems (EDMS): Software solutions that help manage regulatory documents such as applications, regulatory submissions, and approvals.
- Regulatory Information Management Systems (RIMS): Computer programs that facilitate the management of regulatory data, such as product details, submissions, and registrations. Pharmacovigilance Systems: Software solutions that help manage adverse event reporting and safety monitoring.
- Quality Management Systems (QMS): Software solutions that help manage quality-related activities such as document control, change management, and CAPA management.
- Clinical Trial Management Systems (CTMS): Software solutions that help manage clinical trial data and regulatory compliance.
- Electronic Data Capture (EDC): Software solutions that help manage and analyze clinical trial data.

For the implementation of a risk management system and the establishment of a successful information producing operation at the critical phases of the pharmaceutical product life cycle, Regulatory Information Management System(RIMS) must be used in conjunction with technological advances to maintain data coherence. The protection of the coherence and authenticity of data related to such a crucial element of the public healthcare system as pharmaceuticals will be required in the event that registration documents are updated in the future or pharmacovigilance is implemented.[4]



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DISCUSSION

Electronic Document Management Systems (EDMS):

A software solution, Electronic Document Management System (EDMS) enables businesses to efficiently and securely create, manage, and store electronic documents. An crucial tool for handling regulatory documents, such as applications, regulatory submissions, and approvals, in the context of pharmaceutical regulatory affairs is EDMS. Given that regulatory agencies frequently need access to particular documents, EDMS management of regulatory compliance is essential in the pharmaceutical industry. Pharmaceutical firms can secure and control how they store and handle important records including Standard Operating Procedures (SOPs), batch records, clinical trial documentation, and regulatory submissions, thanks to EDMS.[5] Version control, audit trails, access control, and document retention management are just a few advantages that EDMS offers. These features support maintaining document integrity, guaranteeing regulatory compliance, and lowering risks. Pharmaceutical firms can increase productivity in handling documents across their lifecycles, decrease manual processes, and streamline document workflows with EDMS. Due to the growing number of digital records and the necessity for effective management, EDMS has grown in significance for enterprises and organizations.[6]

EDMS helps to improve productivity, reduce paper usage, lower costs, and enhance security and compliance. Due to the increasing volume of electronic documents and the necessity for effective management, EDMS has grown in significance for enterprises and organisations. With EDMS, there is an increase in productivity, less paper usage, money saving, and improved security and compliance.[7]

In the pharmaceutical sector, some of the most popular EDMS programs are:

- **Veeva Vault:** Veeva Vault, a cloud-based EDMS solution, enables pharmaceutical businesses to safely and legally manage their records, data, and operations. Information management, group collaboration, documentation of audits, and compliance monitoring are among the options available.
- **Documentum:** Pharmaceutical companies can manage both their content and processes on a single platform via an enterprise content management system called Documentum. Automation of workflows, document and records management, and compliance monitoring are among its features.
- **OpenText:** OpenText is a software that manages content, processes, and information and offers numerous solutions. It can do tasks including document management, records management, compliance tracking, and archiving.
- **SharePoint:** SharePoint is a platform for online document management and collaboration that was made by Microsoft. Workflow automation, version control, and compliance monitoring are some of its features.
- **Master Control:** Pharmaceutical companies can use Master Control, a cloud-based quality management system, to manage their documents, processes, and quality. There are features offered, including information management, group collaboration, documentation of audits, and conformance monitoring.[8]

Regulatory Information Management System (RIMS)

RIMS is a rapidly expanding software category that makes it easier to submit human medical products, such as drugs, biologics, and medical devices, to regulatory bodies so they can eventually be licensed for sale in a particular market. Regulatory information management systems and software, collectively referred to as "regulatory information management," are computer programmes that:

- a) keep track of product dossiers that contain regulatory product data,
- b) assistance with creating product submissions in authorized forms
- c) oversee the submission of data to regulatory bodies in the appropriate formats.[1]

An effective RIMS organizes the tasks required to get the go-ahead for sale of human medical products. This might not initially appear important, but when you take into account how complicated the other entries are, it becomes





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important. When it created the common technical document (CTD), the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) made considerable strides toward harmonizing product submission processes among agencies. Four of the CTD's five modules are utilized by all accepting agencies, but Module 1 varies per location. There is a lot of support for it. Additionally, for the submission of pharmaceutical and biologic products, the USA, EU, Japan, China, Canada, and a few other nations now recognize the electronic Common Technical Document (eCTD), which consists of a data requirements based on health level seven (HL7) regulated product submissions (RPS).[9] As a result, even though significant attempts at harmonization have made the load of item submission and evaluation lighter, there are still a lot of complex issues. The pharmaceutical industry is heavily regulated by the government, which is one of its characteristics. All pharmaceutical items are tested before being allowed on the market.

Before the pharmaceutical product can be used, the regulatory body must receive information on it in the form of a dossier. One part of the constant data flow carried out in the RIMS is the work on dossier development.[10]. The following features are present in an efficient regulatory information management system:

- Identifies the product data that international regulatory bodies require.
- Create dossiers containing product information, such as eCTD dossiers.
- Incharge of the dossier settings to take product and regulatory agency variance into account.
- Oversee updates and modifications to product information and dossiers.
- Creates submission documents that are compliant.
- Issues and keeps track of submissions through the proper gateways and on the paper.
- Cuts down on the time and effort needed to duplicate regulatory submissions between products and regulatory agencies. Thorough oversight of initial and lifespan submissions is offered.
- Furnish adequate assistance given the complexity and size of the firm.
- Complies with legal specifications for electronic devices.[11]

The following list includes some of the RIM systems used most frequently in the pharmaceutical sector:

- **Aris Global RIMS:** Designed to assist pharmaceutical businesses in managing regulatory information and compliance, Aris Global RIMS is an online regulatory information management system. It has functions including reporting on compliance, tracking submissions, and document management.
- **Veeva Vault RIM:** Developed to assist pharmaceutical businesses in managing regulatory information and compliance, Veeva Vault RIM is an online regulatory information management solution. It has functions including reporting on compliance, tracking submissions, and document management.
- **ISIREgulatory Suite:** Developed to assist pharmaceutical companies in managing regulatory information and compliance, ISI Regulatory Suite is a regulatory information management system. It has functions including reporting on compliance, tracking submissions, and document management.
- **Samarind RMS:** Samarind RMS is a regulatory information management tool created to assist pharmaceutical firms in the administration of regulatory data and compliance. It has functions including reporting on compliance, tracking submissions, and document management.
- **Lorenz Docubridge:** Lorenz Docubridge is a system for managing regulatory information and compliance that is aimed at assisting pharmaceutical companies. It has functions including reporting on compliance, tracking submissions, and document management.[1]

Digital solutions for the production of electronic dossiers enable the sector to efficiently produce an infinite number of child dossiers based on the core list of documents. Collective updates of documents within sets of files are permitted as long as the change is comprehensive and affects both the primary file and any subordinate files. RIMS offers secure storage of both by dependably separating the open and closed components of the documents. By automating the process, digital solutions lower the number of errors associated with the human component. As a result, costs are reduced and the management of regulatory actions is expedited. RIMS enables departments that communicate with regulatory bodies to automate their job, which is a significant fortuity to increase the effectiveness





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of a pharmaceutical firm as a whole.[3]Businesses that deliver consultancy services in the field of circulation of pharmaceutical product might gain a major competitive advantage by utilizing automation technologies. It is difficult to exaggerate how important it is to pass regulatory requirements. The time it takes for the drug to enter the legal market and, subsequently, the profit for the pharmaceutical corporation, determines the fastest and most efficient way through them. Every day that may be saved before the first sales begin is crucial when registering an original medicine or the first generic.[12]By utilising RIMS, we may shorten timelines and prepare the required paperwork and supplies in advance, which will enable a pharmaceutical company to enter the market first.Making the most timely and efficient decisions is dependent on how quickly the required information can be gathered and processed. A large number of items are often presented to the registration department's staff. They could be using different market representations, applying separate regulatory standards, and being at various phases of the regulatory lifecycle. There are a great deal of documents that need to be examined, handled, and organised.[3]This causes human factor errors, which cause delays in the progress of regulatory procedures.When there are differences between the input data and the requirements of regulatory documents, RIMS's analytical capabilities enable you to receive immediate hints. The likelihood of errors is thus decreased to 30%. The passing of regulatory processes is also decreased from 10% to twice.[13]

Many firms use paperwork or, at best, worksheets and other non-specialized solutions for providing information for regulatory processes. In addition to requiring a lot of work from the personnel to maintain the information current, this organisational style also causes gaps between the functional and regulatory information.RIMS is regularly maintained and updated by top authority on regulatory standards to ensure that all current laws and regulations are reflected in the system's algorithms. With projects already loaded into the system, users are instantly informed by prompt notifications when they need to perform particular actions.[14]More efficient software solutions are required for pharmacovigilance as a result of the necessity to control the risks associated with utilizing pharmaceutical goods at all stages of their life cycle in order to safeguard patients and manufacturers and maintain conformance with legal requisites.

The feasibility of performing a clinical trial is evaluated as part of managing clinical research. Additionally, the implementation of the potential for integration with systems for collecting electronic patient data and research findings.[15] The creation of documents and the management of each study subject are done automatically, which improves the effectiveness of the research's execution.Pharmacovigilance data maintenance is a necessary, time-consuming, and expensive procedure that doesn't directly generate revenue for the pharmaceutical business. As a consequence, the larger part of those involved in the distribution of pharmaceuticals feel pressure to keep pharmacovigilance costs as low as possible while still adhering to legal obligations. In this sense, RIMS are collections of specialized software that enable the professional completion of this range of tasks. They allow pharmacovigilance and adverse event reports to be categorized, created, viewed, sent, and maintained in a single application.This programme records information submissions, facilitates multi-user work, and helps users comply with international regulatory authorities' requirements.Information can be securely stored for the long term using a single database. By using a cloud service rather than local files on each employee's hard drive, data storage dependability is increased.[16]It is possible for this application to be connected with other systems and components to obtain information about pharmaceutical goods.

Regulators employ solutions that analyze data uploaded by user to assist pharmaceutical firms in managing their drug portfolio, prepare regulatory measures, and monitor the situation. These solutions are especially crucial for businesses that oversee a sizable project portfolio and have a presence on numerous international marketplaces.The regulatory agencies are the industry's brains, and they ultimately decide what will happen to a pharmaceutical company's assets.[17]To guarantee the accurate evaluation of medications in terms of attribute, efficacy, and safety, it is required to coordinate complicated actions with numerous stakeholders in addition to ensuring adherence to norms and laws. The internal regulatory actions planning, scheduling, and progress monitoring are crucial for the prompt introduction of a pharmaceutical product to the market. The effectiveness of these ostensibly insignificant tasks has a direct impact on the pharmaceutical company's financial success. Each day, the absence of authorization





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of medical use might cost the corporation millions of dollars. Many businesses use MS Excel and other temporal information technology solutions to support these processes, which often rely heavily on labor-intensive manual labour. Enterprise management systems and regulatory product databases are two examples.[18] In addition to endangering general data integrity and dependability, using non-specialized solutions, more critically, causes a gap between operational and regulatory data. This makes it far more difficult to coordinate the testing and manufacturing processes and raises the possibility of documentation inaccuracy. Data continuity planning takes into account both the system's hardware and data from an information technology standpoint. Eliminating points of failure should be the main objective while developing a sustainable system. The emphasis should be on hard drives because they are prone to errors and are employed as storage media.

Using a redundant array of low-cost Redundant Array of Independent Discs (RAID) is the greatest way to increase data storage resilience. The installation of a RAID system, which is a significant part of the deployment of data resiliency systems, will make it feasible to replace the hard drive. Potential power outages must be accounted for when designing a sustainable system.[19] One of the components of the Regulation of Information Technology Risk Management System is solutions for data security and continuity. These rules are meant to ensure and maintain the proper operation of an efficient information technology risk management system, consistent with the size and complexity of the digital support department, with the goal of providing pharmaceutical companies confidence in achieving the goals set by the requirements of data regularity and integrity provision. The tasks of the risk management system include identifying, analyzing, and evaluating information technology hazards that emerge at every level of the pharmaceutical organization. It also includes efficient resource allocation and utilization for risk management. This can be accomplished by building a reliable knowledge basis for operational planning, risk-based management decision-making, and logistical planning for the pharmaceutical organization.[20]

For the implementation of a risk management system and the establishment of an efficient information-generating process at the crucial stages of the pharmaceutical product life cycle, RIMS must be used in combination with technological approaches to preserve data continuity. The maintenance of the continuity and validity of information related to such a crucial element of the public healthcare system as pharmaceuticals will be required in the event that registration documents are updated in the future or pharmacovigilance is implemented.[21]

Pharmacovigilance Software

Pharmacovigilance software is created to assist pharmaceutical firms and regulatory organizations in managing and monitoring the safety of medications and other healthcare items. The research and practices surrounding the Identification, assessment, understanding, and prevention of side effects or any other issues related to drug are referred to as pharmacovigilance.[22] Features like adverse event reporting, signal identification, risk assessment, and safety data management are frequently included in pharmacovigilance software. The software enables the collection and analysis of information from numerous sources, such as clinical trials, post-marketing surveillance, and systems for spontaneous reporting. This makes it easier to spot potential safety problems, keep an eye on the efficacy of medications in real-time, and take the necessary steps to reduce risks to patient health.[23]

Some of the softwares used in Pharmacovigilance are:

- **Oracle Argus safety:** Businesses are shifting their attention in order to adopt a more thorough approach to product safety, beginning with clinical trial development and extending through post-marketing surveillance. The comprehensive platform Oracle Argus safety was created especially to handle the demanding pharmacovigilance needs of the life sciences sector. The Argus safety enhanced database enables sound safety decisions, ensures compliance with international regulations, and unifies management and safety operations.[8]
- **Adverse Reaction Information System Global (ARISg):** Pharmaceutical businesses frequently use ARISg, one of the most popular pieces of pharmacovigilance software. More than 300 businesses utilize it to store important data about the security of pharmaceuticals in ARISg. ARISg offers all the services needed to manage adverse events reporting and the requirements for adverse effects of different regulatory authorities across the world.[24]



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- **Oracle AERS:** Companies that make biological products, vaccines, medical equipment, and contracted research companies frequently struggle to meet time-sensitive regulatory criteria with a finite amount of resources. They must recognise safety occurrences, manage them before they turn into problems, and uphold rigorous adherence to ever-changing regulations. They need a clear view of their data in order to handle crucial business activities.[25]
- **PvNET:** PvNET is a comprehensive pharmacovigilance solution that goes beyond simple compliance with its adverse effect reporting, adverse drug response ,data tracking, and regulatory submission of ICSR (Individual Case Safety Report). PVNET helps consumers make critical decisions by combining safety information from early development through post-marketing. PVNET has successfully completed audits for pharmaceutical safety that were conducted in accordance with GMP standards, 21 CFR compliance, and ICH E2B across the board.[26]
- **RepClinical:** RepClinical is a safe web-based platform that makes it easier to manage crucial pharmacovigilance responsibilities effectively and affordably. One can gather data on adverse events using RepClinical, create regulatory report, and share ICRSs with a variety of regulatory bodies and commercial partners. Everything is carried out in a simple, useful way. It is simple to develop accurate E2B reports using Rep Clinical's clear displays and useful features.[27]

Clinical Data Management System:

“The creation, implementation, and oversight of strategies, plans, policies, programs, and routines for managing, delivering, and enhancing the value of data and information assets” is how Clinical Data Management(CDM) is defined in the context of clinical trials. With its varied interconnectedness, cross-functional capabilities, and scope of responsibilities, CDM has advanced considerably over the past 20 years and is now a recognised profession with growing recognition of its significance in development and research of biopharmaceuticals. Despite how complicated and evolving the field is, CDM persists to develop into an area unto itself, focusing on the management of data connected to clinical trials as a significant resource.[28]

One of the skill sets that CDM is developing as a profession is a background in solid clinical expertise, rigorous science, information technology, system engineering, and excellent communication abilities. It is crucial to comprehend, recognize, work within the structure of worldwide clinical advancement, and employ norms in the establishment and implementation of designs, rules, guidelines, and processes that properly manage the full clinical data lifecycle needs of an enterprise, provided the continuing worldwide harmonization of clinical research and the introduction of regulatory-mandated electronic submissions in the industry.[29] The range of jobs covered by this phrase is fairly broad and may involve a variety of activities unrelated to managing relational databases or other more basic data management methods. Numerous additional subjects, activities, and processes are also pertinent, such as:

- Data management, including standard operating procedures, standards, and policies.
- Management of databases, including data upkeep, administration, and tagging between pertinent clinical or other systems.;
- Aspects of data security management include data access, archiving, privacy, and security as well as information architecture, evaluation, and style, which includes modeling data for a potential clinical data repository or warehouse.
- Data fusion, management of master data , external data transmission, and reference data are all included in reference and master data management.
- Mining information, ETL (extract, transform, and load), and other aspects of business intelligence (BI) administration and data warehousing tools.
- Managing documents, records, and content.
- Metadata management, which includes standardisation, metrics, publication, discovery, and definition of metadata.[30]





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Clinical data management (CDM) has developed and will continue to develop as a consequence of substantially enhanced clinical integration, worldwide standardization, and expected clinical systems interconnection projects. This is in response to the unique multi-functional needs and in line with the distinctive advantages of e-clinical research developments. The subsequent years will go through numerous anticipated reality checks since it is not what it once was. When it comes to clinical trials, there is a lot of paper paperwork. The erroneous expectation of CDM experts was that Electronic Data Capture technology would significantly increase productivity by reducing the amount of printed paperwork required.[31] Although not all sponsor companies perform as well as claimed, some have achieved some of the planned long-term cost savings with claimed clinical efficiencies. Deployment of a computerized document management or clinical trial management system appears to be ineffective in slowing the document buildup, despite the fact that the rate at which paper piles are growing may have slowed for some sponsor firms. Therefore, just as much as clinical science and technology itself, managing change is essential for the effective setup and integration of EDC technology with other critical clinical components. Sponsor e-clinical solutions deployment has never been without challenges, particularly organizational ones.[32]

Electronic Data Capture

It is a computer-based system created for the electronic collecting of clinical data. The conventional paper-based data collecting approach has been replaced by EDC, which helps to streamline data collection and shorten the time it takes for pharmaceuticals and medical devices to reach the market. Many pharma companies and clinical research organisations are now utilising EDC systems for the management of clinical trial data. Users of EDC include Sites (a location where clinical trials are being done), Sponsors (companies or individuals funding clinical trials), CROs (contract research organisations that assist with clinical trial planning and execution), and others. It is used to gather and manage data during all stages of clinical studies. Electronic data capture is a better alternative to traditional paper-based data collecting for clinical trials since it allows information to be entered into the software directly over an internet connection.[33]

- **Goals of using an EDC system in a clinical study:**

- i. Access to real-time data.
- ii. Effective data sharing and a quicker impact on drug marketing.
- iii. Addressing the issue of case report forms being shipped on paper from far-off places.
- iv. Coordination between the data base and major adverse effect reporting.[34]

- **Electronic data capture (EDC) versus paper data capture (PDC):**

Despite the fact that EDC tools have been around for more than 20 years, clinical trials are still conducted using the PDC process. This is due to the fact that technological applications frequently lacked sufficient functionality to enhance the overall data collection process, as well as the expense and maintenance of the electronic systems. The sole variation between the PDC CT Management model and the EDC model is in the sub-processes.[35]

Advantages of EDC

- i. Improved data transfer speed.
- ii. Staff can get data instantly.
- iii. There are fewer queries.
- iv. Data classification and indexing are also options.
- v. Decision-making can be completed more quickly, which will result in time and cost savings.

Disadvantages of EDC

- i. Each PC must have software installed, which is more expensive
- ii. The accessibility of Internet connections in the trial's remote locations
- iii. If a public internet connection is used, data security is a significant issue.
- iv. Electronic device evaluation on a regular basis
- v. Adherence to regulations. [36]





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Some EDC Software used in Pharmaceutical Industry are:

- **Oracle Health Sciences Clinical:** Oracle Health Sciences Clinical provides complete clinical trial management services, including study preparation, monitoring, and documentation. Among the many functions it carries out are site administration, data management, monitoring, and compliance tracking.[37]
- **Medidata CTMS:** Medidata CTMS, an internet-based CTMS program, provides features for managing studies, keeping an eye on sites, and collecting data. Real-time information and analytics are also provided, which improve transparency and decision-making during the trial process.[38]
- **BioClinica CTMS:** The CTMS software BioClinica CTMS offers capabilities for managing, arranging, and tracking studies. Additionally, it has financial management features like invoicing and budget tracking.[39]
- **Veeva Vault CTMS:** Veeva Vault CTMS is an internet-based CTMS that provides tools for managing studies, monitoring sites, and recording data. Additionally, it has tools for financial management, such as tracking and planning budgets.[40]
- **Clinical Conductor CTMS:** Clinical Conductor CTMS offers tools for managing studies, sites, and finances. Additionally, it has elements like document management and compliance tracking.[41]

Manufacturing Execution Systems

Pharmaceutical manufacturing is managed and controlled using software systems called Manufacturing Execution Systems (MES). They give a framework for controlling the entire production process—from raw materials to final goods—and guarantee that it is carried out in accordance with legal standards. MES systems include features for controlling production schedules, monitoring quality control, monitoring batch progress, and managing raw materials and inventory. In order to facilitate seamless data transfer and analysis, they also integrate with other systems, such as Enterprise Resource Planning (ERP) systems.[42]

MES systems are essential for maintaining the consistency and quality of manufactured goods in the pharmaceutical sector. They aid in ensuring that the manufacturing procedure is carried out in line with Good Manufacturing Practices (GMP) and other legal criteria. MES systems can also offer manufacturers real-time insights into the manufacturing process, enabling them to swiftly identify and address problems that could affect the quality or safety of their products. MES systems are a crucial management tool for the pharmaceutical industry's manufacturing process. They support the efficient, correct, and legal execution of the production process, which ultimately results in high-quality products that satisfy the demands of patients and healthcare professionals.[43]

Following the Food and Drug Administration's (FDA) 21 March 1997 issuance of the Final 21 Part 11 Regulations, the MES was determined to be appropriate and recommended for the pharmaceutical sector. The FDA was able to accept digital records, digital signatures, and handwritten authorizations added to electronic records in some circumstances as being equivalent to printed records and written signatures collected on paper due to these standards. People were aware that combining computer technology with mechanical engineering may boost industrial efficiency. As programmable control is a logical extension of mechanical engineering, the Programmable Controller (PLC) was the first to be developed. This allowed the deployment of complicated logic inside machines and in production processes. MES is quickly becoming into one of the key components of the pharma sector's digital manufacturing technology landscape, contributing significantly to pharma digitalization and pharma 4.0.[44]

The MES takes the place of paper-based procedures, which are too slow and prone to mistakes for the modern pharmaceutical sector. Throughout the whole production cycle, it digitally controls, monitors, and documents the processes. Additionally, it increases product quality and production efficiency and may be readily linked with your ERP. Effective communication between the production systems and other departments, such as operations, quality, maintenance, and inventory control, flows through the MES, which serves as a central system.[44]

It enables direct execution of production orders that may respond dynamically to changing orders, machine statuses, quality checks, and much more thanks to real-time data that is available. According to the needs of a business, the MES solution's intelligent software modules assist in managing, analysing, and visualising production data quickly





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and easily. For instance, a MES-based quality assurance module can be introduced to drastically cut batch review durations and lead times if a company is trying to lower its Total Cost of Quality.[45] The Right-First-Time (RFT) is simultaneously improved compared to a paper-based environment. With increased quality, better traceability, maximised lean initiatives, flexible production processes, improved compliance, full business process management, and precise real-time reporting, pharmaceutical companies that have adopted MES enjoy a competitive advantage. Businesses that don't use this technology risk missing out on new and existing market opportunities.[46] Some of the common Manufacturing Execution Systems used are as follows:

- **PAS-X:** The primary MES (manufacturing execution system) for the pharmaceutical and biotechnology sectors is PAS-X. It is operated by more than half of the top 30 pharmaceutical and biotechnology corporations in the world, and it has more than 1000 installations all over the world.[47]
- **Siemens Simatic:** Siemens SIMATIC is a family of automation and industrial control products developed by Siemens AG, a multinational conglomerate headquartered in Germany. The SIMATIC product range includes various components and systems used for controlling and monitoring industrial processes in a wide range of industries, such as manufacturing, energy, transportation, and more.[48]
- **Wonderware MES by AVEVA:** A leader in information management and industrial automation, AVEVA sells the Wonderware MES. Wonderware MES offers manufacturers real-time visibility into production processes, assisting them in enhancing operational effectiveness, quality, and compliance.[49]

SUMMARY AND CONCLUSION

Incorporating software solutions in the pharmaceutical firm has ushered in a transformative era of efficiency, precision, and safety. The multifaceted role of software spans across every stage of the drug lifecycle, from initial discovery to post-market surveillance, revolutionizing how pharmaceutical companies operate. By harnessing computational power, data management capabilities, and advanced analytics, software expedites drug discovery, enhances manufacturing processes, ensures regulatory compliance, and safeguards patient well-being. The pharmaceutical landscape is marked by increasing complexity and rigorous regulatory demands. Software not only addresses these challenges but also propels the industry forward. Collaboration and communication are streamlined, enabling interdisciplinary teams to work seamlessly across geographies. This interconnectedness accelerates decision-making, shortens development timelines, and brings innovative treatments to patients faster. However, it is vital to acknowledge that the rapid pace of technological evolution requires continuous adaptation and a proactive approach to cybersecurity. As software becomes an even more integral part of pharmaceutical endeavors, stakeholders must remain vigilant in safeguarding sensitive data and ensuring robust systems. In essence, software's transformative influence on the pharmaceutical sector is undeniable. Its ability to enhance research, optimize manufacturing, ensure compliance, and monitor safety reaffirms its status as a cornerstone of modern pharmaceutical advancements. The ongoing collaboration between scientific expertise and cutting-edge software promises a future where groundbreaking therapies and improved patient outcomes are not just aspirations but realities.

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Toxicity of *Alangium salviifolium* Leaf Extracts against Two Strains of *Aedes aegypti* and *Culex quinquefasciatus*

Alex Yagoo^{1*}, M.C. John Milton², Jelin Vilvest³, Irine Johnson¹ and Keduki Balakrishna²

¹Research Scholar, Department of Advanced Zoology and Bio Technology, Loyola College (Autonomous), Chennai - 34, Tamil Nadu, India

²HoD and Associate Professor, Department of Advanced Zoology and Bio Technology, Loyola College (Autonomous), Chennai - 34, Tamil Nadu, India .

³Assistant Professor and Research Scholar, Department of Advanced Zoology and Bio Technology, Loyola College (Autonomous), Chennai - 34, Tamil Nadu, India

⁴Emeritus Scientist, Entomology Research Institute, Loyola College Campus, Chennai - 34, Tamil Nadu, India.

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*Address for Correspondence

Alex Yagoo

Research Scholar,

Department of Advanced Zoology and Bio Technology,

Loyola College (Autonomous),

Chennai - 34, Tamil Nadu, India

E. Mail: alexyagoo@loyolacollege.edu



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ABSTRACT

In India, mosquitoes are major carriers of diseases like malaria, dengue, and chikungunya, among others. Researchers have studied various plant extracts and phytoconstituents for their anti-mosquito activity. In this communication, the larvicidal, pupicidal, and ovicidal activities of different extracts of *Alangium salviifolium* (Alangiaceae) leaves were evaluated against *Aedes aegypti* and *Culex quinquefasciatus* mosquitoes. The chloroform extract exhibited the strongest ability to kill larvae in both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, with LC₅₀ values of 156.8 and 131.5 parts per million (ppm), respectively. The methanol extract showed slightly lower larvicidal activity, with LC₅₀ values of 160.2 and 164.7 ppm for *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, respectively. The n-hexane extract was the least effective, with LC₅₀ values of 272.7 and 254.8 ppm for *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, respectively. None of the extracts showed significant activity against the pupal or egg stages of either *Ae. aegypti* or *Cx. quinquefasciatus* mosquitoes. However, the chloroform and methanol extracts of *A. salviifolium* leaves demonstrated strong larvicidal activity against both mosquito species, indicating its potential as a





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larvicide. Nonetheless, further research is necessary to investigate the effectiveness and safety of using this extract as a larvicide.

Keywords: *Alangium salviifolium* leaves, *Aedes aegypti*, *Culex quinquefasciatus*, extracts.

INTRODUCTION

Mosquito-borne diseases, such as filariasis, yellow fever, West Nile virus infection, encephalitis, Zika virus diseases, chikungunya fever, dengue fever, and malaria, are a major public health problem worldwide, particularly in subtropical and tropical regions [1-2]. According to the World Health Organization (WHO), nearly 17 million people suffer from mosquito-borne diseases annually worldwide, and approximately 1-2 million deaths are reported due to malaria every year, while 120 million people are affected by lymphatic filariasis [3]. In India, around 25 million people are reported to be harboring microfilaria, and 19 million people are affected by filarial diseases [4]. The three important mosquito vectors in India are *Aedes*, *Culex*, and *Anopheles*, responsible mainly for dengue fever, filariasis, and malaria, respectively [5]. To reduce the spread of mosquito-borne diseases, mosquito vector control is one of the principal measures. However, persistent synthetic pesticide applications can result in toxic effects on useful insects, such as pollinators and predatory insects, as well as pollution of local habitats and water sources [6-7]. Therefore, there is a need to search for alternative mosquito control agents that are efficient, biodegradable, eco-friendly, and non-toxic. One such alternative is the use of plant-derived compounds, which have been found to be promising alternatives to synthetic insecticides [8].

Alangium salviifolium (L.f.) Wangerin is a flowering plant that belongs to the Alangiaceae family. It is widely distributed throughout tropical Asia, including India, Sri Lanka, Bangladesh, Myanmar, Thailand, Cambodia, Laos, Vietnam, Malaysia, Indonesia, and the Philippines [9]. *A. salviifolium* is known by several common names, including Sage-leaved Alangium and Ankolam. It contains various chemical compounds that are responsible for its pharmacological properties. These include alkaloids, flavonoids, steroids, triterpenoids, lignans, and coumarins. It has been used in traditional medicine to treat a variety of ailments, including diarrhea, dysentery, fever, rheumatism, asthma, and skin diseases. The plant has been reported to possess various pharmacological properties, including antimicrobial, anti-inflammatory, antioxidant, antidiabetic, anti-cancer, anti-ulcer, and hepatoprotective activities [10-12]. The present study aimed to evaluate the larvicidal, pupicidal, and ovicidal activities of different extracts of *A. salviifolium* leaves against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, in order to determine their potential as agents for controlling these mosquito species, which are major carriers of diseases in India.

MATERIALS AND METHODS

Collection of the plant material

In February 2022, the leaves of *A. salviifolium* were gathered from Palayamkottai, a locality in the Tirunelveli district of Tamil Nadu, India. The collected plant material was verified and validated for its identity by Dr. K. N. Sunilkumar, who is a Research Officer at the Department of Pharmacognosy, Siddha Central Research Institute, Chennai. To substantiate the authentication, a voucher specimen was deposited in the institute's herbarium, and it was given an Authentication Code No: A24012302S.

Extraction of the plant material

The leaves of the plant were dried in the shade and then ground into coarse powder. The powdered leaves (1kg) were subjected to successive extraction using n-hexane, chloroform, and methanol in a Soxhlet apparatus. The resulting extracts were filtered and concentrated using a vacuum rotary evaporator. The dry extracts were then stored in airtight containers at a temperature of 4° C until further use. The yield obtained from the three extractions were 30.15g, 32.0 g, and 95.48g, respectively.





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Insect rearing

To raise third instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus*, the larvae were acquired from Entomology Research Institute, Loyola College, Chennai. The larvae were reared in chlorine-free tap water, with a consistent temperature of 27±2°C, relative humidity of 75-85%, and a 13:11 light-to-dark photoperiod. The larvae were fed a diet consisting of dog biscuits and Brewer's yeast in a 3:2 ratio [13].

Larvicidal and pupicidal assays

The larvicidal and pupicidal activities of different extracts were evaluated following the guidelines of the World Health Organization (WHO) [14]. The tests were conducted using various concentrations, ranging from 62.5 ppm to 500 ppm, with five replicates for each concentration for all three activities. The solutions were prepared by creating an emulsion in 1.0% aqueous DMSO. Twenty larvae or pupae were introduced into 100 ml of the solution contained in 150 ml plastic containers. A negative control was set up using 1% aqueous DMSO, while Temephos was used as a positive control. The mortality of the larvae or pupae was determined after 24 hours of incubation, and the specimens were considered dead if there was no movement observed when touched with a glass rod. The percentage mortality and corrected percentage mortality were calculated using the appropriate formulas [15].

Percentage mortality:

$$\frac{\text{No. of dead larvae or pupae}}{\text{No. of larvae or pupae exposed}} \times 100$$

Corrected percentage mortality:

$$[1 - nT/nC] \times 100$$

To calculate the corrected percentage mortality, the number of larvae or pupae alive after treatment (nT) is subtracted from the number of larvae or pupae alive in the control (nC). This value is then divided by the total number of larvae or pupae in the control, and the result is multiplied by 100. The corrected percentage mortality formula is used when the percentage mortality in the control group is less than 5%. This correction factor accounts for any mortality that may have occurred in the control group due to factors other than the treatment being evaluated.

Ovicidal activity

The ovicidal activity was evaluated using a method described by Elango [16], with slight modifications. Freshly laid eggs of *Ae. aegypti* and *Cx. quinquefasciatus* were exposed to different concentrations of the extracts, with five replicates for each concentration. The concentrations used were similar to those for the larvicidal and pupicidal assays. The eggs were monitored for hatchability by examining them under a compound microscope. After 120 hours of treatment, the percent ovicidal activity was calculated using the following formula.

Percentage of Ovicidal activity:

$$\frac{\text{No. of un hatched eggs}}{\text{Total number of eggs exposed}} \times 100$$

The results were compared with those of the standard control, Temephos.





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Statistical analysis

The corrected percentage mortality data obtained from larvicidal, pupicidal, and ovicidal assays for each concentration were analyzed using probit analysis (version 1.5 of the US EPA probit analysis software) to determine the LC₅₀ and LC₉₀ values. A p-value of ≤0.05 was considered statistically significant [17].

RESULTS

The study investigated the larvicidal, pupicidal and ovicidal activities of three different extracts (n-hexane, chloroform and methanol) obtained from the leaves of a plant. The results showed that the chloroform extract exhibited the highest larvicidal activity against both mosquito species (*Ae. aegypti* and *Cx. quinquefasciatus*), with LC₅₀ values of 156.8 and 131.5 ppm, respectively. The methanol extract also showed equally the highest larvicidal activity with LC₅₀ values of 160.2 and 164.7 ppm for both mosquitoes respectively. Based on the results of pupicidal activity, the LC₅₀ values (lethal concentration that causes 50% mortality) for *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes were found to be insignificant. The LC₅₀ values in ppm (parts per million) for the n-hexane, chloroform, and methanol extracts against *Ae. aegypti* were 717.6 ppm, 638.3 ppm, and 563.4 ppm, respectively. For *Cx. quinquefasciatus*, the corresponding LC₅₀ values for the same extracts were 417.0 ppm, 318.4 ppm, and 390.1 ppm, respectively. These results can be confirmed by referring to Table 1 and 2. The results of ovicidal activity for both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes did not show significant activity for any of the extracts. It appears that the methanol extract showed ovicidal activity of 24% and 28.8% at the highest concentration of 500 ppm against *Ae. aegypti* and *Cx. quinquefasciatus*, respectively, when compared to other extracts. These results can be confirmed by referring to Figure 1 and 2 of the study.

DISCUSSION

Plant extracts have been studied for their potential as natural sources of mosquito control due to their low toxicity and eco-friendliness. *A.salviifolium* (Alangiaceae) leaves have been evaluated in this study for their larvicidal, pupicidal, and ovicidal activities against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes. Several studies have reported promising results regarding the antimosquito activities of *A. salviifolium* extracts. One study conducted in India reported that the methanol extract of *A. salviifolium* showed significant larvicidal and pupicidal activities against the dengue fever mosquito, *Ae. aegypti*. The study found that the extract caused a mortality rate of 100% at a concentration of 100 ppm against the larvae of *Ae. aegypti*. The extract also showed pupicidal activity, causing a mortality rate of 100% at a concentration of 100 ppm against the pupae of *Ae. aegypti* [18]. Another study conducted in Malaysia reported that the methanol extract of *A. salviifolium* showed significant larvicidal activity against the filariasis mosquito, *Cx. quinquefasciatus*. The study found that the extract caused a mortality rate of 100% at a concentration of 200 ppm against the larvae of *Cx. quinquefasciatus* [19]. Another research conducted in Bangladesh reported that the ethanol extract of *A. salviifolium* showed significant larvicidal activity against the malaria mosquito, *Anopheles dirus*. The study found that the extract caused a mortality rate of 100% at a concentration of 50 ppm against the larvae of *A. dirus* [20]. Researchers evaluated the larvicidal activity of *A. salviifolium* leaf extract against *Ae. aegypti*, the mosquito species responsible for transmitting dengue, Zika, and chikungunya viruses. The study found that the leaf extract exhibited significant larvicidal activity, with an LC₅₀ (lethal concentration to kill 50% of larvae) of 25.25 ppm [21]. Another study investigated the larvicidal and repellent activities of various plant extracts, including *A. salviifolium*, against *Ae. aegypti* and *An. stephensi*, the mosquito species responsible for transmitting malaria. The study found that the leaf extract of *A. salviifolium* showed significant larvicidal activity against both mosquito species, with an LC₅₀ of 67.51 ppm and 48.44 ppm for *Ae. aegypti* and *An. stephensi*, respectively. The extract also exhibited moderate repellent activity against *Ae. aegypti* mosquitoes [22]. Another study conducted in Thailand investigated the larvicidal activity of the ethanolic extract of *A. salviifolium* against the larvae of *Aedes albopictus*, which is another mosquito species that is known to transmit dengue fever, chikungunya, and Zika virus. The study found that the extract showed dose-dependent larvicidal activity against *Ae. albopictus*, with an LC₅₀ value of 71.32 ppm. The study concluded that the *A. salviifolium* extract could be a potential natural alternative for controlling *Ae. albopictus*





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mosquitoes [23]. Furthermore, a study conducted in Bangladesh investigated the larvicidal and adulticidal activities of the ethanolic extract of *A. salviifolium* against the malaria vector mosquito, *An. stephensi*. The study found that the extract showed significant larvicidal activity against the larvae of *An. stephensi*, with an LC₅₀ value of 34.28 ppm. The extract also showed adulticidal activity against *An. stephensi*, causing a mortality rate of 77.5% at a concentration of 100 ppm. The study suggested that the *A. salviifolium* extract could be a potential source for the development of natural mosquito control agents [24]. Another study found that *A. salviifolium* leaf extract had larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, with the methanol extract showing the strongest activity [25]. The results of the present study indicate that the chloroform and methanol extracts of *A. salviifolium* leaves have strong larvicidal activity against both *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, with the chloroform extract being the most effective. However, none of the extracts showed significant activity against the pupal or egg stages of either mosquito species. These studies suggest that *A. salviifolium* extracts may have potential as natural insecticides for the control of mosquito-borne diseases. It's worth noting that the effectiveness of *A. salviifolium* extracts may vary depending on factors such as geographical location, plant part used, extraction method, and mosquito species targeted. Further research, including in vivo studies and clinical trials, is warranted to validate the potential antimosquito activities of *A. salviifolium* extracts and their safety and efficacy for use in mosquito control strategies.

CONCLUSION

The study found that the chloroform and methanol extracts of *A. salviifolium* leaves exhibited strong larvicidal activity against *Ae. aegypti* and *Cx. quinquefasciatus* mosquitoes, with the lowest LC₅₀ values among the different extracts tested. This suggests that *A. salviifolium* has the potential to be used as a larvicide for controlling these disease-carrying mosquitoes.

Future studies

Further research is needed to identify the active compounds responsible for these activities, as well as to determine the effectiveness of the extracts in different geographical locations and against other mosquito species. Additionally, in vivo studies and clinical trials are needed to validate the safety and efficacy of the extracts for use in mosquito control strategies. It is also important to evaluate the effectiveness and safety of using this extract as a larvicide in field conditions, including its impact on non-target organisms and the environment. Nevertheless, the findings of this study provide valuable insights into the potential of *A. salviifolium* as a natural product for mosquito control, which could be explored further for the development of effective and eco-friendly strategies for mosquito-borne disease control in India and other regions where these diseases are prevalent.

Abbreviations

Ae. aegypti : *Aedes aegypti*;

Cx. quinquefasciatus: *Culex quinquefasciatus*;

A. salviifolium: *Alangium salviifolium*;

ANOVA: Analysis of variance;

LC: lethal concentration;

LL: lower limit;

UL: upper limit;

PPM: Parts per million;

DMSO: Dimethyl sulfoxide.

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Table 1. Lethal concentration (in ppm) of crude extracts of leaves of *A. salviifolium* against the larvae of *Ae. aegypti* and *Cx. quinquefasciatus*

| Species | Extract | LC ₅₀ (ppm) | 95% confidence limit | | LC ₉₀ (ppm) | 95% confidence limit | | Slope ± SE | Intercept ± SE | χ ² |
|---------------------------------------|------------|---------------------------|----------------------|-------|---------------------------|----------------------|---------|------------|----------------|----------------|
| | | | LL | UL | | LL | UL | | | |
| <i>Ae. aegypti</i> larvae | n-Hexane | 272.7 | 152.7 | 891.5 | 853.9 | 417.8 | 64884.7 | 2.5 ± 0.4 | -1.2 ± 1.0 | 6.7* |
| | Chloroform | 156.8 | 36.0 | 499.4 | 391.2 | 209.1 | 724.2 | 3.2 ± 0.6 | -2.0 ± 1.4 | 12.5* |
| | Methanol | 160.2 | 38.0 | 530.7 | 386.4 | 208.4 | 1017.1 | 3.3 ± 0.6 | -2.3 ± 1.5 | 12.9* |
| <i>Cx. quinquefasciatus</i> larvae | n-Hexane | 254.8 | 131.5 | 905.0 | 751.0 | 373.6 | 11077.1 | 2.7 ± 0.5 | -1.5 ± 1.1 | 8.1* |
| | Chloroform | 131.5 | 65.9 | 222.6 | 320.1 | 197.2 | 2105.9 | 3.3 ± 0.5 | -2.0 ± 1.1 | 7.0* |
| | Methanol | 164.7 | 33.6 | 654.3 | 390.7 | 209.4 | 603.0 | 3.4 ± 0.7 | -2.5 ± 1.6 | 13.7* |

LC₅₀-lethal concentration that kills 50% of the exposed larvae; LC₉₀-lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *p ≤ 0.05, level of significance of chi-square values.





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Table 2. Lethal concentration (in ppm) of crude extracts of leaves of *A.salviifolium* against the pupae of *Ae. aegypti* and *Cx. quinquefasciatus*

| Species | Extract | LC ₅₀ (ppm) | 95% confidence limit | | LC ₉₀ (ppm) | 95% confidence limit | | Slope ± SE | Intercept ± SE | χ ² |
|------------------------------------|------------|---------------------------|----------------------|--------|---------------------------|----------------------|--------|------------|----------------|----------------|
| | | | LL | UL | | LL | UL | | | |
| <i>Ae. Aegypti</i> larvae | n-Hexane | 717.6 | 549.7 | 1109.8 | 2882.0 | 1671.3 | 7548.6 | 2.1 ± 0.3 | -1.0 ± 0.7 | 1.1* |
| | Chloroform | 638.8 | 497.4 | 945.8 | 2717.1 | 1607.5 | 6669.9 | 2.0 ± 0.2 | -0.7 ± 0.6 | 1.5* |
| | Methanol | 563.4 | 444.3 | 807.4 | 2581.8 | 1544.3 | 6102.5 | 1.9 ± 0.2 | -0.3 ± 0.6 | 0.7* |
| <i>Cx. quinquefasciatus</i> larvae | n-Hexane | 417.0 | 343.9 | 543.3 | 1854.4 | 1208.3 | 3649.0 | 1.9 ± 0.2 | -0.1 ± 0.5 | 2.5* |
| | Chloroform | 318.4 | 184.6 | 1382.3 | 1009.5 | 474.2 | 1524.5 | 2.5 ± 0.4 | -1.4 ± 1.0 | 6.4* |
| | Methanol | 390.1 | 223.1 | 5578.0 | 1327.7 | 553.1 | 1013.5 | 2.4 ± 0.4 | -1.2 ± 1.1 | 6.2* |

LC₅₀-lethal concentration that kills 50% of the exposed larvae; LC₉₀-lethal concentration that kills 90% of the exposed larvae; LL-lower limit (95% confidence limit); UL-upper limit (95% confidence limit). *p ≤ 0.05, level of significance of chi-square values.

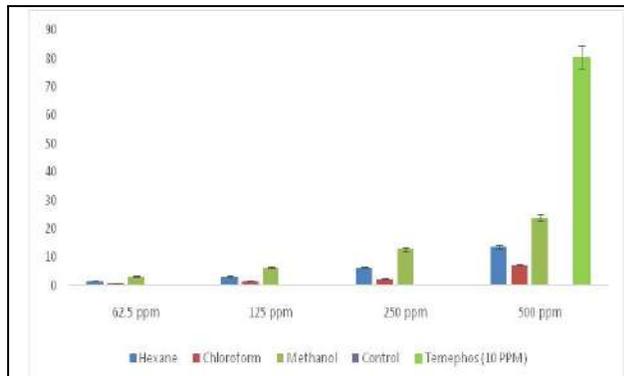


Figure1. Percent ovidical activity of crude extracts of leaves of *A.salviifolium* against *Ae. aegyptieggs*.

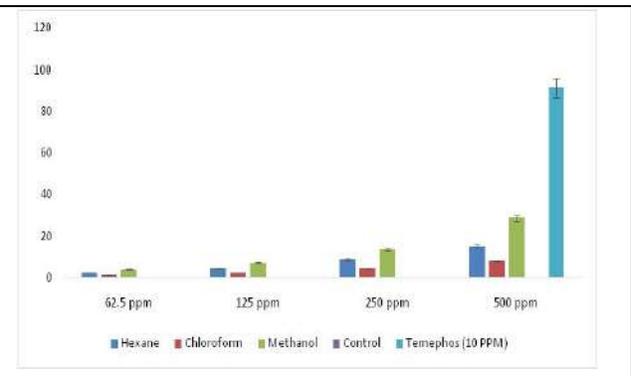


Figure2. Percent ovidical activity of crude extracts of leaves of *A. salviifolium* against *Cx. quinquefasciatus* eggs





Tuberculosis Disease Prediction using Machine Learning Approaches : an Analysis

S. Suresh^{1*} and S. Dhanalakshmi²

¹Assistant Professor, Department of Computer Science, Sri Krishna Arts and Science College, Coimbatore -641008, Tamil Nadu, India.

²Associate Professor, Department of Software Systems, Sri Krishna Arts and Science College, Coimbatore-641008, Tamil Nadu, India.

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*Address for Correspondence

S. Suresh

Assistant Professor,

Department of Computer Science,

Sri Krishna Arts and Science College,

Coimbatore -641008, Tamil Nadu, India.

E.Mail: sureshs@skasc.ac.in



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ABSTRACT

Every hour a human is fading away from the ancient disease Tuberculosis (TB) perhaps it is curable. It is a challenge of predicting a life-threatening disease. TB remains a main destroyer and the pace of TB clinical research is described as bitter. There were more than 1.5 billion deaths by TB but It is not treated as an actual emergency similar to COVID-19. It is a situation to aware and adhere the protocols for the TB disease given by (World Health Organization) WHO. The Science and Technology grew up and reached every edge of the world. There are approaches to prevent, cure, and predict by scientific methods in data mining technique using clinical data, image data and genome data of the TB disease. The objective of this research paper is to study and review of the existing data mining techniques for TB prediction and analysis of the current scenario of TB.

Keywords: Tuberculosis, COVID-19, Prediction, Machine Learning, Clinical data, WHO, Artificial Neural Network.



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INTRODUCTION

Tuberculosis is an actual another pandemic however it is recoverable. Also, the TB is a life-threatening slow killer and it is a fast-spreading disease in human community. Moreover, the COVID-19 is different from TB. Lots of research and guidelines given for TB as well as the recent pandemic situation by COVID-19. The TB disease is not considered as most dangerous than COVID-19 (GLOBAL TUBERCULOSIS REPORT 2021)[1]. Several data mining techniques to track and recover from the disease. This research to study and analyze for TB disease prediction methods using machine learning techniques.

TUBERCULOSIS

Tuberculosis (TB) is a communicable disease by mycobacteria tuberculosis. This disease found from ancient days to till today the data age. It is a situation to know everyone about the TB and its guidelines. It affects all the parts of human body but mostly lungs. It can be cured with specific antibiotics. TB might be in two different categories such as active TB and Latent TB. Typical active TB causes severe illness such as cough more than three weeks, blood-stained sputum, night sweats, loss of appetite, fatigue, fever (more than three weeks) and dispersal. The latent TB does not occur any symptoms and not communication until it become active. If TB is not treated it leads to death. One of the most immediate consequences of COVID-19 was a significant drop in the number of reported tuberculosis cases (Global tuberculosis report 2022. Geneva: World Health organization; 2022. licence: cc BY-NC-SA 3.0 iGo)[2]. In fact, this aspect has had an obvious and direct effect on tuberculosis (TB) and its overall prevalence..

MACHINE LEARNING

Machine Learning (ML) methods are used to study, analyze and predict several areas in real-time. ML methods are used to predict the disease prediction, spread range, patient clinical data analysis, fraud detection, sale prediction, price prediction, and many more. It also helps to a computer system that can give an exact timeline to care a TB patient for recovery from illness. There are numerous techniques but the often used and very familiar methods for predictions are Artificial Neural Network (ANN), Service Vector Machine (SVM), k-Nearest Neighbor (k-NN), Random Forest, Neuro-fuzzy, Naïve bayes, Convolution Neural Network (CNN), k-means, Decision trees and Adaptive-Network-based Fuzzy Inference System (ANFIS).

RELATED LITERATURE

V. Lestari, et.al., 2023 [3] In this research the prediction of Tuberculosis using the back propagation of Artificial Neural Network. The study is done on the application (MATLAB) Nguyen Widrow back propagation method. The network predicted the Tuberculosis cases. The experimented data collected from the North Sumatra Provincial Health Office from 2019 to 2020. Alaa Badawi et.al., 2021 [4] In this work, developed a model for predicting the LTBI (Latent Tuberculosis Infection) from the parameters such weight of body and other risk factors. The latent tuberculosis is caused over 10 million and 1.5 million deaths with both HIV negative and positive persons in the world. The accuracy experimental study with three ANNs predictive percentage is from 75 to 80 percentage and with the sensitivities range is from 85 to 94 percentage. The research study has implemented an application tool to predict the LTBI by a BMI (Body Mass Index). It is beneficial in precise decision making in best of clinical practices and targeting to control the problem of tuberculosis in the world.

Lino Ferreira da Silva Barros et. al., 2021 [5] In this paper, it is described the global problem of TB infection and it remains a main cause of illness and death rate. It is an approach to predict the infectious status of TB using Machine learning technique. The risk of death is increased of inappropriate or delayed treatment. This research makes standard machine learning method to support TB forecast using a real-time data from the confirmed cases and deaths of Brazilian health database related to TB disease. The computation works was completed using an AWS (Amazon Web Services).



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Ibrahim Goni, et.al.,2020[6]In this adaptive Neuro-Fuzzy system for predicting the presence of Mycobacterium tuberculosis. It is a structured system with inputs and one output of which rules made by the system adopted the medical expertise and injected in to the knowledge based which the system would use the rules to make decisions and draw a decision. It is used the MATLAB7.0 to implement and experiment the fuzzy logic and Neural Network toolbox. Diagnosing tuberculosis system is based on Neuro-Fuzzy method. It is designed for diagnosis of tuberculosis disease in human using clinical data.

Salma Jamal, et.al., 2020 [7]In this work, a computational framework to predict the DRTB in the genes. Machine Learning algorithms such as naïve bayes, k-nearest neighbor, Support Vector Machine, and Artificial Neural Network. This classification models examined the genes and evaluated on a dataset and its accuracy average rate of 85 percentage. Moreover, the research study analyzed the simulation of molecular docking and molecular dynamics. It is achieved for wild type and forecasted resistance causing mutant protein and anti-TB drug complexities. S.H. Yoo et al 2020 JINST 15 P10011[8]In this research paper, a binary classifier with deep learning was proposed to diagnose tuberculosis using chest X-ray radiograph. This classifier comprised with two-step binary decision trees, the each tree through a deep learning method with CNN (Convolution Neural Network) implemented on the PyTorch framework. The chest X-ray images were used to classify the normal and TB infected. The study is classified into with TB and without TB disease. The process was split into two steps. The accuracy of the first step with 98 percentage and second step 80 percentage of the proposed algorithms.

Muhammad Asad et.al.,2020 [9]In this paper, ML (Machine Learning) based approach to predict treatment failure of Tuberculosis disease with six countries. It is a case study with a real-time dataset from six burdened countries including India. This work highlighted as 2nd world's deadliest disease is Tuberculosis. This study is suggested that the prediction of the patient's treatment outcome and overcome the incidence of failure in TB treatment using the identified a specific attribute. Khan Muhammad Tahir, et.al., 2019[10]In this research work, a great initiative with real time data. This research constructed on the TB patient data and used Artificial Neural Network algorithm and predicted the Mycobacterium tuberculosis (MTB) positive as well as negative. The algorithm accuracy at the rate of 94%. The precision of the experimental and endorsement also were achieved greater than 93 percentage. This improved accuracy of ANN in the finding of TB projected patients' could be useful for primary management of TB disease to implement the control measures to more transmission and to reduce the drug resistance TB problem.

Akshita Tiwari et. al.,2019 [11]In this research paper, it stated that India is the highest TB incident country among 22 countries including China. TB is the top deadliest airborne disease all over the world. It is tough to cure in case of the patients developed DRTB (Drug Resistance TB) and difficult to further treatment. The process of treatment changes patient to patients. It is a technique to predict the disease by its symptoms with the help of machine learning algorithm. It helps the physician to monitor the patients effectively. Farzad FiruziJahantigh et.al.,(2017) [12]The research work experimented about 600 patients from the specific tuberculosis research center throughout from the year 2015 to 2016. The K-Means clustering algorithm and decision tree were done to achieve the classification and determine common gauges among patients. The Radial Basis Function Neural Network tree achieved 98% of accuracy. Furthermore, the important factors acknowledged are smoking, alcohol consumption, sex, white Blood Cell and Albumin.

COVID-19 IMPACT ON TB

TB diagnosis and treatment were proper and achievable the target before the Covid-19. The Covid-19 pandemic affected the TB diagnosis and treatment to the end TB programme on its time line. The incidence, new TB cases and HIV-Positive global TB incidence are shown in figure1. It shows the stagnation of the TB treatment at the year of 2020 to 2021. The more number of new cases began in that period of time. Figure2 shows that the current situation of TB during on Covid-19in India. It was epidemic but now it should be aware as the unknown pandemic. India is the second largest population country among the world. According to WHO data (World Health Organization), India is the highest TB burden country among the top 10 populated countries shown in thefigure3. WHO identified the global risk factors are consuming alcohol, diabetes, HIV, smoking and under nutrition[13]. The top 5 risk factor



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countries in the world is compared with India. India is most hazard for TB cases in world shown in figure4(a). The figure4 shows that the highest risk factor is under nutrition in India when compared with all other countries in the world top 5 risk factored countries. The Risk factor data is downloaded from WHO's Github repository (<https://github.com/GTB-TME/gtbreport2022>) [13] and Death data is downloaded from WHO's GHO(Global Health Observatory)[14] and visualized by Tableau Public. The risk factor data table is also shown in the figure 4 (b).

Another analysis for death due to TB is compared world top 10 countries and shown in the figure 5. From this analysis study shows that the most mortality rate by TB disease only in India and there were slight down in the year of 2019. But after Covid-19 the death rate is increased till 2021.from the Figure it could understand that the India is the world highest death rate due to the TB, second is Nigeria, Third is Indonesia and so on. The data is excluded TB with HIV. It shows only the death data due to TB in the world.

METHODS AND TOOLS USED

This Analysis is done with various prediction techniques for TB, Latent TB, DRTB. There are different approaches to predict TB diseases. This work is analyzed the previous published research papers on different Machine Learning methods. The table is shown in table5.1, which shows that the ANN is the most used This Analysis is done with various prediction techniques for TB, Latent TB, DRTB. There are different approaches to predict TB diseases. This work is analyzed the previous published research papers on different Machine Learning methods. The table is shown in table5.1, which shows that the ANN is the most used methods and Python framework and MATLAB is utmost used Tools.

CONCLUSION

The global burden compared with WHO data and WHO TB profiles. There are plentiful prediction method published and it should be implemented for future. Numerous machine learning methods are possible to build for future prediction especially for tuberculosis disease. The recent technology such as machine learning and deep learning algorithms in data mining might be developed for quick diagnosis and proper treatment for all types of TB disease. DRTB is still threatening the world. Machine learning algorithms are capable to predict DRTB and improve human health in India as well as the whole world. This work shows that India needs to focus on TB prevention and end TB strategy as much as possible and to be created awareness through out India and to the world. This study is mainly to support end TB strategy for our nation as well as the world. This work scopes the Machine Learning Prediction approach for all types of TB in the Future work.

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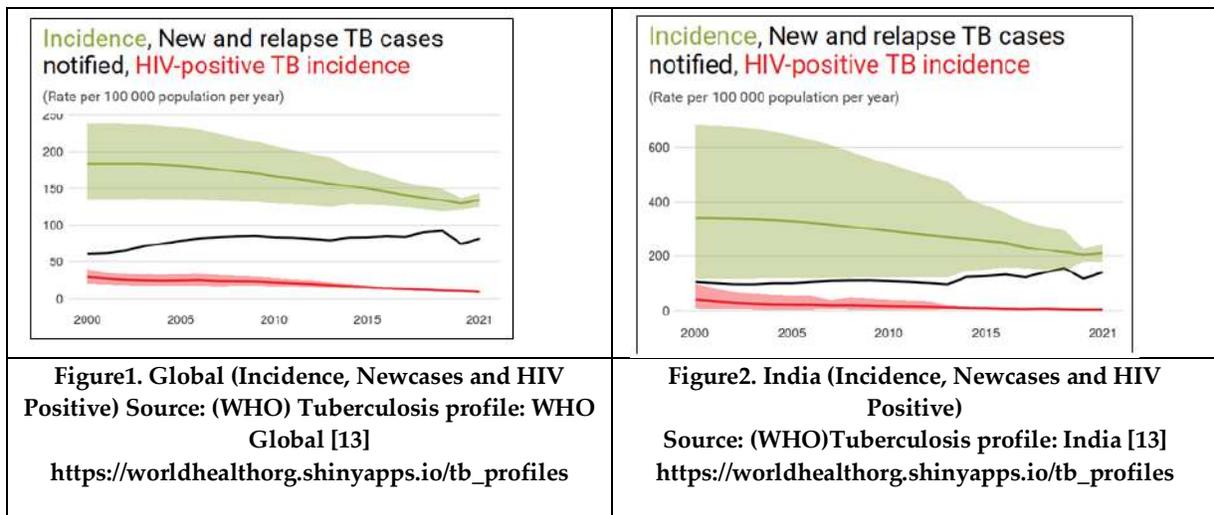
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Table1 – Analysis of various methods and tools

| Year | Author | Methods | Tools/ Programming Language |
|------|--------------------------------------|--|--------------------------------|
| 2023 | V. Lestari et. al | Backpropagation of ANN | MATLAB |
| 2022 | Alaa Badawi et.al | ANN | Python and TensorFlow |
| 2021 | Lino Ferreira da Silva Barros et. al | LR, LDA, KNN, NB, DT, SVM, GB, RF, MLP | AWS instance, i3en.6xlarge |
| 2020 | Ibrahim Goni, et.al | Fuzzy and ANN | MATLAB |
| 2020 | Salma Jamal, et.al | NB, KNN, ANN, SVM, SMO | Weka (Java) |
| 2020 | S.H. Yoo et al | 2D CNN | PyTorch (Python) |
| 2020 | Muhammad Asad et.al | ANN, k-NN, SVM, RF, J48 | Spyder (Python) |
| 2019 | Khan Muhammad Tahir, et.al | ANN | MATLAB |





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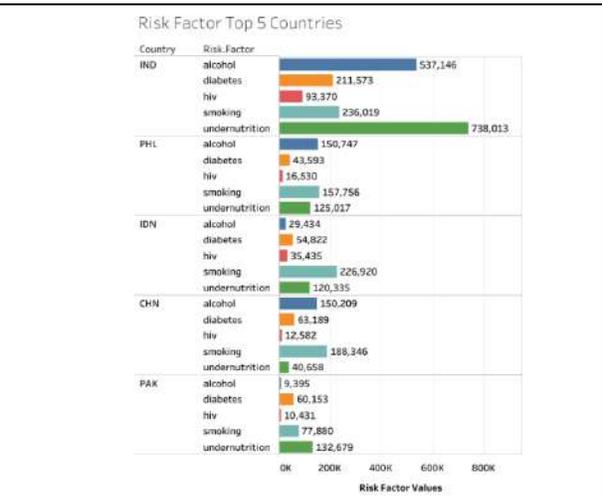
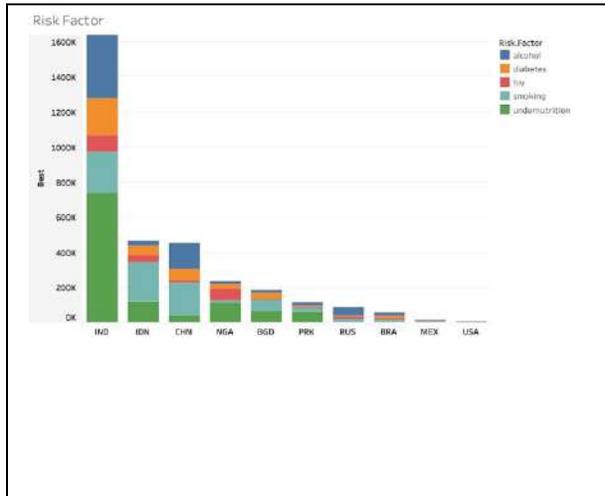


Figure 3 – Risk Factor Compared with World top10 Populated Countries

Figure 4 (a) Top 5 Risk Factor Countries

| Country | Risk.Factor | | | | |
|---------|-------------|----------|--------|---------|----------------|
| | alcohol | diabetes | hiv | smoking | undernutrition |
| IND | 537,146 | 211,573 | 93,370 | 236,019 | 738,013 |
| PHL | 150,747 | 43,593 | 16,530 | 157,756 | 125,017 |
| IDN | 29,434 | 54,822 | 35,435 | 226,920 | 120,335 |
| CHN | 150,209 | 63,189 | 12,582 | 188,346 | 40,658 |
| PAK | 9,395 | 60,153 | 10,431 | 77,880 | 132,679 |

Figure 4(b) Top 5 Risk Factor Countries

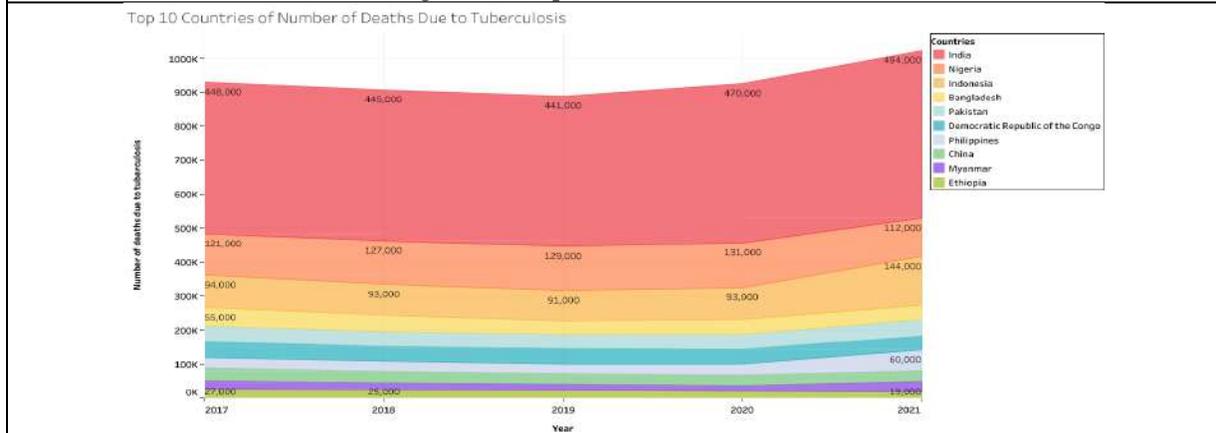


Figure 5 – Deaths Due to Tuberculosis from Top 10 Countries for last 5 Years





Formulation and Evaluation of Herbal Shampoo using Plant Extracts

Chayanika Borah¹ and Jayabrata Saha^{2*}

¹M.Sc.4th SEM (Microbiology) Student, Department of Applied Biology, Block-E, University of Science and Technology, Meghalaya, Techno city, Dist-Ribhoy, 793101, Meghalaya, India.

²Assistant Professor, Department of Applied Biology, University of Science and Technology, Meghalaya (USTM), India

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*Address for Correspondence

Jayabrata Saha

Assistant Professor,

Department of Applied Biology,

University of Science and Technology,

Meghalaya (USTM), India

E.Mail: jayabratha.saha@gmail.com



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ABSTRACT

Hair care is one of the earliest methods of artistic expression in the history of humanity. One of the most prevalent hair care products is shampoo. Herbal shampoos are the most common kind of shampoo because they appear to be more pure, safe and effective. The popularity of herbal shampoo is increasing daily as peoples are becoming more interested in natural and environmentally friendly products. This study's objective is to develop a herbal shampoo out of natural substances such as *Emblica officinalis* (Amla), *Sapindus indica* (Reetha), *Hibiscus rosa-sinensis* (Hibiscus), *Averrhoa carambola* (Star fruit) and meant for cleansing hair and free from side effects, evaluated for their physicochemical properties include determination of p^H, Dirt- dispersion test, skin irritation test, Determination of percentage solid content, Foaming ability and stability. The prepared herbal shampoo demonstrated P^H within the range specified, a comparable amount of solids, constant foam formation and a highly dense nature with satisfactory wetting ability and effective against *S.aureus* and *Aspergillus* sp. The formulated shampoo performed effectively in physicochemical testing. However, more scientific research is needed to improve the product's quality, efficiency and assurance.

Keywords : Herbal shampoo, Reetha extract, star fruit extract, Amla extract, Evaluation





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INTRODUCTION

Hair is an essential component of human beauty . The importance of hair care in daily life cannot be overstated . Since antiquity , Peoples have used herbs for cleaning , beautifying and managing hair (Dubey et al., 2004). Hair care can be improved by using various cosmetic products such as hair oil, shampoo, gel, serum and cream (Sirsat et al., 2022).Shampoo is the most frequent kind of hair treatment that is usually in the form of thick liquid and is applied for cleaning and nourishing purposes because they contain cleansing agents (Revansiddappa et al., 2018). Shampoo acts as a detergent solution which is devoid of harmful chemicals (Manikar and jolly,2001). There are numerous medicated and non medicated synthetic shampoos on the market . Shampoo contains chemically produced surfactants for forming and cleansing , but their continual usage leads to roughness and hair loss, scalp and eye discomfort(Potluri et al.,2013). Toxic chemical agents found in synthetic shampoos, such as parabens and sodium lauryl sulphate , cause hair damage (Sirsat et al.,2022).Synthetic agents have occupied a major share over time but the demand of organic shampoo is increasing daily as they are devoid of synthetic chemicals and have fewer side effects when compared to synthetic cosmetics (Dubey et al., 2004).

Herbal shampoo formulations are considered a potential replacement to chemical-based shampoo ,but producing cosmetics entirely based on organic substances is a challenging endeavour (Shinde et al.,2013). Numerous medicinal plants were identified to have positive benefits on hair and are often utilised in shampoo composition (Firthouse ,2009) .These plant based ingredients can be used as powdery substances ,extracts in crude form , filtered extracts or derivatives (Pooja et al.,2011).The term itself implies that herbal beauty products are organic and devoid of all toxic additives that might be dangerous to the skin. Instead of traditional synthetic products , these products use many different plant components and herbal extracts . Herbs should have anti-oxidant, anti-inflammatory, antiseptic and antibacterial properties. These days, peoples are becoming more and more interested in natural and environmentally friendly products over chemicals for personal care to enhance their beauty. The natural content of the herbs has no damaging impact on the human body and instead benefits it (Warade ,2023). In Indian traditions systems , the exterior layer of *Spindus mukorossi* (Soupnut or reetha), Amla fruits (*Phyllanthus emblica*) and dried *Acacia concinna* (Sheekakai) pods have been used for ages to cleanse hair (Kapoor ,2005). Due to high saponin content of Reetha and Sheekakai , when it is shaking with water , a thick lather forms .Additionally , they are believed to benefit the skin and other biological systems(Khushboo et al.,2010). Amla fruit is used as an antidandruff agent which is high in vitamin C , hair growth promoters and hair strengthening agent in hair preparations (Srivasuki , 2012). It is incredibly tough to create an organic shampoo from single plant-based substance that is less severe and more secure than synthetic ones while also being effective (Badi,2014) . Shampoos usually include 10-30 ingredients , though products containing with as few as four components are available . They are classified as (1) Cleaning agents ; (2) Additives which provide product's stability and comfort ; (3) Conditioning agent used to add softness and gloss.; (4) Special care components , such as dandruff and greasy hair , used to address specific conditions (Gavazzoni, 2015).

The main objectives of this research are - To develop a herbal shampoo incorporating natural components such as *Emblca officinalis* (Amla), *Sapindus indica* (Reetha), *Hibiscus rosa-sinensis* (Hibiscus) , *Averrhoa carambola* (Star fruit) and to evaluate physiochemical properties of the herbal shampoo.

Function of ingredients :

Reetha (*Sapindus indica*) : Reetha is used as a natural shampoo , cleanser and conditioner by South-East Asians. Reetha prevents hair fall and add volume and shine to the hair (Ashwini et al.,2018).Because of its high saponin content , it is employed as a foamy agent and has hair cleaning effects . It is gentle on the scalp and removes any microorganisms that cause infection (Malpani,2020).



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Hibiscus (*Hibiscus rosa-sinensis*) : This is a beneficial flower for hair health , whether dry or fresh . It is high in vitamin C and A , iron and has anti-inflammatory ,antioxidant and antibacterial activities. Hibiscus is also known to prevent premature hair graying (Ashwini et al.,2018).

Amla (*Emblica officinalis*) : It is one of the most widely used Ayurvedic herbs and is a good hair toner . The oil of Amla is widely used hair oil in India and South East Asia . It can help minimise hair loss , graying hair , dry scalp , dandruff , strengthen hair shafts and enhance circulation , all of which promote growth of hair. The high nutrient contents of this fruit adds shine and lustre while conditioning and nourishing the hair (Ashwini et al.,2018).

Starfruit (*Averrhoa carambola*) : Starfruit is a traditional herbal medicine and has antioxidant , antimicrobial , antifungal , antitumor and antiulcer activities (Saha et al.,2018). A significant amount of vitamin B complex in it is also beneficial in terms of hair growth as well as protection to keep is strong and healthy (Chakraborty et al.,2018).

Function of other chemicals

Gelatin – It is used as a thickener .

Potassium sorbate – It is used as a preservative.

Citric acid – It is used to adjust p^H level.

Lavender oil - It is used to impart fragrance.

MATERIALS AND METHODS**Collection of plants**

Plant materials such as Reetha (Fruit) , Hibiscus (Flower), Amla (Fruit) , Star fruit (Fruit) were collected from local area in Assam and other materials were purchased from local market . The plant materials were dried in sunlight and grinded and stored in air tight containers.

Preparation of herbal extracts

10g of each plant powder , namely Hibiscus (*Hibiscus rosa-sinensis*) , Amla (*Emblica officinalis*) , Star fruit (*Averrhoa carambola*) , Reetha (*Sapindus indica*) were mixed with 200ml of distilled water . They were then filtered. The obtained clear extracts were used as herbal extracts .

Formulation of Herbal shampoo

To formulate shampoo , the plant extracts were combined in the amounts listed in the table 1 . Herbal extracts were mixed with 10% (5g in 50ml) gelatin solution and shaken for 20 minutes. Preservative i.e. Potassium sorbate (0.5g in 100ml) was added with stirring. By adding the proper amount of 1% citric acid solution(2g in 10ml of water diluted with water to 30ml) , the solution's P^H was maintained. A few drops of lavender oil were also added to the prepared shampoo to provide scent .

Evaluation of herbal shampoo :

Physical appearance : The obtained product's colour , odour and transparency were assessed(Gaikwad et al.,2020).

Determination of P^H: 1% shampoo solution's P^H was measured using a p^H meter (Sirsat et al.,2022).

Dirt-Dispersion test - In a test tube , place 1% of shampoo solution and 1 drop of Indian ink . The test tube was stopped and shake for about 10 minutes . There were four levels of ink in the foam : None , Light , Moderate and Heavy (Dessai et al.,2016).

Determination of percentage solid content – A dry and clean evaporating dish was weighed and after that the dish was filled with 4g of herbal shampoo. Only the shampoo's weight was calculated .Until the liquid portion of the shampoo dish evaporated ,it was placed on the hot plate .The weight of the shampoo alone (solids) was determined after drying(Pundkar et al., 2020).



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Foaming ability – The ability to foam was evaluated using the cylinder shaking method. 50ml of the 1% shampoo solution was put into a 250ml cylinder with a graduated diameter . The cylinder was shaken for 10 times with one hand covering it. Immediately following the shaking , the amount of foam was measured every minute for 4 minutes (Gaikwad et al., 2020).

Viscosity – The Ostwald viscometer was used to determine viscosity (Dessai et al.,2016).

Skin irritation test - After applying a prepared shampoo solution to the skin for 5 minutes , the skin was examined for redness and irritation (Sirsat et al., 2022).

Microorganisms isolation from human hair : Samples were collected by rubbing the sterile swab sticks on the scalp of six students of University of science and technology , Meghalaya. Nutrient broth was used to inoculate the samples . The samples were then cultured for 24hours at 37C. Mannitol salt agar and MacConkey agar were used to isolate bacteria , while potato dextrose agar (PDA) was utilized to isolate fungal species in the samples. All suspicious *S.aureus* , *E.coli* , *Aspergillus* and *candida* sp. growths were sub-cultured in nutrient agar slant and biochemical tests were performed (Igwebike et al., 2017).

Identification and confirmation of microorganisms : Standard microbiological techniques and biochemical assays such as catalase , indole , oxidase and citrate tests were used To recognise and validate the existence of the numerous microorganisms employed in the investigation (Igwebike et al., 2017).

Antimicrobial sensitivity and resistance patterns : This test was typically used to detect organisms susceptibility or resistance to the designed shampoo. Cheesebrough's method was used for this test. In fresh nutrient broth , the identified microorganisms were sub-cultured. At 37C , broth cultures were incubated until it reached to 0.5 McFarland turbidity standards. The turbidity of the broth cultures that were actively growing then adjusted with NS (Normal Saline) to achieve 0.5 McFarland turbidity criteria. Using sterile swab sticks , this was streaked on solid Muller Hinton agar surface., In the culture media ,wells were punched using a sterile cork borer and dilutions were used to fill the holes . The bacteria plates were incubated at 37C for 24hours and the fungi plates for 72 hours. The zones of inhibition (surrounding the wells)were measured using a ruler(in millimeters) (Igwebike et al., 2017)

RESULT AND DISCUSSION

Herbal shampoo formulation : Admixing all the ingredients in different proportions to formulate a shampoo as shown in the table 1. The plants extracts such as Reetha (*Sapindus indica*) is a naturally occurring surfactant with detergent and foaming properties as it contains phytoconstituents like saponins ; The amino acids (Keratin) in hibiscus (*Hibiscus rosa-sinensis*) make it an conditioning agent; Amla (*Emblica officinalis*) increases the circulation of blood in the scalp , which stimulates the hair growth ;Star fruit (*Averrhoa carambola*) is a nutritious source of antioxidants and vitamin C which makes it a natural hair loss treatment . The gelatin solution (10%) functions as a faux plastic , generating transparent solutions. The citric acid helps to maintain pH and the potassium sorbate which is a preservative that kill and prevent the growth of microorganisms. The lavender oil has a calming effect and divine fragrance to the shampoo (Vijayalakshmi et al.,2018).

Evaluation of formulated shampoo

Physical appearance : Physical characteristics of the shampoo formulation such as colour , aroma and transparency were assessed. As prepared shampoo was transparent , yellow and had good odor , a shampoo should have a pleasing physical look (Malpani et al.,2020).

pH: For enhancing and boosting hair quality ,minimizing eye discomfort and maintaining the ecological equilibrium of the scalp, the shampoo's pH is essential. The pH of the designed shampoo was 5 , which is within the optimal range for shampoo (between 5 and 7.8).pH balanced shampoos keep away fungal infections from the scalp (Badi,2014).



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Dirt –dispersion- Poor quality shampoo causes ink to concentrate in the foam ; dirt should stay in the water . Remaining dirt in the foam will be tough to remove. On the hair , it will deposit again. The amount of ink estimated in foam was light , the result show that prepared formulation is satisfactory (Pundkar et al.,2020).

Percent of solids: Too many solids in shampoo will be difficult to wash out .The percentage of solid components was discovered to be 4%.It implies that it is easily washable(Gaikwad et al.,2020).

Foaming ability : The final formulation produced stable foams, foams were dense and small ,the foam volume is 15ml.The foam volume changed hardly little(Vijayalakshmi et al.,2018).

Viscosity : A great shampoo must have high viscosity . 50milipoise was the determined viscosity of the product , which was sufficient for its application (Malpani et al.,2020).

Skin irritation test : The produced shampoo has no negative effects on the skin. This is owing to the lack of potentially dangerous components (Gaikwad et al.,2020).

Antimicrobial activity : To evaluate the efficacy of the formulated product against two bacterial species i.e. *E.coli* and *Staphylococcus aureus* as well as two fungal species i.e. *Aspergillus* and *Candida* sp. at different concentrations, the antimicrobial sensitivity test was utilised. The result showed antimicrobial activity against *S.aureus* and *Aspergillus* sp (Table 3 and Figure 2). The prepared shampoo was effective against one bacterial species i.e. *Staphylococcus aureus* and one fungal species i.e. *Aspergillus* sp. at all concentrations (10-90%) but with less inhibition . The zone of inhibition of *Aspergillus* species in the range of 11-17mm and 10-14mm for *S.aureus*. At shampoo concentrations (%) of 90 , 80 ,70,60 and 50 (Figure 2) ,the zones of inhibition were most noticeable . The formulated shampoo had no activity against *E.coli* and *Candida* sp. at any concentration since there was no zone of inhibition. Therefore the product is ineffective against the tested strains of *E.coli* and *candida* sp (Igwebike et al.,2017).

CONCLUSION

The development of a reliable and productive shampoo was the primary goal of the current study. This investigation aims at developing a herbal shampoo by using plants extracts i.e. the use of Hibiscus , Reetha , Amla and star fruit. Extracting active compounds from selected plants and incorporating them into a shampoo base was part of the formulation process. The current study focuses on the cosmetic potential of herbal extracts . The formulated shampoo was paraben and sulfate free , Although the formulated shampoo contains the chemical potassium sorbate but the percentage is too low in comparison to the chemical based shampoo in the market. The resulting herbal shampoo formulation was then thoroughly evaluated , including physical appearance , pH, Dirt- dispersion test, skin irritation test , Determination of percentage solid content , Foaming ability and stability. The prepared shampoo had a pH of 5 , which falls within the ideal range for shampoo. It contains less percent of solids so that it is easily washable and has no harmful effects on the skin. The designed shampoo was found to be secure and beneficial because to good froth stability, detergency ,decent cleaning ,tiny bubble size and the prepared shampoo was effective against one of the bacterial species *Staphylococcus aureus* and a fungal species *Aspergillus* sp. The study demonstrated favourable results . More research is needed to investigate a broader range of plants extracts and optimize formulation parameters for improved efficacy and stability . To evaluate long term effects and potential side effects of using herbal shampoos , long term studies are necessary .

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Table 14 : Formulation of herbal shampoo

| Sl no | Ingredients | Quantity |
|-------|--------------------|--------------------------------------|
| 1 | Reetha extract | 5ml |
| 2 | Star fruit extract | 5ml |
| 3 | Amla extract | 5ml |
| 4 | Hibiscus extract | 5ml |
| 5 | Gelatin | 10% solution |
| 6 | Potassium sorbate | 2ml of 0.5% Solution |
| 7 | Citric acid | Few drops of 1% citric acid solution |
| 8 | Lavender oil | Few drops |





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Table 2 : Result and Discussion

| s.no | Evaluation tests | Results obtained |
|------|---------------------------|-----------------------|
| 1 | Physical appearance | Yellow , good foaming |
| 2 | pH | 5 |
| 3 | Dirt dispersion | Light |
| 4 | Percents of solid content | 4% |
| 5 | Foam volume Foam type | 15ml Dense ,small |
| 6 | Viscosity | 50milipoise |
| 7 | Skin irritation | No irritation on skin |

Table 3: The antimicrobial sensitivity and resistance pattern of formulated shampoo (at various concentrations)

| Name of microorganisms | Dilution percentage (%) | | | | | | | | |
|------------------------------|-------------------------------------|----|----|----|----|----|----|----|----|
| | 90 | 80 | 70 | 60 | 50 | 40 | 30 | 20 | 10 |
| | Inhibition zone diameter , IZD (mm) | | | | | | | | |
| <i>Escherichia coli</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| <i>Staphylococcus aureus</i> | 14 | 13 | 12 | 12 | 13 | 10 | 10 | 10 | 10 |
| <i>Aspergillus sp</i> | 17 | 17 | 14 | 12 | 12 | 11 | 12 | 11 | 11 |
| <i>Candida sp</i> | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |



Figure 2: Formulation of Herbal shampoo

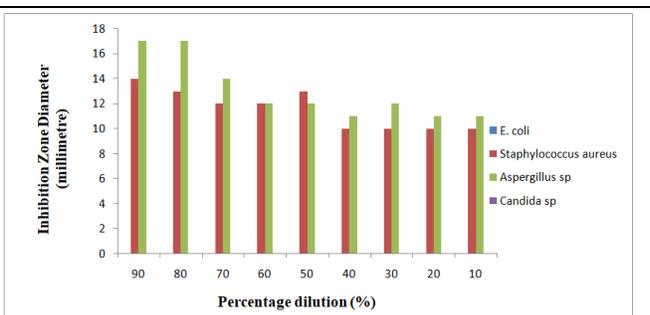


Figure 3 : Plots showing inhibitory zone diameter vs dilution percentage (%) for several bacterial and fungal species.





Vibration and Buckling Analysis of Composite Cantilever Beam with Multiple Cracks

Rajbahadur* and Gaurav Shukla

¹Research Scholar, Department of Civil Engineering, Maharishi University of Information Technology, Lucknow, Uttar Pradesh, India.

²Assistant Professor, Department of Civil Engineering, Maharishi University of Information Technology, Lucknow, Uttar Pradesh, India.

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*Address for Correspondence

Rajbahadur

Research Scholar,

Department of Civil Engineering,

Maharishi University of Information Technology,

Lucknow, Uttar Pradesh, India.



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ABSTRACT

Cracks in structural elements lead to local changes in their stiffness and consequently their static and dynamic behavior changes. The effect of cracks on dynamic characteristics, such as natural frequencies, modes of oscillation of structures, has been the subject of many investigations. However, studies on the behavior of cracked composite structures subjected to in-plane loading are rare in the literature. This work deals with the vibration and buckling analysis of a cantilever beam made of graphite fiber reinforced polyimide with a transverse single-sided non-propagating open crack using the finite element method. The undamaged parts of the beam are modeled by beam finite elements with three nodes and three degrees of freedom at the node. A “total additional elasticity matrix” is added to the elasticity matrix of the corresponding uncracked composite beam element to obtain the overall elasticity matrix and thus the stiffness matrix in agreement with previous studies. The vibration of a cracked composite beam is calculated using the presented formulation and is compared with previous results. The effect of various parameters such as crack location, crack depth, fiber volume fraction, and fiber orientation on the changes in natural frequencies of the beam is studied. It was found that the presence of a crack in the beam reduces the natural frequency, which is more pronounced when the crack is close to the fixed support and the crack depth is greater. The natural frequency of the cracked beam is found to be maximum at about 45% fiber volume fraction and the frequency for any crack depth increases with increasing fiber angle. It was found that the static buckling load of a cracked composite beam decreases with the presence of a crack, and the decrease is more severe with increasing crack depth for any given crack location. Furthermore, the buckling load of the beam decreased with increasing fiber angle and is maximum at 0 degree orientation. Composites as a structural material are used in aerospace, military and



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civil applications due to their tailor-made properties. The ability of these materials to be designed to meet the specific needs of various structures makes them highly desirable. Improvements in design, materials and manufacturing technology are improving the use of composite structures. The suitability of a particular composite material depends on the nature of the applications and needs. This technology has been widely explored for aerospace and construction applications that require materials with high strength and stiffness-to-weight ratio.

Keywords: Composite beam, elasticity matrix, buckling and vibration of beam under dynamic loading, MATLAB environment, etc.

INTRODUCTION

Preventing failure of composite material systems has been an important issue in engineering design. Composites are susceptible to damage such as transverse cracking, fiber breakage, delamination, matrix cracking and fiber matrix debonding under service conditions. Two types of physical failure that occur in composite structures and interact in a complex manner are inter-laminar and intra-laminar failure. Inter-laminar failure is manifested in the micromechanical components of the lamina, such as fiber breakage, matrix cracking, and debonding of the fiber matrix interface. In general, aircraft structures made of fiber-reinforced composite materials are designed so that the fibers carry the majority of the applied load. Inter-laminar failure, such as delamination, means the separation of adjacent layers. The potential for inter-laminar and intra-laminar failure to occur in structural members is considered a design limit and introduces limitations to the full potential of composites. Similar to isotropic materials, composite materials are subject to various types of damage, mostly cracking and delamination. A crack in a composite structure can reduce the stiffness and strength of the structure, redistribute the load so that failure of the structure is delayed, or lead to collapse of the structure. Therefore, cracking is not necessarily a final structural failure, but rather is part of a failure process that may eventually lead to a loss of structural integrity. The importance of inspection in ensuring the quality of manufactured products is therefore well understood. Several methods such as non-destructive testing can be used to monitor the condition of a structure. It is clear that new reliable and inexpensive methods for monitoring structural defects such as cracks should be explored. These variations then significantly affect the static and dynamic behavior of the entire structure. In some cases, this can lead to failure if cracks are not detected early enough. To ensure the safe, reliable and operational life of structures, it is very important to know whether their elements are free of cracks and, if they are present, to assess their extent. The procedures that are often used for detection are called direct procedures, such as ultrasound, X-ray, etc. However, these methods have proven to be ineffective and, in some specific cases, inappropriate, as they require expensive and detailed inspections. To overcome these drawbacks, researchers have focused on more efficient crack detection procedures based on changes in modal parameters such as natural frequencies, mode shapes, and modal damping values introduced by the crack.

LITERATURE

Cracks occurring in structural elements are responsible for local changes in stiffness, which consequently affect their dynamic properties. This problem has been the subject of many papers, but only a few papers have been devoted to changes in the dynamic characteristics of composite structural members. An attempt has been made in this investigation to review the cracked composite beam in the context of this work and the discussions are limited to the following area of analysis. Lu & Law (2009) studied such an effect from multiple cracks in a finite element in dynamic analysis and local damage identification. The finite beam element was formulated using the composite element method with one member – one element configuration with cracks, where the interaction effect between cracks in the same element was automatically included. The accuracy and speed of convergence of the proposed



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model in calculations were compared with existing models and experimental results. It was found that the parameter of the crack model required adjustment using the proposed model. Wang, Inmana & Farrar (2004) investigated the coupled bending and torsional vibrations of a fiber-reinforced composite beam with a crack on the edge surface. The model was based on linear fracture mechanics, Castiglione's theorem and classical lamination theory. The crack was modeled using a local elasticity matrix such that the cantilever beam was replaced by two intact beams with the crack as an additional boundary condition. The combination of bending and twisting can be the result of either material properties or a surface crack. Dimarogonas (1996) gave a comprehensive review of the vibration of structures with cracks. This author covered a wide range of areas that included cracked beams, coupled systems, flexible rotors, shafts, turbine rotors and blades, pipes and casings, empirical diagnosis of machine cracks, and bars and plates with a significant collection of references.

Ghoneam (1995) presented and discussed the dynamic characteristics of laminated composite beams (LCBs) with different fiber orientations and different boundary fixations in the absence and presence of cracks. A mathematical model was developed and experimental analysis was used to study the effects of different crack depths and locations, boundary conditions, and different laminate code numbers on the dynamic characteristics of CLCBs. The analysis showed good agreement between experimental and theoretical results. Krawczuk & Ostachowicz (1995) investigated the natural frequencies of a cantilever beam made of graphite fiber reinforced polyimide with a transverse non-propagating open edge crack. Two beam models were introduced. In the first model, the crack was modeled by Castiglione's massless replacement spring theorem. The second model was based on the finite element method. The undamaged parts of the beam were modeled using beam finite elements with three nodes and three degrees of freedom at the node. The damaged part of the beam was replaced by the finite element of the cracked beam with degrees of freedom identical to the degrees of freedom of the uncracked finished one. The effects of various parameters of crack location, crack depth, fiber volume fraction, and fiber orientation on the changes in natural frequencies of the beam were studied. The results of the calculations showed that the decrease in natural frequencies does not depend only on the crack position and its depth as in the case of an isotropic material, but that these changes strongly depend on the volume fraction of the fibers and the angle of the fibers. composite material. Krawczuk (1994) formulated a new beam finite element with a single non-propagating open crack at one edge located at its midpoint for static and dynamic analysis of composite cantilever-like structures. The element contains two degrees of freedom at each of the three nodes: lateral deflection and independent rotation. He presented exemplary numerical calculations illustrating the changes in static strains and the fundamental natural bending frequency of a composite cantilever beam caused by a single crack.

METHODOLOGY

The governing equations for the vibration analysis of a composite beam with an open unilateral transverse crack are developed. Another elasticity matrix is added to the elasticity matrix of the corresponding element of the composite beam to obtain the total elasticity matrix, and therefore the stiffness matrix is obtained by Krawczuk & Ostachowicz (1995).

The assumptions made in the analysis are:

- i. The analysis is linear. This means that the constitutive relations in the generalized Hooke's law for materials are linear.
- ii. An Euler–Bernoulli beam model is assumed.
- iii. Attenuation was not considered in this study.
- iv. The crack is assumed to be open and of uniform depth a .

Buckling Analysis Study

The mass and stiffness matrices of each beam element are used to create the global mass and stiffness matrix. The dynamic response of the beam for a conservative system can be formulated using the Lagrangian equation of motion, in which the external forces are expressed as time-dependent potentials.





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Then, by performing the required operations, the entire system leads to the governing matrix equation of motion

$$\ddot{q} + K_e - P(t) K_g \cdot q = M$$

Where "q" is the degrees of freedom vector. , and are the matrices of mass, elastic stiffness, and geometric stiffness of the beam. Periodic axial force, where Ω is the disturbing frequency, the static and time-dependent component of the load can be represented as fractions of the basic static buckling load P_{cr} , thus giving.

In this analysis, the calculated static buckling of the coupled beam is considered as the reference load. Next, the above equation reduces to other problems as follows.

i. Free vibrations with $\alpha = 0$, $\beta = 0$ and $\omega = \Omega/2$ natural frequency

$$K_e - \omega^2 M \cdot q = 0$$

ii. Static stability with $\alpha = 1$, $\beta = 0$, $\Omega = 0$

$$K_e - P_{cr} \cdot K_g \cdot q = 0$$

Derivation of element matrices

In this analysis, three nodes of a coupled beam element with three degrees of freedom (axial displacement, transverse displacement and independent rotation) per node are considered. The characteristic matrices of the composite beam element are calculated based on the model proposed by Oral (1991). The stiffness and mass matrices are derived from the procedure reported by Krawczuk & Ostachowicz (1995).

Calculation procedure for a composite cantilever beam

A computer program is developed to perform all necessary calculations in the MATLAB environment. In the initialization phase, the geometry and material parameters are specified. For example, for an Euler–Bernoulli composite beam model with a localized crack, material parameters such as modulus of elasticity, modulus of stiffness, Poisson's ratio and material mass density of the composite beam, and geometric parameters such as dimensions of the composite beam, as well as damage specifications such as crack size, crack location, and extent the cracks are supplied as input data to the computer program. The beam is divided into n number of elements and n+1 number of nodes. The elements of the mass matrix, elastic stiffness matrix, and geometric stiffness matrix are formulated according to the above expression, and the dimensionless natural frequency and buckling load for the uncracked and composite cantilever beam are obtained. The program uses a MATLAB function, "Gauss Quadrature", to perform the integration part. Element matrices are assembled to obtain global matrices. The boundary conditions are determined by the elimination method. For an Euler–Bernoulli composite beam with fixed free end conditions, the first three rows and columns of the global matrices are eliminated to obtain the reduced matrices. The dimensionless eigen frequencies are calculated by solving the eigen value problems in Eq. MATLAB built-in function "eig" is used to calculate eigen values, eigenvectors and mode shape diagram.

CONCLUSION

The following conclusions can be drawn from the present finite element investigations of a composite beam with a transverse non-propagating open crack with one edge. This element is versatile and can be used for static and dynamic analysis of composite or isotropic beam.



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1. From current research, it can be concluded that the vibration natural frequencies of a composite cantilever beam are not only functions of crack location and crack depth, but also functions of fiber angle and fiber volume fraction. The presence of a transverse crack reduces the natural frequencies of the composite beam.
2. The rate of decay of the natural frequency of the composite cantilever beam increases as the crack position approaches the fixed end.
3. The frequency reduction intensity increases as the crack depth ratio increases. This natural frequency reduction, along with the vibration mode shapes, can be used to detect the crack location and crack depth.
4. When the fiber angle (α) increases, the values of natural frequencies also increase.
5. The largest difference in frequency occurs when the fiber angle (α) is 0 degrees. This is due to the fact that the crack yield of a composite beam is a function of the angle between the crack and the reinforcing fibers.
6. The effect of cracks is more pronounced at the fixed end than at the more distant free end. It is concluded that the first, second and third natural frequencies are most affected when the cracks are located near the fixed end, the middle of the beam and the free end.
7. The decrease in dimensionless natural frequencies depends on the volume fraction of fibers. The dimensionless natural frequency is maximum when the fiber volume fraction is approximately 45%. This is due to the fact that the crack ductility of a composite beam is a function of fiber volume fraction.
8. The buckling load of a composite cantilever beam decreases with increasing crack depth for a crack at any particular location due to a reduction in stiffness.
9. As the fiber angle increases, buckling load values decrease. This is due to the fact that for 0 degree fiber orientation, the buckling plane perpendicular to the fibers has the maximum stiffness and for other orientations the stiffness is less and therefore the buckling load is less.

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Evaluation of Avalanche Initiated Harm to Structures : the Agnone Avalanche Contextual Investigation Joshimath (Uttrakhand)

Maaz Allah Khan* and Abhishek Kumar Yadav

Department of Civil Engineering, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh, India

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*Address for Correspondence

Maaz Allah Khan

Department of Civil Engineering,
Babasaheb Bhimrao Ambedkar University
(A Central University), Lucknow,
Uttar Pradesh, India.



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ABSTRACT

Avalanches are among the most significant and successive regular catastrophes that cause serious financial and human misfortunes. After quakes, avalanches are answerable for the best number of losses and the biggest measure of harm to man-made structures. Overall, Joshi Math(Uttrakhand) is impacted by a high spatial thickness of avalanches because of its complicated land setting, which frequently inclines it toward slant flimsiness peculiarities under both regular and anthropogenic impacts. Basically complicated arrangements are far reaching in the southern Apennines and are portrayed by high heterogeneity and exceptionally poor mechanical properties. In this way, these developments address one of the primary variables adding to the inclination of slants to landsliding. In this paper, avalanche prompted harm was explored and examined in a space inside the district of Agnone (Molise locale), which is impacted by a mind boggling avalanche that includes a primarily perplexing development. The methodologies utilized depended on six unique techniques that have recently been portrayed in the writing, and a correlation of the outcomes was made. Information in regards to the harm, which comprises to a great extent of breaks saw in structures and at the ground, were gathered through field reviews. The outcomes were fundamentally broke down to take note of the benefits and imperatives of every order plot. The point of the work was to apply and contrast various methodologies all together with test the best and most precise methodology for evaluating harm because of avalanches at the size of individual structures as well as to give an objective appraisal of the level of avalanche harm to designs and offices.

Keywords: Avalanches . Primarily mind boggling arrangements. Harm characterization .Structures . Agnone



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INTRODUCTION

Avalanches are gravity-controlled regular or anthropogenic cycles that address the most broad geographical peril around the world (Schuster 1996; Schuster and Good country 2001). They make possibly disastrous impacts and cause impressive financial harm as well as fatalities. Mass developments are brought about by a few inclining and driving variables, including both normal and anthropogenic fac-pinnacles, like deforestation or poor metropolitan preparation. Anthropogenic variables are locally more awful than normal ones (Wu and Qiao 2009; Di Martire et al. 2012; Tofani et al. 2013; Wu et al. 2015). In Italy, populace development and the resulting extension of metropolitan regions (Rybár 1997) have frequently actuated individuals to assemble structures in regions over lethargic avalanches or at the edges of dynamic avalanche regions. Also, the negligence of the regulations balanced with a few progressive reprieves be-reason for encroachment, notwithstanding the deficiency of memory of past ruinous avalanche occasions, have prompted the improvement of offices in risky regions, for example, volcanic-or avalanche inclined districts. Metropolitan development can make adjustments hillslope morphology, inciting territory remobilization and re-enactment of old avalanches that were beforehand lethargic or suspended, notwithstanding millennia of information on nega-tive encounters (Chiocchio et al. 1997).

Actual weakness, which characterizes the degree of harm to basic foundations and structures, is a critical boundary in risk evaluation. The event of a few avalanches in metropolitan regions has featured the need to make a standard system to perceive and group various degrees of harm to assist with landing the board specialists (Alexander 1986) to concentration and complete relief procedures. Incline developments make harm structures and different offices and force direct (e.g., substitution, fix or upkeep of harmed struc-tures) and aberrant expenses (any remaining expenses, which are challenging to assess, e.g., misfortunes of administration) (Schuster and Fleming 1986; Schuster 1996; Godt et al. 2000). These expenses rely upon sev-eral factors, including the aspects and speed of the mass development, the extent and kind of the avalanche mecha-nism, the lithology in question, the morphological highlights, and the impacts of anthropogenic movement. Consideration is typically paid to arranging techniques to forestall or lessen metropolitan avalanche calamities, instead of assessing their effects and the subsequent harm. Maps representing the degree of harm to impacted structures could give an instrument to forestall the development of offices in generally avalanche inclined regions. Besides, uneven and geolog-ically complex conditions described by the presence of fundamentally complex developments (Esu 1977), like the southern Apennines, and related with extreme or delayed precipitation occasions, or to solid seismic shocks, are generally exceptionally inclined to avalanches.

This street numbers the depiction and characterization of structures and offices harmed by the reactivation of a sluggish and irregular avalanche, known from the very start of the XIX hundred years, utilizing six different harm evaluation draws near. The avalanche is situated in the district of Agnone (Molise locale, Joshi Math(Uttrakhand)), happened in 2003, and includes Miocene-Pliocene dirt ey-marl lithologies. The degree of harm was evaluated through various existing techniques created in different settings and for various purposes. (I) The principal strategy depends on the recovery of the discernible breaks (Burland 1977; Boscardin and Cording 1989); (ii) the subsequent technique was created after a significant avalanche occasion in Italy (Alexander 1986); (iii) the third is centered around the study of harm to structures in avalanche impacted regions (Chiocchio et al. 1997); (iv) the fourth methodology is a plan acknowledged by consolidating a few harm groupings for use with subsidence, mining-related, and landslide occasions (Cooper 2008); (v) the fifth technique (Baggio et al. 2009) was proposed for post-seismic occasions by the Italian Division of Common Security (DPC henceforth); and (vi) the 6th strategy is an as of late distributed approach in light of the past ones and was created in two ensuing stages to survey the seriousness of breaks and developments (Del Soldato et al. 2017). Avalanche actuated harms at the review site were recorded and evaluated through two field overviews directed in November 2015 and in July 2016. The principal point of this work is to apply and look at changed strategies for characterizing avalanche prompted dam-age on designs and offices to assess the most dependable field technique for the administration and reception of counteraction and healing estimates through an avalanche contextual analysis in Agnone, Italy. The fundamental advan-tages and downsides



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of the various characterizations are investigated and talked about. The paper is organized as follows: the following segment momentarily depicts the geographical and geomorphological setting of the ColleLapponi - Piano Ovetta (CL-PO) avalanche inclined region. The show subtleties of the different harm evaluation approaches applied for the order of the structures and frameworks are given after that. Then, at that point, the subtleties of the field turn out created for the grouping of the harm and the perceived avalanche initiated harm are depicted. Followed by a conversation of the primary outcomes got by applying the six techniques. At last, the principal finishes of the paper are summed up.

Land and geomorphological setting

The review region (Fig. 1a) is sited in the western piece of the region of Agnone (Molise, Joshi Math(Utrakhand)) and is solidly impacted via avalanches and erosional processes. The CL-PO region is situated in the catchment of the San Nicola Valley, a sub-bowl on the hydrographic right half of the Verrino Downpour. The offshoot land units incorporate the Upper Miocene Agnoneflysch (Sannitico-Molisane Developments) (Vezzani et al. 2004, Fig. 1b). This is portrayed by turbiditic siliciclastic stores made out of rotating slim layers of clayey sandstones, sandstones, and arenites (Filocamo et al. 2015). The CL-PO avalanche includes the upper individual from the Agnoneflysch, which is comprised of marl, clayey, and subordinate grayish sandy levels with low mechanical opposition and a few changes. A few lithic intercalations, comprising of sandstone or calcareous levels with exceptionally factor thicknesses, are likewise present. The Agnone Development is covered by a superficial regolith skyline made out of dirt, silty mud, and periodic sand with diffuse change follows, plentiful natural material, and a few clasts. Calcaterra et al. (2008) played out a geotechnical characterization of the avalanche through a few land and geotechnical crusades in which four boreholes were penetrated. They perceived four unmistakable layers (Fig. 2), depicted start to finish as follows:

Level A - hard rock and mudstone pieces circulated all through a tumultuous and plastic earth framework. Direct shear tests performed on network tests demonstrated a pinnacle rubbing point of roughly 19° and a union of 20 kPa.

This skyline was straightforwardly associated with the 2003 reactivation of the CL-PO avalanche; Level B - dark dirt, silty mud, sandy earth, and silty sand. This layer shows medium versatility and has a depleted pinnacle grating point close to 23° and a depleted union of surmised by 28 kPa.

Level C - calcareous levels with thicknesses shifting from 0.1 to 1.0 m;

Level D - marly muds, marls, and clayey marls with residue and earth portions. This skyline shows a pliancy tantamount to that of level B yet with a depleted pinnacle erosion point near 22° and a depleted union of roughly 60 kPa.

The super sliding surface is situated at a profundity of approximately 25 m in the upper and focal piece of the avalanche. The profundity of the outer layer of burst impressively diminishes in the lower part of the avalanche, in which the development appears to develop into an earth stream up to a couple of meters thick. Enduring impacts, which comprise of stained, deteriorated, and debilitated hard shakes, as well as textured designs, were perceived in the borehole logs. An intercalation of cracked and exceptionally penetrable calcareous beds, with thicknesses ranging from decimeters to one meter, were noticed (Calcaterra et al. 2008). The ground morphology is constrained by the offshoot lithotypes. Calcareous slants show the most elevated angles (near $30-35^\circ$), though the argillaceous flysch units exhibit incline slopes of around $5-10^\circ$. In regions where erosional processes are more huge, the steepness increments to $15-20^\circ$. The 2003 CL-PO avalanche created along an incline area that lies somewhere in the range of 850 and 650 m a.s.l. furthermore, has a length of roughly 1500 m down to the conversion with the Verrino Deluge at 590 m a.s.l. The whole avalanche region has a typical slant of around 10° with top qualities in the upper piece arriving at 30° . The environment of the locale is calm with temperatures going from 0 to 27°C in winter and summer, separately. Precipitation falls basically during the blustery period from September to April with a typical month to month collection roughly of 80 mm to which the snow should be added. Throughout the mid year season (from July to August) the accumulated precipitation is roughly 45 mm (<http://www.regione.molise.it/schemiidrici>). The morphology of the area has changed continually over the long run as the reshaping of the earth surface has advanced because of the transformative phases of the avalanche (Fig. 3). After the reactivation happened in 2003, a significant standing water body was distinguished in the center part of the avalanche. This region is delimited by a significant scarp and a counterslope, upstream and downstream, separately, because of the math of the mass development. The



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morphology of the area is compliment because of waste works and the statement of dregs shipped by the surface overflow. Different scarps that have been smoothed by erosional processes are plainly unmistakable inside the avalanche region. These scarps are found near the two flanks, as well as in the focal and upper part of the body of the mass development. The unmistakable ground surface cracks were viewed as in the grouping as an important component for examination of the expansion and development of the gravitational peculiarity.

Outline of the avalanche stock at Agnone

The accessibility of verifiable information, including data re-garding confinement and setting off factors, permits the detailing of future practical situations concerning the evolu-tion of the incline insecurity (e.g., Calcaterra and Parise 2001; Calcaterra et al. 2003) and the recognition of possible reactivations and effects of the peculiarity. Avalanches have been known in the district of Agnone since essentially the start of the 20th hundred years. The most established report portraying a precariousness occasion alludes to a peculiarity that happened in Walk 1905 in the San Nicola Valley because of the blend of a pe-riod of serious precipitation and snowmelt (Calcaterra et al. 2008, Fig. 4a). This occasion harmed the scaffold that vehicle ried the fundamental access street to the authentic focus of Agnone (Almagià1910).The district of Agnone has been progressively impacted by a few little and enormous avalanche occasions. Documented and bibliographical avalanche research, detailed in the cross country AVI Undertaking (Guzzetti et al. 1994), uncovered in excess of 60 avalanches that happened in the region of Agnone and the encompassing domain from 1970 to 1998. In February 1984, a mass move-ment happened near the review region that impacted two mainstays of the viaduct of a State Street (Guadagno et al. 1987), compelling the destruction of a segment of it (Fig. 4b). In 1994, a mass development impacted the Colle-Lapponi region, causing the interference of a country road.At long last, the most recent avalanche stock of the district of Agnone was directed by the IFFI Venture (Italian Avalanches Stock Undertaking) by ISPRA (IstitutoSuperiore per la Protezione e la RicercaAmbientale) in 2008.

Reactivation of the CL-PO avalanche

After an extraordinary precipitation occasion that impacted Joshi Math(Utrakhhand) be-tween the 23rd and the 27th of January 2003, with in excess of 200 mm of downpour falling more than 72 h, the researched region was engaged with a significant reactivation of a lethargic noteworthy avalanche because of an uncommon expansion in pore pressure (Calcaterra et al. 2008). In the district of Agnone, a cu-mulative precipitation of roughly 50 mm was estimated in the meteorological station situated in CL-PO (Lat 41.80° and Long 14.33°) having a place with Provincial Organization for the Farming, Country and Fishing Improvement (AgenziaRegionale per lo SviluppoAgricoloRurale e dellaPesca - ARSARP - www.arsarp.it/agromtorologia) in a similar period in which a reactivation of the avalanche (Calcaterra et al. 2008), with a perplexing style comprising of a progression of huge roto-translational slides by an earth-stream (Cruden and Varnes 1996). The occasion included a few offices, driving the region's organization to embrace prohibitive mea-sures, i.e., the departure of 13 structures situated in the space engaged with the avalanche, wherein 17 families were residing. The civil organization, attributable to the seriousness of the circumstance, reserved assets to play out a few dire interven-tions, for example, the re-molding of the area impacted by the mass development and the unearthing of a channel in the upper piece of the body of the avalanche to block and deplete the water table, which privately arrived at the ground surface.During the years following the primary reactivation, notwithstanding the reception of the previously mentioned medicinal measures, the whole avalanche stayed dynamic, expanding its aspects and making extra harm offices and structures. During Walk 2004 and in the period between December 2004 and January 2005, the avalanche was reactivated by a progression of weighty precipitation occasions (Fig. 4), expanding the region in question and preparing an expected absolute volume of 3.5×10^6 m³ (Calcaterra et al. 2008).

Methodologies for harm appraisal

Removals that happen in avalanche regions are frequently uncovered as breaks on the ground surface or as bursts and breaks in man-made offices. These impacts seem when ground developments that influence the structures are more noteworthy than the pliable burdens that the designs are fit for obliging without evident disfigurement. The



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main components to uncover harm are the most inflexible ones, like walls and façades, or the most vulnerable components, like joints (Bru et al. 2013).

Building harm is usually allotted to three general feline egories: compositional, utilitarian, and underlying harm. These classifications were first scattered by Skempton and MacDonald (1956), who didn't characterize clear limits between them. Engineering harm alludes to the presence of a design (e.g., fine breaks in completions, floor or board walls, breaks more extensive than 0.5 mm in mortar or more extensive than 1 mm in harsh cement and stone work walls). Practical harm af-fects the utilization of the construction and produces broad breaks, shifting of floors and walls, falling mortar, discouraged entryways and windows and other non-underlying harm. Underlying harm lessens the security of the design appearing as cracks and bends in help components (e.g., support points, sections and burden bearing walls). Practically speaking, harm influencing offices must be evaluated by performing field studies, which are exceptionally molded by the models took on and the experience of the administrators.

Mainstream researchers has fostered a few explicit characterizations of harm for regions impacted by normal catastrophic peculiarities, like quakes (e.g., Wood and Neumann 1931; Medvedev 1965; Grünthal 1998), subsidence (e.g., Van Rooy 1989; Howard Humphreys and Accomplices, 1993; Freeman et al. 1994), and avalanches (e.g., Burland 1977; Alexander 1986; Geomorphological Administrations LTD, 1991; Lee and Moore 1991; Chiocchio et al. 1997; Iovine and Parise 2002).

DISCUSSION

A few avalanches influence the district of Agnone (Molise locale, Joshi Math(Utrakhand)) as per the verifiable investigations and recreations depicted in the writing. In 2003, the CL-PO region was impacted by a significant reactivation of an old discontinuous mass development, drawing in open consideration on account of the extreme harm caused to encompassing structures and offices. The neighborhood specialists had to empty a families from their residences because of the serious harm that was caused to certain designs. Notwithstanding the acknowledgment of significant adjustment works in the avalanche body, the follow-ing years displayed an extension of the elements of the avalanche. The progression of the toe and retrogression of the principal scarp, with resulting contribution of the parallel flank to oblige the development, addresses a danger of harm to offices and structures situated in the encompassing region.

This paper zeroed in on the correlation of six unique strategies for avalanche prompted harm order for facilities and structures situated in an avalanche inclined region, a fundamental boundary to focus on additional examinations. The clas-sification step can assist nearby directors with choosing where more definite primary examinations ought to be performed or where further activities should be thought of. Moreover, in crisis cases, this starter structure arrangement might be adequate to help the request for some prohibitive mea-sures, the satisfaction of training fixes or support of the super harmed structures. For this reason, six appraisals of avalanche actuated harm draws near (Table 3) were applied and analyzed. The six arrangement strategies, which appeared to be altogether different from one another, were found to areas of strength for have. Notwithstanding an overall correlation, two form ings, one found upslope as for the crown of the CL-PO avalanche and the second to its left side flank, were decided to examine and examine exhaustively the use of the elements of the various systems for surveying avalanche prompted harm.

CONCLUSION

Six existing characterization techniques for avalanche actuated dam-age were introduced, analyzed, and applied to the designs and framework inside and near the Colle Lapponi-Piano Ovetta avalanche, in the district of Agnone (Molise re-gion, Joshi Math(Utrakhand)). A complete number of 30 structures, two walls, three substantial emplacements, and one electrical pole were explored to characterize the seriousness of harm and to research the primary advantages and downsides of every strategy by a correlation of the subsequent groupings to assess their viability in harm evaluation.





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The methodologies were all concocted to classify the degree of harm that influences structures, and notwithstanding a comparative portrayal of the harm, the contribution of different boundaries permits contrast ences in the structure groupings. The distinctions saw in the subsequent guides were examined in view of the attributes of the methodologies as well as the areas and the elements of the structures in-volved. The most ridiculously complete and fitting technique for the area of interest was incorporated, regardless of whether an improved arrangement could have been to blend various highlights of existing approaches to homogenize the method and to keep away from certain disadvantages. The distinction in characterization of designs and framework, as well as in ground cracks for the two later methodologies, relied upon the various boundaries utilized in the arrangements once the information were gathered. A few contemplations that elaborate the distinguishing proof of the breaks, the chance of reviewing the harm in confidential residences, and the primary data that must be recorded to accomplish great and solid structure characterizations, were likewise examined to extricate the impediments and the qualities of every technique. To sum up, the best technique for depicting the harm level and the genuine circumstance of the designs is by all accounts the new strategy created by the downsides and the advantages of each applied methodology.

The strength of this approach brought about the capacity to research the seriousness of the breaks, taking into account a few elements of past techniques, joined with the extension of the harm and the order of the ground cracks that can assist with characterizing the region engaged with the peculiarity. This grouping offers a less complex evaluation of breaks to be done during field overviews in view of perceptible and obvious indicators as well as considering reference widths and a deduced classification of the designs by thinking about the impacts of the cracks. Eventually, a few contemplations of the significance and the convenience of the structure harm information were performed. The data that can be separated from the subsequent guides can help with overseeing and arranging counteraction and healing actions carried out in various stages and to keep away from potential losses. A few models incorporate executing underlying and non-primary preventive estimates in powerless regions, observing avalanche impacted regions, offering help to neighborhood directors for arranging and declaring regulative restrictions, and assisting private and nearby specialists with remodeling harmed structures or assess the chance of moving a few designs and offices.

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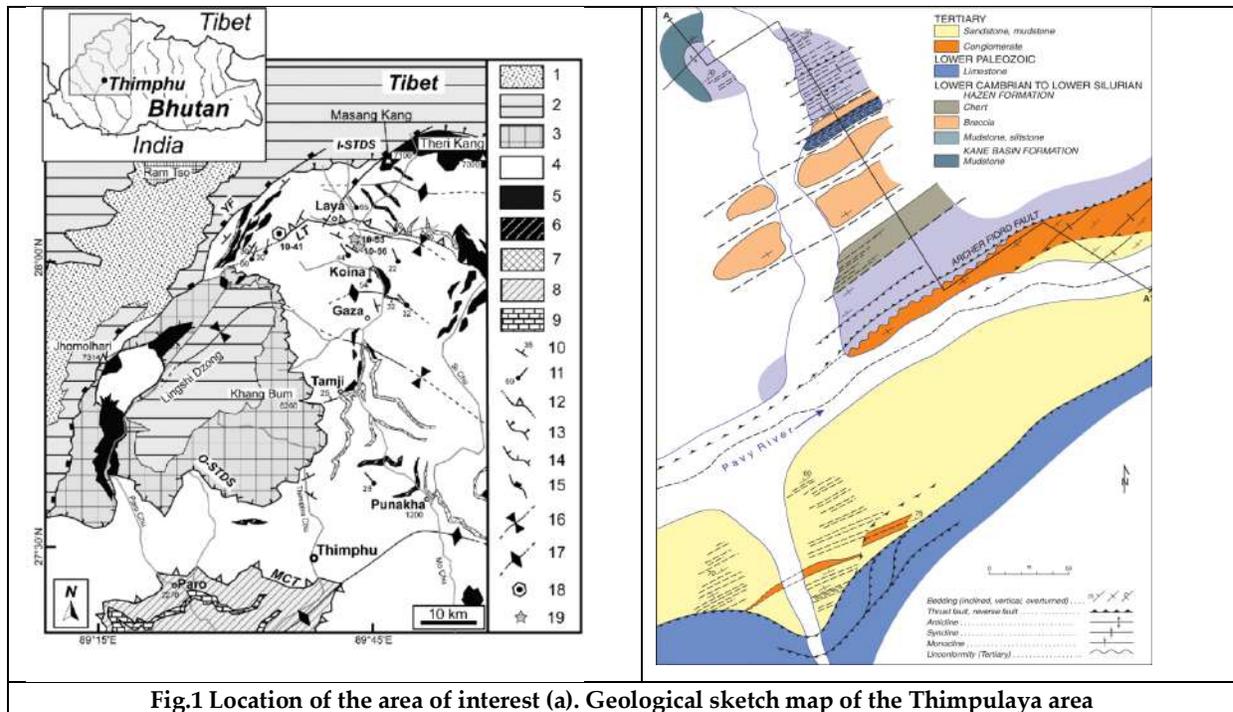
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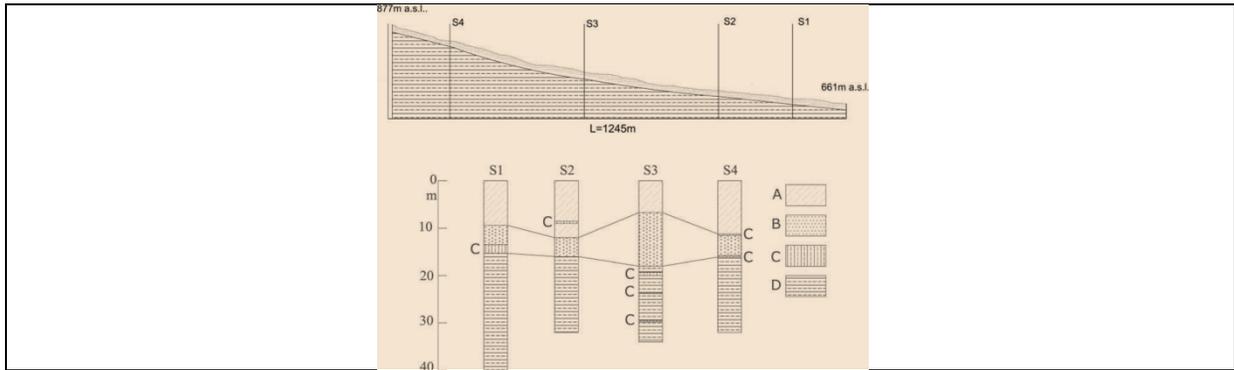


Fig. 2 Engineering geological cross section passing through the median axis of the landslide with the locations of boreholes S1, S2, S3, and S4 modified from Calcaterra et al. (2008). A – hard rock fragments and clays; B – clays and sands; C – limestone



Fig.3 Decadal evolution of the CL-POI and slide. a)Mid-1990s, b)2003, c)2004, d)2005, e)2007, f)2015

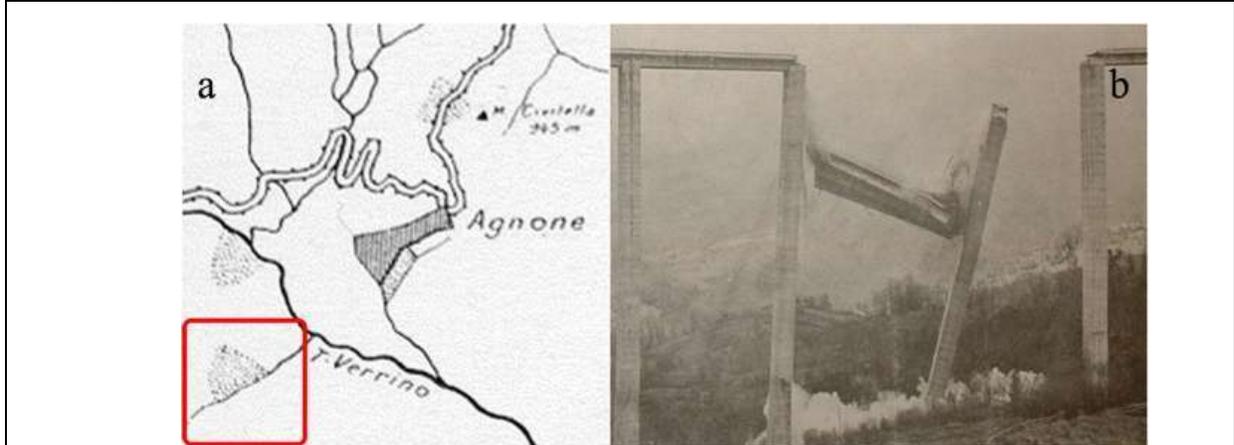


Fig. 4 Excerpt of the map published by Almagià (1910). The red box indicates the location of the CL-PO landslide (speckled in the figure); b) demolition of the State Road viaduct following the 1984 landslide (from Guadagno et al. 1994)





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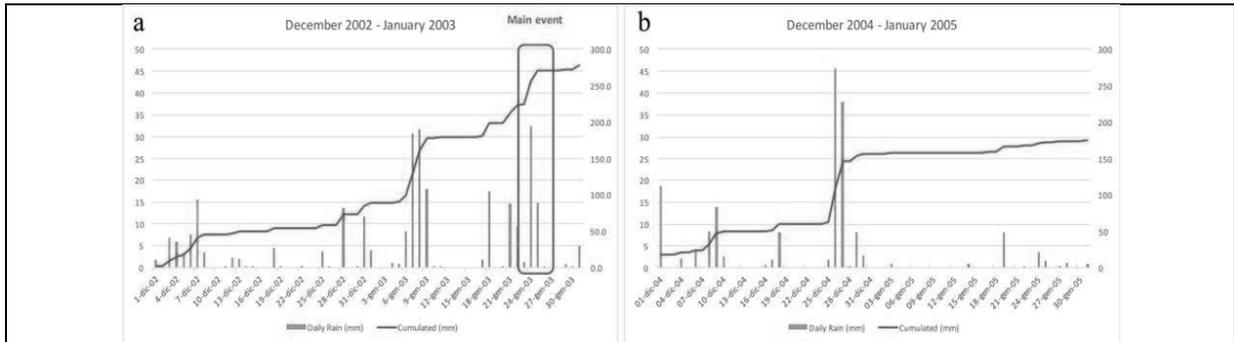


Fig.5 Daily (blue bars) and cumulative (green line) rainfall from (a) December 2002 to January 2003 and (b) from December 2004 to January 2005. The red box highlights the rain event, which caused the main reactivation of the land slide (www.arsarp.it/agromtorologia)

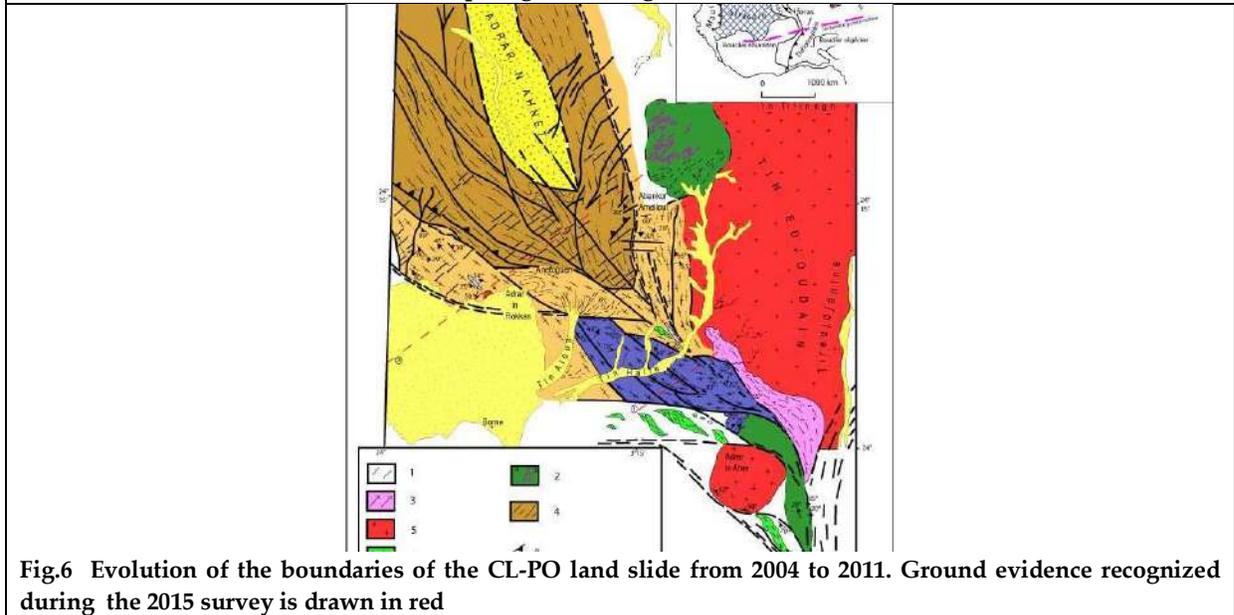


Fig.6 Evolution of the boundaries of the CL-PO land slide from 2004 to 2011. Ground evidence recognized during the 2015 survey is drawn in red





Does Green Marketing Mix Influence Cookware Customers? Empirical Evidence from Chennai Region

A.Appu*

Assistant Professor, MEASI Institute of Management, Chennai-14, Tamil Nadu, India

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*Address for Correspondence

A.Appu

Assistant Professor,
MEASI Institute of Management,
Chennai-14, Tamil Nadu, India.
E.Mail: appumiracle@gmail.com



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ABSTRACT

Growing environmental issues and rising concerns about sustainable development are rapidly shifting traditional marketing and forcing organizations to practice a green marketing mix. This study investigates the relationships between the elements of the green marketing mix and customer's responses toward cookware products in the Chennai region. Descriptive research examined the link between green marketing dimensions and cookware customer's emotions. The study's hypotheses are tested using convenience sampling and structural equation modeling on 340 respondents. The empirical findings reveal that green products, places, and promotions significantly impact cookware customers' responses. Therefore, the green marketing mix elements will allow cookware retail store marketers and managers to understand how their businesses can incorporate sustainability issues into their business activities, improving their relationship with stakeholders and enhancing business performance. This study recommends that cookware manufacturers and retailers use resources more efficiently in manufacturing and distribution to gain a competitive edge that could improve marketing and customer relations. In addition, this paper can influence cookware retailers on green marketing factors to increase customer satisfaction and purchase intention. This paper is one of the first to empirically test the link between green store image, green Price, green Promotion, green Product, green Place, and customer purchase intention in the cookware retail sector context.

Keywords: Green Product, Green Place, Green Store Image, Green Promotion, Green Price, and Purchase Intention





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INTRODUCTION

The environment has emerged as a crucial issue for consumers and producers. Since the traditional marketing mix overemphasizes customers' needs and ignores environmental & social welfare issues, this issue has entered all aspects of corporations, affecting marketing and leading to the concept of a green marketing mix (Abzari M et al., 2013). Subsequently, with increased environmental discourse and concern in societies, businesses can no longer avoid their ecological responsibility. This persistent need compels marketers to incorporate a green concept into their business operations. Consequently, marketers are attempting to align their activities with the environmental requirements of their target segments (Solaiman et al., 2015). Furthermore, Mahmoud TO (2018) stated that developing a green marketing mix was due to organizations' environmental concerns. These companies realized that their survival and continuity depend on aligning their interests with consumer and societal benefits. The green marketing mix encompasses many activities, such as product modification, production process change, advertising change, and packaging change. (Agrawal, Umang & Ansari, Mohammad, 2022) green marketing mix can help a company succeed or fail in a competitive market. As a result, green marketing serves as a link between an organization's eco-friendly activities and the satisfaction of stakeholders. (Keszey, 2020).

Globally, the demand for kitchen cookware appliances has increased over the last decades due to consumers' rising per capita income and busier lifestyles. With strong economic growth and increasing employment opportunities in India, the country's kitchen cookware appliance market continues to grow (Nisha S Tatka, 2016). Supermarkets, convince stores, and hypermarkets are emerging as critical point-of-sale offering a wide range of kitchen cookware appliances from various major appliance manufacturing in India. Even Cookware Retail Stores in India are now subsuming the green concept in their marketing-mix strategies (Kumar et al., 2011). Therefore, green marketing practices have become increasingly crucial for Indian Retail marketers and decision-makers in preserving the natural environment. Several researchers have analyzed how organizations and governments can impose different eco-friendly methods to reduce the impression of industrial activities on the natural environment (Papadas, K. K. et al. 2019).

Prior research, however, examined green manufacturing initiatives and performance relationships. However, most empirical investigations about businesses' green marketing practices have used the traditional marketing theory of the 4P marketing mix model. None of these studies explore whether green marketing mix elements in a cookware retail store context will influence customer environmental responses and intention to use. This is an attempt to ascertain the gap between the mediating variable of customers response' and examine how effectively a green marketing mix, including green Products, green store image, green Prices, green Places, and green Promotion, can impact the green purchase intention. The study is expected to provide valuable insights into the industry's green marketing mix, which can immensely help local and global marketers. Further, it provides a wide-ranging scale for academic researchers exploring the green marketing mix in different organizational settings as a base for future study.

REVIEW OF LITERATURE AND MODEL DEVELOPMENT

Green Marketing Mix

To create a greener economy, there must be a wide variety of innovative, environmentally friendly products and technologies. Many industries have environmental slogans that raise this issue. Successful and green production requires a process with high integration and communications, good information, precise attention to ecological concepts, senior management support, and personality-based measurement and benchmarking. Each company has a unique marketing mix, so marketers must use green 4p creatively while following green marketing principles (Abzari M et al., 2013). Hence, a Green marketing mix can be defined as an organization's pledge to produce harmless, biodegradable goods and services by engaging in recyclable and eco-certified packaging, sustainable manufacturing, and use of energy efficiently (Kotler, 2011). With increased environmental concerns, businesses are





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investigating and evaluating the success of their green marketing mix strategies (Leonidou et al., 2013). These marketing practices reduce the environmental impact of a product over its entire lifetime. (Cronin, Smith, Gleim, Ramirez, & Martinez, 2011). A green marketing mix can also help marketers improve their firms' brand image (Papadas et al., 2017) and market share (Abzari et al., 2013). Like the traditional marketing mix, the green marketing mix has four types: Green Product, Green Price, Green Place or Distribution, and Green Promotion. These are linked (Sinnappan et al., 2015) and aid marketers in persuading their target audience. Since every company has different needs and requirements, its green marketing strategy is a unique mix of these green marketing mix elements. Since every company has different needs and requirements, its green marketing strategy is a unique mix of these green marketing mix elements.

Green Product

Environmental concerns that generate demand for new products have resulted in the revision of existing products and, in some cases, the alteration of their design, formulation, or production (Peattie & Charter, 2003). By incorporating ecological principles into product design, waste and pollution are reduced, and the likelihood that precious resources will be preserved is increased. In light of this, it should be no surprise that green products are central to any green advertising campaign. It is often called sustainable, ecological, eco-friendly, and environmental (Goyal and Mahmoud, 2018). In addition, (Kumar and Ghodeswar, 2015) defined green products as products manufactured with non-toxic ingredients and eco-friendly processes and certified by a recognized organization. (Eneizan et al., 2019) adds to the definition by stating that green products are a strategy for the sustainable consumption of scarce resources through recycling, energy conservation, and natural production. Besides, Green Product conserves energy, resources, harmful substances, pollution, and waste. Therefore, a green product should strike a balance between its environmental features and the satisfaction of its customers (Ottman et al., 2006). Thus hypothesis 1 is proposed as follows:

H₀₁: Green Product positively influences customer's response

Green Price

Green pricing is one of the essential components of the green marketing mix for environmentally conscious products. This concept relates to the production of renewable green energy. According to (Hashem and Al-Rifai, 2011), green pricing refers to prices based on a company's policies about the environment, which are set by rules and company instructions or its efforts in this area. In addition, Larashati et al. (2012) stated that the pricing of green products will be higher due to the cost of incorporating the environmental policy measures prescribed by the company's rules and guidelines or other programs. Some customers only pay extra if they think the Product adds value (Hossain and Khan, 2018). This value may be influenced by a green product's longer lifespan, safety, and other attributes like performance enhancement, efficiency, design, aesthetic appeal, or taste. (Polonsky, Michael & Rosenberger, 2001). Therefore, Green pricing can facilitate a transition in the economy toward eco-friendly and renewable alternatives. Furthermore, a green price indicates a high level of service, which guides customers to anticipate the quality of a particular service (Wilson et al., 2012). As a result of their perceptions of value, modern consumers place a greater emphasis on Price than other factors. Makhitha and Kumalo (2019) confirmed customers' perceptions of prices significantly impact overall satisfaction. Thus hypothesis 2 is proposed as follows:

H₀₂: Green Price positively influences customer's response

Green Promotion

Green Promotion refers to marketing communications informing prospective customers about a service's environmental benefits so that it does not harm the environment (Mahmoud, 2019). Additionally, green marketing involves designing promotional tools like signage, websites, advertisements, marketing materials, videos, white papers, and presentations while considering people, the environment, and profits (Shil, 2012). Further, green Promotion uses advertising strategies that are less harmful to the environment to reduce the negative environmental consequences (Achola and Were, 2018). Therefore, Crucial promotional tools for green advertising are essential to convey communications that could be useful in satisfying consumers' needs and desires. Finally, green advertising





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aims to persuade consumers to buy products that do not pollute the environment by educating them about the benefits of their purchasing choices for themselves and the environment. Thus hypothesis 3 is proposed as follows:

H₃: Green Promotion positively influences customer response

Green Place

Green Place is about managing logistics to reduce transportation emissions, aiming to reduce the carbon footprint (Solaiman et al., 2015). However, green Place is not also concerned with logistics, supply chain management, and distribution. But it is also related to the company's internal environment, business units, and distribution channels. So, the green place concept hopes to instill in these organizations a sense of environmental responsibility that will carry over into their places of employment (Abzari et al., 2013). Moreover, Green places can be promoted by implementing certain in-store practices such as separating green products from systematic products, using eco-friendly materials, and providing recycling facilities at the organization's location (Achola and Were, 2018). Additionally, a well-designed environment can influence customers' purchasing behavior even when they have no intention of purchasing. As a result, cookware retail stores must prioritize location and provide an easily accessible location for customers (Wu et al., 2018). Hence customers will perceive values that reflect their satisfaction or dissatisfaction with the services through a convenient location. Thus hypothesis 4 is proposed as follows:

H₄: Green Place positively influences customer's response

Green Store Image

Wu, Yeh, and Hsiao (2011) described store image as consumers' overall impression of a green store image based on its intrinsic and extrinsic qualities. In addition, (Solomon, Bamossy, & Askegaard, 2002) demonstrated that green store image includes various elements related to store environment, location convenience, product quality, and sales staff's ability to assist customers. However, Naderian (2012) declared that green store image depends on customers' overall impression of the retail store from previous interactions. Therefore, establishing a popular green store image allows a retailer to distinguish itself from competitors and secure a larger market share. As a result, a positive store image helps a retailer grow and strengthen its position in the market by distinguishing itself from competitors through superior product offerings, low prices, and excellent service (Mahmood & Khan, 2014). Hence a positive green store image allows retailers to attract the attention and interest of potential customers. Furthermore, it increases customer satisfaction and influences them to spread positive word of mouth. Thus hypothesis 5 is proposed as follows:

H₅: Green Store Image positively influences customer's response

Green Marketing Mix Elements and Customer Satisfaction

(Ginsberg & Bloom, 2004) Stated that the degree of greenness and size of the green market for a company depends on how well the target customers comprehend green values. The extent to which customers perceive environmental issues and act accordingly impacts the effectiveness of an organization's green policy. (Mahmoud et al., 2017) found their study that all four green Ps (green product, green Price, green Place, & green Promotion) significantly impact the customer's green purchase intention. As a result, to provide an accurate value for the customer satisfaction experience, cookware service providers must first satisfy the customer. Hence, customer satisfaction will increase customer loyalty, repurchase intentions, and word-of-mouth communication, and figure 1 depicts the proposed research model.

RESEARCH METHODOLOGY

The descriptive research study was designed to investigate the effect of a green marketing mix on Chennai city customers' green intention to purchase cookware products. In addition, the primary data related to the constructs were collected using a structured questionnaire. The questionnaire, on the other hand, was divided into two sections. The first section deals with the respondents' demographic information, such as gender, age group, marital status, and annual family income. The second section aimed to collect data on the seven study variables: green Product,





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green Price, green Promotion, green Place, green store image, customer response, and green product purchase intention. Moreover, thirty-five questions in section two were asked to specify their level of (dis)agreement using a five-point Likert scale ranging from 5-strongly agree to 1-strongly disagree. A preliminary survey of 30 respondents was conducted to examine the reliability of the research instrument. After analyzing Cronbach's Alpha of (green product=0.871), (green price=0.889), (green promotion=0.850), (green place=0.882), (green store image=0.863), (customers response=0.866) & (green product purchase intention=0.881). The Cronbach Alpha test was utilized to decide the level of consistency among the various estimations of each factor and found the general score of 0.945 as solid when contrasted with standard alpha above 0.7 is considered adequate (Malhotra & Dash, 2019). As a result, the instrument's internal consistency reliability appears sufficient for collecting the final data. Additionally, 340 responses were gathered using a structured questionnaire based on a convenience sampling technique. Finally, the defined structural model's exogenous and endogenous variables were measured using SEM.

DATA ANALYSIS AND RESULTS

According to the background information provided by the respondent, the number of female respondents (61.77 percent) slightly exceeded that of their male counterparts. However, when it comes to the customers' age range, the group from 26 to 35 years old makes up the most significant proportion (35.60 percent) of the total. In addition, most of the customers who make purchases of green kitchenware items in the sample area are married (77.35 percent). In addition, based on the respondents' annual income distribution, we can see that thirty percent of them fall into the category of 11 to 15 lakhs. In addition, as shown by the type of family that the respondents (55.00 percent) fall under in the sample area, nuclear families make up the majority, are presented in Table 1. The correlation coefficient was calculated to investigate the relationship between the green customer's perception of cookware products and the dimensions of green marketing as indicated in Table 2. The findings show that Karl's Pearson's correlation coefficient between the green store image and the green location had the highest correlation ($r=0.941$, $p=0.000$). In addition, a moderate positive correlation ($r=0.428$, $p=0.000$) exists between green products and green prices at a one percent significance level.

Structural Equation Model

The Casual Modeling Approach was used to determine the relationship between predictor variables such as green product, green Price, green Promotion, green store image, and green Place; mediating factors such as customer's response; and response variables such as intention to use as shown in Figure 2. The structural equation is valid, and the model's goodness-of-fit has been demonstrated by the values of the model's goodness-of-fit. The goodness of fit indexes falls within the allowable ranges for each category to which they belong. Consequently, we can validate the hypothesized connections between the model's variables and the structures.

The results of the Casual Modeling Approach revealed that Green Place ($\text{prob}=0.000$, $\beta=0.696$), Green Promotion ($\text{prob}=0.000$, $\beta=0.257$), Green Product ($\text{prob}=0.000$, $\beta=0.191$), and customer response ($\text{prob}=0.000$, $\beta=0.673$) is positive and highly significant at one percent level of significance as indicated in Table 3. Further, it represents the considerably high level of the positive effect of green places, green Promotion, and green products on customer responses among the respondents by holding all other variables as constant. In addition, the customer's response would increase by 0.696 for every unit value increase in a green place, 0.257 for every unit increase in green Promotion, and 0.191 for every unit increase in a green product. The findings of the evidence study also revealed that Place is the most dominating factor influencing customers' responses to cookware products. This was contributed by emphasizing location, point of purchase, and various modes of payment. Also, a wider distribution of products on the market, payment options that include multiple methods, or the ability to make payments in installments are all factors that can contribute to an increase in the number of purchases made and their total value. On the other side, customer intention to purchase would increase by 0.673 for every unit increase in customer response. As a result, cookware retail stores need to focus on various strategies to generate interest in their cookware products through loyalty programs and improve their brand recognition and recall. The management in cookware retail stores must





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focus on making decisions that revolve around the components of the marketing mix to raise awareness and product value and elicit a favorable response. Similarly, the path coefficient of Green Price ($\text{prob}=0.389$, $\beta=0.034$) and Green store image ($\text{prob}=0.032$, $\beta=-0.242$) is statistically insignificant at one percent and represents the customer's response negative impact. The path revealed that merchandise maintenance, dependability, and store employees are not influencing the green store image. As a result, cookware retail stores must pay close attention to the store environment's controllable elements, such as merchandise and employees, and continuously transmit the promised value through them. This will make customers more reliant on that particular store, benefiting loyalty. Finally, the retail store should categorize the knowledge structure they want to build in customers' minds by specifying the desired level of awareness, strength, and uniqueness of product, functional, and symbolic benefit.

DISCUSSION AND IMPLICATIONS

This study examines the customer's response and green intention to purchase cookware products in Chennai. The results of empirical research found that Green Place has a positive relationship with customer response toward cookware products. Furthermore, it has been revealed that respondents are interested in environmentally friendly shopping areas, and cookware retailer sellers maintain environmentally friendly standards to influence customers' green purchase intention positively. However, it has been discovered that distributional processes must still be quick and simple because customers expect a trouble-free distribution of green products. The result suggests that green cookware retailers should improve to maximize the effectiveness of the distribution method and packaging changes, such as promoting reusable bags, maintaining a closer packaging-to-product ratio, or cube optimization. Besides, It was found that the green promotion element significantly influenced customers' responses to buying green cookware products. However, the results also showed that customers must be aware of the social responsibility of switching to green products through vigorous advertising and promotional campaigns. Further, the Cookware retail stores primarily use environmental concerns to promote their brand by displaying items informing the public about their purchasing decisions' environmental and social implications. Therefore, to cultivate positive customer perceptions, cookware retailers should proactively increase their advertising budgets to convey the advantages of selecting a particular green brand of cookware products over another. Finally, Cookware Retail store policymakers may use digital and social media techniques in target markets to build customer relationships and a good reputation.

The study also showed that the green product attribute favors customers of green cookware products. In addition, the results showed that customers' green purchase behavior is affected favorably by the physical quality of green products when they have a high and optimistic purchase intention. This indicates that the green marketing mix provided by the company Love, atmosphere, and Planet can give customers a positive perception of green products to increase their desire to purchase them. As a result of the findings, green cookware Retail stores should improve products and ensure the highest environmental standards, such as using toxic materials. Hence, green retail stores should keep in mind that by incorporating brand personality into the development of their green products, they can boost sales and entice consumers of non-green products to switch to green ones. Furthermore, the study's findings revealed that the effect of green store image on customers purchasing green cookware is insignificant. The unfavorable impact may be that customers are less likely to shop at stores that have in-store displays for graphics with shared meanings that are less appealing to them. Additionally, it was exposed that a store's ambiance and customer experience could affect customers' thoughts and feelings and the retailer's image. The author added that cookware retail stores should design appealing atmospheric shopping environments to retain customers and encourage positive word-of-mouth through various media channels.

The results of this study add to the literature on customer response and green marketing. This study combines behavioral intentions toward engaging in green behaviors and buying green products with insights from green customer responses. This research also used cross-sectional green marketing mix frameworks to understand customers' pro-environmental response to cookware products. Besides, this study gives us more information about how green customers in Chennai responded positively to green activities and purchases and how their green





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behaviors became part of their daily routines and habits. It is one of the few studies investigating green customer response from a holistic perspective in a single model that includes emotion, motivation, and purchase behavior. The conceptual framework advances knowledge in green consumer behavior and pro-environmental response. The study's significant managerial implications inform policymakers, green product cookware manufacturers, retailers, and marketing managers on customer green purchase response indicators. As a result, cookware retail stores should focus on the Product's green and functional attributes. Also, green cookware manufacturers must introduce innovative products that combine green and functional features. Additionally, cookware retailers should work to increase consumer confidence in eco-labels and introduce products with them. Another significant finding of this research is that customers will buy eco-friendly goods if offered in a wide variety and at convenient locations. Consequently, cookware retailers should ensure that their stores have various green products available for viewing in suitable areas.

The study's practical implications suggest that increasing awareness, willingness to pay a price premium, willingness to spend free time shopping for green cookware products, perceived customer effectiveness, perceived organizational motivation, subjective norm, and the benefits to the individual customer's health and social community can all help to close the response-intention gap in green purchasing.

CONCLUSION AND FURTHER RESEARCH

A green marketing mix is a tool that allows a company to stand out or fall behind in a highly competitive market. The Indian cookware industry is highly competitive, with a significant market share and incorporating the green concept in its marketing-mix strategies. However, for their green strategy to be successful, they must consider the responses of their target customers. Hence, this study tried to analyze the relationship between green marketing mix and customers' green cookware purchase intention. The findings of this study revealed that only green places, green products, and green promotions could influence customer cookware purchases. Apparently, green Place is the only green marketing mix element capable of positively influencing green customer response towards cookware products. By contrast, green promotions and products have the least influence on customers' green purchase response. Notably, green Price was negatively associated with customer response. These findings suggest that customers' reactions and intentions to buy cookware will inevitably decrease as the Price of environmentally friendly products rises. Furthermore, most customers can afford the products if the green Price is reasonable. Additionally, this paper will improve comprehension of the function of the green marketing mix, identify the key constructs that influence consumer purchase intentions, and encourage marketing managers to employ the most effective construct in fostering positive relationships with customers. Thus, green marketing managers have values that guide the development of green marketing practices based on an environmental vision and mission. They spread the green marketing philosophy throughout the company's strategic business functions, consistently committing resources for green product development and rationalizing company processes to deliver consistent consumer value. As a result, this study provided empirical evidence that a green marketing mix can lead to customers' long-term purchase intentions of green cookware products.

The current study examines the role of green marketing mix dimensions and customer green purchase responses to cookware products in Chennai. Similar future studies will explain how these factors contribute to various sectors, including banking, insurance, tourism, and hospitality. In addition, future research could examine the sustainability of cookware retail stores using other elements of the green marketing mix, such as green processes, physical evidence, information practices, and value practices. Moreover, further research can be done to comprehend the impact of a green marketing mix on customers' green purchases of cookware, which are influenced by environmental awareness and concern.



**Appu****Limitations of the study**

This study was restricted to the Chennai region and only analysed the green purchasing behaviour of customers who purchased cookware products. In addition, the current study heavily relied on quantitative data collection techniques, and it was built on a survey approach in which customers were polled using open-ended questions. As a result, more qualitative data collection methodologies should be used to gain additional insights by designing unstructured questions, resulting in a larger pool of information about customer emotions in a retail industry setting. Finally, this study's 340 customer sample and sampling methodology may limit its generalizability without further testing. Therefore, future research should increase the sample size and use different sampling methods to improve conclusions and confirm findings.

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Table 1: Demographic Profile of the Respondents

| Sample Respondents (n = 340) | | |
|------------------------------|----------------------|------------|
| Attributes | Distribution | Percentage |
| Gender | Male (130) | 38.23 |
| | Female (210) | 61.77 |
| Age Group | Below 25 years (58) | 17.05 |
| | 26 to 35 years (121) | 35.60 |
| | 36 to 45 years (96) | 28.23 |
| | Above 45 years (65) | 19.12 |
| Marital Status | Married (263) | 77.35 |
| | Unmarried (77) | 22.65 |
| Type of family | Nuclear family (187) | 55.00 |
| | Joint Family (153) | 45.00 |
| Annual Income | Below 5 lakhs (67) | 19.70 |
| | 6 to 10 lakhs (94) | 27.65 |
| | 11 to 15 lakhs (102) | 30.00 |
| | Above 15 lakhs (77) | 22.65 |

Table 2: Correlation coefficients Results

| Construct | 1 | 2 | 3 | 4 | 5 |
|----------------------|--------|--------|--------|--------|---|
| 1. Green Product | 1 | | | | |
| 2. Green Price | .428** | 1 | | | |
| 3. Green Promotion | .498** | .464** | 1 | | |
| 4. Green Place | .592** | .534** | .631** | 1 | |
| 5. Green Store Image | .608** | .511** | .730** | .941** | 1 |

** . The correlation of coefficient value is significant at the one percent

Table 3: Presenting Model Fit Summary and Regression Weights of Casual Modelling

| Regression Path | Decision | B value | P value | T value |
|--|--------------------|---------|---------|---------|
| Customer Response <--- Green Product | Strongly supported | .191 | 0.000** | 4.594 |
| Customer Response <--- Green Price | Not Supported | .034 | 0.389 | .862 |
| Customer Response <--- Green Promotion | Strongly supported | .257 | 0.000** | 5.129 |





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| | | | | |
|--|--------------------|-------|---------|--------|
| Customer Response <--- Green Place | Strongly supported | .696 | 0.000** | 6.876 |
| Customer Response <--- Green Store Image | Not Supported | -.242 | 0.032 | 2.150 |
| Intention to Use <--- Customer Response | Strongly supported | .673 | 0.000** | 16.734 |

Note: P value=0.054;Chi-square value/df =4.791;AGFI =0.956;GFI =0.903;NFI=0.919;CFI= 0.921; RMSEA= 0.05, RMR= 0.065; and ** indicates highly significant at a 99 percent confidence level.

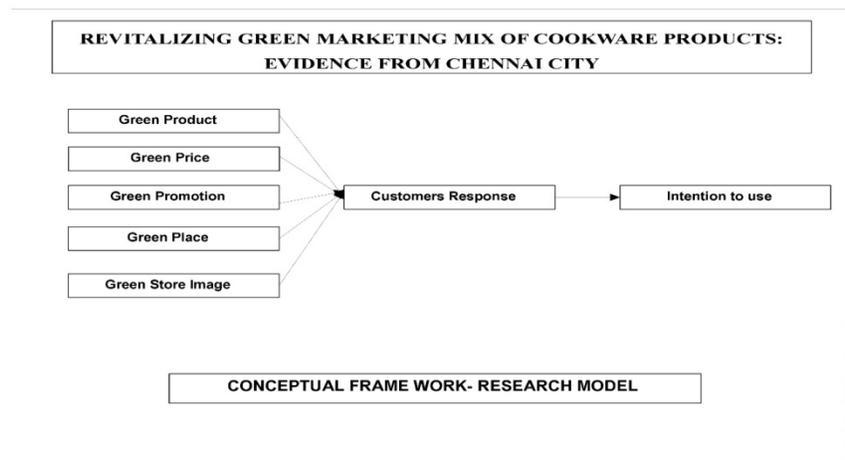


Figure 1: Proposed Research Model

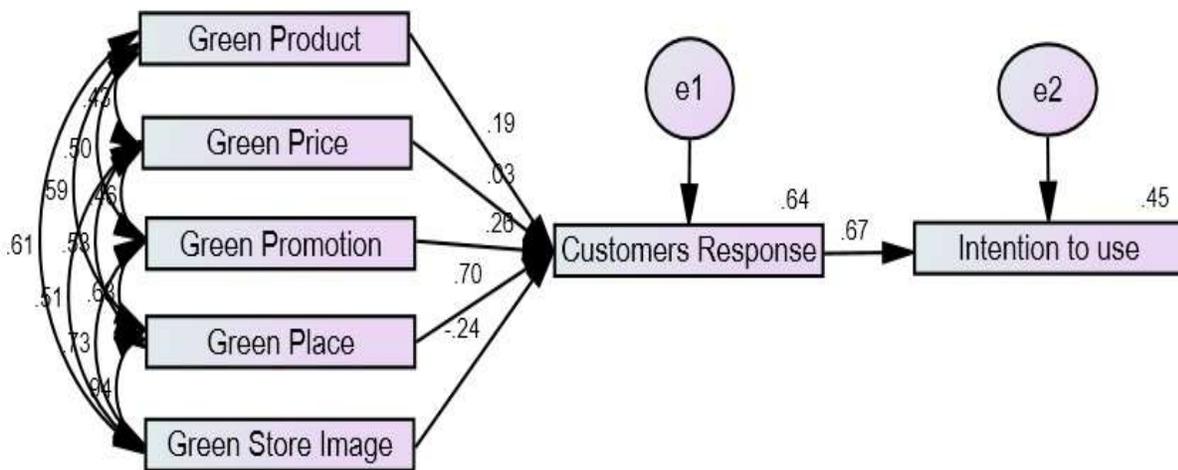


Figure 2: Showing the SEM model summary along with the relationships between constructs





A Novel Approach for Personalized Learning by Mapping Learner to Learning Content using Augmented Bloom's Taxonomy and Employing Resource Description Framework Representation

R.Shinoj Robert^{1*} and M. Maria Dominic²

¹Research Scholar, Department of Computer Science, Sacred Heart College, Tirupattur, Tamil Nadu, India.

²Assistant Professor, Department of Computer Science, Sacred Heart College, Tirupattur, Tamil Nadu, India.

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*Address for Correspondence

R.Shinoj Robert

Research Scholar,

Department of Computer Science,

Sacred Heart College, Tirupattur,

Tamil Nadu, India.

E.Mail: shinojrobert@gmail.com



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ABSTRACT

At present e-learning provides a "one size fits for all" solution to all types of learners. Recently the research is focused on adapting the learning process to become personalized learning. Learner-based desired content according to the learning styles is a tedious approach in the learning scenario. In recent times online tutoring and education have been heightened and educators need to design learning objectives and styles in a codification system. This research is focused on enhancing the personalization of the learning process by employing Revised Bloom's Taxonomy (RBT). This paper's aim aspires to include the revised bloom's concepts under the standard domain of revised blooms. Bloom's taxonomy restricts the hierarchical model under three domains rather the revised Bloom's taxonomy augments the verb. Classifying the learner type and the learning content based on the RBT is of the first kind in any of its ramifications. The primary goal of exhibiting this paper is to sort out Micro-Learning contents concord with the Revised Bloom's Taxonomy included to their subdomain level in the text format. The personalized learning content delivered helps in achieving the desired learning outcomes. Once the learning contents are delivered according to the subdomain levels, mapping to the fit learner is a thought-provoking one. Replacing the investigator, algorithms are brought into effect from the combination of Multinomial Naïve Bayes, Deep Neural Networks and Feed Forward Neural Networks from the respective combinations of Machine Learning, Deep Learning and Quantum Learning Algorithms to sort out the learning contents. From the various combinations used Quantum Learning's Feed Forward Neural Network can produce efficient results. Micro contents are the output of learning

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algorithms. The delivered contents are machinated employing Resource Description Framework (RDF) Graph representation from where the retrieval is made using SPARQL for mapping to the specified learner. Micro contents are organized using file percentage. Personalized learning is achieved by mapping the learner to micro contents.

Keywords: Personalized Learning, Multinomial naive Bayes, Machine Learning, Deep Learning, Quantum Learning, Micro contents, SPARQL, Resource Description Framework.

INTRODUCTION

Learning happens all over and forever. Learner's personal undergoes, knowledge experiences, cultures and environments shape the learning of every individual learner [1]. The act of learning cannot bespeak ant any event instead it's a process. The E-learning environment is on its way by discovering the personalization in each learner type. Personalized Learning is a practice of tailoring the paces of instruction models in accordance to achieve the student learning goal [2]. In recent times many tutors have started to adopt many personalized learning models for the constant engagement of students. This research will present a wholesome technique of research-based framework for delivering a personalized Learning objective in a suit of the learner characteristics. The ratio of delivering the right content to the right person is made simple and easy to approach with the aid of educational taxonomies. The educational taxonomies pave the way to achieve the goals to certain ramification levels for the best interactions. Bloom's taxonomy contribution to the educational domain is mainly in teaching-learning actions [3]. Blooms are powerful tools applied to define the learning objective and the strategies used in specific to the language that needs to be used [4]. It deals the levels of learning and proves with the result in the improvement of higher-order thinking of students by applying the Lower order levels of cognitive skills. This is possible with the target set of hierarchical models; the most applied model is Cognitive in Bloom's. Later lots of researchers were in search of proposing different taxonomies adjoining educator, student, and tutor-designed curriculum in failing to meet rather any other than the cognitive-based domain [5]. Blooms concentrate on the higher-order levels of cognitive skills. The Revised Bloom's Taxonomy is about dealing with action verbs instead the nouns that were listed in the earlier Blooms. Earlier Bloom's is related to the process of thinking levels concentrating on the cognitive-based taxonomy. Cognitive is related to the knowledge that acts as a main source in earlier blooms. In the revised the same knowledge is crossed into different levels as mentioned in the Table.1

Each knowledge type has included the dimensions of student-level understanding. The analysis of these levels made the tutor realize the current student/learner needs more levels of thinking within the knowledge process. The research contribution adapts RBT (Revised Blooms Taxonomy) which paves an extensive measurable augmented verbs track based on six categories that were classified under major three domains. In Revised Blooms, the noun is replaced with action verbs [7]. The RBT covers action verbs that are most decorated today's digital learning environment by not stopping with cognitive extending its level to Affective and psychomotor levels of domains. This includes 6 sub-levels of cognitive, 5 sub-level of Affective and 7 in the psychomotor domain. It helps in identifying the versatile learning styles from the aggregated subdomains of RBT in the categorization of learning contents into micro contents. It helps in identifying the versatile learning styles from the aggregated subdomains of RBT in the categorization of learning contents into micro contents. Achieving an effective personalized learning object is possible by using the verbs in RBT and applying it for classifying the learner and content. The content and the learner classified with this type will be the novel approach in the field of educational domain. This could be preferable and becomes a model for classifying any of the learners and for the learner-preferred content.



**Shinoj Robert and Maria Dominic****TAXONOMIES OF EDUCATIONAL MODELS**

The Blooms Taxonomy objective effectively works on the learning objective specifically assisting tutors in planning and delivering the learning goals towards the learning objective [8]. Table 2 classifies the different educational taxonomy models till recent times from which the extension of the verb list is made. The recent taxonomy models are collected to enhance the verb list for better augmentation in classifying the learner's skills and behaviour. Table 2 classifies the different educational models from where the Revised Blooms are updated and kept up to date for classifying the learner.

AUGMENTED VERB LIST

Table 3 includes the augmented verb list from the taxonomy educational models. To classify the learner and content bloom's taxonomy is to be updated to serve better personalized learning. By strengthening the verb list from these models a better personalization of learning is served. In the educational field, Taxonomy has become ubiquitous in practice in many countries; the search for earlier blooms to Revised Blooms has increased in recent search engines resulting in 21000 plus searches [14]. The recent Interference of Bloom's Taxonomy in online courses resulted exceedingly well in blended learning. It proves to be more systematic, efficient and promoted in proper linguistic utilization with blooms terminology for a new generation of online learners. The importance of blended learning increased the number of online learners promoting concentration on different online courses and learning activities among the learners. The keywords used in the contents (verbs) help in delivering the expected learning outcome by mapping the particular behavior of each learner which is getting expressed via the learning style of the learner. Gearing up any given content that is grounded on RBT will help in improving the personalized learning process of the learner. The verb list of Revised Blooms Taxonomy 2001 includes the basic verbs which need improved technical terms for digital kids [15]. In pursuit of learning significant changes have been brought in the verb terminologies and in this research work augmentation of bringing from various related taxonomies has been classified under the three domains.

Table 4 portrays the augmented verb list from the actual formation of the existing one. The in-depth contribution of 144 possible verbs including technical from different related taxonomies helps to form a much-needed educational taxonomy domain from where the expected learning outcome is achieved measurably. This helps in finding the learner's behaviour and achieving the learning objective concerning the learning outcome.

LEARNER CLASSIFICATION

Classifying the learner and the content related to it is the first of its kind in applying RBT. The augmented verb list will help in finding the content and the learner in a suit of their skills and behaviour. The revised verbs under the 18 categories help the tutor and the learner to achieve the expected outcome. Figure 1 shows the Learning Object achievement by the learner classification and content classification. Learner classification is done by learning content based on the RBT verbs. The content is based on any domain; to be specific the C++ domain is chosen. Preprocessed content is taken as the input; since the contents are preprocessed it is prepared specific to the learner as a brief one. Table 5 Shows a questionnaire model prepared to find the learner type with the verblist to the cognitive domain. Totally 50 questions were prepared from the 18 subcategories. The learner is classified accordingly to the Revised Blooms Hierarchical Level. A questionnaire was set to exhibit the learner characteristics from their responses with the MOODLE as a quiz activity with the RBT. The questions were pruned fitting in the 18 sub-level domains from the Cognitive, Affective and Psychomotor domains.

Figure 2 illustrates the multiple-choice questionnaire models with the RBT verb list. With these, the learner gets classified under the 18 subcategories of the RBT verb list. The questionnaire classified in Figure.2 was prepared to exhibit the learner characteristics from their responses with the MOODLE as a quiz activity according to the Revised Bloom's Taxonomy. The questions were pruned fitting in the 18 sub-level domains from the Cognitive, Affective and Psychomotor domains. The response was collected from the learner set of nearly 100 students in MOODLE. The answer representation was framed as 0 and 1. 0 represents incorrect and 1 represents the correct answer.



**Shinoj Robert and Maria Dominic****CONTENT CLASSIFICATION****Text Preprocessing**

The web-based learning approach breaks the one-size fits all conception since the learners inevitably could not concentrate on the specific content that is widely common because of the diverse backgrounds that are distinct from each learner type [16]. Tailoring the content according to the learner's behaviour by understanding the learning levels of the learner with the RBT will pave a better way to achieve personalized learning outcomes. The proposed Content preprocessing methodology will help in achieving a better-personalized outcome. The methodology is classified as phase works. Figure. 5 shows the proposed content preprocessed Framework with the classification phases involved. Preparing the content based on enhanced verbs collection of RBTs is to be the first kind of classification for learners. The phases of methodology are classified in Table 6. The preprocessing steps are classified as phase works. Starting with that the raw content is converted to the phase of content-cleaning techniques

Learning content cleansing technique**Lowercase**

The Lowercase is a noise removal process removing the lowercases and treating the given content in an equal format and uniformity in content by avoiding unnecessary uppercases. Content-based on OOPS is taken as a sample raw content for applying text preprocessing techniques. Figure 2 projects the given content is converted into the lowercase order.

Digits to string conversion

The digit-to-string conversation technique converts the numbers to strings format. The digits that give the learner content may cause trouble in reading or understanding for the learner. In Figure.4, the content including digits is removed and preprocessed without digits. For example the "domain 4 you" and "domain four you" resembles the same meaning but are difficult in processing.

Punctuation and Accent Removal

The next process after the digits removal is punctuation and accent removal. The punctuation will not result in additional language data [17]. It is recommended process in text preprocessing since it is the source of redundancy and sentiment analysis.

Spell Check

The spellcheck is the far-flung in the learner content. It is common while tailoring, forming or arranging content to occur. It needs to be eradicated in the initial phase. The learning content is getting transformed to a consistent format for advanced analysis of phase II.

Content Diminution Technique

In the content diminution process, the size of the content is reduced by eradicating repeated sentences.

Sentence Tokenization

The word diminution means reduction. Sentence Tokenization is the separation process of breaking the paragraph into small units as tokens and it is made by the punctuations and spaces as an initial process sentence tokenization handles the given content or text into a purposeful one [18]. It breaks the given content as a paragraph and makes meaningful units as tokens.

Stopword removal

The Stopword removal techniques handle the invaluable texts to be removed from the content. The learning content becomes more precise in suit to the learner.



**Shinoj Robert and Maria Dominic****Feature Extraction Technique**

Before the process of content classification, the given learning content is to be separated as a model or vector. The cleaned text or content is transformed and used for modelling in the feature extraction Technique. The feature extraction technique is carried out to understand the content to be made understood by the machine [19].

POS (Parts of Speech Tagging)

The accuracy of the classification is made stronger by the POS classification. The featured model is classified and labelled or tagged according to the appropriate parts of speech. The POS appropriates into a verb, noun, conjunction, or pronoun [20]. The appropriate POS dealt with in our research part is based on verbs. The tutor finds an easy process since the text is tagged by the respective verbs at the end of the particular phase.

LEARNING ALGORITHM MODELS

The automated phase reaches the content classification phase where the content is classified from the chosen learning algorithm models. Working with learning content text-grounded learning content is suggested for a better learning experience for learners. The preprocessed content includes the Revised Bloom's Taxonomy verbs. The researcher has chosen taken selective algorithms from different learning models that are classified in Table 5.

The Naïve Bayes classification is best suited for classification techniques [21]. MNB (Multinomial naïve bayes) is used better in textual data analysis. it is handled for the NLP-based approach. Multinomial Naive Bayes falls under supervised learning from the Machine learning Model. The reason for choosing Multinomial it is very effective in performing with discrete data. The research type dealt with is about the learning content and it is bound to the specific domain level of each learner. It is good at managing discrete data. Text categorization and word counts are the type of discrete data handled in multinomial naïve Bayes [22]. The Feature Extraction technique processes the text as a tag. The tag is considered in the MNB frequency. The frequency states the total times the particular word occurs in the document [23]. The accuracy is increased with the multinomial naïve Bayes classifier.

DNN (Deep Neural Network) is a trained model in Deep Learning. DNN is much explored in hierarchically organized multi-labelled text categorization and is better visualized for text representations [24]. The tutor's choice of choosing this specific algorithm in the Deep learning model is text classification is primal of data mining that handles data as long-term fluctuations, short term evaluations which overcomes the traditional method bestowing the outclass performance by applying network models for better accuracy and stable result [25]. FNN (Feed Forward Neural Network) in Quantum Machine Learning is a multi-layer network model. The importance of bringing the quantum learning model is to bring a fast performance. The two algorithms stated above for classification require binary code to encode the data [26]. In quantum learning, bits are replaced by qubits including multiple states at the time [27]. The performance of the quantum learning algorithm is compared over the DNN and FNN on the information processing and the search of the given learner content. Quantum learning is to be an interpretation of Quantum Computing and Machine Learning.

The evaluation process was made with the students with the OOPS-based learner content. The micro-content was fed on the MOODLE. The researcher conducted an online quiz for the delivery of results. For the experimented results, the random sample keywords taken from the three respective domains are exactly 59. The keywords help in identifying the particular content or text-based sentences Figure 13. Visualizes the total keywords used randomly by the three domains. The better keywords are much utilized for the cognitive domain. It ranges high compared to the affective and psychomotor. Totally 290 learning content types from the OOPS-based domain are chosen with the keywords (verbs). Each data is separated as meaningful sentences covering the domain. The micro-content was stored in CSV file format for easy representations according to the learning algorithms.

The Table9 format exemplifies the total learning content count included and classified according to the domain level. Figure 18 shows the implementation of the machine learning algorithm. Contents are preprocessed and stored in the



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CSV file formatted. The CSV file format of the preprocessed data is uploaded and gets classified according to the selected multinomial naïve Bayes algorithm. The classified algorithm is stored in the text file format arranged by the name MC1, MC2, etc. The delivered content is precise by the earlier preprocessing techniques that shaped more values to it. Figure 19 List the Micro contents file format saved in text files. Each content type is respectively stored in the domain levels. The implementation model is followed the same for the Deep Learning and Quantum Learning Models. As displayed in Figure 19, for each learning algorithm model contents are classified under the separate folder created.

Accuracy, Precision, Recall and F1 score are the evaluation metrics utilized for the classification of learning contents to the micro contents. Accuracy stands for the total accurate forecasting from the total inputs. At least an equal number of samples should be there for the better functioning of a result. Precision stands on the correct positive results. The recall is a positive result collected from the total relevant samples. The confusion Matrix tabulates the whole performance model with the layout for predictions [28]. The total classifier analysis can be brought under the confusion matrix. In this classification, the information about the miscategorized data is also placed in the tabulated results. Confusion matrix classification work is based on binary mode. The evaluation taken for the study is based on the RBT.

EXPERIMENTAL RESULTS OF CONTENT CLASSIFICATION

The experiment results of the above-mentioned Figure are based on the Revised Blooms Taxonomy Keywords (verbs). Utmost of the 59 keywords was carefully weighted for the classification of learning content into the micro-content. Performance metrics help in understanding well the model has performed for the given data in Machine learning, deep learning and quantum learning. Performance metrics play a vital component in the evaluation frameworks of learning Models. The specific benchmark dataset task consorted to the learning models are measured with the selected pipelining processes that are visualized in Figure.21 respectively. Accuracy, the most frequently used performance metric from the learning models, the Feed Forward Neural Network has increased to 1.38 % of the other benchmark rather compared with Machine learning and Deep Learning Models and still, it will be progressive in the inclusion of more keywords. The Analysis of precision, Recall and F1 Score has Feed Forward Neural network has performed well in comparison with the Deep neural Network and Multinomial Naïve Bayes Algorithm.

Provided with the benchmark data task of the learning models quantum learning's Feedforward neural network has topped in all the performance metrics evaluations. The categorization of learning content fitting to the keywords of Revised Bloom's Taxonomy is better classified with Quantum learning's Feedforward Neural Network.

CONTENT ORGANIZATION AND RETRIEVAL FOR PERSONALIZATION

Once the micro contents are classified it needs to be sorted and stored. The contents can be sorted using the file percentage. A formula is derived to calculate the file percentage; the contents are classified according to the subdomain. The cognitive domain is taken for the calculation of file percentage. Figure 22 shows the file percentage calculation. MLC represents the Micro-Learning Content, C stands for content and TC stands for Total Content. The 33% weightage is assigned to each level of the content in remember level in the cognitive domain. Totally 9 contents representing the cognitive domain are tabulated in Table 9.

Remember level file percentage is distributed for 33% of the contents in table 10.

The Learning algorithm derives the Micro content. Exemplification of content organization can be done in many forms. The graph model is one of the forms for storage and retrieval. Once the Micro content is delivered from the learning algorithm the next phase is organizing the content. The reason behind choosing RDF is it stores the data in the triples format as Subject – Predicate – Object that exactly matches the results of RBT. The RBT level of the domain is exactly matched to the RDF representation.



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The micro-content is organized according to the RDF Format which can be retrieved to the suit or the characteristic of the learner. Using Protégé ontology representation for Micro Content is created. The representation includes the creation of annotations of each individual with the microcontent as MC1, MC2 etc. The annotation properties include the Label, Comment and File Percentage. Once the contents are organized, the retrieval is performed using the SPARQL query and it is get visualized using the RDF visualization tool. Learner's learning ability is better ameliorated by delivering the content as Micro content. Ontology has a better representation of micro contents. Using Protégé ontology representation for Micro Content is created. The representation includes the creation of annotations of each individual with the micro-content as MC1, MC2 etc. The annotation properties include the Label, Comment and File Percentage. The micro-content is defined in the annotations created as shown in Figure 23.

Once the contents are organized, the retrieval is performed using the SPARQL query and it is get visualized using the RDF Visualization tool. Figure shows the SPARQL query to retrieve the content based on the File percentage assigned to it. Each micro-content is related to its class types with the object properties. The MC1, MC8 and MC9 are assigned to the domain cognitive. In specific MC1 falls under the sub-domain Remember . The personalization is accomplished once the personalized content is mapped to the fitted learner. To each one, the micro-content with the learning content is assigned by the file percentage with the annotation properties. On the same, it is retrieved by the file percentage. From the score secured by the learner, the content was classified by the tutor according to their learning behaviour.

RESEARCH EFFECTIVENESS

The efficiency result is proven by the learning algorithm models; hence the quantum learning way of classifying the content is been the more efficient way. The performance metrics of Accuracy, Precision, Recall and F1 Score proved the result in which the quantum learning algorithm is best to use. The effective results are produced by the learner by using RBT. Each subdomain score level has been raised by using the RBT. A novel research of classifying the learner and learning content type based on the Revised Bloom's Taxonomy is been made by the researcher. Despite classifying the learners by the blooms, the proposed is a novel type of it. The classification is made from the three main domains of Bloom and 18 sub-level of its sub-category type. A better learning outcome is achieved by producing learner-centred content for the learner

CONCLUSION

The objective of classifying the learner in the suit to the learner's characteristics of the learner and concordant to the domain type and its sub-level is made. The learner classification is based on the RBT. The text-formatted learning content was represented by the RDF representation and in same the retrieval is done through SPARQL. The personalization of assigning learners to the learning content is achieved. The efficiency result is proven by the learning algorithm models; hence the quantum learning way of classifying the content is been a more efficient way. The performance metrics of Accuracy, Precision, Recall and F1 Score proved the result in which the quantum learning algorithm is best to use. The effective results are produced by the learner by using RBT. Each subdomain score level has been raised by using the RBT. A novel research of classifying the learner and learning content type based on the Revised Bloom's Taxonomy is been made by the researcher. Despite classifying the learners by the blooms, the proposed is a novel type of it. The classification is made from the three main domains of Bloom and 18 sub-level of its sub-category type. A better learning outcome is achieved by producing learner-centred content for the learner.

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Authors



Dr. M. Maria Dominic obtained his B.Sc., M.Sc., and M.Phil. and PhD in Computer Science. He has been working in Sacred Heart College, from 1996 onwards at various capacities He has also worked in Multimedia University, Malaysia on a Contractual Basis. He has co-authored a book on OOP using C++ published by Pearson education. He has published more than 20 research articles in International Journals. He has 4 PhD Research scholars working under him in the field of Artificial Intelligence especially in Machine learning and deep Learning.



Mr. Shinoj Robert is a part time research scholar in the Department of computer Science, Sacred Heart College, Tirupattur District, and working as an Assistant professor from 2014 in the Department of Computer Application, Don Bosco College, Yelagiri Hills, and His area of research in the field of Machine learning and E-learning.

Table 1: Knowledge level classifications listed in Revised Blooms Level [6]

| Knowledge Type | Dimensions |
|----------------|----------------------------|
| Factual | Terminologies, disciplines |
| Conceptual | Classifications, models |
| Procedural | Inquiries, methodologies |
| Metacognitive | Solving skills |

Table 2: Taxonomy of educational Models with verb list pattern [9]

| Taxonomy Model | Year | Colour Pattern |
|---------------------------------------|------|----------------|
| TPACK Revised Model | 2021 | |
| Revised ADDIE’s Model | 2021 | |
| The Marzano Teacher Evaluation Model | 2017 | |
| Bigg’s 3P Revised | 2017 | |
| UBD Six facets of design | 2014 | |
| Jerrold Kemp’s Shopping list | 2014 | |
| 6 Models of Blended Learning | 2013 | |
| Merril’s Principles of Instruction | 2012 | |
| Successive Approximation Model (SAM) | 2012 | |
| SAMR Taxonomy | 2010 | |
| Bloom’s Digital Taxonomy | 2008 | |
| Marzano and Kendall/Taxonomy | 2007 | |
| Flinks Taxonomy | 2003 | |
| Norman’s Depth Of Knowledge Framework | 2000 | |





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| | | |
|---|------|--|
| Bloom’s Revised Taxonomy | 2001 | |
| SOLO (Structure of Observed Learning and Outcome) | 1982 | |
| Krathwohl’s Taxonomy of Affective Domain | 1965 | |

Table 3: Augmented verb list from the related taxonomies [10] [11] [12]

| Domain | Category | Verbs (keywords) |
|-----------|------------------|--|
| Cognitive | Creating | Create, constant, find, formulate, produce, prepare, efficacy, summarize, categorize, reconstruct, write, edit, prove, overcome, decide, suitable, inquiry, animate, code, cope, debug, correspond, enhance, facilitate, portray, incorporate, mind map, devise, blog, podcast, remix, wiki, Film, program, website, lead, rate, analogies, imagine, idealize, adequate, invent |
| | Evaluating | Appraises, concludes, contrast, critique, criticizes, defend, discriminate, diagnose, assess, support, justifies, experiment, generate, judge, rank, rate, release, counsel, argue, grade, theories, uncover, dispute, debate, post, pivot, iterate, review, inherent, document, make meaning, make sense, provide a metaphor, self-asses, rebuild, mastery, onboard, simplify, jigsaw, reveal, draw a conclusion, timeline, polish |
| | Analyzing | Analyze, compare, connect, deduce, estimate, discriminate, differentiate, breakdown, illustrate, infer, outline, relates, separate, specify, sort, picked, sequence, deconstruct, classify, subdivide, correlate, dissect, ensure, Figureure out, file, reasoning, tag, moderate, feedback, content, hyperlink, divide, reverse-engineering, crack, Puzzle, instruct, induce, teach, strategize, anatomize, target, surface, meet, point out |
| | Applying | compile, utilize, dramatize, operates, manipulate, show, modify, chart, integrate, apply, fabricate, use, happen, alphabetize, ascertain, avoid, attain, depreciate, diminish, employ, expose, factor, interconvert, round off, train, solveproblem, publish, load, hack, paint, keywords, Play, diverse, speak, test, involve, speed up |
| | (3)Understanding | Comprehends, converts, estimates, extends, infer, interpret, recount, explain, express, spot, group, illustrate, depict, draw, translate, predict, converts, generalize, paraphrases, estimates, represent, prioritize, point, diagram, model, articulate, associate, extrapolate, give, question, transform, storyboard, tweet, gather, subscribe, explicit, subject |
| | Remembering | define, identify, know, label, list, name, outlines, recalls, reproduce, select, state, match, recite, draft, complete, exemplify, recognize, enumerate, reflects, relate, name, quote, tabulate, trace, pattern, absorb, restate, curate, google, bookmark, network, annotate, brainstorm |
| | Characterizing | Act, qualify, serve, propose, verify, perform, influence, let, discriminate, listen, modifies, internalize, look and feel, |





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| | | |
|------------------------------------|----------------------------------|--|
| Affective (16) | | tolerate, strive, reject, promulgate, profess, praise, persevere, object, Empathize, disagree, commit, challenge, advocate, administer, oversee, plan, recommend, submit, |
| | Organizing | relate, synthesize, systematize, clarify, examine, adhere, alter, integrates, order, organize, generalize, identifies, develop, unit, gap, gather, anticipate, confer, consider, consult, coordinate, follow through, investigate, merge |
| | Valuing | appreciate, cherish, treasure, demonstrate, promote, invite, believe, join, justify, propose, respect, complete, differentiate, follow, read, report, select, work, share, follow, alter, generalize, visualize, codify, weigh, devote, peruse, seek, direct, endorse, care, complement, foster, query, motivate, value, ability |
| | Responding | answer, assist, aid, comply, conform, discuss, greet, help, label, perform, present, tell, practice, rite, report, cooperate, Game, tool, expertise, insight |
| | Receiving | acknowledge, ask, attentive, follow, give, understand, attend, recognize, names, sit, reply, erect, identifies, use, attend, locate, choose, hold, replies, point, fail, miss, accept, point to, experience, deliberate, Keep, studies, names |
| Psychomotor (Elizabeth Simpson) | Perception | describe, detect, differentiate, distinguish, identifies, isolate, relates, select, be like |
| | Set | begin, display, explains, moves, proceeds, show, state, volunteer |
| | Guided Response | copies, trace, follow, react, responds |
| | Mechanism (basic proficiency) | calibrates, constructs, fasten, fix, grind, heat, manipulate, mend, mix, pipet, stop, wash, wipe |
| | Complex (or) overt response | assembles, dismantle, measure, sketch, percuss, remove, suture |
| | Adaptation | adapt, alter, change, rearrange, reorganize, revise, vary, recover, avoid, |
| | Origination | arranges, build, combine, form, shake, design, initiate, make, originate, compose, script |

Table 4: Revised Blooms Taxonomy Augmented verb list

| RBT Domain Level | Actual verbs | Extended Verbs | Total verbs |
|------------------|--------------|----------------|-------------|
| Cognitive | 101 | 71 | 172 |
| Affective | 66 | 17 | 83 |
| Psychomotor | 56 | 56 | 112 |
| Total | 223 | 144 | 367 |





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Table 5: OOPS-based questionnaire classification for the cognitive domain

| Domain | Subdomain | MCQ Questions | Question no |
|-------------------------------------|---------------|---------------|-------------|
| Cognitive (Total MCQ -18) | Creating | 3 | 1-3 |
| | Evaluating | 3 | 3-6 |
| | Analyzing | 3 | 7-9 |
| | Applying | 3 | 10-12 |
| | Understanding | 3 | 13-15 |
| | Remembering | 3 | 15-18 |

Table 6: Proposed text content preprocessing phases

| | |
|-----------|---------------------------------------|
| Phase I | Learning content cleansing technique |
| Phase II | Learning content Diminution technique |
| Phase III | Content Feature Extraction Technique |
| Phase IV | Content Classification technique |
| Phase V | Content Organization and Retrieval |

Table 7: Learning algorithm models used for content classification

| Algorithm Model | Name |
|------------------|-----------------------------|
| Machine Learning | Multinomial Naïve Bayes |
| Deep Learning | Deep Neural Network |
| Quantum Learning | Feed Forward Neural Network |

Table 8: Respective keywords (verbs) from the domain

| Domain | Keywords |
|--------------|-----------|
| Cognitive | 25 |
| Affective | 19 |
| Psychomotor | 15 |
| Total | 59 |

Table 9: Total learning content count

| Domain | Keywords |
|--------------|------------|
| Cognitive | 131 |
| Affective | 82 |
| Psychomotor | 77 |
| Total | 290 |

Table 8: Evaluation metric results for learning algorithm models as a table format

| Learning Algorithm | Classification Model | Accuracy | Recall | Precision | F1 Score |
|-----------------------------|----------------------|----------|--------|-----------|----------|
| Multinomial Naïve Bayes | Machine Learning | 0.3 | 0.4285 | 0.5 | 0.4615 |
| Deep Neural Network | Deep Learning | 0.312 | 0.5 | 0.6285 | 0.5615 |
| Feed Forward Neural Network | Quantum Learning | 1.3816 | 1.3725 | 1.3670 | 1.3642 |





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Table 9: RDF Representation of the contents

| Subdomain Level | Sub domain | Content-Type | | | | | | | | | Total | Content % |
|-----------------|------------|--------------|-----|-----|-----|-----|-----|-----|-----|-----|-------|-----------|
| | | MC1 | MC2 | MC3 | MC4 | MC5 | MC6 | MC7 | MC8 | MC9 | | |
| Level 1 | Remember | ✓ | | | | | | | ✓ | ✓ | 3 | 33% |
| Level 2 | Understand | | | | ✓ | | | ✓ | | | 2 | 22% |
| Level 4 | Analyze | | | | | | | ✓ | | | 1 | 11% |
| Level 5 | Evaluate | | | | ✓ | ✓ | | | | | 2 | 22% |
| Level 6 | Create | | ✓ | | | | | | | | 1 | 11% |

Table 10: Remember the level file percentage

| Remember | MC1 | MC8 | MC9 |
|----------|-----|-----|-----|
| 33% | 11% | 11% | 11% |

Table 11: RDF Representation of the contents

| Subject | Predicate | Object |
|-------------------|--|---|
| Domain: Cognitive | The content includes the keywords: The actual verb in the content | Target node: Eg MC1, MC2 The known class in the object |

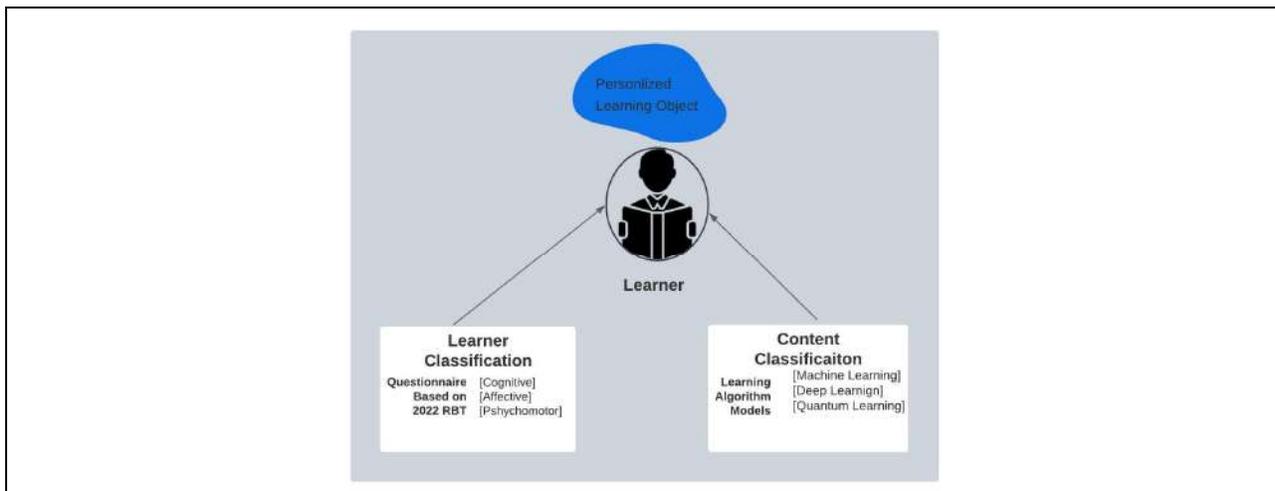


Figure.1 Learning objects deliverance from the learner and content classifications

1. Class can be **defined** as a container containing data variables and functions.
2. Class is a user **defined** data type holding variables and member functions together
3. class can only be accessed by **using** objects
4. objects are **created** from classes
5. declaration of objects is made in the same way as we **make** a declaration of variables
6. object can be **defined** as a real-world entity existing physically in the world with some behavior and state
7. abstraction is a **practice** of hiding irrelevant details from the user
8. encapsulation is **keeping** the data and function into a single unit like a capsule

Figure.2 Questionnaire content model based on the cognitive RBT verbs





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| S | T | U | V | W | X | Y | Z | AA | AB | AC | A |
|-----------------|-----------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-------|
| Time taken | Grade/10. | Q. 1 /0. | Q. 2 /0. | Q. 3 /0. | Q. 4 /0. | Q. 5 /0. | Q. 6 /0. | Q. 7 /0. | Q. 8 /0. | Q. 9 /0. | Q. 10 |
| 14 mins 35 secs | 9.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 13 mins 38 secs | 9.8 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 17 mins 21 secs | 9.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 22 mins 21 secs | 7.8 | 0.2 | 0.2 | 0.2 | 0.2 | 0 | 0 | 0.2 | 0.2 | 0.2 | 0.2 |
| 23 mins 13 secs | 7.4 | 0.2 | 0.2 | 0.2 | 0 | 0 | 0 | 0.2 | 0.2 | 0 | 0 |
| 19 mins 24 secs | 7.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0 | 0 | 0.2 | 0.2 | 0.2 | 0.2 |
| 23 mins 13 secs | 7.8 | 0.2 | 0.2 | 0.2 | 0.2 | 0 | 0 | 0.2 | 0.2 | 0.2 | 0.2 |
| 9 mins 30 secs | 8.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 20 mins 52 secs | 9.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 5 mins 38 secs | 7.4 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0 | 0.2 | 0.2 | 0.2 | 0.2 |
| 19 mins 30 secs | 6.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0 | 0.2 | 0.2 | 0.2 | 0.2 |
| 6 mins 55 secs | 8.6 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| 7 mins 58 secs | 9.8 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

Figure.3 shows the score obtained by the learner

| BT | BU | BV | BW | BX | BY | BZ | CA | CB | CC | CD |
|-----------------|-----------------|---------------------|-------------|--------|----------|---------|----------|-----------|---------|-------|
| Cognitive level | Affective Level | Psychomotor Level 3 | TOTAL (100) | Rememb | Understa | Applyin | Analyzir | Evaluatir | Creatin | Total |
| 20 | 18 | 28 | 66 | 0.6 | 0.4 | 0.6 | 0 | 0.2 | 0.2 | 2 |
| 36 | 32 | 18 | 86 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 3.6 |
| 36 | 32 | 30 | 98 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 3.6 |
| 20 | 22 | 20 | 62 | 0.6 | 0.2 | 0.6 | 0.2 | 0.2 | 0.2 | 2 |
| 30 | 24 | 24 | 78 | 0.6 | 0.2 | 0.6 | 0.6 | 0.4 | 0.6 | 3 |
| 28 | 32 | 30 | 90 | 0.6 | 0.2 | 0.6 | 0.4 | 0.4 | 0.6 | 2.8 |
| 24 | 22 | 26 | 72 | 0.6 | 0.2 | 0.6 | 0.2 | 0.6 | 0.2 | 2.4 |
| 28 | 32 | 30 | 90 | 0.6 | 0.2 | 0.6 | 0.4 | 0.4 | 0.6 | 2.8 |
| 26 | 26 | 28 | 80 | 0.6 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 2.6 |
| 24 | 28 | 26 | 78 | 0.6 | 0.2 | 0.6 | 0.2 | 0.6 | 0.2 | 2.4 |
| 26 | 18 | 16 | 60 | 0.6 | 0.4 | 0.6 | 0.2 | 0.4 | 0.4 | 2.6 |
| 12 | 14 | 12 | 38 | 0.2 | 0.2 | 0 | 0.2 | 0.2 | 0.4 | 1.2 |
| 24 | 26 | 30 | 80 | 0.6 | 0.4 | 0.6 | 0.4 | 0.2 | 0.2 | 2.4 |
| 26 | 24 | 6 | 56 | 0.6 | 0.4 | 0.2 | 0.6 | 0.6 | 0.2 | 2.6 |
| 22 | 22 | 20 | 64 | 0.6 | 0.2 | 0.4 | 0.2 | 0.4 | 0.4 | 2.2 |
| 36 | 32 | 32 | 100 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 3.6 |

Figure.4 Learner score table from the three domains of RBT.





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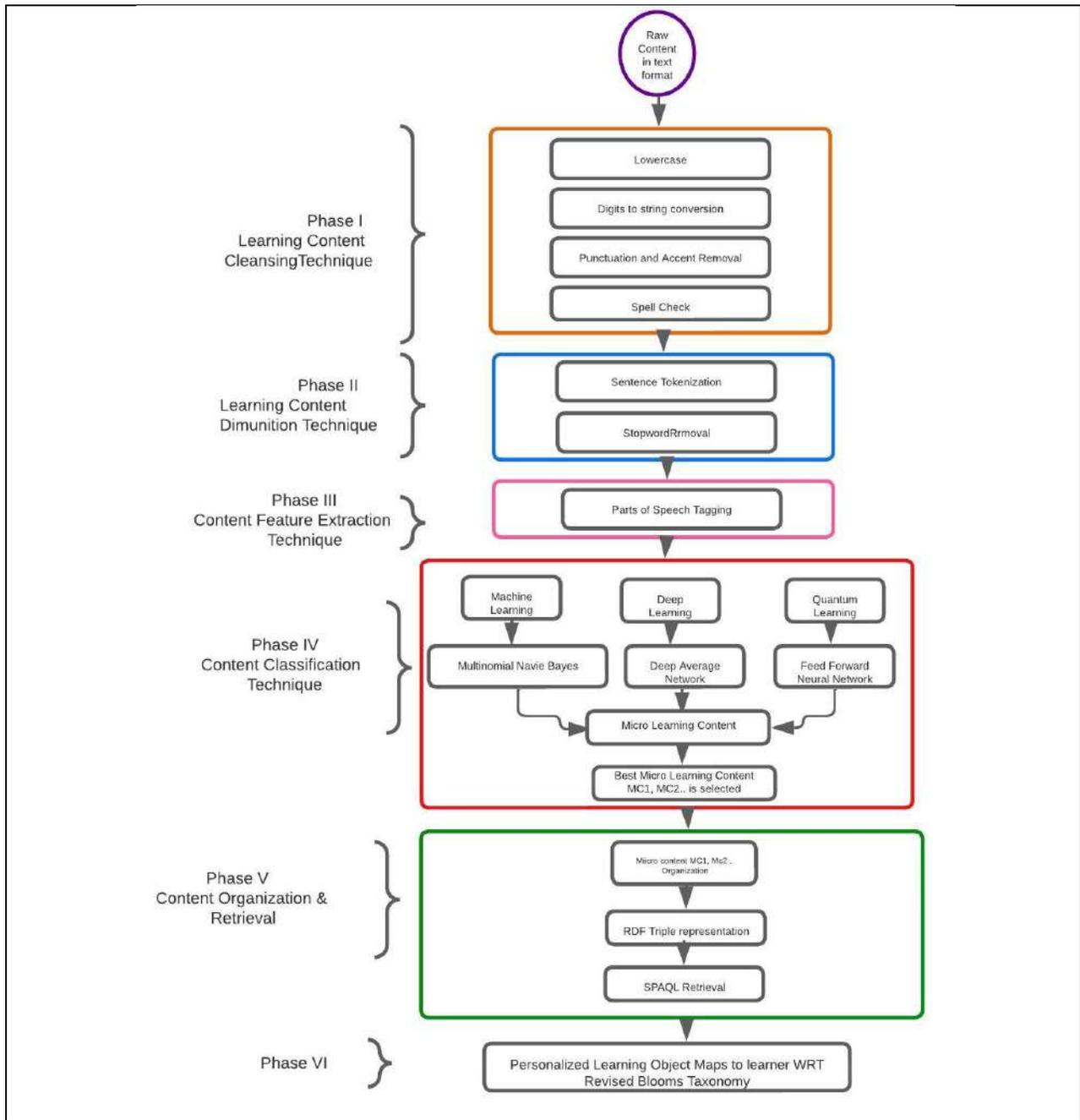


Figure.5 Text PreprocessingFramework





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```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
===== RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase I\lowercase.py =====
polymorphism can be defined as the ability of a message to be displayed in more than one form. polymorphism in c++ allows us to reuse code by creating one function tha
t's usable for multiple uses. a real-life example of polymorphism, a person at the same time can have different characteristics. the quality or state of existing in or
assuming different forms gives the exact meaning representing polymorphism. we can also make operators polymorphic and use them to add not only numbers but also combin
e strings. this saves time and allows for a more streamlined program. in c++ polymorphism is classified into 2 types of polymorphism: compile time polymorphism this is
also known as static (or early) binding. runtime polymorphism known as dynamic (or late) binding.
>>>
    
```

Figure 6: Lowercase conversion

```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
= RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase I\digits-removal-v1.py
polymorphism can be defined as the ability of a message to be displayed in more than one form. polymorphism in c++ allows us to reuse code by creating one function tha
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. in c++ polymorphism is classified into two () types of polymorphism: ). compile time polymorphism this is also known as static (or early) binding. ) runtime polymorp
hism known as dynamic (or late) binding.
>>>
    
```

Figure.7 Digit to normal text converted process

```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
= RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase I\par-v1.py
polymorphism can be defined as the ability of a message to be displayed in more than one form polymorphism in c++ allows us to reuse code by creating one function that
's usable for multiple uses a realife example of polymorphism a person at the same time can have different characteristics the quality or state of existing in or assu
ming different forms gives the exact meaning representing polymorphism we can also make operators polymorphic and use them to add not only numbers but also combine stri
ngs this saves time and allows for a more streamlined program in c++ polymorphism is classified into two types of polymorphism compile time polymorphism this is also
known as static or early binding runtime polymorphism known as dynamic or late binding
>>>
    
```

Figure.8 Punctuation and Accent Removal

```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
= RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase I\spellcheck-v2.py
polymorphism can be defined as the ability of a message to be displayed in more than one form polymorphism in c++ allows us to refuse code by creating one function tha
t's unable for multiple uses a realize example of polymorphism a person at the same time can have different characteristics the quality or state of existing in or assu
ming different forms gives the exact meaning representing polymorphism we can also make operators polymorpho and use them to add not only numbers but also combine stri
ngs this saves time and allows for a more streamlined program in c++ polymorphism is classified into two types of polymorphism compile time polymorphism this is also k
nown as static or early binding auntie polymorphism known as dynamic or late binding
>>>
    
```

Figure.9 Redundancy-avoided contents are produced in the spell-check technique





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| |
|---|
| polymorphism can be defined as the ability of a message to be displayed in more than one form. |
| polymorphism in c++ allows us to reuse code by creating one function that's usable for multiple uses. |
| a real life example of polymorphism a person at the same time can have different characteristics. |
| the quality or state of existing in or assuming different forms gives the exact meaning representing polymorphism. |
| we can also make operators polymorphic and use them to add not only numbers but also combine strings. |
| this saves time and allows for a more streamlined program. |
| in c++ polymorphism is classified into two types of polymorphism compile time polymorphism this is also known as static or early binding. |
| runtime polymorphism known as dynamic or late binding. |

Figure.10 Tokenized sentences as paragraph

```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
= RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase II\sr-v2.py
['runtime', 'polymorphism', 'known', 'as', 'dynamic', 'or', 'late', 'binding']
['runtime', 'polymorphism', 'known', 'dynamic', 'late', 'binding']
>>>
    
```

Figure 11: Stop word removed content

| | |
|---|--|
| 1 | polymorphism defined ability message displayed one form |
| 2 | polymorphism c++ allows us reuse code creating one function ' usable multiple uses |
| 3 | real life example polymorphism person time different characteristics |
| 4 | quality state existing assuming different forms gives exact meaning representing polymorphism |
| 5 | also make operators polymorphic use add numbers also combine strings |
| 6 | saves time allows streamlined program |
| 7 | c++ polymorphism classified two types polymorphism compile time polymorphism also known static early binding |
| 8 | runtime polymorphism known dynamic late binding |

Figure.12 the content is placed as sentences for easy representation

```

IDLE Shell 3.9.13
File Edit Shell Debug Options Window Help
Python 3.9.13 (tags/v3.9.13:6de2ca5, May 17 2022, 16:36:42) [MSC v.1929 64 bit (AMD64)] on win32
Type "help", "copyright", "credits" or "license()" for more information.
>>>
= RESTART: E:\Research\11-Implementation\Text Preprocessing Results\Python 3.9.13 Results\Phase III\pos-lemmatize.py
[nltk_data] Error loading averaged_perceptron_tagger: <urlopen error
[nltk_data] [Errno 11001] getaddrinfo failed>
[['runtime', 'JJ'], ('polymorphism', 'NN'), ('known', 'VBN'), ('dynamic', 'JJ'), ('late', 'JJ'), ('binding', 'NN')]
[['runtime', 'a'], ('polymorphism', 'n'), ('known', 'v'), ('dynamic', 'a'), ('late', 'a'), ('binding', 'n')]
runtime polymorphism know dynamic late binding
>>>
    
```

Figure.13 POS converted content





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| Sno | Featured content | POS Tagging |
|-----|---|--|
| 1 | polymorphism defined ability message displayed one form | [('polymorphism', 'n'), ('defined', 'v'), ('ability', 'n'), ('message', 'n'), ('displayed', 'v'), ('one', None), ('form', 'n')] |
| 2 | polymorphism c++ allows us reuse code creating one function ' usable multiple uses | [('polymorphism', 'n'), ('c++', 'n'), ('allows', 'v'), ('us', None), ('reuse', 'v'), ('code', 'n'), ('creating', 'v'), ('one', None), ('function', 'n'), ('', 'n'), ('usable', 'a'), ('multiple', 'a'), ('uses', 'n')] |
| 3 | reallife example polymorphism person time different characteristics | [('reallife', 'n'), ('example', 'n'), ('polymorphism', 'n'), ('person', 'n'), ('time', 'n'), ('different', 'a'), ('characteristics', 'n')] |
| 4 | quality state existing assuming different forms gives exact meaning representing polymorphism | [('quality', 'n'), ('state', 'n'), ('existing', 'v'), ('assuming', 'v'), ('different', 'a'), ('forms', 'n'), ('gives', 'v'), ('exact', 'a'), ('meaning', 'v'), ('representing', 'v'), ('polymorphism', 'n')] |
| 5 | also make operators polymorphic use add numbers also combine strings | [('also', 'r'), ('make', 'v'), ('operators', 'n'), ('polymorphic', 'a'), ('use', 'v'), ('add', 'a'), ('numbers', 'n'), ('also', 'r'), ('combine', 'v'), ('strings', 'n')] |
| 6 | saves time allows streamlined program | [('saves', 'n'), ('time', 'n'), ('allows', 'v'), ('streamlined', 'v'), ('program', 'n')] |
| 7 | c++ polymorphism classified two types polymorphism compile time polymorphism also known static early binding | [('c++', 'n'), ('polymorphism', 'n'), ('classified', 'v'), ('two', None), ('types', 'n'), ('polymorphism', 'n'), ('compile', 'n'), ('time', 'n'), ('polymorphism', 'n'), ('also', 'r'), ('known', 'v'), ('static', 'a'), ('early', 'a'), ('binding', 'n')] |
| 8 | runtime polymorphism known dynamic late binding | [('runtime', 'a'), ('polymorphism', 'n'), ('known', 'v'), ('dynamic', 'a'), ('late', 'a'), ('binding', 'n')] |

Figure.14 POS converted sentences with the verb tags

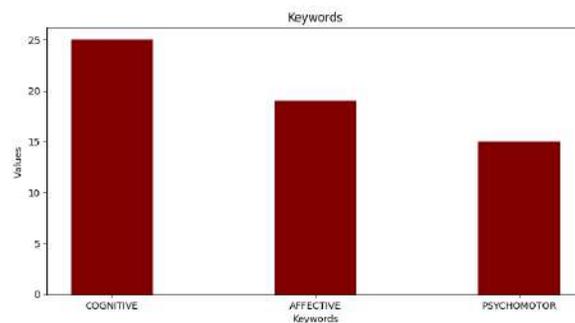


Figure.15 Keywords representation from the RBT domains

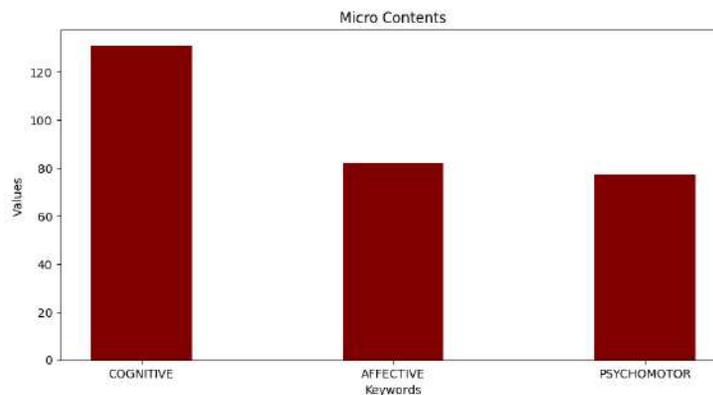


Figure 16. the total Micro content delivered in data representation from the domains





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← → ↻ ⓘ 127.0.0.1:5000

WebVOWL Staging-DBIMA Live-DBIMA

Upload new File

Choose File train.csv Upload

Figure.17 Content classification based on the Multinomial Naïve Bayes in ML

| Name | Date modified | Type | Size |
|----------|------------------|---------------|------|
| mc1.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc2.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc3.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc4.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc5.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc6.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc7.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc8.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc9.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc10.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc11.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc12.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc13.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc14.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |
| mc15.txt | 5/2/2023 8:33 PM | Text Document | 1 KB |

Figure.18 Classified Learner content according to the Learning Algorithm





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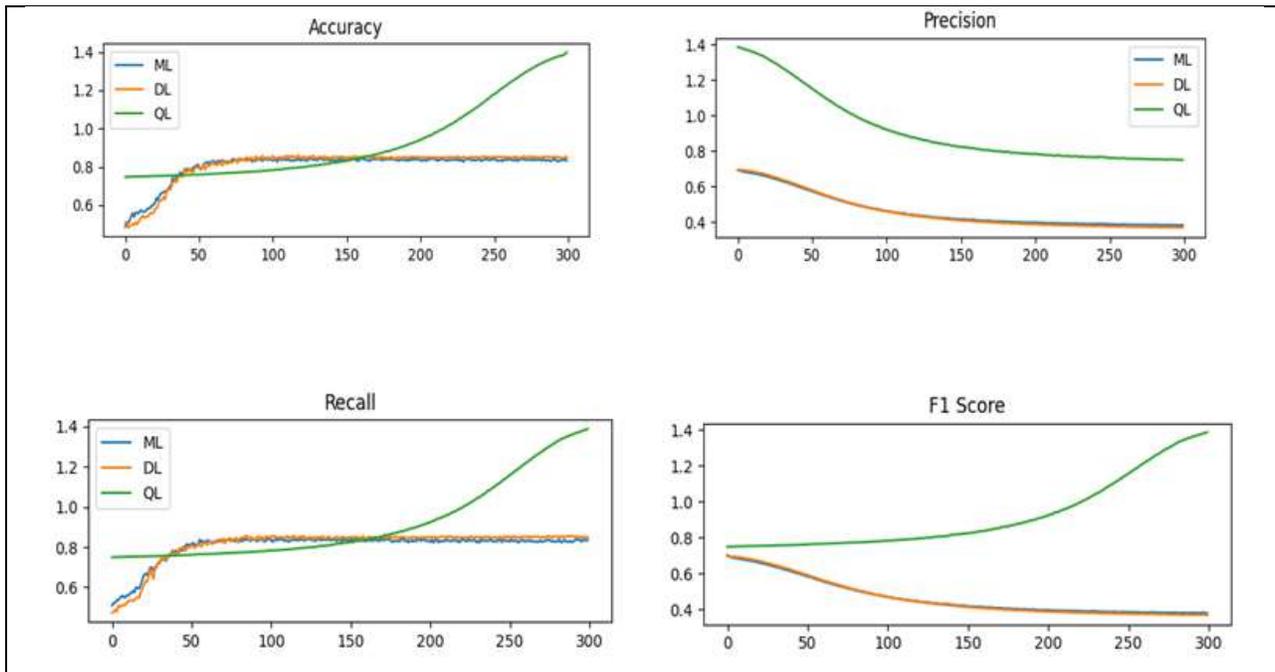


Figure.19 Visualization results of Accuracy, Precision, Recall and F1 score

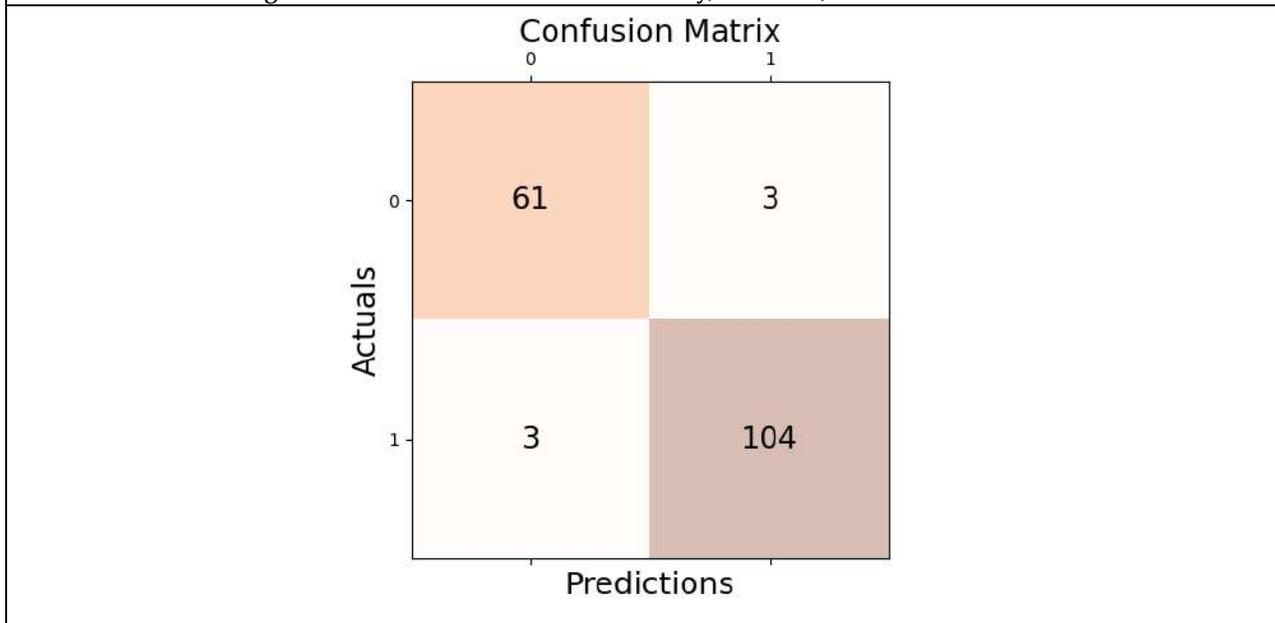


Figure.20 Visualization of the evaluation metrics of the confusion matrix for the three learning Models

```

=====
Accuracy      Recall      Precision    F1 Score
ML:  0.3      0.42857142857142855    0.5          0.4615384615384615
DL:  0.312    0.5                0.6285714285714286    0.5615384615384615
QL:  1.3816924095153809    1.3725695610046387    1.3670632243156433    1.3642128705978394
=====
    
```

Figure. 21 Evaluation metric results for learning algorithm models





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MLC= Micro-Learning Content, C= Content, TC= Total Content
 $MLC = C/TC * 100$
 $3/9 * 100 = 33\%$
 Remember Level Content = 33 %

Figure.22 Formula to calculate the file percentage for the remembering level

The screenshot shows the Protege interface. On the left, a class hierarchy for 'Remember' is displayed, including subclasses like 'Analyzing', 'Applying', 'Creating', 'Evaluating', 'Remember', 'Understanding', and 'Psychomotor'. Below this, direct instances for 'Remember' are listed: 'define', 'MC1', 'MC8', 'MC9', 'recall', and 'select'. On the right, the 'Annotations' tab for 'MC1' is shown, with annotations for 'rdfs:label' (MC1), 'rdfs:comment' (By default data is not defined to outside world), and 'rdfs:FilePercentage'. The 'Description' tab also shows 'MC1'. The 'Types' section shows 'Remember' as a type.

Figure.23 Creation of Annotation and class hierarchy in Protege

SPARQL query:

```
PREFIX rdf: <http://www.w3.org/1999/02/22-rdf-syntax-ns#>
PREFIX owl: <http://www.w3.org/2002/07/owl#>
PREFIX rdfs: <http://www.w3.org/2000/01/rdf-schema#>
PREFIX xsd: <http://www.w3.org/2001/XMLSchema#>
SELECT *
WHERE {?MC rdfs:FilePercentage ?FilePercentage
}
ORDER BY DESC(?FilePercentage)
```

| MC | FilePercentage |
|-----|----------------|
| MC8 | "13%"@en |
| MC1 | "11%"@en |
| MC9 | "10%"@en |

Figure.24 Personalized learning content retrieval using SPARQL





Various Treatment Modalities for Indirect Sinus Lift

Manish Dev Sejwal^{1*} and Amit Bhardwaj²

¹Ph.D Scholar, Senior Lecturer, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, New Delhi-NCR, India.

²Professor and Head, Department of Periodontology, Faculty of Dental Sciences, SGT University, Gurugram, New Delhi-NCR, India

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*Address for Correspondence

Manish Dev Sejwal

Ph.D Scholar, Senior Lecturer,
Department of Periodontology, Faculty of Dental Sciences,
SGT University, Gurugram,
New Delhi-NCR, India.
E.Mail: manish23041981@gmail.com



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ABSTRACT

Pneumatization and Vertical bone loss of maxillary sinus are the typical physiological changes that mainly occur in the edentulous maxillary posterior region. These physiological changes create a great problem for the operator to place dental implants in this region. Therefore, various treatment modalities have been introduced to raise the floor of the maxillary sinus and place dental implant safely. The indirect sinus lift is one of the simplest and safest techniques that has been introduced into clinical implant dentistry. In this review article, various treatment modalities have been presented for indirect sinus lift.

Keywords: Pneumatization; Maxillary Sinus; Sinus Lift; Dental Implants; Augmentation; Vertical Bone.

INTRODUCTION

The maxillary sinus, also referred to as Highmore's antrum, is a bilateral pyramidal-shaped paranasal cavity. It is lined by a delicate respiratory ciliated epithelium that facilitates fluid movement towards the ostium [1]. The Schneiderian membrane, covering the maxillary sinus cavity, holds crucial importance for the success of sinus augmentation techniques and the avoidance of potential complications [2,3]. In the field of implant dentistry, the posterior maxillary area without teeth presents distinct challenges [4]. This is due to extensive and enduring bone resorption resulting from prolonged partial or complete edentulism. The atrophic maxilla poses several issues, including poor bone quality and inadequate vertical bone volume, varying between individuals, which leaves insufficient bone for placing endosseous implants [5].



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Various treatment approaches have been proposed to address the loss of bone volume in the posterior maxillary region:

Short implant placement: This strategy involves using shorter implants and is the most conservative and minimally invasive option. It offers less complex procedures, reduced costs, fewer surgical interventions, and decreased complications [6-10].

Zygomatic or pterygoid implant insertion: Another approach involves using the available residual bone in anatomical buttress areas [11] like the frontomaxillary, frontozygomatic, and pterygomaxillary buttresses. This method combines zygomatic or pterygoid implants with conventional anterior implants, showing favorable survival and success rates [12-15].

Regenerative treatments: Maxillary sinus augmentation employs two main regeneration techniques: the lateral window or direct approach and the crestal or indirect approach. Boyne and James [16] were pioneers in publishing the sinus augmentation technique in 1980, endorsed by Tatum [17] for the direct approach. This approach is often chosen when substantial bone resorption impedes the placement of short implants.

**Tools available for Indirect sinus lift.
Summers Osteotome Technique****A set of Summers Osteotome**

Summers [18] introduced the crestal technique, also known as the transalveolar approach, for the first time in 1996. This method is typically chosen when there is a moderate level of bone resorption, and it becomes feasible to place dental implants using Summer osteotomes. Studies have revealed highly favorable survival rates for this procedure [18,19]. In 1994, Summers pioneered the sinus lift technique, employing osteotomes to elevate the sinus membrane. He later refined the procedure by eliminating the need for hammering, which increased its patient-friendliness. This enhanced method involved the application of graft material around the implant [20]. Another term used to describe this technique was the bone-added osteotome sinus floor elevation procedure [21]. This approach was considered less traumatic and invasive for patients. However, certain limitations existed, such as the requirement for a remaining bone height of over 5mm to prevent sinus membrane perforation and the potential for decreased primary stability of the implant [21]. Nonetheless, when the remaining bone height ranged from 5mm to 6mm [20,22] this technique stood out as a highly viable surgical alternative.

ii)Hydraulic Sinus Lift Technique with CAS KIT.

The Crestal Approach Sinus KIT (CAS-KIT) is specifically designed to easily and safely lift the sinus membrane in edentulous posterior maxilla. The unique component of the CAS kit is the CAS-Drill. The CAS-Drill forms a conical bone barrier, protecting the membrane, the atraumatic design of the drill tip allows the clinician to perform sinus lift. The CAS-Drill tip has an inverse conical shape this shape will form a conical bone chip while drilling, which assists with safely lifting the membrane. In addition, bone particles generated when drilling discharge upwards, producing a Membrane Auto-Lift Function. Now the sinus membrane or Schneiderian membrane is lifted indirectly by CAS-drill. The hydraulic dissociation of the sinus mucosa by injecting a normal saline via continuous expulsion or aspiration that creates a pocket between sinus membrane and crestal bone and concurrent filling of the sub Schneiderian space with solid or semisolid bone substitute would be done [23].

Osseodensification with Densah® burs for indirect sinus lift.

Osseodensification is an innovative bone drilling technique pioneered by Dr. Salah Huwais for placing implants in toothless alveolar ridges. This approach involves compacting the osteotomy walls within the bone bed. To perform osseodensification, specialized drills known as Densah® burs are employed. These drills, developed by Versah LLC, were created by Dr. Salah Huwais, a periodontist, in 2014 [24]. The process of osseodensification entails gently



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deforming the cancellous bone using a Densah® bur. The bur is designed with specific lands and flutes to densify the bone as it drills into the bone bed. When used in densification mode (which doesn't involve cutting), the bur is rotated anticlockwise at speeds ranging from 800 to 1500rpm. This rotation generates a powerful compression wave within the osteotomy, leading to a plastic expansion of the cortical plates of the alveolar ridges. This technique capitalizes on the inherent flexibility of bone, which can absorb energy and change shape when subjected to compression or loading. Consequently, bone can undergo gradual plastic deformation over time. Densification is a method of implant site preparation that doesn't excavate the bone but instead creates a densified layer of bone around the implant site [25]. This is achieved by both plastically expanding and compacting the bony ridge, effectively autografting it. This autografting process takes place along the entire length of the osteotomy, utilizing a hydrodynamic mechanism that involves substantial irrigation to elevate the Schneiderian membrane of the maxillary sinus in a crestal direction.

Intralift Technique by Piezosurgery unit.**Piezosurgery unit**

Torrella et al. introduced piezoelectric technology for lateral osteotomy procedures [26]. By harnessing piezoelectric surgery, the sinus lift technique has been streamlined, aiming for minimal invasiveness. Troedhan and his team devised the Intralif approach, utilizing piezoelectric surgery and a specialized ultrasound tip set for sinus floor elevation. This technology's high-powered ultrasonic tools enable precise osteotomies, even in denser cortical bone, without affecting soft tissues. One notable benefit is the preservation of soft tissue integrity, allowing sinus membrane elevation without perforation. The piezoelectric surgical kits encompass a variety of inserts, including osteotomy and diamond-cutting tools. Following the creation of the bone window, the sinus membrane is gently detached from the bone, and controlled hydrodynamic pressure using saline solution induces piezoelectric cavitation [27]. In Italy, Vercellotti [28] and co-researchers employed the Piezo Surgery System to perform [21] bony window osteotomies on 15 patients. The inserts vibrated at 60-210 mm and surpassed 5W in power. Autogenous bone grafts and platelet-rich plasma were used for all sinus augmentations in the study. Out of the [21] osteotomies, only one resulted in membrane perforation, achieving a remarkable success rate of 95% [28]. Thus piezosurgery unit can be used for direct window preparation or indirect sinus lifting procedures.

DISCUSSION

Sinus lifting is a fundamental technique for managing deficient areas at the back of the mouth lacking teeth. In 1980, Boyne and James conducted bone augmentation exceeding 10mm using a lateral approach in the deficient upper jaw, resulting in considerable post-surgery complications and a heightened risk of membrane perforation. Consequently, an alternative procedure, the indirect sinus lift, has been developed utilizing various bone grafting materials such as autogenous bone, heterologous bone materials, allografts, and platelet derivatives [29]. The advantages of the indirect method are numerous, including its conservative nature, the potential for implantation immediately after the lift, and a reliably positive bone healing process. Additionally, the survival rates of implants over a five-year period are impressive, reaching 92.7% for ridge heights below 5mm and 94.9% for ridge heights exceeding 5mm after employing the indirect sinus lift technique. The vertical distance between the alveolar bone's crest and the sinus floor significantly influences the survival rate of implants placed in augmented sinus sites. This factor is also pivotal for achieving primary implant stability. Literature supports a direct correlation between available bone quantity and implant survival rates. Rosen et al. [30] concluded that survival rates are closely tied to the remaining bone height, starting at 96% when there is 5mm or more bone and dropping to 85% when there is 4mm or less. The grafted material experiences a reduction in volume during the first three months of bone remodeling, but this decrease stabilizes throughout the entire follow-up period of 60 months. In terms of grafting materials, several authors report consistent bone formation (averaging 6.51mm ± 2.49mm) even without grafting materials, following a minimum one-year observation. Conversely, some authors argue for their necessity, as the use of only a blood clot or platelet concentrates may yield unpredictable outcomes. While autologous bone grafts were once considered the benchmark, they are prone to substantial resorption and contamination within the sinus due to oral pathogens. Ultimately, the





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reliable predictability of indirect sinus lift procedures depends on various factors, including remaining bone height, primary stability, careful elevation of the Schneiderian membrane without tearing, minimal membrane detachment force, elasticity, deformability, elevation extent, and the number of implant sites.

CONCLUSION

In an atrophic maxilla the bone height can be gained by different sinus augmentation techniques in implant dentistry. When compared to the lateral approach technique, the indirect sinus lifting techniques show higher safety and excellent success rates.

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Fig.3. Densah®bur



Fig.4. Piezosurgery unit





On $\alpha w g$ Closed Set in Topological Spaces

Ramachandran.S*

Associate Professor of Mathematics, GTN Arts College, Dindigul-624005, Tamil Nadu, India

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*Address for Correspondence

Ramachandran. S

Associate Professor of Mathematics,
GTN Arts College, Dindigul-624005,
Tamil Nadu, India
E. Mail: srrdass@gmail.com



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ABSTRACT

The aim of this paper is to introduce a new class of sets called $\alpha w g$ - closed sets in topological spaces and to study their properties. Further, we define and study $\alpha w g$ - open sets $\alpha w g$ - continuity.

Keywords: $\alpha w g$ - closed sets, $\alpha w g$ - open sets, $\alpha w g$ continuous.

INTRODUCTION

In 1970, Levine [9] introduced the concept of generalized closed set in the topological spaces and a class of topological spaces called $T_{1/2}$ spaces. Extensive research on generalizing closedness was done in recent years by many mathematicians. In 1990, S.P. Arya and T.M. Nour [2] define generalized semi-open sets, generalized semi-closed sets. In 1993 Maki H, Devi R and Balachandran K [21] introduced generalized alpha closed ($g\alpha$ -closed) sets. In 2000, A. Pushpalatha[16] introduced a new class of closed sets called weakly closed(w - closed) sets .In 2007, S.S.Benchalli and R. S. Wali[3] introduced the class of set called regular w -closed(rw -closed) sets in topological spaces. Recently Viswanathan,A., and Ramasamy,K., (2009),introduced the concept of generalized closed sets and weakly closed sets in topological spaces. In this paper, we introduce a new class of sets called alpha weakly generalized - closed sets (briefly $\alpha w g$ -closed sets) and we study their basic properties. We recall the following definitions, which will be used often throughout this paper.

Preliminaries

Throughout this paper, X, Y, Z denote the topological spaces $(X, \tau), (Y, \sigma)$ and (Z, η) respectively, on which no separation axioms are assumed.

Definition 2.1: A subset A of a space X is called

(1) a pre-open set if $A \subseteq \text{int}(\text{cl}(A))$ and a pre-closed set if $\text{cl}(\text{int}(A)) \subseteq A$.





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(2) a semi-open set if $A \subseteq \text{cl}(\text{int}(A))$ and a semi-closed set if $\text{int}(\text{cl}(A)) \subseteq A$.

(3) an α -open set if $A \subseteq \text{int}(\text{cl}(\text{int}(A)))$ and a α -closed set if $\text{cl}(\text{int}(\text{cl}(A))) \subseteq A$.

(4) a semi-preopen set ($=\beta$ -open) if $A \subseteq \text{cl}(\text{int}(\text{cl}(A)))$ and a semi-pre closed set (β -closed) if $\text{int}(\text{cl}(\text{int}(A))) \subseteq A$.

The semi-closure (resp. α -closure) of a subset A of (X, τ) is denoted by $\text{scl}(A)$ (resp. $\alpha\text{cl}(A)$ and $\text{spcl}(A)$) and is the intersection of all semi-closed (resp. α -closed and semi-pre closed) sets containing A .

Definition 2.2: A subset A of X is called

1. a generalized closed (briefly g -closed) [9] set iff $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in X .
2. Strongly generalized closed (briefly g^* closed)[20] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is g open in X
3. a regular open [18] set if $A = \text{int}(\text{cl}(A))$ and regular closed[18] set if $A = \text{cl}(\text{int}(A))$.
4. a semi generalized closed (briefly sg – closed)[4] if $\text{scl}(A) \subseteq U$ whenever $A \subseteq U$ and U is Semiopen in X .
5. a generalized semi closed (briefly gs – closed)[2] if $\text{scl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in X .
6. a generalized semi-pre closed (briefly gsp – closed)[5] if $\text{spcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in X .
7. a regular generalized closed (briefly rg – closed)[15] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is regular open in X .
8. a generalized preclosed (briefly gp – closed) [10] if $\text{pcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in X .
9. a generalized pre regular closed (briefly gpr – closed)[7] if $\text{pcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is regular open in X .
10. a weakly closed (briefly w – closed)[16] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is semiopen in X .
11. a regular weakly closed (briefly rw – closed)[3] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is regular semiopen in X .
12. a weakly generalized semi closed (briefly wg – closed) [13] if $\text{cl}(\text{int}(A)) \subseteq U$ whenever $A \subseteq U$ and U is open in X .
13. a regular weakly generalized semi closed (briefly rwg – closed)[13] if $\text{cl}(\text{int}(A)) \subseteq U$ whenever $A \subseteq U$ and U is regular open in X .
14. a regular generalized weakly semi closed (briefly rgw – closed)[17] if $\text{cl}(\text{int}(A)) \subseteq U$ whenever $A \subseteq U$ and U is regular semi-open in X .
15. a regular[^] generalized closed (r^g closed)[22] if $\text{gcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is regular open.
16. g^{\wedge} - closed set [23] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is semi-open in (X, τ) .
17. ag^{\wedge} - closed set [24] if $\alpha\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is g^{\wedge} -open in (X, τ) .
18. ag^* - closed set [25] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is α -open in (X, τ) .
19. sag^* - closed set [26] if $\alpha\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is g^* -open in (X, τ) .
20. $wg\alpha$ -closed set [27] if $\alpha\text{cl}(\text{int}(A)) \subseteq U$ whenever $A \subseteq U$ and U is α -open in (X, τ) .
21. wag -closed set [27] if $\alpha\text{cl}(\text{int}(A)) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ) .
22. ψ -closed set [28] if $\text{scl}(A) \subseteq U$ whenever $A \subseteq U$ and U is sg -open in (X, τ) .
23. ψg -closed set [29] if $\psi\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ) .
24. $g^*\psi$ -closed set [30] if $\psi\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is g -open in (X, τ) .
25. ψg^{\wedge} - closed set [31] if $\psi\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is g^{\wedge} -open in (X, τ) .
26. $\alpha\psi$ - closed set [32] if $\psi\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is α -open in (X, τ) .
27. $g\alpha^*$ - closed set [25] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is α -open in (X, τ) .
28. ag - closed set [33] if $\alpha\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is open in (X, τ) .
29. $g\alpha$ - closed set [21] if $\text{cl}(A) \subseteq U$ whenever $A \subseteq U$ and U is α -open in (X, τ) .
30. r^g -closed set [35] if $\text{gcl}(A) \subseteq U$ whenever $A \subseteq U$ and U is regular open in (X, τ) .





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The complements of the above mentioned closed sets are their respective open sets.

31. (gsp)*-closed set [36] if $cl(A) \subseteq U$ whenever $A \subseteq U$ and U is gsp open.
32. α^g closed set [37] if $gcl(A) \subset U$, whenever $A \subset U$ and U is α -open in X .
33. $g^\#$ -closed set [38] if $cl(A) \subset U$, whenever $A \subset U$ and U is αg -open in X .
34. gp^* -closed set [39] if $cl(A) \subseteq U$ whenever $A \subseteq U$ and U is gp-open in (X, τ) .
35. r^*g^* closed set [40] if $rcl(A) \subseteq U$ whenever $A \subseteq U$ and U is g-open in (X, τ) .
36. Mildly generalized closed (briefly mildly g closed) [29] if $cl(int(A)) \subseteq U$ whenever $A \subseteq U$ and U is g-open in X .

Definition 2.3: A map $f: X \rightarrow Y$ is said to be

1. a continuous function [1] if $f^{-1}(V)$ is closed in X for every closed set V in Y .
2. a pre continuous [11] if $f^{-1}(V)$ is pre closed in X for every closed set V in Y .
3. a α -continuous function [34] if $f^{-1}(V)$ is α -closed in X for every closed set V in Y .
5. a gs -continuous [1] if $f^{-1}(V)$ is gs closed in X for every closed set V in Y .
6. a αg -continuous [7] if $f^{-1}(V)$ is αg -closed in X for every closed set V in Y .
7. a rwg-continuous [13] if $f^{-1}(V)$ is rwg- closed in X for every closed set V in Y .
8. a rgw-continuous [13] if $f^{-1}(V)$ is rgw- closed in X for every closed set V in Y .
9. a swg -continuous [13] if $f^{-1}(V)$ is swg- closed in X for every closed set V in Y .

Alpha Weakly Generalized Closed Sets (α wg- closed sets)

Definition 3.1: A subset A of (X, τ) is called a **alpha** Weakly generalized closed (**briefly α wg closed**) if $cl(int(A)) \subset U$, whenever $A \subset U$ and U is α -open in X .

We denote the family of all α wg closed sets in space X by α w GC(X).

Theorem 3.2: Every closed set of a topological space (X, τ) is α wg closed set.

Proof: Let $A \subset X$ be a closed set and $A \subset U$ where U be α -open. Since A is closed, $cl(int(A)) \subset cl(A) = A \subset U$. Hence A is an α wg closed set.

Remark 3.3: The converse of the above theorem need not be true as seen in the following example.

Example 3.4: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$. Let $A = \{b, c\}$ then A is an α wg closed set but it is not a closed set.

Theorem 3.5: Every g^\wedge -closed set is α wg closed.

Proof: Let A be a g^\wedge -closed set. Let $A \subset U$ where U is α -open. Since every α -open set is semi open and A is g^\wedge closed, $cl(A) \subset U$. Therefore $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.6: The converse of the above theorem need not be true as seen in the following example.

Example 3.7: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a g^\wedge -closed set.

Theorem 3.8: Every (gsp)*-closed set α wg is closed.

Proof: Let A be (gsp)*-closed. Let $A \subset U$ and U be α -open. Since every α -open set is gsp open set and A is (gsp)*-closed set, $cl(int(A)) \subset cl(A) \subset U$, Hence A is α wg closed.





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Remark 3.9: The converse of the above theorem need not be true as seen in the following example.

Example 3.10: Let $X = \{a, b, c, d\}$, $\tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$. Let $A = \{c\}$ then A is α wg-closed set but it is not a $(gsp)^*$ -closed set.

Theorem 3.11: Every $g^\#$ -closed set is α wg closed.

Proof: Let A be $g^\#$ -closed in (X, τ) . Let $A \subset U$ where U is α open. Since every α open set is α g-open set and A is $g^\#$ -closed, $cl(A) \subset U$. Then $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.12: The converse of the above theorem need not be true as seen in the following example.

Example 3.13: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a $g^\#$ -closed set.

Theorem 3.14: Every αg^* -closed set is α wg closed.

Let A be αg^* -closed in (X, τ) . Let $A \subset U$ where U is α open. Since A is αg^* -closed, $cl(A) \subset U$, then $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.15: The converse of the above theorem need not be true as seen in the following example.

Example 3.16: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a αg^* -closed set.

Theorem 3.17: Every gp^* -closed set is α wg closed.

Let A be gp^* -closed in (X, τ) . Let $A \subset U$ where U is α open. Since A is gp^* -closed, $cl(A) \subset U$, then $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.18: The converse of the above theorem need not be true as seen in the following example.

Example 3.19: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a gp^* -closed set.

Theorem 3.20: Every w -closed set is α wg closed.

Let A be a w -closed set. Let $A \subset U$ where U is α -open. Since every α -open set is semi open and A is w -closed, $cl(A) \subset U$. Therefore $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.21: The converse of the above theorem need not be true as seen in the following example.

Example 3.22: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a αg^* -closed set.

Theorem 3.23: Every αg^* -closed set is α wg closed.

Let A be αg^* -closed in (X, τ) . Let $A \subset U$ where U is α open. Since A is αg^* -closed, $cl(A) \subset U$, then $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.





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Remark 3.24: The converse of the above theorem need not be true as seen in the following example.

Example 3.25: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a αg^* -closed set.

Theorem 3.26: Every gp^* - closed set is α wg closed.

Let A be gp^* - closed in (X, τ) . Let $A \subset U$ where U is α open. Since every α -open set is gp -open set and A is gp^* - closed, $cl(A) \subset U$, then $cl(int(A)) \subset cl(A) \subset U$. Hence A is α wg closed.

Remark 3.27: The converse of the above theorem need not be true as seen in the following example.

Example 3.28: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$. Let $A = \{b\}$ then A is α wg closed set but it is not a gp^* - closed set.

Example 3.29: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$. Then

1. rwg closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{a, b\}\{a, c\}, \{b, c\}\}$
2. rgw closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{a, b\}\{a, c\}, \{b, c\}\}$
3. αg^* closed $= \{X, \phi, \{b\}\{c\}, \{a, c\}, \{b, c\}\}$
4. wag closed $= \{X, \phi, \{b\}\{c\}, \{a, c\}, \{b, c\}\}$
5. ψg^* closed $= \{X, \phi, \{b\}\{c\}, \{a, c\}, \{b, c\}\}$
6. r^*g closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{a, b\}\{a, c\}, \{b, c\}\}$
7. α^*g closed $= \{X, \phi, \{c\}, \{a, c\}, \{b, c\}\}$
8. r^*g^* closed $= \{X, \phi, \{c\}, \{a, c\}, \{b, c\}\}$
9. αwg closed $= \{X, \phi, \{b\}, \{c\}, \{b, c\}\}$

Example 3.30: Let $X = \{a, b, c, d\}$, $\tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$. Then

1. ψ closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
2. rg closed $= \{X, \phi, \{c\}, \{d\}, \{a, b\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, b, c\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
3. αg closed $= \{X, \phi, \{c\}, \{d\}, \{a, d\}, \{b, d\}, \{c, d\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
4. gpr closed $= \{X, \phi, \{c\}, \{d\}, \{a, b\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, b, c\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
5. $sa g^*$ closed $= \{X, \phi, \{c\}, \{d\}, \{a, d\}, \{b, d\}, \{c, d\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
6. gp closed $= \{X, \phi, \{c\}, \{d\}, \{a, d\}, \{b, d\}, \{c, d\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
7. sg closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
8. gsc closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
9. pgb closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
10. g^*b closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
11. gb^* closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
12. sgb^* closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
13. pg^*b closed $= \{X, \phi, \{a\}, \{b\}\{c\}, \{d\}, \{a, c\}\{a, d\}, \{b, c\}, \{b, d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$
14. α^*g closed $= \{X, \phi, \{d\}, \{a, d\}, \{b, d\}\{c, d\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
15. g^* closed $= \{X, \phi, \{d\}, \{a, d\}, \{b, d\}\{c, d\}, \{a, b, d\}, \{a, c, d\}, \{b, c, d\}\}$
16. αwg closed $= \{X, \phi, \{c\}, \{d\}, \{c, d\}, \{a, c, d\}, \{b, c, d\}\}$

Theorem 3.31

1. Every αwg closed set is rwg closed.





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2. Every α wg closed set is α rgw closed.
3. Every α wg closed set is α - α g $^{\wedge}$ closed.
4. Every α wg closed set is α -wag closed
5. Every α wg closed set is α - ψ g $^{\wedge}$ closed
6. Every α wg closed set is α -r $^{\wedge}$ g closed
7. Every α wg closed set is α - ψ closed
8. Every α wg closed set is α -rg closed
9. Every α wg closed set is α -ag closed
10. Every α wg closed set is α -gpr closed
11. Every α wg closed set is α -sag * closed
12. Every α wg closed set is α -gp closed
13. Every α wg closed set is α -sg closed
14. Every α wg closed set is α -gs closed
15. Every α wg closed set is α -pgb closed
16. Every α wg closed set is α -g * b closed
17. Every α wg closed set is α -gb * closed
18. Every α wg closed set is α -sgb * closed
19. Every α wg closed set is α -pgb * closed

Proof: Straight forward.

Remark 3.32: The converse of the above theorem need not be true as seen in the following examples.

In Example 3.29, $B = \{a,b\}$, then A is α rgw closed but not α wg closed

In Example 3.29, $B = \{a,b\}$, then B is α rgw closed but not α wg closed set.

In Example 3.29, $B = \{a,c\}$, then B is α g $^{\wedge}$ closed but not α wg closed.

In Example 3.29, $B = \{a,c\}$, then B is α wag closed but not α wg closed.

In Example 3.29, $B = \{a,c\}$, then B is α - ψ g $^{\wedge}$ closed but not α wg closed.

In Example 3.29, $B = \{a,b\}$, then B is α -r $^{\wedge}$ g closed but not α wg closed.

In Example 3.30, $B = \{a,b,c\}$, then B is α - ψ closed but not α wg closed.

In Example 3.30, $B = \{a,b,c\}$ then B is α -rg closed but not α wg closed.

In Example 3.30, $B = \{a,b,d\}$, then B is α -ag closed but not α wg closed.

In Example 3.30, $B = \{a,b,d\}$, then B is α -gpr closed but not α wg closed.

In Example 3.30, $B = \{a,b,d\}$, then B is α -sag * closed but not α wg closed

In Example 3.30, $B = \{a,b,d\}$, then B is α -gp closed but not α wg closed

In Example 3.30, $B = \{b,d\}$, then B is α -sg closed but not α wg closed

In Example 3.30, $B = \{b,d\}$, then B is α -gs closed but not α wg closed

In Example 3.30, $B = \{a,d\}$, then B is α -pgb closed but not α wg closed

In Example 3.30, $B = \{a,d\}$, then B is α -g * b closed but not α wg closed

In Example 3.30, $B = \{a,d\}$, then B is α -gb * closed but not α wg closed

In Example 3.30, $B = \{b,d\}$, then B is α -sgb * closed but not α wg closed

In Example 3.30, $B = \{a,d\}$, then B is α -pgb * closed but not α wg closed

Remark 3.31: α wg closed sets and α - α g $^{\wedge}$ closed sets are independent to each other as seen from the following examples.

Example 3.32:

* Let $X = \{a, b, c, d\}$, $\tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$. Let $A = \{a, b, d\}$, A is α - α g $^{\wedge}$ closed but not α wg closed and





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the subset $B=\{c\}$ in X is αwg closed but not α^g closed.

Remark 3.33: αwg closed sets and g^* closed sets are independent to each other as seen from the following examples.

Example 3.34:

* Let $X = \{a, b, c, d\}, \tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$ Let $A = \{c\}$, then A is αwg closed but not g^* closed.

* Let $X = \{a, b, c, d\}, \tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$. Let $A = \{d\}$, then A is g^* closed but not an αwg closed set.

Remark 3.35: αwg closed sets and r^*g^* sets are independent to each other as seen from the following example.

Example 3.36:

Let $X = \{a, b, c\}, \tau = \{X, \phi, \{a\}, \{a, b\}\}$. The subset $\{a, c\}$ is r^*g^* closed but not αwg closed and the subset $\{b\}$ is αwg closed but not r^*g^* closed.

Remark 3.37: αwg closed sets and r^*g^* closed sets are independent to each other as seen from the following examples.

Theorem 3.38: Let A be an αwg closed set in a topological space X . Then $gcl(A) - A$ contains no non-empty α -closed set in X .

Proof: Let F be a α -closed set such that $F \subset cl(int(A)) - A$. Then $F \subset X - A$ implies $A \subset X - F$. Since A is α^g closed and $X - F$ is α -open, then $gcl(A) \subset X - F$. That is $F \subset X - cl(int(A))$. Hence $F \subset cl(int(A)) \cap (X - cl(int(A))) = \phi$. Thus $F = \phi$, whence $cl(int(A)) - A$ does not contain nonempty α -closed set.

Theorem 3.39: The finite union of two αwg closed sets are αwg closed.

Proof: Assume that A and B are αwg closed sets in X . Let $A \cup B \subset U$ where U is α -open. Then $A \subset U$ and $B \subset U$. Since A and B are α^g closed, $cl(int(A)) \subset U$ and $cl(int(B)) \subset U$.

Then $cl(int(A \cup B)) = cl(int(A) \cup cl(int(B))) \subset U$. Hence $A \cup B$ is αwg closed.

Theorem 3.40: If A is an αwg closed subset of X such that $A \subset B \subset cl(int(A))$, then B is an αwg closed set.

Proof: Let $B \subset U$ where U is α open. Then $A \subset B$ implies $A \subset U$. Since A is αwg closed, $gcl(A) \subset U$. By hypothesis $cl(int(B)) \subset cl(int((cl(int(A))) \subset U$. Hence B is αwg closed.

Remark 3.41: The converse of the above theorem need not be true as seen in the following example.

Example 3.42:

Let $X = \{a, b, c, d\}, \tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$. Let $A = \{d\}$ and $B = \{c, d\}$. Then A and B are αwg closed sets. But $A \subset B$ is not a subset of $cl(int(A))$.

Regular Weakly Generalized Open Set:

Definition 4.1: A set $A \subset X$ is called alpha weakly generalized open (αwg open) set if and only if its compliment is alpha weakly generalized closed. The collection of all αwg open sets is denoted by $\alpha wgO(X)$.

5. αwg Continuous and αwg Irresolute Functions:

Definition 5.1: A function $f : (X, \tau) \rightarrow (Y, \sigma)$ is called αwg continuous if every $f^{-1}(V)$ is αwg closed in X for every closed set V of Y .

Definition 5.2: A function $f : (X, \tau) \rightarrow (Y, \sigma)$ is called αwg irresolute if every $f^{-1}(V)$ is αwg closed in X for every αwg closed set V of Y .





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Example 5.3: Let $X = \{a, b, c\}$, $\tau = \{X, \phi, \{a\}, \{a, b\}\}$ and $Y = \{a, b, c\}$, $\sigma = \{Y, \phi, \{a\}\}$. Define $f : (X, \tau) \rightarrow (Y, \sigma)$ by $f(a)=a$, $f(b)=b$, $f(c)=c$. Here the inverse image of the closed sets in Y are α wg closed sets in X . Hence f is α wg continuous.

Example 5.4: Let $X = \{a, b, c, d\}$, $\tau = \{\phi, X, \{a, b\}, \{c\}, \{a, b, c\}\}$ and $Y = X$, $\sigma = \{Y, \phi, \{a\}, \{c\}, \{a, c\}, \{a, b\}, \{a, b, c\}\}$.

Define $f : (X, \tau) \rightarrow (Y, \sigma)$ by $f(a)=a$, $f(b)=b$, $f(c)=c$, $f(d)=d$. The inverse image of every α wg closed set in Y is α wg closed set in X . Hence f is α wg irresolute.

Remark 5.5: Every α wg irresolute function is α wg continuous but the converse is not true as seen in the following example.

Example 5.6: In example 5.3, f is α wg continuous but not α wg irresolute.

Remark 5.7: Every continuous function is α wg continuous. But the converse is not true as seen in the following example.

Example 5.8:

Let $X = \{a, b, c, d\}$. $\tau = \{X, \phi, \{a\}, \{b\}, \{a, b\}, \{a, b, c\}\}$ and $Y = X$,

$\sigma = \{Y, \phi, \{a\}, \{c\}, \{d\}, \{a, c\}, \{a, d\}, \{a, c, d\}\}$. Define $f : (X, \tau) \rightarrow (Y, \sigma)$ the identity mapping. Then f is α wg continuous but not continuous.

Theorem 5.9: Let $f : (X, \tau) \rightarrow (Y, \sigma)$ and $g : (Y, \sigma) \rightarrow (Z, \eta)$ be any two functions. Then

- (i) $(g \circ f)$ is α wg-continuous if g is continuous and f is α wg -continuous
- (ii) $(g \circ f)$ is α wg-irresolute, if g is α wg -irresolute and f is α wg -irresolute.
- (iii) $(g \circ f)$ is α wg continuous if g is α wg continuous and f is α wg -irresolute.

Proof:

(i) Let V be any closed set in (Z, η) . Then $f^{-1}(V)$ is closed in (Y, σ) , since g is continuous. By hypothesis $f^{-1}(g^{-1}(V))$ is α wg closed in (X, τ) . Hence $g \circ f$ is α wg continuous.

(ii) Let V be α wg closed set in (Z, η) . Since g is α wg irresolute, $g^{-1}(V)$ is α wg closed in (Y, σ) . As f is α wg irresolute, $f^{-1}(g^{-1}(V)) = (g \circ f)^{-1}(V)$ is α wg closed in (X, τ) . Hence $g \circ f$ is α wg irresolute.

(iii) Let V be closed in (Z, η) . Since g is α wg continuous, $g^{-1}(V)$ is α wg closed in (Y, σ) . As f is α wg irresolute, $f^{-1}(g^{-1}(V)) = (g \circ f)^{-1}(V)$ is α wg closed in (X, τ) . Hence $(g \circ f)$ is α wg continuous.

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Strengthening Local Democracy in India - a Focus on Robust Governance

P. Sakthivel^{1*} and Showkat Ahmad Dar²

¹Professor, Department of Political Science and Public Administration, Annamalai University, Annamalai University -608002, Tamil Nadu, India

²Department of Political Science and Public Administration, Annamalai University, Annamalai University-608002, Tamil Nadu, India

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*Address for Correspondence

P. Sakthivel

Professor,

Department of Political Science and Public Administration,

Annamalai University, Annamalai University -608002,

Tamil Nadu, India

E.Mail: sakthiraji@rediffmail.com



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ABSTRACT

Local democracy in rural India emphasizes robust Local Governance through decentralized structures like Panchayat Raj Institutions. It promotes citizen participation, transparency, and accountability, empowering individuals to contribute to their village's development through democratic means. The present study aims to analyze the impact of the 73rd and 74th Constitutional amendments to the Indian Constitution on strengthening Local Democracy in India. The study focuses on assessing the effectiveness of the amendments in promoting robust Local Governance at the grassroots level and its implications for democratic development in the country, particularly in the state of Tamil Nadu. Further, the study examines the extent to which the amendments have empowered local communities, especially the marginalized and enhanced citizen participation in local Governance. The study investigates the impact of the amendments on Citizens participation in the affairs of the PRIs, ensuring transparency, inculcating accountability and bringing the local communities in the ambit of decision-making processes at the local level. By identifying the challenges and limitations faced in implementing the provisions of the 73rd and 74th Constitutional Amendments, the study aims to suggest measures for further enhancement of the working of local governance. Through, the analysis of various dimensions of local democracy, the study seeks to provide valuable insights into the strengths and weaknesses of the amendments and propose research-based solutions and strategies for promoting citizen empowerment, inclusivity, and accountability in local Governance. In the end, the study aims to contribute to the existing knowledge on strengthening local democracy in India by offering evidence-based recommendations obtained from Tamil Nadu for policymakers, practitioners, and researchers.

Keywords: Local Democracy, Governance, Decentralization, Transparency, Accountability





INTRODUCTION

India has a three-tier system of local Government institutions, namely, Panchayats at the village level, Panchayat Samitis at the block level, and Zila Parishads at the district level. These institutions play a crucial role in (Maddick, H. 1970) ensuring decentralized Governance and bringing the marginalized communities in the ambit of decision-making process. The Panchayati Raj refers to the system of local self-Government in rural areas. It involves elected representatives at the village, block, and district (Mitra, S. K. 2001) levels who are responsible for local development, planning, and the implementation of various Government schemes. In the aftermath of 73rd and 74th constitutional amendments, the Local democracy in India has been flourishing primarily based on regular elections held at various levels, including the state of Jammu and Kashmir Elections are (Baba, N. A. 2012) conducted to elect representatives for Panchayats, Panchayat Samitis, Zila Parishads, and municipalities at an regular interval, for instance 5 years. These elections allow citizens to participate (Tokekar, P. 2019) in political decision-making processes and exercise their democratic rights. Local Government institutions in India have been assigned specific powers and functions to ensure effective local Governance. These powers include planning and implementing (Gordon, G. L. 2013) local development schemes, collection of local taxes, maintenance of essential services, promotion of education and healthcare at the local level, etc. To enable local democracy, the Indian Government has implemented the system of devolution of funds (Thavaraj, M. J. K. 1978) to local bodies. Financial resources are allocated to Panchayats and municipalities to carry out their functions effectively and promote local development.

Local democracy in India encourages citizen participation and engagement in local decision-making processes. It provides a platform for (Heller, P. G. (2009) citizens to voice their concerns, participate in public meetings, and hold elected representatives accountable for their actions. Local democracy focuses on community development by involving local communities in decision-making and development planning. It emphasizes the importance of grassroots-level initiatives, social inclusion, and empowering marginalized sections of society. Local Government institutions are responsible for implementing various centrally sponsored schemes and programmes at the grassroots level. These schemes cover a wide range of sectors such as agriculture, rural development, health, education, poverty alleviation, and more. Local democracy in India promotes (Corbridge, S. (2005) transparency, accountability, and good Governance at the local level. It encourages the adoption of information technology, e-Governance, and the use of social audits to ensure (Kumar, P., Kumar, D., & Kumar, N. 2014) efficient service delivery and prevent corruption. Major developments in local Governance have been witnessed in recent years. Decentralization and empowerment of local bodies have gained prominence, granting them greater autonomy and resources. This has led to increased citizen participation, transparency, and accountability (Gaventa, J., & Valderrama, C. 1999) in decision-making processes, especially at the local level. The digitization of Local Governance has improved service delivery and accessibility, benefiting rural communities. These developments have contributed to the efficient administration, inclusive development, and empowerment of local communities in India.

Local democracy forms the cornerstone of any vibrant and inclusive democratic system. In the case of India, the world's largest democracy, strengthening local (Ananth Pur, K., & Moore, M. 2007) democracy assumes even greater significance. Local Governance institutions are responsible for delivering essential services, representing the interests of communities, and ensuring citizen participation in decision-making processes. However, despite the democratic ideals enshrined in the Constitution, local democracy in India often faces numerous challenges that hinder its effectiveness and hinder (Kumar, S. (2014) the realization of robust Governance. Issues such as penetration of caste and religion at the grassroots affairs and thereby it has prevented the marginalized communities, especially the Sc, ST, minorities, Women etc, from political participation at the local level. The problem at hand is the need to strengthen local democracy in India, with a specific focus on robust Governance. Weak Governance systems, lack of citizen engagement, limited transparency, and inadequate institutional capacity present significant obstacles to the realization of participatory and accountable (Ahmad, R. 2008) Governance at the grassroots level. These challenges contribute to the marginalization of communities, inefficiencies in service delivery, and a growing disconnection



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between citizens and local authorities. Thus, it becomes imperative to explore ways to enhance local democracy, empower citizens, and promote inclusivity and effective decision-making processes.

Objectives

1. To assess the extent to which the 73rd and 74th Constitutional Amendments has empowered the local communities and enhanced citizen participation in local Governance.
2. To evaluate the current state of local democracy in India, especially in the state of Tamil Nadu, and identify the challenges and limitations faced in implementing the provisions of the 73rd and 74th amendments and suggest measures for improvement.
3. To explore successful case studies and best practices that have resulted in robust Local Governance and unearth how it has enhanced the quality of working of local Democracy.
4. To analyze the scope for improvement in local Governance systems to strengthen democratic practices.

METHODOLOGY

The methodology employed in the study is a combination of descriptive and analytical approaches, as well as qualitative study methods. Secondary sources such as books, scholarly articles, and state and Union Government reports are extensively utilized to gather relevant information and insights. These sources provide a comprehensive understanding of the historical context, policy frameworks, and best practices in local Governance. The descriptive and analytical approach, combined with qualitative research methods and thematic analysis, enables researchers to explore the complexities and dynamics of local Governance in India. It allows for an in-depth examination of issues related to citizen participation, accountability, decentralization, and service delivery. Collection of a wide range of secondary sources certainly prompted the researchers to collect evidence-based insights, thereby enriching the study of local Governance in India.

RESULT AND DISCUSSION**Empowering Local Communities: 73rd and 74th Constitutional Amendments**

The 73rd and 74th Constitutional Amendments, passed in 1992, aimed to empower local communities and enhance citizen participation in local governance by devolving power to grassroots-level institutions, namely Panchayats (in rural areas) and Municipalities (in urban areas). These amendments sought to decentralize (Tokekar, P. 2019) authority and decision-making, providing citizens with opportunities to actively engage in the development and management of their localities.

Provisions of the 73rd and 74th Constitutional Amendments: The 73rd Amendment applies to rural areas and introduced three-tier Panchayati Raj institutions – Gram Panchayats, Panchayat Samitis, and Zila Parishads. Similarly, the 74th Amendment applies to urban areas and established municipalities (Gaiha, R., et al 1998) as local self-governing bodies.

Extent of Empowerment and Citizen Participation:**Decentralization of Power**

The 73rd and 74th Constitutional Amendments brought a transformative change in India's governance by decentralizing power to local bodies, fostering (Pathy, J. 1980) participatory decision-making. Empowering Gram Panchayats and Municipalities, these amendments allowed communities to address their unique issues effectively. At the rural level, Gram Panchayats gained control over crucial sectors like rural (Reddy, G. R. 1970) development and education, while Municipalities took charge of urban planning and public health. Citizen participation through regular elections and participatory mechanisms such as Gram Sabhas and Ward Committees further strengthened the democratic (Vaidya, C. 2009) process at the local level, ensuring elected officials remain accountable to the people they serve.



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Financial empowerment, reservations for marginalized sections, and participatory planning played key roles in this process. With funds devolved to local bodies, resources were distributed more equitably, allowing for locally relevant projects. The reservation of seats for marginalized groups and women promoted better representation (Kalirajan, K., & Otsuka, K. 2010) and inclusion in decision-making. Participatory planning through Gram Sabhas and Ward Committees provided citizens with active platforms to shape their communities' future, fostering a deeper sense of (Pur, K. A. 2006) ownership and collaboration. This inclusive approach of participatory governance has led to more responsive, efficient, and sustainable development initiatives, building trust and mutual (Nambiar, M. 2001) understanding between officials and the public.

Strengthening Grassroots Democracy

The 73rd and 74th Amendments aimed to promote democratic principles at the local level. Regular elections ensure that local representatives are accountable to the people. For instance, frequent elections in Panchayats have led to greater responsiveness from elected officials. With the introduction of frequent elections in Panchayats and Municipalities, a significant change has (Tandon, R. 1997) been observed in the responsiveness of elected officials. By facing the electorate at regular intervals, local representatives are incentivized to be more attuned to the needs and concerns of their constituents. This heightened accountability fosters a sense of duty among elected officials to address the local community's issues and (Palanithurai, G. 2007) implement policies that align with their interests. Moreover, these frequent elections also encourage a competitive democratic environment. With the potential for change in leadership after each election, it becomes imperative for local representatives to perform effectively and (Thirupathi, & Bhaskar, 2020) demonstrate tangible progress in their areas of responsibility. This competitive aspect fosters a spirit of healthy competition among different candidates, often leading to a diverse pool of representatives, each with unique perspectives and ideas. The concept of local accountability not only ensures that the citizens' voices are (Mookherjee, D. 2014) heard but also holds representatives answerable for their actions and decisions during their tenure. As a result, the focus shifts from a top-down approach to a bottom-up one, where the priorities of the local population take center stage in the decision-making process.

The 73rd and 74th Constitutional amendments have undoubtedly made significant strides in empowering local communities and enhancing citizen participation in local governance. The examples highlight the positive outcomes, such as increased access to resources, better representation for (Sharma, B. M. 2010) marginalized groups, and participatory planning. Citizens now have a greater say in shaping their localities and can actively engage in decision-making processes. However, challenges persist in certain regions. In some cases, states have been slow to devolve funds and authority to local bodies, limiting their true empowerment. Political interference and bureaucratic hurdles (Mitra, S. K. (2001) can hinder the effective functioning of these institutions. While the 73rd and 74th Amendments have significantly empowered local communities and boosted citizen participation in local governance, further efforts are needed to fully realize their potential. Continuous support from the government, public awareness campaigns, and capacity-building measures for elected representatives can further strengthen grassroots democracy and lead to more inclusive and sustainable development at the local level.

Local Democracy in Tamil Nadu

Local democracy in India is governed by the 73rd and 74th Constitutional Amendments, which introduced Panchayats and Municipalities as constitutionally mandated local self-government bodies for rural and urban areas, respectively. The Panchayati Raj system comprises three tiers: Gram Panchayat at the village level, Panchayat Samiti at the block level, and Zila Parishad at the district level. Similarly, Municipalities operate at the urban level, including municipal corporations in larger cities and municipalities and town panchayats (Jayal, N. G. 2006) in smaller urban areas. The success and effectiveness of local governance can vary across states, depending on factors like political will, administrative capacity, financial resources, and citizen engagement.

Local Democracy in Tamil Nadu

Tamil Nadu has a vibrant political landscape and a relatively robust system of local democracy. Historically, Tamil Nadu has been known for its active citizen participation in politics, and this spirit extends to the local (Narayana, D.



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2005) governance level as well. The state has a well-established Panchayati Raj system, with elected representatives at the village, block, and district levels. Gram Sabhas, the village-level assemblies, are an essential feature of the Panchayati Raj system in Tamil Nadu. They serve as forums for villagers to participate in decision-making, prioritize (Karpagavalli, V., & Mohanasoundari, R. 2017) development projects, and hold local officials accountable. Tamil Nadu's urban local bodies, including municipal corporations and municipalities, have also played a crucial role in the state's development. These bodies are responsible for providing essential services like water supply, waste management, public health, and urban planning.

One of the notable aspects of Tamil Nadu's local democracy is its strong focus on social welfare programmes and inclusive development. The state has implemented various schemes for poverty alleviation, women's empowerment, education, healthcare, and infrastructure development at the local level. However, like any other state, Tamil Nadu faces some challenges in its local democracy. These challenges include issues of administrative capacity, financial constraints, and political interference. Sometimes, local bodies might lack the resources and autonomy necessary to address the (Biswas, A. 2020) diverse needs of their communities effectively. Moreover, there have been instances of political patronage and corruption at the local level, which can undermine the principles of genuine decentralization and citizen participation. Tamil Nadu has made significant strides in establishing and strengthening local democracy through the Panchayati Raj and urban local bodies systems. Active citizen participation, the role of Gram Sabhas, and a focus on social welfare (Bhagat, R. B. 2005) have contributed to the state's progress in local governance. However, addressing challenges related to administrative capacity, financial resources, and political interference will be crucial to further improve the state of local democracy in Tamil Nadu.

Challenges & Improving the 73rd Amendment

The 73rd Constitutional Amendment Act of 1992 introduced transformative changes in India's local governance system, devolving power (Rao, P. 2018) to the grassroots level through the establishment of Panchayati Raj institutions. Tamil Nadu, being a significant state in India, also witnessed the implementation of these provisions. While the amendment aimed to empower local communities and (Behera, S. 2007) enhance citizen participation, several challenges and limitations have been faced in its implementation in Tamil Nadu. This comprehensive assessment explores the key challenges and limitations faced in effectively implementing the provisions of the 73rd Amendment in Tamil Nadu.

Uneven Devolution of Powers

The implementation of the 73rd Amendment faces a critical challenge due to the uneven devolution of powers from the state Government to local Panchayati Raj institutions. While certain functions have been delegated, several crucial matters, including administrative control and financial resources, remain under the purview of the state government. This disparity limits the autonomy of local bodies and hinders their (Singh, M. (2003) capacity to make independent decisions in areas crucial to local development. As a result, the true potential of decentralized governance is hindered, and the effective delivery of services to citizens may be compromised. Addressing this issue by ensuring a more balanced and comprehensive devolution of powers is essential to strengthen local democracy and promote participatory decision-making at the grassroots level in Tamil Nadu.

Financial Dependence

Financial dependence is a significant challenge faced by local bodies in Tamil Nadu due to their heavy reliance on financial assistance from the state government. While financial support is necessary for local governance, overreliance can result in fiscal constraints, limiting the autonomy and effectiveness of these bodies. With limited financial resources, local bodies may face difficulties in implementing independent development initiatives and addressing the unique needs of their communities. Moreover, such dependence can lead to delays in decision-making, as local bodies (Mitra, S. K. 2001) need to wait for state funds, hindering prompt and effective service delivery. To strengthen local democracy and empower local bodies, it is essential to explore avenues for enhancing their revenue generation and financial autonomy. By reducing financial dependence and ensuring more sustainable



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financial models, local bodies in Tamil Nadu can become more proactive in meeting the diverse needs of their communities and driving inclusive development.

Lack of Financial Autonomy

Despite the mandate for financial devolution under the 73rd Amendment, a lack of financial autonomy remains a challenge for local bodies in Tamil Nadu. While they receive funds from the state government, they often lack control over local revenue generation sources. This limitation can impede their ability to fund and prioritize (Boetti, L., Piacenza, M., & Turati, G. (2012) development projects based on local needs. Without sufficient financial autonomy, local bodies may face challenges in addressing immediate concerns and delivering services efficiently. To strengthen local governance, it is crucial to empower local bodies with greater control over revenue generation and budget allocation, enabling them to be more responsive to the specific requirements of their communities. By promoting financial autonomy, the 73rd Amendment's objectives of decentralization and empowering local communities can be more effectively realized in Tamil Nadu.

Bureaucratic Interference

Bureaucratic interference and political patronage present a significant challenge in the effective functioning of local bodies in Tamil Nadu. When bureaucrats or Government officials exert undue influence or control over the decisions and operations of local bodies, it can lead to compromised decision-making processes (Mukhopadhyay, A. 1999) and reduced responsiveness to the needs of the community. Such interference may hinder the autonomy of local bodies, impeding their ability to address local issues promptly and independently. Political patronage can also result in the allocation of resources and projects based on political considerations rather than the actual needs of the community, leading to misallocation of funds and services. To strengthen local democracy and ensure the effective functioning of local bodies, it is crucial to minimize bureaucratic interference (Islam, M. T., & Fujita, K. (2012) and political influence. Creating a transparent and accountable system that empowers local representatives and ensures citizen participation in decision-making can help mitigate these challenges and promote more responsive and accountable local governance in Tamil Nadu

Weak Revenue Generation

Weak revenue generation presents a significant challenge for many local bodies in Tamil Nadu. The limited tax base, low collection efficiency, and inadequate revenue-generating opportunities contribute (Mohapatra, B. P. (2013) to their financial constraints. In rural areas, the predominantly agrarian economy and a substantial informal sector result in many potential taxpayers remaining outside the tax net, reducing revenue potential. Tax evasion and low collection efficiency exacerbate the problem, leading to revenue leakages. The lack of revenue-generating assets and insufficient user fees and local economic activities further hamper local bodies' financial resources. Insufficient funds limit their ability to finance development projects and deliver essential services effectively. Addressing this challenge requires a multifaceted approach. Local bodies must explore innovative revenue sources, such as property taxes and user charges for services. Strengthening tax administration and collection mechanisms is essential to maximize revenue collection. Providing technical support and capacity-building in financial management can help optimize (Jha, S. (2002) resources and fund development projects aligned with the community's needs. By enhancing revenue generation, local bodies in Tamil Nadu can achieve greater financial independence and more effectively address the diverse needs of their communities.

Capacity Building Challenges

Capacity building challenges pose a significant obstacle to the efficient functioning of local bodies in Tamil Nadu. The lack of adequate training and development programmes for elected representatives and officials hinders their (Tandon, R., & Bandyopadhyay, K. K. (2004) ability to effectively carry out their roles and responsibilities. Elected representatives at the local level play a crucial role in decision-making and governance. However, many of them may lack the necessary skills and knowledge to address complex issues, formulate effective policies, and oversee development projects. This deficit can result in suboptimal decision-making, potentially hindering the development of the community. Similarly, officials working within local bodies may face capacity constraints, affecting their



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ability to implement policies and deliver services efficiently. Inadequate training in areas such as financial management, project implementation, and public administration can lead to inefficiencies and delays. Capacity building challenges requires comprehensive training programmes tailored to the specific needs of local representatives and officials. Such programmes can focus on governance, public administration, financial management, and other essential areas. Workshops and seminars can be (Venkatesu, E. (2016) organized to facilitate knowledge sharing and skill development. By investing in capacity building initiatives, Tamil Nadu's local bodies can empower their representatives and officials with the skills and expertise necessary to make informed decisions, implement effective policies, and address the diverse needs of their communities. Strengthening capacity at the local level will contribute to more efficient and responsive governance, fostering sustainable development and inclusive growth across the state.

Unequal Representation

Unequal representation poses a critical challenge in achieving inclusive decision-making in local governance in Tamil Nadu. Marginalized sections, including women, and SC/ST communities, may not have adequate (Jayal, G. N. (2005) representation in local bodies, which hampers their ability to voice their concerns and influence policy decisions that affect their lives. Historically, these marginalized groups have faced social and economic disadvantages, leading to their underrepresentation in local governance structures. The lack of diverse perspectives and experiences in decision-making can result in (Kalaramadam, S. (2018) policies and projects that do not adequately address the unique needs and challenges faced by these communities.

Measures must be taken to promote and ensure the adequate representation of marginalized groups in local bodies. Reserving seats for women and members of Scheduled Castes (SC) and Scheduled Tribes (ST) is one way to enhance their participation. Creating awareness about the importance of inclusive governance and empowering marginalized communities to actively engage in local politics is essential. Moreover, building the capacity of elected representatives from marginalized groups and providing them with opportunities for (Ananth Pur, K. 2007) leadership and skill development can empower them to effectively advocate for their communities interests. By addressing the issue of unequal representation and promoting diversity in local governance, Tamil Nadu can foster more inclusive decision-making processes and advance equitable and sustainable development for all its citizens.

Political Fractionalization

Political fractionalization presents a significant challenge to local governance in Tamil Nadu. The state's political landscape is characterized by (Munshi, K., & Rosenzweig, M. 2008) frequent changes in political leadership and a fragmented party system. As a result, local bodies may experience frequent shifts in administration and policy direction, disrupting the continuity and effectiveness of governance. With changing political leadership, priorities and development agendas may vary, leading to inconsistent policy implementation and delayed projects. Moreover, frequent transitions can hinder the long-term planning and execution (Wacziarg, R. 2003) of development initiatives, impacting the progress of local communities. The fragmented party system can further compound these challenges. Coalition governments at the state level can lead to varied ideologies and conflicting interests within local bodies, making it challenging to achieve consensus and smooth decision-making. Fostering political stability and promoting constructive dialogue between different parties is crucial. Emphasizing long-term development planning beyond electoral cycles can help ensure continuity and sustainability in local governance. By mitigating the effects of political fractionalization, Tamil Nadu can strengthen the effectiveness of local bodies and promote more coherent and consistent governance for the betterment of its communities.

Limited Women's Participation

Limited women's participation poses a significant challenge in promoting inclusive and gender-responsive local governance in Tamil Nadu. Despite the reservation of seats for women in local bodies, there are still barriers that hinder (Everett, J. (2009) their meaningful engagement in decision-making processes. Cultural norms and gender stereotypes can perpetuate the perception that women are less capable or knowledgeable in matters of governance, limiting their opportunities to actively participate in local politics. Women may face social and familial pressures that



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discourage them (Bauri, A., & Basu, A. (2022) from taking on leadership roles in local bodies. Limited access to education and resources may hinder women's capacity to engage effectively in decision-making processes and contribute to local development. It is essential to promote gender-sensitive policies and measures that go beyond just reserving seats for women. Encouraging women's political empowerment through targeted capacity-building programmes, mentorship initiatives, and leadership development can help equip them with the skills and confidence needed to participate actively in local governance. Creating a supportive environment that challenges gender biases and encourages women's full participation in decision-making can lead to more inclusive and representative local governance in Tamil Nadu. By breaking down barriers and fostering gender equality in local politics, the state can harness the potential of all its citizens, contributing to more equitable and effective governance at the grassroots level.

Lack of Awareness and Participation

Limited awareness and participation among citizens in Gram Sabhas and local body meetings present a significant challenge to the effectiveness of participatory democracy in Tamil Nadu. Despite the provision for citizen engagement in decision-making processes, many people may not be fully aware of their rights and (Singh, M. K. (2021) opportunities to participate actively. The lack of awareness can result from factors such as limited access to information, illiteracy, or a lack of civic education. As a consequence, citizens may not fully understand the significance of their involvement in local governance or the potential impact of their inputs on development initiatives. Low participation can hinder the democratic process at the local level. When citizens are not actively engaged in Gram Sabhas and local body meetings, the voices of the community may not be adequately heard, and important perspectives and suggestions may be missed.

Efforts must be made to enhance citizens' awareness of their rights and roles in local governance. Public awareness campaigns, community outreach programmes, and civic education initiatives can help empower citizens and encourage their active participation in decision-making processes. Ensuring greater accessibility to information through transparent communication channels and simplifying procedures for public engagement can facilitate citizens' involvement in local democracy. By promoting increased awareness and participation, Tamil Nadu can strengthen the foundations of participatory governance and ensure that local bodies are more responsive to the needs and aspirations of their communities.

Corruption and Mismanagement

Corruption and mismanagement pose significant challenges to effective local governance in Tamil Nadu. Instances of corruption, where public officials misuse their power for personal gain, and mismanagement, characterized by (Bardhan, P., & Mookherjee, D. (2006) inefficiencies and lack of accountability, can lead to the erosion of public trust in local bodies. When corruption is prevalent within local bodies, resources meant for development projects and public welfare may be diverted or misused, leading to the misallocation of funds and delayed or incomplete projects. This not only hampers the development of the community but also creates a sense of disillusionment among citizens. Mismanagement, on the other hand, can lead to the inefficient delivery of services, delayed decision-making and ineffective implementation of policies. This may result from a lack of transparency, inadequate monitoring mechanisms, or a failure to (Ardigo, I. A. (2019) adhere to best practices in governance.

To combat corruption and mismanagement, promoting transparency, accountability, and ethical behavior is essential. Implementing effective anti-corruption measures, such as stringent audits and whistleblower protection, can help deter corrupt practices. Strengthening monitoring and evaluation mechanisms can enhance the efficiency and accountability of local bodies. Building a culture of ethical leadership and promoting a merit-based approach in appointments can also contribute to reducing corruption and improving governance. By addressing these challenges, Tamil Nadu can foster a more transparent and accountable local governance system that enjoys the trust and support of its citizens.



**Sakthivel and Showkat Ahmad Dar****Overlapping Institutions**

Overlapping institutions present a notable challenge in the effective functioning of local governance in Tamil Nadu. Sometimes, different tiers of local governance, such as Gram Panchayats, Panchayat Samitis, and Zila Parishads, may have overlapping functions and responsibilities. This redundancy can lead to confusion and inefficiencies in decision-making and resource allocation. When there is an unclear division of responsibilities, it becomes challenging for local bodies to (Blair, H. 2000) perform their functions effectively. Duplication of efforts and a lack of coordination may result in wasted resources and delays in implementing development projects. Moreover, disputes may arise between different tiers of local governance, further hampering effective decision-making and delivery of services. It is essential to establish clear and distinct roles for each level of local governance. Devolution of powers should be complemented by proper coordination mechanisms and collaborative efforts to ensure seamless functioning. Enhancing communication and collaboration between different tiers can promote efficient resource allocation and better service delivery. Capacity-building programmes can empower elected representatives and officials to understand their roles and responsibilities better, enabling them to fulfill their functions more effectively. By streamlining functions and enhancing coordination among different levels of local governance, Tamil Nadu can optimize resources and improve the effectiveness of its decentralized governance system, benefiting its communities with more efficient and inclusive development.

Infrastructure Deficits

Infrastructure deficits pose a significant challenge to local bodies in Tamil Nadu. Inadequate infrastructure and limited resources can hinder the implementation of essential development projects and effective service delivery. Insufficient infrastructure, such as roads, bridges, schools, healthcare facilities, and sanitation systems, can impede the development of communities. Without proper infrastructure, local bodies may (Rao, M. G. (200) struggle to provide basic services and amenities to their residents, impacting their quality of life and hindering economic growth. Moreover, the lack of resources can limit local bodies' capacity to undertake ambitious development initiatives. A shortage of funds can result in delayed or incomplete projects, affecting the timely delivery of services and development programmes. Infrastructure deficits, it is crucial to prioritize infrastructure development in local planning and budget allocation. Seeking financial assistance from the state Government or exploring public-private partnerships can help mobilize resources for critical infrastructure projects. Strengthening administrative capacity and technical expertise within local bodies can ensure more effective project management and implementation. By addressing infrastructure deficits, Tamil Nadu's local bodies can improve the living standards of their communities, boost economic growth, and foster sustainable development across the state.

Political Control of Funds

Political control of funds presents a critical challenge in local governance in Tamil Nadu. Elected representatives often have discretionary powers over the allocation of funds, which can lead to non-priority allocations and resource diversion. When funds are subject to political influence, there is a risk that development projects and resources may be allocated based on political considerations rather (Mitra, S. K. (2001) than the actual needs and priorities of the community. This can lead to the misallocation of resources, hindering the implementation of essential development projects and undermining the progress of the region. It is crucial to establish transparent and accountable mechanisms for fund allocation. Implementing participatory budgeting processes, where citizens have a say in deciding how funds are allocated, can promote a more inclusive and citizen-centric approach to resource allocation. Promoting financial transparency and oversight can help ensure that funds are used efficiently and in accordance with established priorities. Strengthening mechanisms for monitoring and auditing fund utilization can deter misuse and enhance accountability. By reducing political control of funds and promoting more transparent and accountable financial practices, Tamil Nadu's local governance can better prioritize development projects and ensure resources are directed toward initiatives that truly benefit the community.

Low Revenue Collection

Low revenue collection poses a significant challenge to the financial independence of local bodies in Tamil Nadu. Insufficient revenue from local taxes and user fees can limit their capacity to fund development projects and deliver



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essential services effectively. Local bodies heavily depend on revenue generated through property taxes, sales taxes, and user fees for services such as water supply, waste management, and public facilities. When revenue collection is low, it can lead to budgetary constraints, hindering the (Aijaz, R. (2007) implementation of crucial development initiatives. Several factors contribute to low revenue collection, including tax evasion, non-compliance, weak tax administration, and resistance to user fee payments. The informal economy and limited tax base in certain areas can further impact revenue generation. Local bodies can focus on enhancing tax compliance through improved tax administration and enforcement measures. Public awareness campaigns can also be employed to highlight the importance of paying taxes and user fees for the sustainable development of their communities. Exploring innovative revenue-generating sources and optimizing existing revenue streams can bolster the financial independence of local bodies, enabling them to undertake more comprehensive and impactful development projects. By addressing the issue of low revenue collection, Tamil Nadu's local bodies can become more self-reliant and better positioned to address the diverse needs of their communities.

Resource Allocation Disparities

Resource allocation disparities pose a significant challenge to local governance in Tamil Nadu. When resource allocation from the state Government does not align with the actual needs and priorities of local communities, it can hinder the effective implementation of development projects and service delivery. Centralized resource allocation may not fully consider the unique challenges and requirements (Dash, B. B., & Raja 2018) of different regions within Tamil Nadu. As a consequence, some areas may receive disproportionately less funding, while others may be favored, leading to regional disparities and unequal development outcomes. There is a need to adopt a more decentralized and participatory approach to resource allocation. Engaging local communities in the budgeting process and conducting needs assessments can help identify the most pressing priorities and allocate resources accordingly. Furthermore, promoting fiscal decentralization and providing local bodies with greater financial autonomy can empower them to make decisions that best serve their communities' interests. By addressing resource allocation disparities and embracing a more decentralized approach, Tamil Nadu's local governance can ensure that resources are allocated in a more equitable and efficient manner, promoting balanced development and inclusive growth across the state.

Capacity Constraints in Rural Areas

Capacity constraints in rural areas present a significant challenge to effective local governance in Tamil Nadu. The limited availability of skilled personnel and professionals can impede planning and implementation capabilities, hindering the development of rural communities. In rural settings, there may be a scarcity of qualified individuals with expertise in fields such as public administration, engineering, finance, and project management. This can result in a lack of technical knowledge and skills required to design and execute development projects effectively. The absence of specialized professionals may lead to delays in project approvals and inadequate monitoring and evaluation of ongoing initiatives, impacting the quality and timely delivery of services to rural residents. To address capacity constraints, it is crucial to invest in capacity-building programmes and provide training opportunities to individuals working in rural local bodies. Strengthening technical and managerial skills can enhance their ability to plan, implement, and monitor development projects efficiently. Encouraging knowledge-sharing networks and collaborations between rural and urban areas can facilitate access to expertise and resources, bridging the gap in capacity. By addressing capacity constraints in rural areas, Tamil Nadu's local governance can empower rural communities with the skills and knowledge necessary to drive sustainable development, foster innovation, and improve the quality of life for its rural residents.

Political Interference in Local Elections

Political interference in local elections is a significant concern in Tamil Nadu's local governance. When political parties and influential figures exert undue influence over the electoral process, it undermines the democratic principles of free and fair elections. Interference can manifest in various ways, such as candidate nominations, campaign support, and the use of money and muscle power to influence voters. Such actions can distort the election outcome and result in the selection of representatives who (Rao, N., & Kumar, D. B. 2022) prioritize party interests



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over the needs of the community. This interference hampers the election of capable and committed individuals who genuinely seek to serve the public. Competent candidates may face unfair disadvantages, leading to a loss of trust in the electoral system. To protect the democratic process, it is crucial to strengthen election regulations and ensure their enforcement. Transparent funding mechanisms and impartial electoral commissions can promote fairness and accountability. Raising awareness among voters about the importance of independent decision-making and voting for capable representatives can empower citizens to make informed choices. By curbing political interference in local elections, Tamil Nadu can foster a more democratic and responsive local governance system, ensuring that elected representatives is truly committed to the welfare of their communities.

Ineffective Grievance Redressal Mechanisms:

The absence of robust grievance redressal mechanisms poses a significant challenge in Tamil Nadu's local governance. When citizens encounter issues or have concerns related to local services or projects, the lack of effective mechanisms to address grievances can lead to frustration and a sense of helplessness. Without reliable avenues to voice their problems, citizens may feel disconnected from the (Olowu, D., & Wunsch, J. S. (2004) decision-making process and may not be able to hold local bodies accountable for their actions. This lack of accountability can lead to a breakdown in trust between the public and local representatives. It is crucial to establish transparent and accessible grievance redressal mechanisms. Local bodies should create channels for citizens to submit their complaints, suggestions, and feedback. These mechanisms should be responsive, timely, and impartial in handling grievances. Promoting awareness among citizens about the existence and functioning of grievance redressal mechanisms can encourage greater public participation in local governance and foster a sense of ownership over local issues.

By strengthening grievance redressal mechanisms, Tamil Nadu's local governance can enhance citizen engagement, ensure accountability, and improve the responsiveness and effectiveness of local bodies in addressing the needs and concerns of their communities. The 73rd Constitutional Amendment sought to empower local communities and enhance citizen participation in Tamil Nadu's local governance, several challenges and limitations have been encountered in its implementation. The state must address these issues to strengthen local democracy and foster inclusive and sustainable development. Efforts to promote financial autonomy, capacity building, and citizen awareness, while curbing bureaucratic and political interference, will be crucial in realizing the true potential of decentralized governance in Tamil Nadu.

Successful Case Studies

India's local governance system plays a crucial role in promoting grassroots democracy and facilitating inclusive development. Over the years, various successful case studies and best practices have emerged, leading to robust local governance and enhanced local democracy. This study explores some of these exemplary initiatives and the strategies that contributed to their success.

Case Study 1: The e-Panchayat Project The e-Panchayat Project aimed to strengthen the functioning of Panchayati Raj Institutions (PRIs) in India through the use of Information and Communication Technology (ICT). It streamlined (Ali, M. P. 2020) administrative processes, improved service delivery, and enhanced transparency and accountability at the local level. Janbhagidari in Rajasthan The Government of Rajasthan initiated a process called "Janbhagidari" to encourage people's participation (Kumar, A. 2019) in local governance. It involved conducting Gram Sabhas (village assemblies) regularly, where villagers could voice their opinions and actively participate in local decision-making

Case Study 2: Kudumbashree is a poverty eradication and women empowerment program in Kerala. It focuses on the formation of neighborhood (Nidheesh, K. B. (2009). Study on the changing process of Kerala women through Kudumbashree in Kerala. Self-help groups at the grassroots level, enabling women to actively participate in local governance and decision-making processes.



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Case Study 3: *Karnataka's Gram Panchayat Development Plan (GPD)* involved preparing and implementing participatory plans at the gram panchayat (village) level. It empowered (Dhavaleshwar, C. U., & Ali, S. 2012) local communities to identify their development priorities and allocate resources accordingly.

Case Study 4: The *Pani Panchayat* initiative in Maharashtra empowers villagers to manage their water resources through local governance structures. This decentralized approach to water management has been effective in promoting community involvement and ownership of water-related decisions, leading to better water conservation and equitable distribution. Participatory budgeting in Pune allows citizens to have a say in how a portion of the city's budget is spent, enhancing local democracy and prioritizing projects that address residents' needs.

Case Study 5: *Bhagidari System* in Delhi involved citizens in governance through various forums and platforms, facilitating their active (Chakrabarty, B. 2012) participation in shaping local policies and developmental projects.

Case Study 6: *Participatory Planning* in Himachal Pradesh Himachal Pradesh has implemented a bottom-up approach to development planning, where Gram Sabhas and local committees actively participate in the formulation and execution of development plans.

Case Study 7: Some municipalities and panchayats in Tamil Nadu have implemented participatory budgeting, where citizens are actively involved in the decision-making process regarding budget allocation and public projects. This practice allows residents to prioritize projects based on local needs and ensures a (Narayana, D. 2005) transparent and accountable use of public funds. Tamil Nadu introduced participatory budgeting practices, allowing citizens to have a say in local budget allocations. This inclusive approach ensures that community needs and priorities are taken into account while planning and executing development projects. Tamil Nadu has made significant strides in implementing e-governance initiatives to improve service delivery and citizen engagement. For example, various municipalities and panchayats have adopted online platforms to handle citizen grievances, issue birth and death certificates, and manages other administrative tasks. These initiatives have increased accessibility and reduced bureaucratic hurdles. Tamil Nadu has taken steps to increase women's representation in local governance. The state has implemented a 50% reservation (Buch, N. 2009) for women in local bodies, which has resulted in increased female participation and leadership in decision-making processes. Tamil Nadu has been lauded for effectively implementing various social welfare schemes at the grassroots level. Initiatives like the Nutritious Meal Program for children and pregnant women, and the Amma Unavagam (Amma Canteens) offering subsidized food have positively impacted vulnerable communities.

The successful Indian national case studies and best practices that have resulted in robust local governance and enhanced local democracy demonstrate the potential for effective decentralization and community empowerment. Through the implementation of schemes like the Panchayati Raj system and the Right to Information Act, India has witnessed significant improvements in local governance. These initiatives have fostered greater citizen participation, transparency, and accountability in decision-making processes at the grassroots level. The success of local governance in India can be attributed to the engagement of local communities in developmental projects and the allocation of funds based on local needs and priorities. By encouraging the active involvement of citizens in the planning and execution of projects, the Government has been able to ensure better utilization of resources and foster a sense of ownership among the local populace.

The adoption of technology in local governance practices has played a pivotal role in streamlining administrative processes and promoting citizen-centric services. Initiatives like e-governance and mobile-based platforms have facilitated easier access to information and services, promoting efficiency and transparency. The Indian experience of promoting robust local governance and enhanced local democracy serves as a valuable model for other countries seeking to empower their communities and improve governance at the grassroots level. By prioritizing citizen engagement, transparency, and technological advancements, India has set a commendable example of effective decentralization and participatory democracy, leading to socio-economic progress and inclusive development.



**Sakthivel and Showkat Ahmad Dar****Scope for improvement in Local Governance**

Strengthening democratic practices at the local level is crucial for ensuring citizen participation, transparency, and accountability in governance. There are several areas where local governance systems can be improved to enhance democratic practices:

Citizen Participation and Engagement: Local governments should actively encourage citizen participation and engagement in decision-making processes. This can be achieved through mechanisms such as public consultations, town hall meetings, participatory budgeting, and digital platforms for feedback and suggestions. Empowering citizens to have a say in local policies and projects can foster a sense of ownership and accountability.

Transparency and Access to Information: Transparent governance builds trust between citizens and their local governments. Enhancing access to information through open data initiatives, online portals, and clear communication (Anand, P. B. 2014) channels can enable citizens to be better informed about Government actions and expenditures. This transparency allows citizens to hold their leaders accountable and make more informed choices during elections.

Decentralization of Power: Devolving power and resources to local authorities and communities is essential for effective local governance. Decentralization allows decision-making to be closer to the people it affects, making governance (Bellinger, N. 2022) more responsive and tailored to local needs and preferences.

Capacity Building: Strengthening the capacity of elected representatives and local Government officials are very essential for improving governance practices. Training programmes, workshops, and knowledge-sharing platforms can enhance their (Narayana, D. (2005) understanding of democratic principles, administrative skills, and policy implementation techniques.

Anti-Corruption Measures: Implementing robust anti-corruption measures is essential to safeguarding democratic practices. Creating independent oversight bodies, enforcing strict ethical codes, and promoting (Ravallion, M. 2012) transparency in procurement processes can help curb corruption and promote fair practices in local governance.

Inclusive Representation: Local governments should strive for inclusive representation to ensure that diverse voices and marginalized groups are adequately represented in decision-making processes. Promoting gender equality and inclusivity can lead to more comprehensive and representative policy outcomes.

E-Governance: Leveraging technology to improve e-governance can streamline administrative processes, increase efficiency, and make services more accessible to citizens. Digital platforms can facilitate (Monga, A. 2008) online services, e-voting, and real-time tracking of projects, enhancing transparency and public participation.

Strengthening Local Institutions: Building strong and independent local institutions is essential for sustainable democratic practices. This includes empowering local legislative bodies, strengthening the rule of law, and (Joseph, T. M. (Ed.). (2007) ensuring an independent judiciary to protect citizens' rights and resolve disputes.

Civic Education: Promoting civic education in schools and among communities can cultivate a culture of democratic values, civic responsibility, and active citizenship. Educating citizens about their rights and responsibilities as well as the workings of the local Government can foster a more informed and engaged electorate.

Evaluation and Feedback Mechanisms: Regular evaluation of local governance policies and projects is crucial to identify areas of improvement and (Gopi, M. 2016) make necessary adjustments. Feedback mechanisms that allow citizens to express their opinions and evaluate Government performance can help course-correct policies and actions when needed.



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By addressing these areas of improvement, local governance systems can become more inclusive, accountable, and responsive to the needs of citizens, thereby strengthening democratic practices at the grassroots level.

Implications of the Study

Democracy lies at the core of India's political fabric, with its rich diversity and vast population demanding an inclusive, accountable, and participatory governance system. Over the years, India has made significant strides in promoting democratic values at the national level. However, the effectiveness of democracy largely depends on its local implementation, where citizens' voices find resonance in the decisions that directly impact their daily lives. Strengthening local democracy has become imperative for India to address socio-economic disparities, promote inclusive development, and ensure efficient service delivery to its vast population spread across diverse regions. The study on "*Strengthening Local Democracy in India - A Focus on Robust Governance*" delves into the critical factors that contribute to improving governance at the grassroots level. It investigates the challenges faced by local institutions, explores potential avenues for citizen engagement, and emphasizes the need for empowering local governments to take on more responsibilities in decision-making and administration.

Major Findings

1. Improved local governance can enhance citizen participation and engagement in decision-making processes.
2. Strengthening local democracy may lead to greater accountability and transparency in administration.
3. Effective decentralization can promote efficient service delivery and resource allocation at the grassroots level which would certainly benefit the marginalized communities.
4. Empowering grassroots level institutions can help address regional issues and foster inclusive development.
5. Robust local governance might lead to better coordination between different levels of local governments and reduce bureaucratic undue interference also.
6. Enhanced local democracy may create a fertile ground for social and economic development initiatives.
7. A focus on robust governance can empower marginalized communities and ensure their representation in decision-making processes.
8. Strengthening local democracy may lead to a reduction in corruption and misuse of power at the local level.
9. Robust governance at the local level can attract investment and promote economic growth in the region.
10. Improved local governance may lead to better management of natural resources and environmental conservation.
11. Engaging citizens in decision-making processes can lead to greater social cohesion and harmony among diverse communities.
12. Strengthening local democracy can serve as a foundation for political stability and reduced conflicts.
13. Enhanced citizen awareness and involvement can lead to more informed voting and better electoral outcomes.
14. Local democracy can act as a training ground for nurturing future national leaders.
15. Emphasizing robust governance can inspire other regions and countries to adopt similar decentralized approaches.

CONCLUSION

The imperative of strengthening local democracy in India, with a focus on robust governance, cannot be overstated. Local governance plays a crucial role in the development and progress of the nation. By empowering local bodies and ensuring their effective functioning, India can address several pressing challenges, such as efficient public service delivery, equitable development, citizen participation in decision-making process, fostering inclusive growth etc. The decentralization of power and resources to local governments enables them to respond more effectively to the specific needs and aspirations of their communities. It fosters a sense of ownership and accountability among local leaders, making them more attuned to the concerns of their constituents. Moreover, local governance provides a fertile ground for experimentation and innovation, allowing for tailored solutions to unique regional problems.





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A key aspect of strengthening local democracy is enhancing the capacity and capability of elected representatives and bureaucrats who are working at the local institutions. This involves providing them with adequate resources, technical expertise, and administrative autonomy. Training and capacity-building programmes can also empower local representatives to discharge their duties more effectively and efficiently. Transparency and accountability are crucial pillars of robust governance. Implementing mechanisms to ensure open access to information and encouraging citizen participation in decision-making processes can help in building trust between the Government and the people. Promoting citizen engagement through participatory budgeting and regular public consultations, for instance through Grama Sabha meetings, can create a more inclusive and responsive governance structure.

There is a need to bridge the gap between urban and rural governance, as well as address issues related to gender and minority representation at the local level. By ensuring inclusivity and representation, local governments can better address the needs of all sections of society. The role of ICT and mobile or e-governance in bolstering local democracy cannot be overlooked. Utilizing digital platforms for governance, service delivery, and citizen engagement can significantly enhance the efficiency and transparency of local institutions. India has made significant strides in strengthening local democracy; challenges persist, such as bureaucratic resistance, financial constraints, and political interference. These challenges requires a concerted effort from all stakeholders, including policymakers, civil society organizations, and citizens. A robust local governance system is fundamental to India's progress and sustainable development. By empowering local institutions, fostering transparency, encouraging citizen participation, and leveraging technology, India can create a vibrant and inclusive democracy at the grassroots level. Strengthening local democracy is not only a constitutional mandate but also a crucial step towards building a more prosperous, equitable, inclusive growth and harmonious nation for all its citizens.

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Thrombospondin - Related Anonymous Protein (TRAP) as a Vaccine Antigen Candidate against Neglected *Plasmodium vivax* Malaria

Jyoti Acharya^{1*}, Sanjay Kumar Kochar² and Dharmesh Harwani³

¹Ph.D Scholar, Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan, India.

²Senior Professor, Department of Medicine, S.P. Medical College and A.G. of Hospitals, Bikaner, Rajasthan, India.

³Assistant Professor, Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan, India.

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*Address for Correspondence

Jyoti Acharya

Ph.D Scholar,

Department of Microbiology,

Maharaja Ganga Singh University,

Bikaner, Rajasthan, India.

E.Mail: dharmesh@mgsbikaner.ac.in



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ABSTRACT

The World Health Organization (WHO) reported worldwide approximately 241 million malaria cases in 2020. In India, this year, out of the 83% reported malaria cases, nearly 28% of cases were observed due to *P. vivax* infection. It is difficult to control the spread of *P. vivax* malaria due to the formation of latent hypnozoites and the presymptomatic appearance of gametocytes. WHO has recommended the first-generation pre-erythrocytic vaccine RTS,S against *P. falciparum* malaria. However, to develop a highly efficacious vaccine and elicit sterile immunity against *P. vivax* malaria, a few candidate antigens belonging to different life cycle phases of the parasite are under consideration in clinical trials. One of these candidate antigens is known as Thrombospondin Related Adhesive Protein (TRAP) which plays a vital role in sporozoite motility and sporozoite-host interaction. However, to date, we have limited knowledge about its natural antigenicity in the population susceptible to *P. vivax*-associated malaria. The purpose of this review is to emphasize and highlight the role of the pre-erythrocytic vaccine candidate antigen TRAP in a multi-antigen construct to decrease the burden of *P. vivax*-associated malaria.

Keywords: *P. vivax* malaria; Vaccine candidate antigen; TRAP antigen candidate





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INTRODUCTION

Malaria is an acute febrile illness caused mainly by the four species of *Plasmodium* parasite. Among these, the most common two species are *Plasmodium falciparum* and *Plasmodium vivax* followed by the less common *Plasmodium malariae* and *Plasmodium ovale* species [1][2]. Malaria continues to be a major cause of mortality and morbidity in the tropical and subtropical areas of the world. However, the burden of malaria cases has been observed to decrease in many countries [3]. In parallel to this reduction in overall incidence, a major shift in species composition has been also observed with *P. vivax* [4]. Although the mortality rate caused by *P. vivax* is rare compared to *P. falciparum*, *P. vivax*-associated malaria has a large socioeconomic impact and therefore it is now recognized as a reason for severe malaria [4]. *P. vivax* causes an estimated 14.3 million malaria infections every year and is considered to be a leading cause of malaria in Latin America and Asia [5]. In the past, it was recognized as benign tertian malaria but currently, *P. vivax* is identified as a public health threat leading to severe morbidity and mortality[6]. It is also recognized that *P. vivax* can stay dormant in the liver and reemerge years later to elicit new infections[7]. There are an increasing number of cases of severe disease, including respiratory distress, severe anemia, renal failure, jaundice, and thrombocytopenia due to *P. vivax* infection [8]. Vaccines are the most reliable alternative to elicit sterile immunity against malaria. The development of a safe and affordable vaccine would be an important addition to increasing immunity in the case of human malaria. The World Health Organization (WHO) recommends, a newly developed, RTS,S malaria vaccine among children in sub-Saharan Africa and other countries with mild to severe transmission of *P. falciparum* malaria[9]. This was the first human parasite vaccine that passed the vaccine development program with very high efficacy. But still, we do not have a safely accessible and broadly effective vaccine to control *P. vivax*-mediated malaria which is required for the success of WHO initiated malaria eradication program. Moreover, vaccine development efforts made so far have focused on *P. falciparum* and to a lesser extent on *P. vivax* [9, 10].

The polymorphisms exhibited by *Plasmodium* antigens and their antibody targets are poorly understood [10, 11]. Therefore, a vaccine-candidate antigen that has the least genetic diversity and small variation in multiple human parasite species can prove to be a potential vaccine candidate antigen of major interest. Several candidate antigens of the *P. falciparum* parasite have been characterized as the targets to confer natural immunity but the research about the most promising *P. vivax* candidate antigens has not expanded. For this reason, there are a limited number of active vaccine-candidate antigens undergoing clinical trials. Thrombospondin-related adhesive protein (TRAP) is a transmembrane protein that participates in the invasion of the *Plasmodium* parasites both in the mosquito salivary glands and into the liver cells. TRAP is a pre-erythrocytic stage antigen that plays important role in sporozoite gliding and invasion of the human hepatocyte. TRAP mediates the induction of immune response in human and animal models and is recognized as a pre-erythrocytic vaccine candidate antigen[8, 9-11]. This review considers the estimated global burdens of *P. vivax* malaria and the most recent progress to eliminate the linked morbidity and mortality through an effective vaccine development program using pvTRAP.

The Intricate Life Cycle of Plasmodium parasite

The life cycle of the malaria parasite is complex. The process has three phases in the mosquito and two in the human host (Fig. 1). The parasite undergoes a development stage in the mosquito and the mother requires a blood meal to mature her eggs. She bites a human and injects material from salivary glands, which contain primitive malarial parasites called sporozoites. These sporozoites circulate in the blood for a short time and then settle in the liver where they enter the parenchymal cells and multiply; this stage is known as pre-erythrocytic schizogony. After about 12 days there may be many thousands of young parasites known as merozoites in the liver cell, the cell ruptures and the free merozoites enter red blood cells[12]. In *P. vivax*, some of these are differentiated into the dormant stage and can delay the process of schizogony. These are termed hypnozoites, which will stay in the liver and can cause relapse after weeks, months, or years later[13]. *P. falciparum* on the other hand does not have a continuing liver cycle. In the red blood cells, the parasites develop into two forms, a sexual form, and an asexual stage. The sexual stage consists of male and female gametocytes, which circulate in the blood and are taken up by a female mosquito when taking a





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blood meal. The male and female gametocytes fuse in the mosquito's stomach and form oocysts in the stomach lining. These oocysts develop over days that contain large numbers of sporozoites, which move to the salivary glands and are ready to be injected into a human host when the mosquito next takes a blood meal. In the asexual cycle, the developing parasites form schizonts in the red blood cells, which develop into merozoites. The infected red cells rupture and release a batch of merozoites, which invade new red blood cells. In *P. vivax*, *P. ovale* and probably *P. malariae*, all stages of development after the liver cycle can be observed in the peripheral blood. However, in the case of *P. falciparum* only ring forms and gametocytes are usually present in the peripheral blood. Developing forms appear to stick in the blood vessels of the large organs such as the brain which can restrict the blood flow with serious consequences[14]. The above described four different stages of the life cycle of malaria parasites with each stage providing potential vaccine-antigen targets[15].

***P. vivax* Vaccine Candidate Antigens**

The complexity of the parasitic life cycle, little understanding of host-parasite interactions, and several other mechanisms are operative in favor of the *P. vivax* parasite to evade immune responses. Thus, the development of an anti-parasite vaccine seems to be a very difficult task. However, recently several trials with recombinant antigens have shown a high level of protection against the challenge posed by the *P. vivax* parasite [16, 17]. In general, if the whole parasitic life cycle is considered, there can be essentially six targets for a malaria vaccine which include sporozoites, liver, merozoites, infected RBC, parasite toxins, and sexual stages (Table 1). The sporozoite remains in the bloodstream for a very short period before invading hepatocytes, making it an unlikely target for an effective protective response. Circumsporozoite protein (CSP) is one of the most studied malaria vaccine candidate antigens for this stage. Other vaccine candidate antigens for this stage include Thrombospondin-related adhesive protein (TRAP), Sporozoite and liver-stage antigen (SALSA), Sporozoite threonine and asparagine rich protein (STARP)[18]. The liver-stage antigens represent the second target for the development of a malaria vaccine. During this stage, immunity is most likely mediated by cellular-dependent mechanisms involving CD8+ T cells, CD4+ T cells, natural killer (NK) cells, and $\gamma\delta$ T cells. CSP is also a constituent of most multiantigen DNA vaccines under trial. Other identified liver-stage antigens include liver-stage antigen-1 (LSA-1), and LSA-2, LSA-3 among others[19].

The merozoite is the only stage in the human host wherein the malaria parasite is in the extracellular phase. This makes it a 'visible' target for antibodies. Main vaccine candidate antigens of this class are merozoite surface protein (MSP)-1, -2, -3, -4, -5, erythrocyte-binding antigen (EBA), apical membrane antigen (AMA)-1, rhoptry-associated protein (RAP)-1, 2, acidic-basic repeat antigen (ABRA), and Duffy-binding protein (DBP) of [20-23]. Once the parasite gains entry into the RBC several antigens could elicit an antibody response. One of the best-studied molecules of such type is the ring erythrocyte surface antigen (RESA)[24]. Other vaccine antigens are serine-rich protein (SERP), erythrocyte membrane protein (EMP)-1, -2, and -3, and glutamate-rich protein (GLURP)[25-27]. A malaria infection results in severe symptoms in non-immune individuals at the time of merozoite release from the RBCs infected with mature schizonts. These symptoms seemingly result from a wave of toxemia owing to molecules released by synchronic parasites. This cytokine, in turn, has important roles not only in the protection but also in the pathogenesis of malaria disease[28]. The last possible target for a malaria vaccine is the sexually differentiated parasite stages; vaccine preparations for this stage are transmission-blocking vaccines[29]. Several candidate antigens such as Ps25, Ps28, Ps48/45, and Ps230 have been identified and evaluated for this stage[30]. Table 1 summarizes the main malaria vaccine candidate antigens of each stage identified so far[18].

It must be determined before that which life stage of the parasite has to be targeted. It is also crucial to know whether the vaccine needs to combine elements that target more than one life stage. Many *P. vivax* vaccine development trials have generally followed *P. falciparum* vaccine development efforts. For example, the CSP-based Vivax-1 vaccine that is produced in *E. coli* as a chimeric protein with the advantage of comprising the central repeat regions of two major circulating *P. vivax* strains, VK210 and VK247, thus has the potential to provide wide coverage to confer protection against a large number of *P. vivax* strains [31]. Similarly, *P. vivax* blood-stage vaccine development programs focus on Duffy-binding protein (PvDBP) which binds the Duffy antigen receptor for chemokines (DARC) on erythrocytes needed for merozoite invasion. Two DBP vaccine candidates have completed phase-1 trials[32].





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Two important surface molecules that play key roles in liver infection are Circumsporozoite protein (CSP) and Thrombospondin-related anonymous protein (TRAP)[33]. As stated earlier, CSP is a multifunctional protein that is involved in sporozoite development in mosquitoes, and invasion into mosquito salivary glands and human liver cells[34]. While TRAP is a pre-erythrocytic vaccine candidate antigen found in both *P. falciparum* and *P. vivax* species. TRAP is stored in the micronemes and exposed to the surface at the sporozoite anterior tip when the parasite comes into contact with the host cells. *Plasmodium* sporozoite and liver stages antigens are targeted in pre-erythrocytic vaccines (PEV). These are symptomatically silent forms that initiate infection in humans following the inoculation of sporozoites by mosquitoes into the skin. Antibodies produced against surface antigens clear sporozoites from the skin, and bloodstream and block their invasion to hepatocytes. A major concern in *P. vivax* vaccine development is the genetic polymorphism usually observed among *P. vivax* strains in numerous geographical areas across the world [35].

The genetic polymorphisms have been identified in *P. vivax* isolates for apical membrane antigen-1 (AMA-1), Duffy-binding protein (DBP), Merozoite surface protein-1 (MSP-1), Circumsporozoite protein (CSP), and Thrombospondin related anonymous protein (TRAP). For example, the genetic variability in various vaccine-candidate antigens has been observed in 106 *P. vivax* isolates from the amazon region of Loreto, Peru. Similarly, genetic diversity has been also determined for the Peruvian isolates. Applying population studies the observed diversity was compared with the isolates from other geographical locations[35]. Polymorphism in PvTRAP gene has been also reported from isolates of Brazil and Thailand[36].

A pre-erythrocytic vaccine that prevents infections would not only reduce the number of primary infections by preventing the establishment of hypnozoites but also reduces the risk of multiple relapses contributing to continued transmission. Given that, a single mosquito bite may lead to one primary infection and up to four relapses mediated by *P. vivax*. A pre-erythrocytic vaccine would have a larger effect on *P. vivax* transmission compared to *P. falciparum* transmission[37]. Therefore, vaccine-candidate antigens from the pre-erythrocytic stages could be the target of antibodies that can prevent sporozoite invasion to hepatocytes or these could be the putative target of cellular immune responses restricting infected hepatocytes. In this way, an effective pre-erythrocytic vaccine would inactivate the parasite before it leaves the liver to confer sterile immunity. Fig. 1 summarizes antigens expressed at different stages of the parasite life cycle. These candidate antigens have been found to have potential roles for their use to design and develop a subunit vaccine against *P. vivax*[38-41].

TRAP: a crucial protein for *Plasmodium* sporozoite invasion

Thrombospondin-related adhesive protein (TRAP) is a conserved microneme protein important for parasite motility and infection of mosquito salivary glands. It is a transmembrane protein that belongs to the TRAP/Micronemal protein 2 super family of adhesions. In combination with the Circumsporozoite protein (CSP), it is found to be essential for malaria sporozoite infection to hepatocytes[42, 43]. It has a molecular weight of 90 kDa. TRAP is found on the surface and micronemes of sporozoites and lasts up to an initial four days in the liver stage. It produces humoral as well as cytotoxic T-lymphocyte response in rodent models and is mainly used in multi-antigen constructs. This protein is extremely conserved among *Plasmodium* species. In *P. vivax*, towards its N-terminus, a conserved fragment (WTPCSVTC-GVGVRVRRR) is found at 307-324 position that binds to sulfated glycoconjugates[44]. Similar to CSP, TRAP binds to the heparan sulfate molecules of the basolateral domain of hepatocytes throughout the initial contact between hepatocytes and the invading sporozoites[45]. TRAP is a type 1 transmembrane protein whose ectodomain consists of (i) aA domain, (ii) a thrombospondin type 1 repeat (TSR), and (iii) a repeat region of different lengths and sequences, depending on the *Plasmodium* species (Fig. 2).

The A domain is 200 residues long adhesive module that was first recognized in the plasma protein as a von Willebrand factor[46]. It consists of complement protein factors B and C2, extracellular matrix proteins, including many types of non-fibrillar and FACIT (fibril-associated collagen with interrupted triple helix) collagens, and integral membrane proteins with seven integrins and chains[47]. The crystal structure of the A domain of TRAP has





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been determined[48-51]. It comprises alternating amphipathic helices and hydrophobic B strands conforming to the classic B “Rossmann” fold. In the A domain of TRAP, 5 conserved residues that coordinate a bivalent cation (Mg^{2+} or Mn^{2+}) and define the metal-ion dependent adhesion site (MIDAS) motif, have been identified[51, 52]. TRAP has been observed to be highly polymorphic and with a particular allelic variant, it induces a more effective immune response. So far, few preclinical studies have evaluated the efficacy of a PvTRAP vaccine. One such study includes the induction of antibodies reactive to *P. vivax* sporozoites which utilize long synthetic peptides within the N-terminal region of the PvTRAP binding motif involving both Freund and Montanide ISA 720 adjuvants to BALB/c mice and *Aotus* monkeys [53]. In *Aotus* monkeys, compared to montanide ISA 720, a vaccine formulated in CFA/IFA induced higher titers and partial protection upon intravenous challenge with 2×10^4 *P. vivax* sporozoites. In a related study, a heterologous prime-boost vaccine induced high titer antibodies and high T cell response in immunized mice with recombinant ChAd63 and MVA both expressing PvTRAP which partially protected against the chimeric *P. berghei* expressing PvTRAP challenge[54].

Potential Roles of PvTRAP in Vaccine Development

The possible role of TRAP in the sporozoite invasion of hepatocytes and merozoite invasion of erythrocytes makes this molecule a potential malaria vaccine candidate. TRAP is considered one of the most conserved malaria antigens across the *Plasmodium* species which was characterized initially in *P. cynomolgi*[55]. The potential of this antigen and its fragments in protection against the disease have been already tested in animal models. TRAP also helps sporozoites to glide and recognize the host receptors on the mosquito salivary gland and hepatocytes[56]. The highly polymorphic (especially at T cell epitope) PfTRAP gene from the *Plasmodium* isolates of Bioko island and other malaria-endemic countries has provided the genetic information and necessary background to develop a PfTRAP-based universal vaccine [57]. PvTRAP could also act as a potential pre-erythrocytic vaccine candidate antigen to operate synergistically to block sporozoite invasion. Different antibody binding specificities of PvTRAP could function at different stages of hepatocyte invasion. Consequently, PvTRAP was accepted mutually as a strong candidate for developing an effective malaria vaccine.

A Korean study discovered just one major allelic type and restricted sequence variations of PvTRAP among Korean *P. vivax* isolates than the isolates identified in the returned travelers from India, Indonesia, and Papua New Guinea. Numerous polymorphisms have been also observed in the strains from returned travelers[58]. In mice and monkeys models, PvTRAP induced high levels of specific antibodies, proving its potential for its important role in vaccine development program[51]. As mentioned earlier, after immunization with PvTRAP-derived long synthetic peptide (LSP) both in mice and *Aotus* monkeys, the specific anti-peptide antibodies cross-reacted with the parasite in indirect fluorescent antibody (IFAT) assays. Only two out of six immunized animals became infected in the *P. vivax* sporozoite challenge as compared with four out of six animals from the control group. These results suggest that this TRAP fragment has protective potential against *P. vivax* malaria and deserves further studies as a vaccine candidate antigen.

In non-human primates, mice, and humans, the potential of TRAP as a vaccine candidate is supported by many observations associated with immunogenicity and protection. For instance, co-immunization using CSP in combination with TRAP in the murine model conferred complete protection against a parasite challenge, whereas vaccine dosage using only CSP provided partial protection. TRAP-specific CD8⁺ T lymphocytes seem to be key mediators in the protection against sporozoite challenge in mouse vaccination assays[59].

CONCLUSION

How to eliminate the burden of malaria disease in the regions devastated by it? The ongoing research has expanded our current understanding of the role of vaccine-candidate antigens for their use in the effective vaccine development against *P. falciparum* and *P. vivax* associated malaria. These various approaches have revealed the potential role of PvTRAP (*P. vivax*- Thrombospondin Related Anonymous Protein) as a promising vaccine candidate





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antigen. The key role in gliding motility, invasion in two different hosts, and erythrocytic binding properties confer PvTRAP as a good target. Additionally, two pre-erythrocytic vaccine candidate antigens of *P. vivax*, CSP, and TRAP could also function synergistically to block sporozoite invasion, as they appear to have different antibody binding specificities that could function at different stages of hepatocyte invasion. In this way, to achieve acceptable malaria protection, multiple vaccine subunits can also be deployed. In conclusion, the present review provides firm bases to evaluate the PvTRAP alone and or in combination with other *P.vivax* subunit vaccine candidate antigens in preclinical studies. PvTRAP-derived vaccine development programs should also consider the influence of putative antigenic diversity to avoid vaccine escape.

Conflict of interest statement

The authors declare no competing interests.

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Authors' contributions

JA and SK contributed to conceptualization; DH was responsible for supervision, literature search, writing, and original draft preparation. JA, SK and DH contributed to the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

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Table 1 Vaccine candidate antigens according to the life cycle of *Plasmodium malaria* parasites

| Sporozoite | Liver stage | Merozoite | Blood stage | Toxin | Sexual stage |
|--|---|--|---|--|--|
| <ul style="list-style-type: none"> • Circumsporozoite protein (CSP) • Thrombospondin-related adhesive protein (TRAP) • Sporozoite and liver-stage antigen (SALSA) • Sporozoite threonine- and asparagine-rich protein (STARP) • CSP | <ul style="list-style-type: none"> • Liver-stage antigen (LSA)-1 and -3 • SALSA • STARP • Merozoite surface protein (MSP)-1, -2, -3, -4 and -5 • Erythrocyte-binding antigen (EBA)-175 | <ul style="list-style-type: none"> • Apical membrane antigen (AMA)-1 • Rhoptry-associated protein (RAP)-1 and -2 • Acidic-basic repeat antigen (ABRA) • Duffy-binding protein (DBP) • Ring erythrocyte surface antigen (RESA) • Serine-rich protein (SERP) | <ul style="list-style-type: none"> • Erythrocyte membrane protein (EMP)-1, -2 and -3 • Glutamate-rich protein (GLURP) | <ul style="list-style-type: none"> • Glycosylphosphatidylinositol (GPI) | <ul style="list-style-type: none"> • Ps25 • Ps28 • Ps48/45 • Ps230 |





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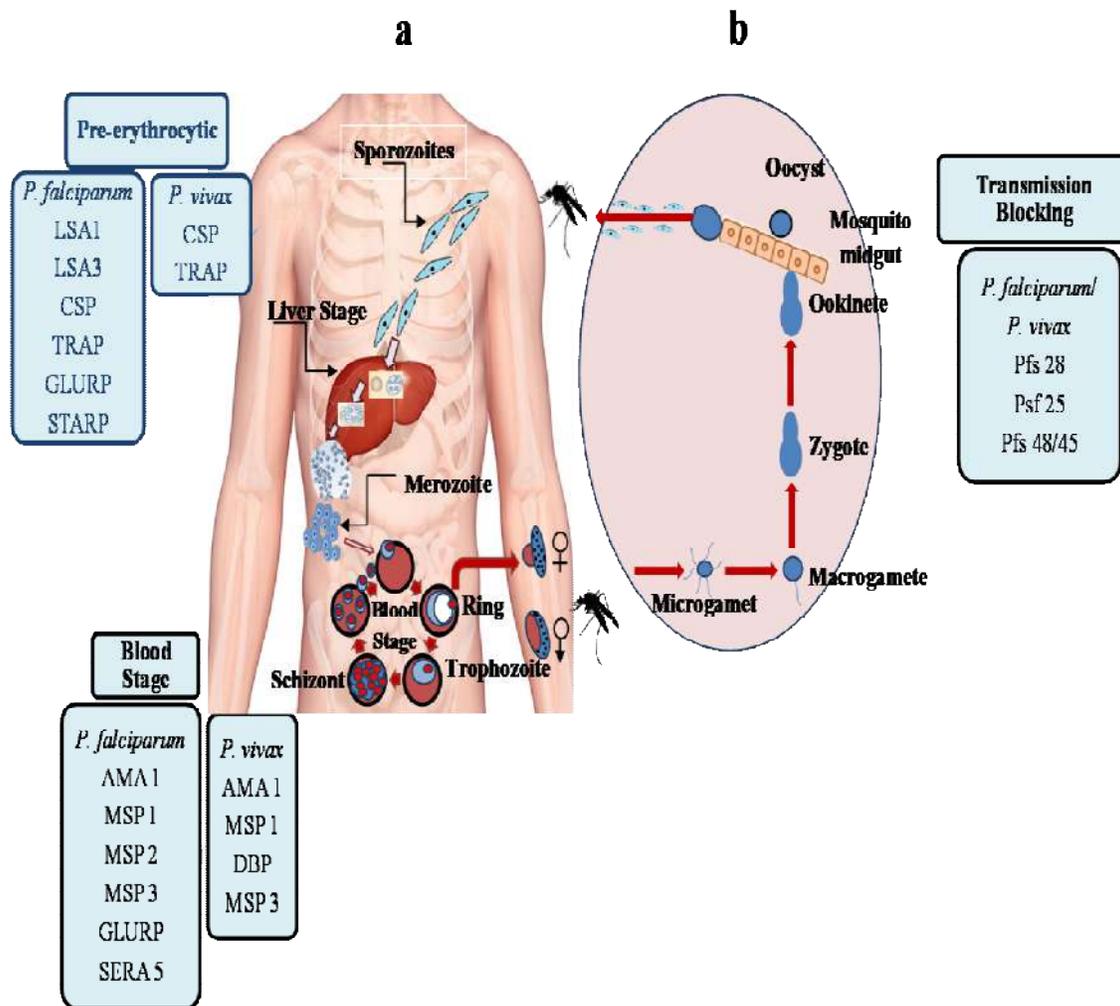


Figure 1. Different malaria vaccine antigen candidates of *P. falciparum* and *P. vivax* according to their lifecycle stage targeted. a Human, and b Mosquito





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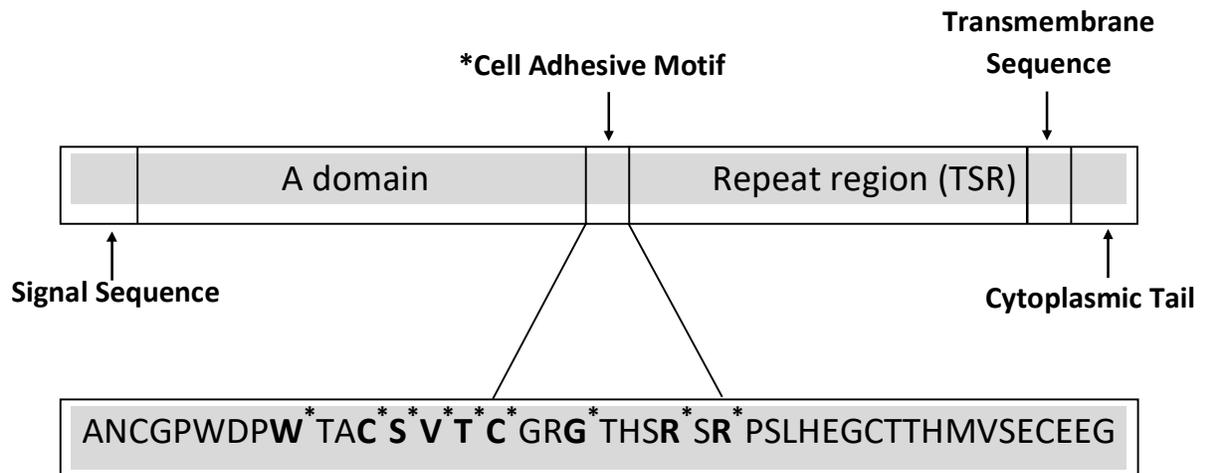


Figure 2. *Plasmodium vivax* Thrombospondin related anonymous protein (TRAP) protein





Prospection of Antifungal Loaded Nanoparticulate Topical and Transdermal System for Enhanced Therapy

Ranjitha B^{1*}, N Raghavendra Naveen², Goudanavar Prakash³, Biradar Nikita¹ and Asha B R¹

¹M.Pharm, Dept. of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar-571448, Karnataka, India.

²Assistant Professor, Dept. of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, Adichunchanagiri University, B G Nagar-571448, Karnataka, India

³HoD, Dept. of Pharmaceutics, Sri Adichunchanagiri College of Pharmacy, B.G. Nagar, Karnataka, India.

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*Address for Correspondence

Ranjitha B

M.Pharm, Dept. of Pharmaceutics,
Sri Adichunchanagiri College of Pharmacy,
Adichunchanagiri University,
B G Nagar-571448, Karnataka, India.
E.Mail: gowdaranjitha118@gmail.com



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ABSTRACT

Human fungi can infect the skin, mucosal membranes, internal organs, or cause more severe, invasive, and systemic disorders. There are several factors that affect how therapeutic agent formulations are developed, designed, and how they are administered. For the efficient creation of appropriate drug delivery systems, the relationships between formulations, modes of administration, pharmacokinetics, toxicity, and therapeutic indications must be carefully investigated. There are numerous NP formulations that make effective antifungal drug delivery methods. This study examines a number of kinds of nanoparticles used in the administration of antifungal drugs, including dendrimers, polymeric nanoparticles, inorganic nanoparticles, and vesicles based on phospholipids (nanovesicles). Both their benefits and disadvantages are highlighted. Many NP formulation-based in vitro or cell culture investigations manage to attain a sufficient strong drug loading capacity but fall short of the clinically significant concentrations expected for in vivo studies. Hence, investigations that focus on finding answers to problems with drug loading, nanoparticle stability and high production and standardisation costs may aid in the translation of these nano-formulations from the laboratory to the clinic.

Keywords: polymeric nanoparticles, inorganic nanoparticles, antifungal drugs, nanovesicles, and nanoscaled.



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INTRODUCTION

The number of antifungal agents is quite small in comparison to antibiotics. There are currently about 80 different types of antifungal drugs being utilised in clinics. Azole, allylamine, and morpholine are three main groups of clinical antifungal drugs, along with polyenes and β -glucan prochloroglucan synthesis inhibitors, which prevent the synthesis of ergosterol (echinomycin). Additionally, the pyrimidine analogues, which are frequently used as adjuvant drugs, prevent the synthesis of fungal nucleic acid; Antifungal resistance, however, has become a severe issue in clinics for treatment due to the overuse of antibiotics and antifungal drugs. Consider the recent increase in *Candida albicans* resistance. Research shows that from 2003 to 2005, the resistance to *C. albicans* raised from 36.36% to 63.98%. Fungal resistance is a severe hazard to human health and makes clinical treatment much more difficult. Moreover, invasive fungal infections have become more common over time. There are more than one invasive fungal infection for every 100,000 people, (1) and the death rates for *C. albicans*, *Aspergillus fumigata*, and *Cryptococcus neoformans* are 20% to 40%, 20% to 70%, and 50% to 90%, respectively. (2) Finding new treatment plans and creating new therapeutic drugs are thus urgently needed and very significant. The latter faces difficulties including the need for fungus-specific drugs (3).

Nanoparticles (NPs) are now highly valued for their numerous uses in a variety of biological, pharmaceutical, and medical disciplines. Their size hardly reaches the range of 100 nm when taken structurally. These NPs are capable of controlling a variety of drugs, including micro hydrophobic and hydrophilic chemicals, vaccines, and biological molecules. (4) NPs can be used for a variety of purposes, including scaffolds for tissue engineering, targeted drug delivery, and disease diagnosis. (5) NPs have been widely used as cellular scaffolds, drug carriers, nanoliposomes, carbon nanotubes, nanofibers, and nanocapsules. To effectively deliver a given drug at a certain time and place for maximal efficacy, it is important to manage particle size, surface characteristics, and other aspects of NP production as a drug delivery system. (6) The NPs utilised for drug administration should have the following qualities in addition to biocompatibility and biodegradability: timely release, ideal mechanical properties, and ease of manufacture. Surface modification allows for tracking of NPs that become caught in the body through the circulatory system or phagocytosis and are subsequently saved in the circulation system (7).

In terms of their dimensions, design, and material composition, NPs can be divided into a number of different categories. Even NP preparation techniques can produce a range of NPs, each of which has a unique loading capacity, delivery, and shelf-life. (8) Dendrimers, nanotubes, nanospheres, nanocapsules, liposomes, micelles, and polymersomes are the different categories based on how they appear. They have been divided into organic and non-organic groupings in other investigations. Within the organic group, organic molecules make up the majority of the NPs, but in the mineral category, metals (such as iron, gold, etc.) and other mineral elements are crucial to the structure of the NPs. (9) The term "organic particle" refers to substances like solid lipid nanoparticles, dendrimers, carbon nanotubes, polymers, and liposome whereas "mineral nanoparticles" have a core comprised of metal or mineral components that is encased in an organic shell. The cores show magnetic, electrical, and fluorescent characteristics. (10) In a different classification. Based on how they are generated, NPs are split into two types: nanosphere and nanocapsule. These NPs are produced from macromolecular or synthetic polymeric components. The drug is positioned in the centre of nano capsules, which resemble sac-like structures and are surrounded by a polymeric layer. The drug and polymer are either uniformly disseminated or surface-absorbed within a nanosphere, which is a matrix system. Drugs with specialised therapeutic properties for particular diseases, such as malignancies, are added to polymers when they are used as nanoparticles (NPs). Two methods exist for attaching these NPs to nanocomposites: The drugs are twofold: 1. they are enclosed in nanocarriers, and 2. they are conjugated on the surface of nanoparticles (11).

In comparison to alternative delivery techniques, topical therapy modalities provide a number of benefits, such as non-invasiveness, usability, targeting infection locations, less side effects and drug interactions, better patient compliance, and more affordable treatment. (12) In contrast to oral drug therapy, these qualities make topical therapy



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a desirable therapeutic choice for superficial cutaneous fungal infections. If sufficient drug release and penetration are controlled, Targeted skin areas can simply get sufficient concentrations of antifungal drugs through topical therapy.(13)Moreover, because much smaller levels of the drugs are created in the blood, topical antifungal administration reduces side effects related to drug therapy. To attain comparable local drug concentrations, greater oral doses are needed compared to alternative treatment approaches, which can have unfavourable effects. Additionally, oral administration is linked to harmful adverse effects, such as severe liver damage, and drug-drug interactions (14).

The most effective approach against the most prevalent skin dermatophytes is probably topical use of anti-fungal drugs since it ensures direct access and a higher retention rate at the target. Moreover, topical treatment reduces pre-systemic metabolism and reduces systemic toxicity. Various drugs, including clotrimazole, itraconazole, and ketoconazole, are applied topically to the skin by rubbing or distributing them.(15–17)Site-specific drug administration, less systemic toxicity, increased patient compliance, increased treatment effectiveness, and improved bioavailability are further benefits of topical delivery.(18)The topical application of anti-fungal drugs, however, has the potential to result in unfavourable skin reactions, such as allergic rashes and itching.(19–21) Additionally, standard formulations require high doses and frequent administration, which is linked to a higher risk of both local and systemic toxicity. In order to decrease local side effects and improve treatment efficacy, a revolutionary drug delivery mechanism is envisioned. The topical nanocarrier techniques used in the current review for cutaneous application of anti-fungal drugs are advanced. Novel drug delivery systems have attracted a lot of attention in pharmaceutical research as a topic of topical formulation (NDDS).Due to its outstanding ability to regulate the release kinetics of encapsulated drugs, encapsulate a wide variety of pharmaceuticals, and enhance disease specific localization, NDDS reduces dose frequency and increases clinical efficacy. Understanding the specific mechanism of antifungal drugs is necessary to achieve good therapeutic effectiveness when utilising an appropriate topical formulation.(22,23)

ANTIFUNGAL DRUGS**AMPHOTERICIN B**

Amphotericin B is the only polyene that has been authorised for use in systems (AMB). Its main benefits include the minimal incidence of resistance, despite decades of usage, and its fungicidal effectiveness against the majority of clinically relevant infections. Nephrotoxicity has been AMB's main drawback, although new lipid formulations that address this problem have just received approval. With far less side effects than AMB, The efficacies of Ambisome, Abelcet, and Amphocil/Amphotech are roughly comparable.(24)The small variations in effectiveness that come from the specific release of active AMB from the complex and realignment of the antifungal drug to tissues appear to be influenced by the type of lipid, the molar ratio of lipids, and the size of the vesicle in the bilayer membrane containing the polyenes.(25,26).

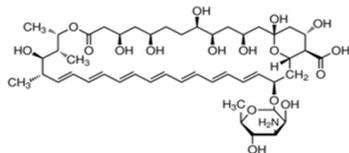
A macrocyclic polyene known as amphotericin B (AmB) is widely regarded as the best medication for treating invasive fungal infections, particularly pathogenic yeasts. devised and created SLNs to improve AmB's effectiveness and lessen its adverse effects on the kidneys.(27)AmB should be administered in lipid formulations due to its limited water solubility. At present, the drug is offered in three lipid formulations: liposomal AmB(Ambi-some), AmB lipid complex (Abelcet), and AmB colloidal dispersion(Amphocil), however they are all pricey.(28–30) Recently, SLNs were created to enhance topical application of AmB.(31)The preparation of the lipid particles in particular was done to address the issue of poor local action and improve drug absorption through the skin.(32)Clinical mucocutaneous leishmaniasis, a form of cutaneous leishmaniasis that is resistant to antimony therapy, has also been treated with amphotericin B, and experimental cutaneous leishmaniasis.(33)In order to solve the problem of restricted local activity in dermatopharmacotherapy, a selective delivery method that enhances the uptake of the bioactive component into the skin is required.By serving as a local depot or reservoir and reducing the effective dosing frequency and dose, and systemic adverse effects associated with conventional topical therapy, this may help localise the medicine to the application site. The stratum corneum is the main barrier preventing topical drugs from being





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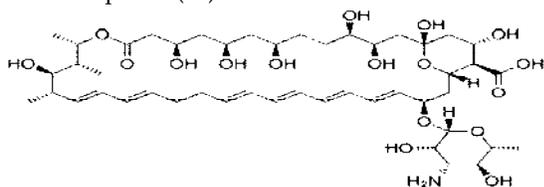
absorbed via the skin. Site-specific transport of these medications to the skin is made possible by SLN's small size and rather narrow size distribution, which increases their concentration there.(34)



NYSTATIN

Just like its structurally related relative AMB, nystatin bound ergosterol and inhibits fungal membranes. Its toxicity has limited its application to topical diseases, but Aronex (TX), motivated by the popularity of AMB's liposomal formulations, has created a novel formulation of this conventional medicament to improve the therapeutic index of the substance. Phase III trials for liposomal nystatin (Nyotran) are being conducted in neutropenic patients with suspected candidemia. Nyotran was 67% successful at treating candidiasis, according to a phase II research. What was most impressive was its ability to treat infections in 9/15 patients whose conditions had previously shown resistant to other antifungals. Although this compound's therapeutic index has increased as a result of formulation advancements, its use may be constrained by its high price as well as challenge from the well-established liposomal AMB industry.(35,36)

One of the first polyene antifungal drugs, nystatin (Nys),(37) is frequently used to treat *Candida* species-caused cutaneous and mucocutaneous fungal infections.(38) In order to prevent and treat candidiasis Ergosterol, a crucial component of the fungal cell membrane, is where Nys binds. Due to the development of barrel-shaped spanning channels that prevent the membrane from functioning as a selective barrier, cellular components are lost, and finally the fungus dies.(39) Due to the fact that it exhibits a more diverse range of antifungal action than other polyene antifungal drugs,(40) In addition to the previously mentioned resistance against the conventional antifungal treatments, Nys represents a promising and effective candidate for the treatment of a variety of infections caused by *Candida* species.(41)



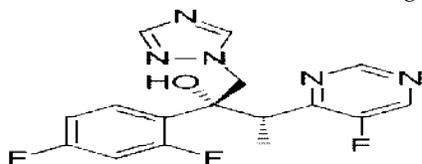
VORICONAZOLE

Voriconazole is a second-generation fluconazole derivative made synthetically. Phase 3 trials are being carried out, and research on them is progressed in both the United States and Europe. It has a potency 16 and 160 times stronger than fluconazole, respectively, in inhibiting the lanosterol 14-demethylase of *Candida albicans* and *Aspergillus fumigatus*. In addition to preventing certain yeasts and filamentous fungi from demethylating 24-methylene dihydrolanosterol, voriconazole also does this. It combats mycoses that fluconazole can not effectively treat, which explains why.(42) As a result, Since it can be faster absorption orally and is not affected by gastric pH, it is a more potent azole than fluconazole, has a wider antifungal range, and superior pharmacokinetic properties than itraconazole. It is advisable to use caution when administering the substrates of various CYP isoforms together because the cytochrome P450 enzymes, particularly CYP2C9 and CYP3A4, significantly metabolise it. Urinary excretion is the main method used to get rid of voriconazole's metabolites. Parenteral voriconazole should not be administered to individuals with moderate to severe renal insufficiency because the inactive components can build up over time. Transient and dose-related visual problems, hepatotoxicity, and the need to stop treatment in some circumstances are just a few of the pertinent side effects.(43) Acute renal failure, rash, peripheral oedema, and stomach discomfort have also been reported along with photosensitivity and the emergence of lesions that are compatible with discoid lupus vulgaris. Although there have been reports of cross-resistance strains,(44) it is effective



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in vitro against *Candida* species that are fluconazole-susceptible or resistant.(45)It is more powerful than amphotericin B at preventing the growth of *Candida* species that are both azole-susceptible to and resistant to azoles, having up to 16 times the efficacy of fluconazole in vitro.(46)Voriconazole has also shown significant in vitro activity against *Cryptococcus neoformans*, as well as against dimorphic and filamentous fungi, including *Ascomycetes*, *Aspergillus* species, *Fusarium* species, *Coccidioides immitis*, *Bipolaris* species, *B dermatitidis*, *H capsulatum*, dermatophytes, *Malassezia* species, *Scedosporium apiospermum* (*Pseudoallescheria boydii*), *S schenckii*, and *Absidia corymbifera*, although its in-vitro activity against the last two organisms is less potent than against the others.(47,48) Voriconazole, a second-generation triazole, is available orally and intravenously.(49)It is effective against a variety of *Candida*, *Aspergillus*, and *Cryptococcus* species, even those that are resistant to other widely used antifungal medications. Nevertheless, oral dosing of voriconazole is challenging due to complex pharmacokinetics, significant drug interactions, and very significant adverse effects.(50)Furthermore, difficulties with heart rhythm are said to be brought on by its intravenous use.(51) In order to get around these issues and improve the antifungal activity of voriconazole against cutaneous candidiasis, a topical delivery mechanism is required.(52)Over the past 20 years, a lot of work has been paid to developing a unique drug delivery mechanism. The major goal of this work is to create a unique topical drug delivery system that will change the pharmacologic and pharmacokinetic properties of voriconazole-loaded nanoparticles to enhance their therapeutic safety and efficacy. Colloidal delivery systems are well-known among many medication administration methods. Polymeric NPs make up the basic colloidal drug delivery method. This method is primarily being researched as a controlled site-specific drug and for the enhancement of low water-soluble drug bioavailability.(53)

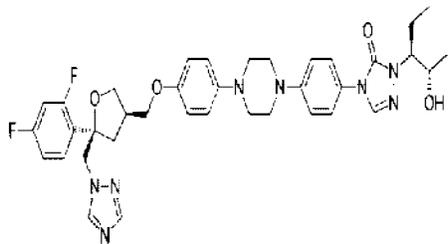


POSACONAZOLE

Posaconazole (SCH-56592) is an itraconazole analogue that has been hydroxylated. Its potency in inhibiting the enzyme lanosterol demethylase of *Aspergillus flavus* and *Aspergillus fumigatus* is ten times more than that of itraconazole. Despite the fact that its CYP metabolism is less than that of voriconazole, it inhibits CYP3A4. Although it seems to be more effective against *Rhizopus* species and slightly less effective against yeasts,(54)It has an activity spectrum in vitro that is similar to voriconazole's.(55)Additionally, it has proven successful in the management of coccidioidomycosis. It has demonstrated its effectiveness against filamentous fungus in animal studies.(56)In a limited group of patients who had failed prior antifungal treatments, a non-comparative open research found that 75% of patients with fusariosis and candidiasis and 53% of those with aspergillosis responded to the treatment. It is exclusively offered as an oral administration preparation.(57)

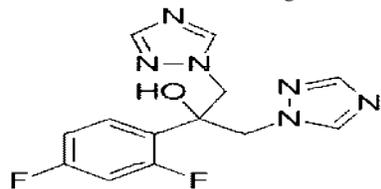
The drug posaconazole is in BCS Class-II and has a high lipid solubility but a low water solubility. Posaconazole, an antifungal medication, comes in a variety of dose forms, including as injections, oral suspensions, and delayed release tablets. When administered orally, these formulations may result in patient non-compliance, decreased stability, site-specific administration, decreased bioavailability, nausea, and stomach pain. Making Posaconazole-loaded nanostructured lipid carriers gel for topical distribution is therefore necessary to prevent these therapeutic adverse effects, to increase bioavailability, and to increase patient compliance for various topical fungal diseases.(58,59)



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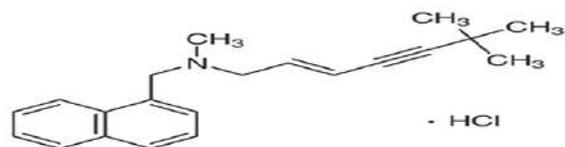
FLUCONAZOLE

A triazole antifungal medication called fluconazole (FLZ) is used in particular to treat severe skin infections.(60) Unfortunately, systemic absorption and skin irritation from FLZ's topical application meant that mycological eradication was not successful.(61)It has been recommended to use drug delivery devices to combat the negative effects of FLZ.(62) Using SLNs could be an useful alternate strategy to help FLZ penetrate the skin. Fluconazole is a synthetic antifungal medication of the imidazole class. It works by slowing the growth of fungus that causes infections. It is employed in the treatment of fungal infections. Fluconazole continue to be one of the most often prescribed triazoles because of its high level of tolerance, good absorption, and low risk of side effects.It eliminates all the negative effects of other antifungal medications like Amphotericin B,ketoconazole, Miconazole,and Clotrimazole.While it undergoes first pass effect through the liver and elimination through the kidneys after overcoming the unfavourable effects of other antifungal medications, fluconazole has some side effects whether administered orally and parenterally.Fluconazole nanoparticles were added to freshly made gel for transdermal distribution when these negative effects of the tablet dosage became apparent.(63,64)



TERBINAFINE HCL

The synthetic allylamine terbinafine (TB), which inhibits squalene epoxidase, has strong, broad-spectrum fungicidal effects. Both methods of administering TBby mouth and topicallyare generally well tolerated;Nonetheless, after oral administration, about 40% of the dosage undergoes first-pass metabolism and is strongly bound to plasma proteins. The required concentrations for antimycotic activity at the target skin region may actually be easier to reach after topical treatment if appropriate drug release and penetration are ensured.(65)So this antifungal drug could be delivered throughout the skin using SLNs loading TB. Treatment for many skin conditions such dermatophytosis, candidiasis, seborrheic dermatitis, and onychomycosis is successful when terbinafine HCl is used.Because it is simpler to administer, topical treatment is frequently used.As they promote drug penetration into the skin without causing first-pass metabolism or negative side effects such allergic reactions, SLNs for topical administration have a variety of advantages. They also direct the active component to the affected areas of the skin.(66,67)Therefore, create and describe SLNs formulations of TFH for topical drug delivery system.



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FUTURE PROSPECTIVES

Fungal infections of the skin that affect people frequently affect them. Different antifungal medications have been sold to treat superficial fungal infections. An excellent method for treating these localised infections is topical administration of antifungal drugs. Antifungal drugs can be administered topically to prevent the unintended systemic side effects that are frequently associated with oral dosing. The traditional topical formulations, which include cream, gels, ointments, powders, and other similar substances, have largely controlled the market for these antifungal drugs. There are various difficulties associated with conventional antifungal agent distribution through the skin for the treatment of superficial fungal infections in terms of its safety, effectiveness, penetration, etc. The use of nanocarriers may be the way to enhance medicine distribution and get around the problems that occur with using traditional treatments for superficial fungal infections. To facilitate the skin administration of antifungal drugs, a wide range of nanocarriers have been developed. Lipid-based nanocarriers are one of the most popular types of nanocarriers for the topical application of antifungal drugs. Due to their chemical composition, lipid-based nanocarriers are favoured for the topical administration of medications. The main factor allowing for the secure administration of antifungal medicines is a variety of biocompatible lipids. Topical delivery of antifungal drugs has been extensively studied using lipid-based nanocarriers, and many are still being studied.

CONCLUSION

Worldwide, fungal infections are a substantial cause of morbidity and mortality associated with skin illnesses. Several potent antifungal drugs exist, but their therapeutic value is constrained by either excessive toxicity or subpar physicochemical qualities. Some of these limitations can be overcome by nanoparticles thanks to their advantageous traits, such as tiny size, versatility, and biocompatibility. The greatest research has been done on lipid-based nanocarriers liposomes, solid lipid nanoparticles, and nanostructured lipid carriers for the delivery of antifungal drugs. Many of these nanocarriers, including liposomes, have successfully completed numerous clinical studies for the treating of invasive mycoses. In fact, the commercialization of liposomal amphotericin B represents a significant advance because it permits the use of this highly effective antifungal drug in medical settings with next to no harm. Various other nanoparticle formulations have shown encouraging results when it comes to improving the water soluble of antifungal drugs, their antifungal efficacy, improving stability, and concentrating on sick tissue. Nonetheless, Amphotericin B is almost the only antifungal medication to have successfully finished clinical tests and been produced in nanoformulation. Since these issues hinder formulations based on nanoparticles from being used in clinical settings, efforts in the field of antifungal drug delivery should focus on finding answers to these issues.

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Table 1 . List of Antifungal drugs with Nano system

| Sl.no | Antifungal drugs | Nano system | Dosage form | References |
|-------|------------------|-------------------------------|-----------------|------------|
| 1. | Amphotericin B | Solidlipid nanoparticles | Topical gel | (31) |
| 2. | Nystatin | Nanocapsular hydrogel | Topical gel | (68) |
| 3. | Voriconazole | Nanoparticles | Topical gel | (69) |
| 4. | Posaconazole | Nanostructured lipid carriers | Topical NLC gel | (67) |
| 5. | Fluconazole | Solidlipid nanoparticles | Topical gel | (70) |
| 6. | Terbinafine HCL | Solidlipid nanoparticles | Topical | (71) |





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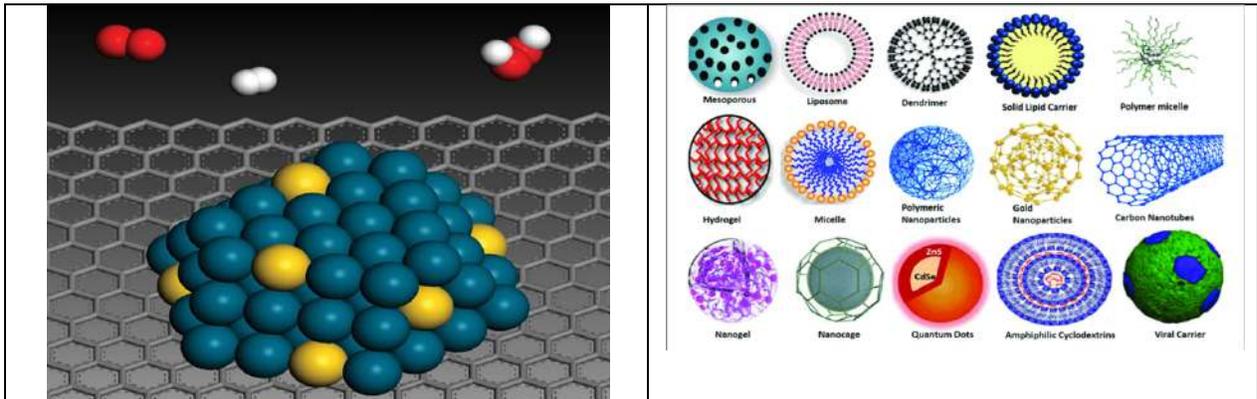


Figure1:Nanoparticles (7)

Figure 2: Different types of nanoparticles (11)

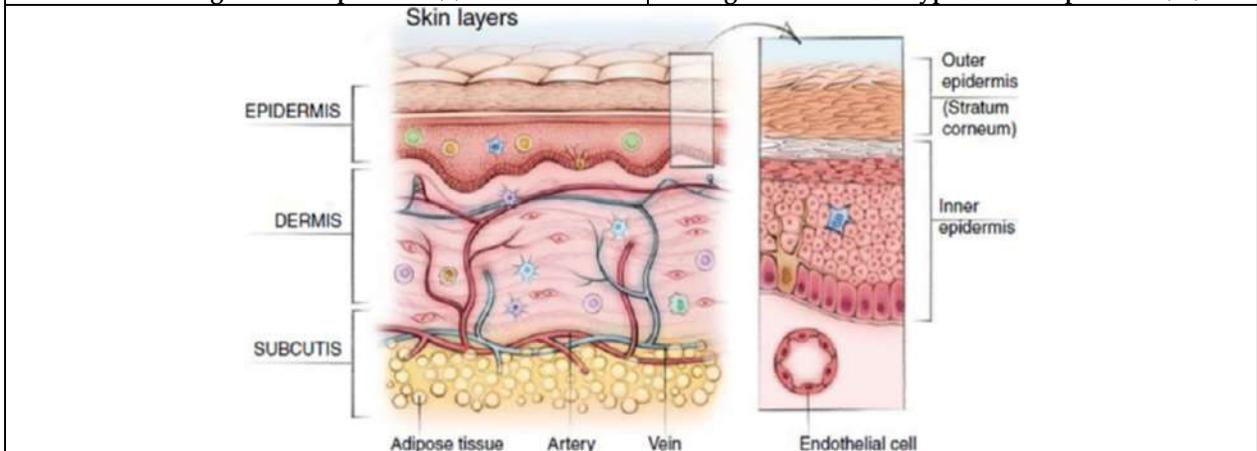


Figure 3: Skin structure(22,23)





Academic Achievement among Adolescent Students of Kashmir Valley in Relation to their Academic Procrastination

Aadil Hussain Mir^{1*} and R.Sivakumar²

¹Ph.D, Research Scholar, Department of Education, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

²Associate Professor, Department of Education, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India

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*Address for Correspondence

Aadil Hussain Mir

Ph.D, Research Scholar,
Department of Education,
Annamalai University, Annamalai Nagar,
Chidambaram, Tamil Nadu, India.
E.Mail: mirshahid916@gmail.com



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ABSTRACT

The current investigation was undertaken to know the academic achievement among adolescent students of Kashmir valley in relation to Academic procrastination. The investigator had selected 200 adolescent students from Kashmir valley. Stratified random sampling were used in order to collect the sample. Self constructed scale of academic procrastination were adopted to know the level of procrastination tendency among adolescent students and Academic achievement scores has taken from school. Pearson's product moment of correlation was applied to find out the relationship and 't' test was applied to find out the significance of the difference between different variables. The findings of the study revealed that is significant and negative relationship between academic achievement and academic procrastination among adolescent students of Kashmir valley. The study also found that there is significant difference in the academic procrastination of boys and girls, urban and rural adolescent students.

Keywords: Academic procrastination, Achievement, relation, Adolescent, student

INTRODUCTION

In the present era everyone strives for excellence and perfection, success is something that every person aspires to. For students, success entails academic achievement, which ought to be properly matched with the pillars on which their future success in life will be built. In order to accomplish the child's ideal and harmonious growth, academic success is crucial. it has always been a key issue and the focal point of educational research despite varied statement about the aim of education. Excellence in all academic fields, in the classroom, and in extracurricular activities can be



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regarded as academic achievement. Academic success is a performance indicator that shows how well a person has achieved particular objectives. Academic achievement refers to the outcomes in the classroom that show how well a student has met their learning objectives. Exams or ongoing evaluations are frequently used to gauge it. According to Good (1959), Academic achievement is defined as “the information obtained or abilities developed in the academic areas, typically assessed through test results or instructor evaluations.” Achievement of the student may be influenced by several factors like study habits, intelligence, Academic Procrastination, socio-economic status, parental support .one of the main factor affects the achievement of the student is academic procrastination. Academic procrastination is a phenomenon where students unnecessarily postpone or delay academic tasks until the last moment. Academic procrastination is the practice of delaying completion of required academic work such as exam and term paper writing, managing school-related administrative tasks, and maintaining attendance obligations. (Özer, Demir, & Ferrari, 2009). Academic procrastination has been identified in educational studies as an unreasonable propensity to put off starting or finishing a work (senecal et al. 2003). It has been demonstrated that procrastination occurs frequently in academic contexts, particularly among college students. According to estimates, between 50% to 95% of college students, regularly putt off doing their work (steel and Ferrari,2013). This has a considerable impact on the students’ performance.

NEED AND IMPORTANCE OF THE STUDY

Academic success plays a significant role in both education and the learning process. In this fiercely competitive society, it has evolved into a predictor of a child’s future. It is the primary objective that every person in every society is supposed to achieve. Teenagers learn about their talents, abilities, and competences through academic accomplishment, which is a crucial component in developing job aspirations. Student accomplishment is one of the most crucial results of any educational establishment. Individuals are classified as high, average, or poor achievers based on their degree of success. But in this cutthroat dynamic world students are disposed to innumerable distractions. These distractions result in academic procrastination, where a student frequently delay or postpone their academic tasks until the last moment which may put an adverse impact on academic achievement of the students as it is associated with a wide range of academic issues .Studies shows that there is a negative correlation between Academic achievement and Academic procrastination.(Ojo Adenlyl, 2019, Das A. 2016, Akpur U.2020,Ucar H.2021, Jaafar N. 2022).The related literature indicated the evidences regarding the association of students Achievement and Academic procrastination but none of the study was found pertaining to Adolescent students from Kashmir valley .so a need was felt to study academic achievement in relation to academic procrastination of adolescent students of Kashmir valley.

REVIEW OF RELATED LITERATURE

The researcher becomes familiar with existing information about the study being investigated after reviewing relevant literature. It lessens the possibility of repetition. It aids the researcher in determining the nature of the variables, the topic of study, the breadth and boundaries of the issue. It provides reassurance to the researcher that the issue under examination has antecedents in published literature and necessitates additional research. It Provides the researcher with extensive background information on the correlations between the variables and the results of previous studies that are relevant to the issue under examination. The researches that has been done in the past , it can be said that higher the academic procrastination of the students, the lower will be the academic achievement(Kim and Seo, 2015). Kader ahmad (2014) conducted a study on academic procrastination and student achievement in an introductory economics course. Findings showed that procrastination has a significant and negative impact on student achievement. Kuftyak E (2022) investigated procrastination, stress and academic performance and it was found that academic Procrastination hinders students’ ability to learn, puts an adverse impact on their performance, increases stress, and surely has an effect on how future specialists will develop professionally. Balhara & Mittal (2022) revealed in their study that the students having high and low academic procrastination is significantly different as far as their academic achievement is concerned. Male students were more prone to procrastinate than female students. It was also reported that significant negative correlation were found



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between academic achievement and academic procrastination of secondary school students. Bashir & Gupta (2018) made a correlation study between academic procrastination and academic performance. Findings of the study depicted that there exists a significant negative relationship between academic procrastination and academic performance of university students. The study also revealed that male and female university students are significantly different in their academic procrastination and academic performance. Above given studies are related to the academic procrastination and its effects on the academic achievement, several studies were conducted to know the impact of academic procrastination on the academic achievement of adolescent students. Unfortunately, not a single work was found on the adolescent students of Kashmir valley. So, this study represents a feeble effort to close the current gap.

OPERATIONAL DEFINITION

Academic achievement: Academic achievement is the end result of education; it measures how well a student, teacher, or institution has accomplished short- and long-term educational objectives. Exams or ongoing evaluations are frequently used to gauge it. In the present study it refers to the score obtained by 12th standard students in their previous annual examination (11th) conducted by the school affiliated to JKBOSE.

Academic procrastination: it is a phenomenon where students unnecessarily delay their academic tasks until the last moment. In this study it is defined as the tendency of a learner to intentionally postpone academic chores afflicted by the behavioral, institutional and social nuances.

Adolescent students: It refers to the students who are studying in class 12th

OBJECTIVES OF THE STUDY

- To study the relationship between academic achievement and academic procrastination of students of Kashmir valley.
- To study if any significant difference exists between academic procrastination of adolescent girls and boys.
- To study if any significant difference exists between academic procrastination of rural and urban adolescents.

HYPOTHESIS:

- There exists no significant relationship between academic achievement and academic procrastination of adolescent students of Kashmir valley.
- There exists no significant difference between academic procrastination of adolescent boys and girls.
- There exists no significant difference between academic procrastination of rural and urban adolescents

METHODOLOGY

Observational method, case study method, and survey research are the various approaches of the descriptive method. From the different approaches that may be employed in descriptive research, normative survey method was adopted for collecting data relevant for the study, considering the objectives.

SAMPLE

A sample is a portion of the population from which the researcher hopes to extrapolate the findings. For the purpose of choosing the sample, stratified random sampling was used. 200 adolescent students of Kashmir valley studying in 12th standard were chosen as a sample out of which 100 boys and 100 girls.

TOOL USED

Academic procrastination scale: Academic procrastination scale was constructed and validated by Aadil hussain and R. sivakumar (2022) in order to know the level of academic procrastination among adolescent students of Kashmir valley. The scale is having 30 items and is based on three dimensions on 5- point likert format, each statement is rated on five-points, (strongly agree=5, agree= 4, undecided= 3, disagree= 2 and strongly disagree= 1).



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Academic achievement: The score that the adolescent students received in their previous class (the 11th) were gathered from school records and used as an indicator of their academic performance.

STATISTICAL TECHNIQUE USED:

In order to determine the difference between two groups, the investigator employed the statistical technique known as the “t” test. Pearson’s product moment of correlation technique were also adopted to study the relationship between academic achievement and academic procrastination of adolescents of Kashmir valley.

ANALYSIS AND INTERPRETATION:

Results were systematically presented in the below mentioned tables.

Hypothesis 1- There is no significant relationship between academic achievement and academic procrastination of adolescent students of Kashmir valley as shown in Table no. 1.

Hypothesis 2:

There exists no significant difference between academic procrastination of adolescent boys and girls as shown in Table no. 2.

Hypothesis 3:

There exists no significant difference between academic procrastination of rural and urban adolescents shown in Table no. 3.

FINDINGS

There exists significant and negative correlation between academic achievement and academic procrastination of adolescent students of Kashmir valley, which means the higher be the academic procrastination, the lower will be the academic achievement and vice versa. Significant difference was found between adolescent boys and girls of Kashmir valley with respect to their academic procrastination. Adolescent boys are more prone to procrastinate the academic tasks than girls.

Significant difference was found between urban and rural adolescent students of Kashmir valley with respect to their academic Procrastination. Urban adolescent students have more procrastination tendency than rural one.

DISCUSSION OF THE RESULTS

One of the most crucial elements of the educational system and the main determinant of the evaluation of educational excellence is student academic accomplishment. Academic procrastination is one of several factors that affect academic accomplishment, and it is a significant one. So, the goal of the current study was to draw conclusions about how academic accomplishment and academic procrastination of teenage students in the Kashmir valley relate to one another. Academic procrastination is obviously a severe problem that has to be addressed because it has a negative impact on students' academic progress. Some characteristics of procrastinators include their preference for low-level assignments, poor time management, reluctance to exhibit their skills and potential, difficulties making decisions, and a tendency to dwell on the past. Hence they pass up chances, postpone academic chores and leave their tasks incomplete. Like Bashir and Gupta (2018) examined that there is a negative relationship between academic procrastination and academic achievement. Our result showed the same. As per this study students having low procrastination had good academic success than those who are having high procrastination tendency in the academic chores. Our findings have also support from different researches which conforms that academic procrastination is adversely related with academic achievement (Ahmad, 2014; Kufiyak, 2022; Kim & Seo, 2015; Balhara & Mittal, 2022).



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CONCLUSION

The study focused on the correlation between academic procrastination and academic achievement. The results revealed that there exists significant difference between adolescent boys and girls of Kashmir valley with respect to their academic procrastination; there exists significant difference between urban and rural adolescent students of Kashmir valley with respect to their academic Procrastination. Negative significant relationship was found between academic achievement and academic procrastination of adolescent students of Kashmir valley.

EDUCATIONAL IMPLICATIONS

Academic achievement is essential because it directly decides the positive results of the students and has always served as a yardstick to measure the learning outcomes. It allows the students to enter competitive fields. But in a competitive dynamic world adolescent students are disposed to innumerable distractions. These distractions result in Academic procrastination which is a serious issue that needs to be addressed. Therefore it is the responsibility of the stakeholders especially teachers to come forward in order to play a constructive role to curb the tendency of procrastination among adolescent students.

- Instructors who function as role models must explain to the pupils the consequences of procrastination so that they, too, are aware of its detrimental effects.
- Positive self-talk, relaxation techniques, and meditation should all be promoted by teachers.
- Teacher should acknowledge and identify the students' achievement wholeheartedly.
- Teachers can inspire students to put forth their most prized endeavor by providing them with honest comments and guidance as they complete tasks.
- Teachers should provide ample opportunities to the students to take part in the process of making classroom decisions.
- Teacher should use transformational education model as it will improve students' self-efficacy.
- The school can host a variety of seminars and workshops on topics like how to manage stress, the advantages of meditation, leading a healthy lifestyle, and preparing for exams, etc.
- Students should prioritize their efforts to handle assessment scenarios by completing academic work as soon as possible, commit to their studies with the assistance of their teachers, and have faith in their abilities.
- Students should create a decent schedule so they may finish their job on time and live with less stress.
- Students should prepare themselves before for examination instead of waiting for the last moment.
- A student should divide their tasks into minor parts to complete it on a time instead of giving up.
- A student should never feel lethargic towards studies and remember the worth of time.
- A student should never feel low by comparing themselves with their batch mates and friends.
- Parents should never pressurize their children to achieve more while comparing them with others.
- Parents should not quarrel before their children as it will negatively impact the child's personality development.

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Table -1 Showing the variables, r value and their significant level

| SL.NO | Category | No. of students | Df(N-2) | R value | P Value | Level of significance |
|-------|--------------------------|-----------------|---------|---------|---------|-----------------------|
| 1 | Academic achievement | 200 | 198 | -.532 | 0.000* | Significant |
| 2 | Academic Procrastination | 200 | 198 | | | |

Table 1 depicted that the calculated r value is greater than table value at 0.01 level, hence the null hypothesis stands rejected .Thus there is significant negative relationship between academic achievement and academic procrastination, which means that the higher will be the academic procrastination ,the lower will be academic achievement and vice versa.

Table -2 : Result of Mean, SD, t- value of adolescent boys and girls with respect to Academic procrastination

| SL.No | Gender | No. of students | Mean | S.D | 't' value | P value | Level of Significance |
|-------|--------|-----------------|-------|------|-----------|---------|-----------------------|
| 1. | Boys | 100 | 86.1 | 14.1 | 2.42 | 0.017* | Significant |
| 2. | Girls | 100 | 81.82 | 9.95 | | | |

Table 2 depicted that the calculated 't' value is greater than the table value at 0.05 level. Hence the null hypothesis stands rejected. So we can say that there is a significant difference between academic procrastination of adolescent boys and girls.



**Aadil Hussain Mir and Sivakumar****Table – 3: Result of Mean, SD, t- value of urban and rural adolescent students with respect to Academic procrastination**

| SL.No | Gender | No. of Students | Mean | S.D | 't' value | P Value | Level of Significance |
|-------|--------|-----------------|------|------|-----------|---------|-----------------------|
| 1. | Urban | 100 | 88.2 | 11.2 | 5.08 | 0.000* | Significant |
| 2. | Rural | 100 | 79.6 | 12.3 | | | |

Table 3 depicted that the calculated 't' value is higher than the table value at 0.01 level. Hence the null hypothesis stands rejected. This shows that there is significant difference between urban and rural adolescent students of Kashmir valley with respect to their academic Procrastination.





A Study to Find the Effect of Inverse Ratio Breathing on Chest Expansion in Post Upper Abdominal Surgery Patients - An Interventional Study

Krishna Sheth^{1*}, Pragna Gondaliya², Haseena Makrani¹ and Devki Mehta¹

¹MPT Scholar at Shree K.K. Sheth Physiotherapy College, Rajkot, Gujarat, India.

²Associate Professor at Shree K.K. Sheth Physiotherapy College, Rajkot, Gujarat, India.

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*Address for Correspondence

Krishna Sheth

MPT Scholar at

Shree K.K. Sheth Physiotherapy College,

Rajkot, Gujarat, India.

E.Mail: krisha202c@gmail.com



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ABSTRACT

Patients who underwent upper abdominal surgery usually develops pulmonary complications, with changes to pulmonary mechanics in the first postoperative days. Respiratory exercises during hospitalization helps to prevent postoperative pulmonary complications. The Inverse Ratio Breathing is the breathing technique in which inspiration is prolonged, such that inspiration time is double than expiration time in order to increase the volume of the thoracic cavity than in normal spontaneous breathing and thus reduces the post operative pulmonary complications. 30 patients were included and were divided into 2 groups. Group A received Inverse ratio breathing along with conventional physiotherapy, and Group B received conventional physiotherapy alone once a day for Postoperative day 1 to post operative day 3 and chest expansion at xiphisternal level was measured pre and post treatment. The study showed a statistically significant difference in Interventional group ($p < 0.05$) Inverse ratio breathing along with conventional physiotherapy helps to increase chest expansion among post upper abdominal surgery patients.

Keywords: Chest expansion, Inverse ratio breathing, post upper abdominal surgery, post operative pulmonary complications.



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INTRODUCTION

Upper abdominal surgery is defined as any surgical procedure performed through an incision above or extending above the umbilicus [1]. Abdominal Surgery has become an important part of global health care, which is estimating about 234 million patients operating per year [2]. In India, the overall incidence of post-operative complications was 41.6% after upper abdominal surgeries [3]. General anesthesia and peri-operative drugs affect breathing regulation by altering neural drive, which causes the reduction in functional residual capacity and disruption of ventilation-perfusion ratio due to the decrease in pulmonary compliance and eventually causing hypoxemia and increase in the respiratory rate [4,5]. Due to reflex inhibition of the phrenic nerve and nerve innervating abdominal muscles, surgical incisions near the diaphragm and abdominal muscles leads to postoperative pain and limit respiratory movement. So, Due to these reasons, there is chance of the development of the post operative pulmonary complications and one such post operative complication is the reduction in chest expansion [6].

The normal inspiration to expiration ratio is 1:2. The subject can prolong the duration of inspiration such that it becomes double the duration of expiration. This is called inverse ratio breathing. By prolonging inspiration in such manner, the contraction of the diaphragm and external intercostal muscles increases, which further increases the volume of the thoracic cavity than in normal spontaneous breathing. The accessory muscles of inspiration are also put into the use [7]. Chest expansion measurement are used to evaluate a patient's base line status, treatment effectiveness, and progression of disease with regard to chest mobility and respiratory muscle function, in clinical setting; a simple and inexpensive technique for measuring chest expansion is a tape measure which has been shown to be reliable in healthy volunteers [8]. The reliability of this technique shows an interclass correlation coefficient of 0.81 to 0.91, proving it reliable in clinical settings [9]. Major upper abdominal surgeries is primarily endangered by postoperative complications and can lead to morbidity and mortality. The physiotherapy treatment should be such that it facilitates the recovery from surgery by preventing or treating the complications and aiding the patient in returning to pre-morbid physical function. So, the purpose of the study was to find the effect of Inverse ratio breathing to improve chest expansion after upper abdominal surgeries.

Aim of the study

The aim of the study was to find the effect of Inverse ratio breathing on chest expansion in post upper abdominal surgery patients.

Objectives of the study

To find out the effect of Inverse ratio breathing on chest expansion in post upper abdominal surgery patients.

METHODOLOGY

- Study setting- Hospitals in and around Rajkot.
- Study population- Post upper abdominal surgery patients.
- Source of data- Hospitals in and around Rajkot.
- Sampling technique- purposive sampling
- Sample size- 30
- Study duration- once a day for Post operative day 1 to post operative day 3

The study was conducted on 30 upper abdominal surgery patients between the age group of 35-50 years. Subjects were taken from hospitals in and around Rajkot city. Prior to the participation, all the subjects were explained briefly about the aims and objectives of the study, health benefits and procedures for measuring chest expansion. Informed consent signed by the subjects for their voluntary participation was taken. Then the following information was recorded for each subject- Name, age, gender, occupation, address, weight, height, BMI, heart rate, respiratory rate and chest expansion.





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Inclusion Criteria

- Age group: 35-50 years
- Both male and female patients were included.
- BMI range from 18 to 25 kg/m²
- Patients who underwent upper abdominal surgery (midline, transverse and Para-median incision)

Exclusion Criteria

- Patient who underwent abdominal surgery in the past
- Patients with infection, active inflammation, sepsis
- Patients on mechanical ventilation.
- Heavy smoker patients.
- Patients having any chest or cardiovascular diseases in the past.
- Patients having any neurological disorders and orthopedic disorders which are affecting pulmonary function.

MATERIALS

- Measure tap
- Pillows
- Pen and Paper
- Assessment form
- Consent form
- Data collection form

PROCEDURE

30 Patients with upper abdominal surgery were selected by purposive sampling, and the study was explained to them then, patients were randomly assigned into two groups.

1. Group-A: 15 patients had received Inverse ratio breathing and conventional physiotherapy.
2. Group-B: 15 patients had received conventional physiotherapy alone.

Treatment protocol

For group A [7]

- Subjects were asked to lie comfortably.
- An Auditory commands were given to the subjects.
- The subjects were instructed to carry out inspiration for 4 seconds at a constant speed and to carry out expiration for 2 seconds.
- This breathing technique was performed for 1 minute, then 1 minute rest was given, again breathing technique was given for 1 min followed by 1 min rest. Likewise, for total of 10 minutes. Also, conventional physiotherapy in the form of bed mobility, positioning, breathing exercises and splinted coughing has been given
- One session per day for POD-1 to POD-3
- Before and after performing the breathing technique, the chest expansion measurements was taken.

For group B

- Bed mobility:-Bed mobility and independence following abdominal surgery were encouraged with a rope attached to the end of the bed by which patients can pull themselves up.
- Positioning: - Advice for Fowler's position (facilitates the relaxing and tension of the abdominal muscles, allowing for improved breathing), alternating from side to side and, if possible, sitting out of bed.
- Breathing exercises: - deep breathing, apical breathing and lateral costal breathing exercise were given.
- Splinted coughing: - In semi fowlers position, supporting the incision with the hand



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RESULTS

Statistical Analysis

Study design: Comparative interventional study.

Test: The Shapiro-Wilk test was used to check normality, and the data followed normal distribution. The collected data were analyzed by Paired t- test to compare the result within the group and Unpaired t- test to compare the result between groups with a 0.05 level of significance with a 95% of the confidence interval.

Table. 1 Intra group comparison of chest expansion at xiphisternal level

Table. 2 Inter group comparison of chest expansion at xiphisternal level

Graph: 1 Intra group comparison of chest expansion at xiphisternal level of Group A

Graph: 2 Intra group comparison of chest expansion at xiphisternal level of Group B

Graph: 3 Inter group comparison of chest expansion at xiphisternal level – Group A and B

DISCUSSION

The intent of the study was to find the effect of inverse ratio breathing on chest expansion in post upper abdominal surgery patients. The result of these study supports the alternate hypothesis. In group-A and group-B significant difference was found in pre and post intervention. Also Inter group comparison shows the significant difference that Group A showed more significant improvement than group B. W.P. Zhang et al. evaluated that in the patient during one-lung ventilation, Inverse ratio ventilation with PEEP improves hypoxemia, promote oxygenation and improve dynamic compliance of the respiratory system [10]. During Inverse ratio ventilation, inspiration time is more than expiration time, which increases Mean Airway Pressure (Average airway pressure over the entire respiratory cycle). An Increase in Mean Airway Pressure, increases alveolar stability and recruitment, and decreases the dead space ventilation thus improves the oxygenation and improves dynamic compliance. Along with these, as expiration time is less than inspiration time, so alveoli may not get sufficient time to empty completely during exhalation and the gas gets trapped in the lung. This trapped gas creates pressure in the alveoli, which is known as Auto-PEEP. These will recruit those alveoli which require more time to get recruited. Due to this, alveolar surface area increases, and more surface area is available for gaseous exchange, thus ventilation increases [11,12,13]. The present study shows statistically significant results for inverse ratio breathing, supporting to these W.P. Zhang et al. demonstrated that Inverse ratio ventilation increase Mean airway pressure improves oxygenation and dynamic compliance of respiration in the obese patient undergoing gynecological laparoscopy and also stated that Inverse ratio ventilation is superior to the conventional ratio ventilation [14].

Clinical implication

Reduction in chest expansion after upper abdominal surgery is common. Inverse ratio breathing is helpful in improving chest expansion. Thus Inverse ratio breathing with Conventional physiotherapy is prescribed for the improvement and maintenance of chest expansion.

Limitations of the study

- Small sample size
- Study duration of the treatment protocol was short.
- Anesthesia may hamper the performance of the patient; it was not taken into consideration.

Further recommendation

- Study can be done with the larger sample size.
- Length of stay and pain can also be taken for consideration.
- Study can be done with other types of incision (e.g., Thoracic).
- Study can be done by taking the follow up after one or two weeks.





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CONCLUSION

From this study, it can be concluded that three days of combined Inverse ratio breathing technique with conventional physiotherapy showed more significant improvement in chest expansion than conventional physiotherapy alone for upper abdominal surgery patients.

Abbreviations

- **POD:** Post operative day
- **BMI:** Body mass index

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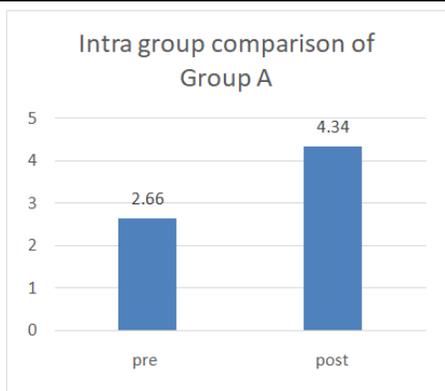
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Table 1

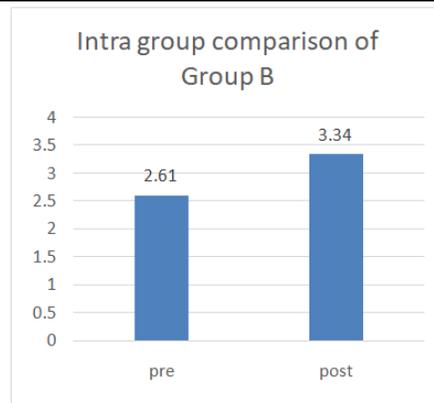
| Group | Pre mean ± SD | Post mean ± SD | t value | p value | Result |
|-------|---------------|----------------|---------|---------|-------------|
| A | 2.66 ± 0.77 | 4.34 ± 0.69 | -12.78 | < 0.05 | Significant |
| B | 2.61 ± 0.79 | 3.34 ± 0.74 | -9.65 | | |

Table 2

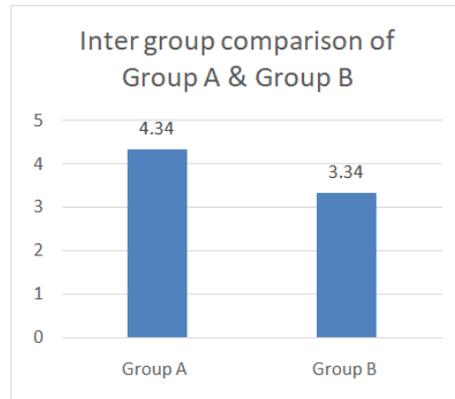
| Group | Mean ± SD | t value | p value | Result |
|-------|-------------|---------|---------|-------------|
| A | 4.34 ± 0.69 | 3.81 | <0.05 | Significant |
| B | 3.34 ± 0.74 | | | |



Graph 1 Intra group comparison of Group A



Graph 2. Intra group comparison of Group B



Graph 3. Inter group comparison of Group A & Group B





Natural Growth Promoter Effect of Garlic, Ginger and fenugreek on *Fenneropenaeus indicus*

Renupriya Kumar¹, Kalaiselvam Murugaiyan^{2*} and Shantanu Jana³

¹Research Scholar, CAS in Marine Biology, Annamalai University, Parangipettai-608502, Tamil Nadu, India.

²Director and Professor, CAS in Marine Biology, Annamalai University, Parangipettai - 608502, Tamil Nadu, India.

³Student, CAS in Marine Biology, Annamalai University, Parangipettai-608502, Tamil Nadu, India.

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*Address for Correspondence

Kalaiselvam Murugaiyan

Director and Professor,

CAS in Marine Biology,

Annamalai University,

Parangipettai - 608502, Tamil Nadu, India.

E.Mail: sweetrenupriya24@gmail.com



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ABSTRACT

Sea food has excellent nutrient value such as proteins, fats, lipids, amino acids and numerous minerals which benefit the healthy human diet. Aquaculture is the major industry in production of shrimp, fishes and many more sea food-based supplements. The major goal in aquaculture production of sea food is to have enhanced immunity, environment and growth rate in prawn and fishes. The use of medicinal herbs to increase immunity has been discussed in various studies. In this study, the effects of garlic, ginger, fenugreek seeds on the growth of the *Fenneropenaeus indicus* have been classified and reviewed. In this way, these medicinal herbs enhance the aquaculture production with natural benefits and its harmless in animal diet. The research studies on natural growth promoters effects on *F.indicus* was analyzed based on increase in weight, intake rate, survival rate, growth performance rate which gave significant results that justifies the beneficial use of these herbs as growth promoters in aquaculture.

Keywords: *Fenneropenaeus indicus*, Garlic, Ginger, fenugreek, growth promoter

INTRODUCTION

Mankind is losing ground in the struggle to feed itself. Today the limited quantity of food calories is a great concern to many parts of the underdeveloped world, but the quality, notably of proteins is more crucial. Supplies of proteins



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are particularly scarce and costly in most nations. For over one-third of their populations, the protein problem is reaching a crucial point (Sukhatme.,1974). Fish and Fishery products are considered superior to red meat in many parts of the world (Pigott., 1990). As a result, the consumption of fishery products is on the increase among the health-conscious world population, especially in developed countries. Besides, as a source of food, fishery products also find some significant industrial uses. Fishery products are either exploited from the natural water bodies through various methods of fishing or raised artificially in a suitable environment through different kinds of aquaculture. India is considered one of the world leaders in shrimp production and expertise. Considering the requirement of precious foreign exchange for the country for the national prosperity.

The Ministry of Commerce has identified marine products export as a thrust area for development. Among the marine products, shrimp production is the dominant item. Sustainable Aquaculture of shrimp partially depends on the environmentally friendly and economically feasible and fully balanced meeting of the full requirements. It is worth noting that the production rate exceeding 1 ton/ha/ crop cannot be achieved without the involvement of well-balanced artificial feeds. At high stocking densities, inadequate feeding leads to poor growth. Appropriate feed formulations should meet an adequate level of dietary requirements. The dietary requirement can be considered under different groups such as energy, protein, lipids, carbohydrate, vitamins, and minerals. Shrimps are aquatic organisms inhabiting the Sea and estuaries. Nearly, 5,64,800 tons from a total culture area of 10,92,300 ha from a total culture area of 10,92,300 ha, of which 8-9% of shrimp from Asian countries, China topping the list recorded an average production of 1,138 kg/ha 1.65 lakh ton from 1,45,000 ha (The GOAL 2016 shrimp production survey).

For the high production, people started to incorporate antibiotic growth promoters in feed. But due to its negative impacts (residual accumulation in fish tissue, emergence of antibiotic-resistant microbes), natural compounds are more acceptable to the public. The herbal immunostimulants have been reported to enhance the efficiency of feed utilization and animal productive performance (Levis et al., 2008). The herbal plants have a wide variety of properties such as: antioxidant, antimicrobial, anticarcinogenic, analgesic, insecticidal, antiparasitic, anticoccidial, growth promoters, appetite enhancement, and stimulant of secretion of bile and digestive enzyme activity, laxatives and antidiarrhea, hepatoprotection (Coutteau et al., 2011). Garlic, *Allium sativum* L., has been used for the treatment of many diseases since ancient times as reported in the Codex Ebers (1550 BC) where an Egyptian medical papyrus described several therapeutic formulas based on the garlic as a useful remedy for a variety of diseases such as heart problems, headache, bites, worms, and tumors. Garlic (*Allium sativum*) has several beneficial effects for humans and animals, exhibiting antimicrobial, antioxidant, and antihypertensive properties (Sivam 2001). Garlic can help in the control of pathogens, especially bacteria and fungi, and increase the welfare of fish (Corzo., 2007). Ginger (*Zingiber officinalis*) belongs to Zingiberaceae family. The part of the plant used is the rhizome, an important spice. The use of spices as food and feed additives has been practiced widely since ancient times. To date, no study has been carried out on the shrimp with *Z. officinalis* as an herbal appetizer. Therefore, in the present study, *Z. officinalis* was chosen and the stimulatory effect was verified and fed to postlarvae (PL-1–30) of *Penaeus monodon* through the live feed *Artemia franciscana*, because of its versatile characteristics, such as taste, high nutritive value, non-selecting filter-feeding capability, and non-contamination of the culture water. Ginger increases the pancreatic and intestine lipase (Platel and Srinivasan., 2000). Fenugreek (*Trigonella foenumgraecum*) is an annual herb that belongs to the family Leguminosae widely grown in Pakistan, India, Egypt, and Middle Eastern countries (Alarcon- Aguilara et al., 1998). Fenugreek has also been reported to exhibit pharmacological properties such as antitumor, antiviral, antimicrobial, anti-inflammatory, hypotensive, and antioxidant activity (Cowan et al., 1999 Shetty et al., 1997).

MATERIALS AND METHOD

Tank setup

The aquarium tanks used for experiments were of size 80x50x50 cm. Four aquariums including control were stalked on a wooden rack. Aquariums were located in a secured place where there is no direct sunlight and covered all the



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sides with black paper to avoid algal growth in the tank. Water in the aquarium was connected to the air compressor. The Vellar estuaries water was taken into the tank and allowed to aerate for 48 hours and used for filling the aquarium.

Sample collection

Healthy shrimp seeds of *Fenneropennaeus indicus* were collected from Vellar estuary Parangipettai (Latitude 11°30'08"N & Longitude 79°45'14"E), Cuddalore district. The shrimp seeds were visually observed and then the various size was stocked in a glass tank and acclimatized to laboratory condition for one day only. A total of 100 no's seeds were collected. In each aquarium tank, 25 numbers of shrimp were introduced. The water exchange of 25% was done in all shrimp tanks every day. Leftover feed, excreta, and other debris were siphoned off from the bottom of the tank without disturbing the shrimps.

Pellet preparation

Garlic, ginger, and fenugreeks seed were purchased in sufficient quantities from the local market. The ingredients were sun-dried for 2 weeks and powdered at required quantities before feed preparation. Four experimental diets were prepared by supplementing a basal formulated diet with different levels of 0% (Control), 4%, 2.5%, and 1% of garlic, ginger, and fenugreek powders respectively. The growth parameters of all the shrimps of each aquarium were individually estimated by taking their total body length and weight at every 7 days. Diet of known size (2mm) were taken and gently dropped into a rectangular fiberglass tank (size 80x50x50 Cm) with a water level of 30cm. The time taken for its disintegration was taken as the stability period of the feed diet. It is expressed hours. The sinking rate of diet was determined in a measuring jar of 1-liter capacity. Five uniform-sized diets were gently dropped into water individually and the time taken by each to travel the known depth of water was noted using a stopwatch. The average time taken by each test diet was calculated separately and the values were expressed in cm/second.

In the present study, the weight and growth rate of shrimp were calculated in experimental and control tanks during the incubation period of 45 days. During the culture of shrimps, the water quality parameters such as salinity(ppt), dissolved oxygen (ml/litter), temperature (c), and pH were measured. The salinity of the water is measured by a salinometer, Atago, co. Ltd Japan and is expressed in part per thousand. Dissolved oxygen of water was estimated by Winkler's method and expressed in ml/liter. The temperature of the water was measured using a celsius thermometer. The hydrogen ion concentration of the water was measured using pH pen.

RESULTS AND DISCUSSION

The stability of the pellet diet (shrimp feed) is expressed in hours. The stability of the diet was less than 4-5 hours. The result is shown in Table 1. stability of the pellets

ENVIRONMENTAL PARAMETERS

During the experimental period, the lowest Salinity of water was 15ppt, and the highest was 20ppt. The dissolved oxygen concentration of the water was between 4.5 to 5.5 ml/l. During the experiment, the lowest and highest water temperatures were 24°C and 28°C. respectively. The pH values were also recorded during the experiments. The pH of the water ranged from 7.6 to 8.4.

SURVIVAL RATE OF SHRIMPS

It was interesting to note that the survival rate of shrimps in the experimental tanks fed by (Garlic, Ginger, and Fenugreek) \cong 92%, 96%, and 92% whereas it was founded at 80% in the control tank. A total of 100 no's seeds were collected. In each aquarium tank, 25 numbers of shrimp were introduced.



**Renupriya Kumar et al.,****GROWTH PERFORMANCE OF SHRIMP**

The growth performance of shrimp fed by growth promoters was studied. Data analyzed on the growth performance of shrimp in three experiments fed shrimps and control, including initial length and final length, initial weight and final weight, FCR, and survival rate, during the experiment are described below. No significant difference was measured for initial length and weight among control shrimps experiment fed shrimps during initiation of the experiment. At the end of the experiment, statistical analysis showed that shrimps fed ginger feed diet grew significantly faster than the control. During the culture of the shrimp *F. indicus* with the experimental diet, some interesting observations on molting were also made. The shrimp stopped the feeding for about two to three days before molting and the exoskeleton was broken along the swimming legs. Shrimp came out from the old case leaving the exoskeleton of the carapace almost interacting on the surface. The process of molting took place only at night and was completed in 10-15 minutes. The body was very soft and depressible on the first day morning. In the molted shrimp 55% of the body hardened on the second day 70% on the third day, 90% on the fourth day 100% on the fifth day. After that, the shrimp were normal in the activities subsequent and molting. The molting occurred quickly in the young ones' carapace and afterward the process became slow.

CONCLUSION

This investigation was carried out to study the effect of formulated dry pellet feed mixed with three different ingredients (garlic, ginger, and fenugreek) on the growth of shrimp *F. indicus* the experiment was carried out in the laboratory for 45 days. Three types of pellet feed are prepared by garlic, ginger, and fenugreek at 4%, 2.5%, and 1%. The water quality parameter was measured. The physical evaluation of the shrimp diet suggests that this diet was consumable and highly acceptable. During the experiments shrimp's final body weight was observed at 1.07 gm, 2.54 gm, 2.81 gm, and 1.21 gm in the control tank, garlic experiment tank, ginger experiment tank, and fenugreek experiment tank. During the experiments, shrimps final body lengths were observed at 3.9 cm, 4.5 cm, 5cm, and 4.3 cm in four different tanks (control, garlic, ginger, fenugreek). In four experiments tank (control, garlic, ginger, fenugreek) survival rate at 80%, 92%, 96%, 92%. During the experiment the shrimps *F. indicus* fed the experiment diets, some interesting observations on molting were also made these shrimp stopped feeding for about two to three days before the molting exoskeleton broke long the swimming legs. The process of molting took only at a night and was complete in 10 to 15 minutes. The Growth promoter and survival rate of *F. indicus* were done using herbs. The shrimps were fed with herbal feeds. This study revealed that *F. indicus* fed with ginger feed 2.5% diets showed the highest survival and found significant ($P < 0.001$) compared to other treatments. Growth-promoting substance digestive enzymes activators and other bioactive principles of garlic might have contributed to the increase of feed utilization in the garlic-supplemented diet. The constituents of zinger, have anti-microbial factor and anti-stimulant factor, may have contributed to the evaluated survival in the experimental group compared to control upon the biochemical evaluation protein shows the highest value followed by other biochemical constituents like amino acids, carbohydrates, and lipids. The information generated from the present investigation might contribute to the incorporation of herbs in commercial aquaculture as a supplement in formulated shrimp feed to achieve a good growth rate and survival.

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Table 1: Indicates the stability of the pellets

| Prepared Pellet Diet | Stability (in hours) |
|----------------------|----------------------|
| Garlic mixed feed | Less than 4.5 |
| Ginger mixed feed | Less than 4.5 |
| Fenugreek mixed feed | Less than 4.3 |

Table 2: The table shows the sinking rate of the different pellets

| Prepared pellet diet | Stability (cm /sec) |
|----------------------|---------------------|
| Garlic mixed feed | 7 |
| Ginger mixed feed | 7 |
| Fenugreek mixed feed | 7 |

Table 3 : Environmental Parameters of the water

| | |
|-----------------------------------|----------------|
| salinity | 15 -20 ppt |
| Dissolved oxygen | 4.5 -5.5 ml /l |
| Temperature | 24°C –28°C |
| Hydrogen -ion- concentration (pH) | 7.6 – 8.4 |

Table 4: Survival % of shrimps in 4 tanks.

| Prepared diet | Initial survival | Final survival | Percentage (%) |
|----------------------|------------------|----------------|----------------|
| Simple Feed | 25 | 20 | 80 |
| Garlic mixed feed | 25 | 23 | 92 |
| Ginger mixed feed | 25 | 24 | 96 |
| Fenugreek mixed feed | 25 | 23 | 92 |

Table 5: Result of weight gained, FCR, Survival rate values of shrimp, *F. indicus*

| Parameters | Control | Garlic | Ginger | Fenugreek |
|---------------|---------------|----------------|----------------|----------------|
| Weight gain | 1.07gm/shrimp | 2.54 gm/shrimp | 2.81 gm/shrimp | 1.21 gm/shrimp |
| FCR | 1:1 | 1:15 | 1:2 | 1:2.5 |
| Survival rate | 80% | 92% | 96% | 92% |





Beyond Cute and Cuddly: A Scientific Review on Behavioral Ecology, Distribution and Conservation Status of Red Panda in India

Dhruvajit Kalita and Manab Jyoti Kalita*

Assistant Professor (Contr.), Department of Zoology, Pandu College, Guwahati-781012, Assam, India

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*Address for Correspondence

Manab Jyoti Kalita

Assistant Professor (Contr.),

Department of Zoology,

Pandu College, Guwahati-781012,

Assam, India.

E.Mail: kalitamanabzoo@gmail.com



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ABSTRACT

Red Panda is a small, arboreal mammal native to the Himalayan region with distinctive chestnut fur colour, a bushy tail with dark-light patterns. From India, Red Panda is mainly reported in Sikkim, Arunachal and West Bengal. This study mainly reviewed the distribution pattern of Red Panda in India as well as emphasized the information about their behavioural ecology, threats along with conservation. The summary comprises 42 peer-reviewed and relevant articles on Red Panda using scientific research platforms like Google Scholar and Research Gate. Maximum reports from India were found elaborating on Red Panda's behaviour during captive conditions but very few reports have been gathered from the studies that were carried out in the wild and researchers have to dig up more information about the species from the wild for their better conservation.

Keywords: Red panda, distribution, Review, Threats, Conservation, India.

INTRODUCTION

The sole representative of the family Aliuridae[33] the Himalayan Red Panda (*Ailurus fulgens* F.G. Cuvier, 1825), also known as 'fire cat' [41], has been reported to be distributed in India, Nepal, Bhutan, Northern Myanmar, Tibet, and the western Yunnan province of China as well [36,18,10]. Except for their breeding period, Red Pandas are generally solitary [32] and during breeding season they are found as a 3-5 membered family within a small group. Despite having a charming appearance, this arboreal creature has chestnut coloured body with glossy brown eyes and six light and dark chestnut and buff bands in the tail region as peculiar appearance[24]. Red Panda's diet is strictly based on plants despite being a member of the Carnivora order like young bamboo shoots, mushrooms, roots, and acorns



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[38]. In India, Badola et al., 2020, include the species distribution as occurring solely in Sikkim, Arunachal Pradesh, West Bengal, and Meghalaya [3], however, Duckworth (2011) recounts sightings of red pandas in the hills above Haflong in the Barail range, Assam [13]. Moreover, about 90% of the total habitat is contributed by Arunachal Pradesh [26]. In India, this mammal is protected under schedule-I of the wildlife protection act, 1972 and most of its population resides in small and isolated protected areas (less than 500 sq km) and about 50% of Red Panda habitat has been lost in the last three generations, bringing it as endangered in the red list category [10,5,34]. These pandas are protected under Schedule I of the Indian Wildlife (Protection) Act, 1972 and also listed in Appendix I of the Convention on International Trade in Endangered Species (CITES) [19, 2, 25, 20]. Due to the declining population of Red Pandas, it has been placed on the Red List of Threatened Species in 2015 by IUCN and listed as Endangered (IUCN-2015).

HABITAT AND DISTRIBUTION

In India, Red Panda prefers dense moist temperate forests dominated by bamboo trees along with rhododendron vegetation [1, 24]. Red Panda appears to like ancient and large tree growth with abundance of hollows and dead snags, as well as near to water body, moreover, the slopes pointing the south and east are preferred by this animal. The altitudinal range has been reported of Red Pandas in Himalayas from 1500-4800m and in Meghalaya from 700-1400m [25]. The animal is also observed at a range of 200m in Balpakram National Park, Meghalaya [39]. India has Red Panda populations on around 11,778 square kilometres spread over 20 protected areas [8]. Despite the infrequent encounters, Red Panda was sighted in many areas of Eastern Himalayan Region:

Sikkim

Sikkim is the second largest habitat that accommodates the red panda [16]. Among the protected areas of Sikkim: Singma Rhododendron Sanctuary, Pangolakha Wildlife, Kyongnosla Alpine Sanctuaries, Maenam Wildlife Sanctuary, Barsey Rhododendron Sanctuary, Fambong Lho Wildlife Sanctuary and Khangchendzonga Biosphere are considered to be the wandering ground of the Red Pandas [10, 15].

Darjeeling (West Bengal)

Pradhan et al., 2001, confirmed the presence of Red Panda in the Singhalila National Park, Darjeeling which is situated on the north-west border of Darjeeling, India with a less number of encounter records with the animal by using direct and indirect evidence [28]. Apart from the Singhalila NP, the red panda has also been recorded from Neora Valley as well as Senchal Wildlife Sanctuary in the Darjeeling district [23]. In the lower portion of Neora valley, red panda was recorded at an altitude of 1311 m in the Mouchowki forest (East Nar Block) [23].

Arunachal Pradesh

Because of its highly rich diversity by virtue of geographical position, altitudinal variations and climatic conditions of Arunachal Pradesh is considered as most suitable habitat and Red Panda has been reported from 11 districts of this state which are namely: Changlang, Dibang valley, East Kameng, East Siang, Upper Siang, Lohit, lower Subansiri, Upper Subansiri, West Kameng, West Siang and Tawang [3, 7, 12]. Red Panda has also been recorded from protected areas like Moulting National Park, Namdapha National Park, Namdapha Tiger Reserve, Dibang, Eaglenest, Mehao, Sessa Orchid, Kamlang Wildlife Sanctuaries and Taley Valley Wildlife Sanctuary [7].

Meghalaya

Choudhury 2001, reported the sighting of Red Pandas from two protected areas: Nokrek National Park and Balpakram National Park in the Meghalaya state. [8]. However, Gosh and Dutta, 2011, stated that authentic sightings records or scientific investigations of red panda have not been made in this state [15].

Assam

Apart from the colder regions mentioned above related to red panda sightings, Assam and some districts of Meghalaya are exceptions. According to Duckworth 2011, the author received information of two sightings of Red Panda from North-East Cachar and Haflong but without any primary evidences [13, 14].



**Dhruvajit Kalita and Manab Jyoti Kalita****BEHAVIOURAL ECOLOGY****Reproductive behaviour**

The long-day breeders, Red Pandas are used to engage in reproductive activities between the months of January and March, resulting in offspring being born from May to August. In the southern hemisphere, captive pandas experience a delay of six months in their reproductive cycle [9]. Northrop and Czekala, (2011) reported that the breeding season of red pandas from different hemispheres (North and South) is different but these results are recorded in captive condition, hence further study is required in details. At the beginning of the breeding season the social interactions were seen to be significantly increased between the two separate sexes, and new born individuals attained the sexual maturity at the age of 18 to 20 months for both the sexes (in captive) [27]. Just before the mating period, the female one is acting more like a beneficiary by leaving scent marks surrounding her territory and walking around exposing the genitals [35, 27, 6]. The male becomes more active as an oestrus instigator [42]. Before mating and the beginning of the oestrus period, the females started a chirping sound and increases interaction with the male with high-pitched vocalization exchanged between the two sexes [27]. Red pandas experience a day-long period of oestrous, during which females can go through multiple cycles within a breeding season. However, the duration of the intervals between each cycle remains uncertain [9]. After successful mating and completion of gestation period that lasts about 131 days [30], the new born comes out. The newborn is clothed in thick buff fur at birth, and around day 15, just before the eyes open, reddish hairs develop. Their chestnut coloration of the adult fur is attained in around 2 months of age [24].

Feeding behaviour

Though the red panda was placed under order Carnivora and has a short digestive tract, it is a herbivorous animal and feeds on fresh leaves of bamboo [39, 31]. Their microhabitat is composed of fallen logs, tree stumps, and high shrub densities so that they can easily access the bamboo leaves using those objects [40]. Red pandas exclusively feed upon young bamboo leaves at the base of the stalk, unlike the Giant Panda of China, which eats the stalk itself [24, 11].

THREATS AND CONSERVATION

Apart from anthropogenic pressures, Red Pandas are influenced by some natural phenomena also. During studies related to Red Pandas, researchers learned that due to lack of awareness, these animals were hunted down and people got directly or indirectly benefited. Threats of Red Panda are broadly classified into two categories namely biotic and abiotic threats [23]:

Threats related to habitat

Threats those are directly influenced to the habitat of red pandas. These threats include habitat destruction for farming purposes as well as fragmentation of habitat, firewood demand etc. Habitat fragmentation impacts the movement of red pandas, and leads to loss of genetic variability [8].

Poaching and illegal trade

Poaching and trading is also one of the greatest threats of red pandas. Due to habitat destruction and shrinkage of forest area, the Red Panda becomes easy target to hunters, reducing the red panda's population. It has been reported that live trapping and trading of red pandas from their native place and hand them over to particular zoos which was an active practice before the Wildlife Protection Act 1972 [23, 29]. Ghose & Dutta, 2011, described the killing of this creature has been reported from different areas of Arunachal Pradesh like West Siang, West Kameng, and Dibang Valley [15].

Livestock grazing

Disturbances caused by the grazing of livestock mainly cattle, sheep etc restrict the free movement of red pandas. According to a report published in 2020, due to unregulated grazing behaviour shown by the livestock threaten the habitat and long-term survival of red pandas in the eastern side of Nepal [21, 36].



**Dhruvajit Kalita and Manab Jyoti Kalita****Encroachment**

Encroachment to the natural habitat of Red Panda may leads to habitat fragmentation. In Singalila National Park, there are still some villages resides inside the national park, which directly impact the movement of Red Panda and hence the threat persist [22].

Threats from ethnic beliefs

Some tribal people of Arunachal Pradesh in India considered wearing Red Panda's tail as good luck charm [17]. Apart from the India region these believes are also popular among the Tribes of other parts of the world. For example, In Nepal, there are some socio-cultural believes regarding Red Panda including medicinal use as well as making decorative items. These believes also considered the animal as a frightful one and predator to their livestock[4].

Forest fire

In Singalila and Neora Valley fire outbreak had been reported in the late 19th century which impacted the habitat as well as food of red pandas. [23].

Conservation

The Red Panda population faces high threats in Himalayan regions including India, Bhutan and Nepal [37]. Due to the increase in human population, the habitat of Red Panda is overlapped by human residential area which in turns increases the inter specific conflict between them. To deal with the declining population status of Red Panda and habitat degradation the protected area network especially in Arunachal Pradesh additional area need to be taken under forest departmental enforcement. Illegal deforestation, jhum cultivation in the high altitudinal area where Red Panda prefer to reside need to be controlled. Forest dwellers and neighbouring people of the villages are less aware about the legal conservation status of Red Panda, hence massive awareness campaign among local people, NGOs, and volunteers will be beneficial to the conserve of the species [8]. Anti-poaching squads and Community-based red panda monitoring squads have been developed in habitat of Red Panda in the Himalayan region to keep an eye on animals were formed, where there are sufficient people resources[37]. Workshops focussing on species conservation strategies were held in countries such as India (in 2013), Nepal (in 2010) and China (in 2012) within the Himalayan region [37].

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Phytobiotics as Potential Natural Supplements on Growth, Intestinal Histology and Biochemical Indices of Fish – A Review

Kabir Alom Ahmod¹, Shuraim Zoha¹ and Krishnakumar Velayudhannair^{2*}

¹M.Sc Student, Department of Life Sciences, CHRIST (Deemed to be University), Bangalore Central Campus, Hosur Road, Dharmaram post, Bengaluru -560029, Karnataka, India.

²Assistant Professor, Department of Life Sciences, CHRIST (Deemed to be University), Bangalore Central Campus, Hosur Road, Dharmaram Post, Bengaluru -560029, Karnataka, India.

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*Address for Correspondence

Krishnakumar Velayudhannair

Assistant Professor,

Department of Life Sciences,

CHRIST (Deemed to be University),

Bangalore Central Campus, Hosur Road,

Dharmaram Post, Bengaluru -560029, Karnataka, India.

E.Mail: krishnakumar.v@christuniversity.in



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ABSTRACT

Plant extracts have been shown to support a variety of processes, including anti-stress, growth promotion, appetite stimulation, improvement of tonicity and immune stimulation, maturation of culture species, aphrodisiac and anti-pathogen properties in fish, due to the presence of active compounds, such as steroids, glycosides, alkaloids, terpenoids, tannins, flavonoids, phenolics, saponins, or essential oils. However, the long-term effects of these compounds on fish physiology is not much known. Moreover, there is a lack of homogeneity in the manufacturing of the extract and the delivery of the plant extracts to the fish. This review aims to provide an insight about the effect of different plant extracts on different kinds of fishes with special emphasis on growth, biochemical indices and intestinal histology of fishes.

Keywords: Plant extracts, Microvilli, Aquaculture, Metabolites, Growth.

INTRODUCTION

Fish accounts for a significant amount of the world's food supply and is the most affordable source of easily digestible animal protein. The globe currently has around seven billion people, with that figure expected to rise to nine billion by 2050. Since output from marine capture fisheries has nearly stalled in recent years, the aquaculture sector has become the primary source of fish for the food supply [Prasanta Jana *et al.*,2018].





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Seafood, unlike other muscle meals, is extremely vulnerable to microbial and chemical degradation. Because of its high amounts of moisture, nutrients, and pH, fish is a highly perishable commodity that frequently spoils quickly after death [Viji *et al.*, 2017]. Plant extracts, also known as phytobiotics, contain active components like as polyphenols, flavonoids, terpenoids, and others that are responsible for growth promotion, appetite stimulation, antistress, tonicity and immune stimulation [Reverter *et al.*, 2014]. When fed to farmed fish, several plant extracts have been found to stimulate hunger and weight gain [Reverter *et al.*, 2014]. Phytobiotics often stimulate the release of digestive enzymes and have a direct impact on gut microbiota. There is evidence that plant extracts improve digestion and nutrient absorption, which ultimately increases feed conversion, protein synthesis, and fish development [Prasanta Jana *et al.*, 2018]. This review gives an insight about the properties of different plant extracts and how it affects the growth, intestinal histology and carbohydrates and protein content in the fishes.

Growth parameters

Specific growth rate (SGR): SGR is used to compare the growth rate such as length, size and weight between species for a particular time period. It is calculated by the following equation,

$$\text{Specific growth rates (\% day}^{-1}\text{)} = [(\ln W_1 - \ln W_0) / T] \times 100$$

where, W_1 = final weight, W_0 = initial weight and T = time in days.

The weight gain rate (WGR%) = (final weight, g – initial weight, g) / (initial weight, g)

Protein Efficiency Ratio (PER) = weight gain of fish / crude protein in diet.

The PER is utilized as an indicator for fish diets and are used for the evaluation of protein utilization and turn over.

When fed dietary stevia, red hybrid tilapia did not show much growth rate, indicating that dietary stevia was not metabolised as an energy source in tilapia fish [Choy *et al.*, 2018], whereas when the same fish was supplemented with dried basil leaves (DBL) as a feeding attractant, the WGR and SGR increased significantly in all diets compared to the control diet [El-Dakar *et al.*, 2008]. The maximum SGR was found in the high diet, while the lowest was found in the control diet. These results suggest that plant extracts can induce growth through improving the olfactory effect of diet [El-Dakar *et al.*, 2008]. When the red Tilapia fish [Trullàs *et al.*, 2022] and Nile tilapia fingerlings [Boonanuntanasarn *et al.*, 2018] was fed with 5g/kg and 10g/kg of *Jerusalem artichoke* (JA) prebiotic, showed a higher weight gain and SGR when compared to the control diet. This beneficial effect of JA on the growth performance might be due to the compounds such as inulin, FOS, carbohydrates, proteins, vitamin C and minerals. [Trullàs *et al.*, 2022; Boonanuntanasarn *et al.*, 2018]. In the juvenile mirror carp fish, the WGR was increased linearly as the stevia concentration increased. [Wang *et al.*, 2021], Similarly, there was no much effect on the growth rate as well as WGR of rainbow trout when they were supplemented with histamine and stevia extract compared to control treatment [Wang *et al.*, 2021]. When rainbow trout fish were fed with dietary lemon balm extract, SGR and WGR were considerably greater in the 0.5 and 1g kg⁻¹ diet groups compared to the control group.

The rapid growth of rainbow trout may give the animal with an early protective immunity [Bilen *et al.*, 2020] When the hybrid Catla fish were supplemented with varying quantities of *Lasia* extract (LSE), the SGR and WGR of fish from all treatment fishes increased significantly when compared to the control. Among the different concentrations of LSE, the 3% dietary level showed the highest values for both the parameters. [Munglue *et al.*, 2019]. Similarly, when the same fish was supplemented with rice paddy herb extract (*Limnophila aromatica*) it was observed that the diets mixed with 3 and 5% LAE decreased the values of SGR compared to the diet of 1%. This would have occurred due the toxicity and the supplementation period of LAE. Generally, the effect of LAE on diets increases the SGR compared to the control group which enlightens the enhancement of digestive enzymes by LAE. Whereas there was significant increase in the WGR which might be attributed by the phytochemical substances such as flavonoids and essential oils. [Munglue *et al.*, 2020] The effect of the fish *Labeo chrysophekadion* fed for 150 days by the different concentrations (0, 0.25, 0.50 and 0.75 gkg⁻¹) of ethanolic extract of *Apium graveolens* showed there was significant difference in SGR on day 30, higher values of SGR was obtained in higher concentration of feed and lowest values were found in groups fed with 0.50 and 0.75g kg⁻¹.



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The weight gain of *L. chrysophekadion* showed that there was significant difference, at day 60 highest WG was observed in 0.50gkg⁻¹ test group compared to the control, but on day 120 and 150, it showed a highest WG values in fishes supplemented with 0.75 gkg⁻¹ [Sutthi et al, 2020]. The dietary effect of *Chenopodium album* extract on juvenile *Cyprinus carpio* on its SGR was significantly increased in all the experimental groups (0.01, 0.05 and 0.1g kg⁻¹) compared to control group when the diet was fed for a period of 45 days. But WGR was enhanced only in diets supplemented with 0.01 and 0.1g kg⁻¹ of extract. This increase might be due to the secretion of digestive enzymes or through modulating the beneficial intestinal microflora which plays an important role during the digestive enzyme's secretion. This study conducted by Iman Daw Amhamed et al. also showed that there was increase in the secretion of digestive enzymes which eventually led to enhancement of growth parameters [Iman Daw Amhamed et al.,2018]. Similarly when the fish was supplemented with 1 and 2 % of *Phyllanthus niruri* L diet, it was observed that the 2% fed diet showed the highest growth performance both in terms of SGR and WGR which resulted due to the unstressful, more palatable and digestible nature of *P.niruri*. [Sunitha et al., 2017] The common carp fish when supplemented with dried, prepared rehmannia root powder (DP, PP) and dried, prepared rehmannia root extract (DE, PE) it was observed that the growth parameters such as SGR was significantly higher in all the 4 groups whereas WGR was higher in PP and DE supplemented diets [Wang et al., 2015] The same fish supplemented with aloe vera extract with the concentrations of 0.2, 0.4, 0.8 and 1.6%. The estimation was carried out on 30th, 60th and 90th days of feeding diets and was observed that there was a significant change in SGR and WGR and PER in all treated groups, but the increase in growth rate, weight gain and protein efficiency ratio was much clearer in the fishes supplemented with 0.4 and 0.8% diet. [Khanal et al., 2021] whereas the fish supplemented with ethanolic extract of oak leaf extract with the concentrations of 0.5, 1 and 2 g kg⁻¹ showed no significant effect on the growth parameters; concluding that the extract may not stimulate digestive enzymes and gut absorption. [Paray et al., 2020]

The growth parameters were calculated when the fish golden pompanos were fed with experimental diets of dandelion extracts at the concentration of 0, 0.5, 1, 2, 4 and 10 g kg⁻¹. It was observed that group 3 diet showed the highest SGR. The fish fed with diet 2 and 6 also showed a higher SGR compared to diet 1. The weight gain was significantly higher when the extract levels were increased from 0 to 1 g kg⁻¹, further increase in the concentration of extract there was a decrease in weight gain. This is due to the utilization of extra energy towards metabolism when supplemented with excess dandelion extract which may lead to some toxic effects and hence results in adverse effects in growth. The PER was also enhanced in all the diets except the group 4 diet (4g kg⁻¹). [Tan et al., 2017] whereas, the hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) when supplemented with ginkgo biloba leaf extract (GBE) showed a quite opposite results wherein there was no change in SGR and WGR of fish but the PER was significantly lower in diets 5 and 6 compared to diet1 [Tan et al., 2018] Similar results for SGR and WGR were obtained when they were fed with *Astragalus membranaceus* extract of concentrations 0%, 0.1%,0.2%,0.4% and 0.8%. The reason for this might be due to the absorption and utilization of the extract by different kinds of aquatic animals, the different cultural environment and also the different concentrations of the extract used. [Sun et al.,2022] Whereas, when the same hybrids were fed with 0%,0.1% and 0.4% of *Senecio scandens* Buch- Ham extracts (SSBE) there was significant increase in the WGR and SGR at the initial days of feeding which eventually became constant after a particular number of days [Sun et al.,2020].

Sciaenochromis fryeri was supplemented with 0,2,4 and 8% Wood betony extracts for a period of 56 days. The best growth rate was observed when the fish was fed with 3.9% of diet whereas the highest levels of wood betony diet showed a reduction in growth performance. [Mohammadi et al.,2019]. The juvenile Asian sea bass fish was fed with fermented lemon peel (FLP) along with basal diets in the concentrations of 1, 3 and 5%. It was observed that the fish fed with FLP did not show any negative effects on growth parameters i.e all the diets showed a similar values for growth parameter. Whereas, the protein efficiency ratio of fish when fed with 5% of FLP diet showed the lowest response after feeding for 8 weeks. By this it was demonstrated that 1 and 3% diets can be used for evaluating fish functions without any negative effect on growth. Certain other fishes which were supplemented with dietary lemon peel had a significant negative effect on the growth parameters which might have resulted due to the presence of fibre, tannin or limonin. [Zhuo et al.,2021]. Nile tilapia fish supplemented with dietary ZLP extract (*Ziziphus mauritiana* leaves) in the concentrations of 5, 10 and 20g per kg diets showed a significant change in growth



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parameters [Amin et al., 2019] As in case of aqueous and ethanolic extracts of guava leaf, the growth performance was high as the concentration of guava leaf extract increased. [Gobi et al.,2016] The maximum growth rate was obtained in the fish supplemented with 10g per kg of diet while the lowest growth was observed in the control group. The growth rate was significantly lower in 20g/kg treated fish due to the formation of tannin protein complex in stomach and prevented the absorption of amino acids from intestine. The tannin also reduces nitrogen availability in digestive tract by reacting with its outer layer and thereby reducing the nutrients passage. Similarly, the PER was enhanced in the fishes which were supplemented with 10 and 20 g/kg diet when compared to the control which is related to the improvement in the enzymatic digestion of sugars, organic phosphorous utilization, and fibre digestion due to the presence of ZLP. [Amin et al., 2019] The same fish when supplemented with 1500mg/kg of Astragalus polysaccharides (APS) showed a higher SGR and WGR [Zahran et al., 2014]. The dietary supplementation of peppermint extract (1,2 and 3%) on SGR and WGR of Caspian white fish observed an increment in the fish supplemented with 3% diet. [Adel et al., 2015] The effect of *Vitex agnus-castus* extract on zebra fish showed a significant change in the growth parameters. The treatment was carried out in different concentrations (0g kg⁻¹ , 5g kg⁻¹, 10g kg⁻¹), SGR and weight gain was significantly higher in the 15gkg⁻¹ treated fish. This indicated that *Vitex agnus castus* was beneficial and nutrient materials positively affect the growth parameters. [Enayat Gholampouret al., 2020]

Intestinal histology

Red tilapia fish [Trullàs et al., 2022] and Nile Tilapia fingerlings [Boonanuntasarn et al., 2018] showed certain changes in its intestinal morphology when it was fed with JA (prebiotic) supplemented diet. The villous height and width along the intestine were higher than those fed with control diet. However the absorptive area in the distal part of the intestine was also higher along with the number of goblet cells in proximal as well as distal parts of intestine. this indicates that acid, neutral and double staining mucous cells in fish fed with the diet was higher. Whereas in the middle part of the intestine, only the number of double stained and neutral mucus cells were higher compared to the control diet. Due to the higher absorption area and higher microvilli densities, it results in higher nutrient absorption thereby increasing the weight of the fish [Trullàs et al., 2022 ,Boonanuntasarn et al. 2018]. Certain fishes such as rainbow trout when supplemented with stevia showed a protective effect against the abnormalities caused such as exfoliation and distension of cardiac mucosal epithelium, Atrophy of lamina propria and gastric mucosa. The muscles of fish contains histamine which is derived from L-histidine undergoing decarboxylation reaction in the presence of histidine decarboxylase enzyme. The plentiful amount of histidine as a free amino acid present in fish undergoes this decarboxylation reaction after the death of fish to produce histamine which is toxic to fishes and causes allergy or some kind of gastric abnormality in them. This abnormality is protected when the fishes are supplemented with stevia in their diet. To check these abnormalities researchers studied the histology of fishes by supplementing few fishes with histamine and few fishes with stevia and compared it with the fish that was neither fed with histamine nor the stevia extract (control). They observed that the histamine supplemented fish showed few abnormalities in tissues and fishes fed with stevia did not show much differences among the treated fishes. With this it was concluded that stevia had some anti-medicinal properties which protected them against such abnormalities [Shiozakiet al., 2004].

The supplementation of different levels of LSE on hybrid catla fish resulted in the increase of Goblet cells in all parts of fish intestine, whereas the structure of enterocytes, goblet cells and microvilli arrangement did not show any change in the intestine enteritis. The fish supplemented with 5% LSE, the proximal segment of fish significantly decreased the subnucleus height and enterocyte nucleus diameter but the microvilli height were increased. When the middle segment of fish intestine was studied, it showed that fish fed with 1 and 3% of LSE decreased the enterocyte height and subnucleus height whereas fish supplemented with 1 and 5% of LSE decreased the supranucleus height compared to control. The 1% LSE supplemented diet decreased the enterocyte number and there was no difference in the microvilli height among the dietary treatments. Similarly, when the studies was conducted on distal segment of fish intestine, 1% and 3% supplemented diet increased the supranucleus height whereas the 1% and 5% supplemented diet decreased the subnucleus height when compared to control fish. There was no significant differences in enterocyte height and enterocyte number among the dietary treatments.



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[Munglue *et al.* 2019] Similar studies on the same fish was conducted by LAE extract and it was observed that the intestinal villi height and width was higher in 3 and 5% diet compared to control. It was also noted that there was no any damage or tissue degradation. The enterocyte heights and supranucleas heights of middle and proximal regions of fish intestine was enhanced when they were fed with LAE extract. The goblet cell number was also increased in 3 and 5 % LAE supplemented diets in middle and distil intestinal regions. Moreover the subnucleas height, enterocyte nucleus height and width of fish was increased in 5% LAE diet. But there were no changes in all these in the proximal part of fish intestine.[Munglue *et al.*, 2019] The fish *Sciaenochromis fryeri* supplemented with 2, 4 and 8% of WB extract did not show any changes in the intestinal morphologies such as hmV, intestinal diameter and hF/D ratio.

However, fishes supplemented with low dose as 2% showed an increase in the hF and hE in comparison with control groups which led to the improvement on the gut function and growth rate. [Mohammadiet *et al.*, 2019] the common carp fish when treated with dietary aloe vera extract found that the villi were spotted in the anterior gut of all the experimental groups this was due to the presence of aloe vera. It was also observed that in these dietary supplemented fishes the villi gave a more prominent surface area for absorption by providing taller, narrower, and routinely moulded villi and also due to higher number of intestinal villi. Similarly, there was morphological changes in the distil intestine in response to aloe vera supplemented diet which is responsible for the growth. [Khanalet *et al.*, 2021] The intestine of Asian sea bass when supplemented with different diets (0,1,3 and 5%) of FLP, a tight lamina propria at the end of microvilli was evident in 1 and 3% FLP diets whereas extended lamina propria was observed in control and 5% diet. There was no change in the microvilli height of all the dietary fishes, but the area of microvilli was higher in fishes fed with diets 1 and 3 % FLP. Similarly, the width was higher in diets fed with 0 and 5% FLP than with 1 and 3% diets. The estimation was of anterior intestine since the digestion and absorption is much greater in anterior intestine compared to the posterior intestine.

The studies conducted by li-chao Zhuoet *al.* showed that the organic acids produced due to fermentation process maintained the intestinal health of fish. [Zhuoet *al.*, 2021] The intestinal muscular coat was varaiable among the Nile tilapia fish supplemented with different concentrations of ZLP. Whereas there was no variations when the same fish was treated with APS (Astragalus polysaccharides) [Zahranet *al.*, 2014] but the length of intestinal villi was significantly higher in the ZLP[Amin *et al.*, 2019] and APS [Zahran *et al.*, 2014] treated fish compared to the control which indicates the improvement in the absorptive surface area resulting in the better nutrient utilization by fish. [Amin *et al.*, 2019]. Supplementation of *Phyllanthus niruri* L. diets to fish, *Cyprinus carpio* L showed a positive effect on the intestinal histology. There was no much changes observed as the intestine showed no Leukocyte Infiltration in epithelium, no mucous production or damage to the villi between the experimental and control diets. [Sunithaet *al.*, 2017] The hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) was supplemented with different concentrations of SSBE [Sun *et al.*, 2020] and *Astragalus membranaceus* extract (AE)[Sun *et al.*, 2022] The villus length, villus width, intestinal wall thickness and cross-sectional area of the foregut, midgut and hindgut was increased in 0.05 and 0.1% of SSBE diet [Sun *et al.*, 2020] whereas only villus length increased in the hindgut as the concentration of AE increased which indicates that AE promote the development of hindgut to a certain extent. The villus structure of foregut had no changes on the AE extract. [Sun *et al.*, 2022].

Biochemical indices

Proteins

The hybrid tilapia when fed with different concentrations of dried basal leaves, resulted in an increase in protein content of fish. This explains increasing the voluntary feed intake and palatability index of diets containing graded levels of DBL. [El-Dakar *et al.*, 2008] When the tilapia fish was supplemented with JA extract, the total protein content in the serum of the diet fishes did not show much significant effect compared to the control fish. [Trullàs *et al.*, 2022] The total protein content in the fish *L. chrysophekadion* when they were supplemented with 0.50 and 0.75g kg⁻¹ was observed to be highest compared to other dietary groups whereas the lowest protein content was found in 0.25g kg⁻¹ supplemented feed.[Sutthiet *al.*, 2020]. The protein content in the muscle and whole body of golden pompanos supplemented with different levels of dandelion extract for 8 weeks had a significant effect; the higher the extract



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level (diet 6 with the concentration of 10 g kg⁻¹), higher was the protein content in whole body. The lowest protein content was observed in muscles of fishes fed with diet 1 which was observed to be significantly lower than diet 3 and diet 4 without any statistical differences. [Tan et al., 2017] But the protein content in hybrid grouper (*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) fed with ginkgo biloba leaf extract (GBE) showed a lower values in diet 2 and 3 [Tan et al., 2018]

Similarly, the total protein content was estimated and it was observed that fish fed with diet 2, 3 and 4 had an increased levels of total protein than the fish fed with diet 1 without the statistical differences, GBE extract in hybrid grouper fish showed a significant increase only in diet 2, [Tan et al., 2018] this increase in total protein levels is thought to be due to stronger innate immunity response [Tan et al., 2017]. Whereas the plasma protein content in the hybrid grouper fed with 0.1% SSBE showed a significantly high levels which indicates that the SSBE in diet regulates the protein metabolism in the fish. [Sun et al., 2020] The same hybrids when they were fed with *Astragalus membranaceus* root extract, the total protein content in serum and whole fish body had no effect on the supplement, whereas there was an increasing trend of crude protein content in the muscle of the fish. [Sun et al., 2022]. Dhas SA et. al worked on different herbal extracts such as *Mucuna pruriens*: *Withania somnifera*: *Moringa oleifera* in the ratios 1:1:1, 2:1:2, 1:2:1 was fed to *Etroplus suratensis*. The total protein content was highest in blood of an animal which was supplemented with diet 3 compared to the other diets. Similarly, the total protein content in the body tissue was maximum in diet 3 followed by diet 2 and then diet 1. Whereas the protein content in the liver was significantly higher in diet 3 and diet 2, but did not show any significant difference in diet 1 compared to the control group.

The reason for the high protein content in the fishes supplemented with experimental diets is due the hormone production during maturation which induces the synthesis of protein mechanism which eventually enhances the sperm and egg production in fish. [Smj et al., 2017] Indian major carp was fed with *Cynodon dactylon* mixed diet with 0.05% (group A) ,0.5% (group B) and 5% (group C) extract and observed that total protein levels was significantly increased from 30 to 60 days of feeding. [Kaleeswaran et al., 2012]. The protein content estimated in common carp when they were supplemented with aloe vera with different concentrations (0.2,0.4,0.8%) showed a significant higher level in 0.4 and 0.8% diets. [Khanal et al., 2021]. The total protein content in Nile tilapia fed ZLP was significantly increased in 20g/kg diet compared to the control. This indicates that ZLP supplementation plays an important role in feed intake with subsequent effects on fish body composition. [Amin et al., 2019] The supplementation of *P. nirurion* the fish *Cyprinus carpio* L resulted in an increase of total serum protein due to the anabolism of *P.niruri* and less wastage of energy. Determination of serum protein denotes the capacity of protein synthesis of liver and the osmolarity of blood and renal impairments and therefore serum protein is used as a bio-marker to assess any toxic changes in the fishes. [Sunitha et al., 2017].

The supplementation of peppermint at 1,2 and 3 % did not show any changes in the protein content of Caspian white fish; whereas the protein content in the skin mucus of fish increased as the dose of the extract increased. The highest protein content was found to be in 3% peppermint supplemented diet. Similar results were obtained in the serum total protein content of fish supplemented with peppermint enriched diets. [Adelet et al., 2015]. The sublethal doses i.e. 40% and 80% of *Euphorbia tirucalli* plant extract when exposed to fish *Channa punctatus* over 24h or 96h , it was observed that the 24h exposure period reduced the total protein content upto 60 and 63% similarly the 96h exposure period also reduced the total protein content upto 80 and 63%. The depletion of proteins was in both the liver and muscle tissues which suggests the high protein hydrolytic activity due to increase in the protease enzyme activity in both the tissues. [Tiwari and Singh, 2006]

Carbohydrates

Carbohydrates are usually considered to be the sensitive biochemical indicator of environmental stress, stress induced by handling, feeding, forced activity, thermal shock and contact with chemical pollutants. [Sunitha et al., 2017]. The effect of red tilapia fish on the JA supplemented diet increased the blood glucose levels when compared to control set of experimental fishes, but the plasma glucose levels showed no effect on the diet. [Trullàs et al., 2022] The effect of ethanolic extract of *Apium graveolans* on fish dramatically increased the serum glucose levels as the



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concentration of the extract was increased and the highest values were obtained for 0.25 and 0.5gkg⁻¹, however the researchers observed that the glucose levels rapidly decreased in group fed with 0.75gkg⁻¹[Sutthiet *et al.*, 2020]. The plasma glucose levels were estimated when the golden pompano fish[Tan *et al.*, 2017] and hybrid grouper fish(*Epinephelus lanceolatus* × *Epinephelus fuscoguttatus*) [Tan *et al.*, 2018] were supplemented with dietary dandelion extracts [Tan *et al.*, 2017] and GBE extracts [Tan *et al.*, 2018] respectively and was observed that there was no significant increase in plasma glucose levels. As the diet concentration increased the glucose levels decreased which indicates that adding dietary extracts of dandelion and ginkgo biloba leaf leads to hypoglycemia in fishes [Tan *et al.*, 2017, 2018]. whereas the hybrid grouper when supplemented with 0.1% SSBE [Sun *et al.*, 2020] and *Astragalus membranaceus* root extract [Sun *et al.*, 2022] showed higher plasma glucose levels [Sun *et al.*, 2020, 2022]. The *Etroplus suratensis* showed a highest blood glucose level from the diet 3 of herbal extracts (1:2:1). Similarly, the carbohydrates level in liver, gonad and body tissue showed a significant increase in experimental diet 3 followed by diet 2 and then diet 1 compared to the controlled group. The increase in carbohydrates level is directly influenced by digestion and absorption. Studies have also proven that carbohydrates level increases during maturation process as it provides the energy for egg and sperm cells and rapidly decreases after ovulation. [Smjet *et al.*, 2017] The serum glucose levels was significantly high in all the experimental groups of Catla fish. Meanwhile the carbohydrates level in group B and group C reached the optimum at day 30 and 40 of feeding. Later the glucose levels are reduced in day 50 and day 60 of feeding diets [Kaleeswaran *et al.*, 2012] the dietary supplementation of *P. niruri* on fishes increased the carbohydrate content which resulted due to the unstressful and good nutritive nature of the herb. Therefore it is observed that this herb is non - toxic to fish. [Sunitha *et al.*, 2017] The plasma glucose levels was measured in common carp fish after feeding for a period of 60 days (before stress, 6h after stress and 24h after stress termination. The oak extract treated fish showed a lowest plasma glucose level. [Parayet *et al.*, 2020]. The carbohydrate content in the fish under the stress condition of *E. tirucalli* plant extract is depleted to meet the energy demand. The reduction is upto 63% and 60% in 24h exposure period and similarly for 96h exposure period the reduction was upto 70% and 65% in both liver and muscle tissues. The depletion might be due to the direct utilization of energy generation which is caused by aqueous extract induced hypoxia [Tiwari and Singh, 2006].

CONCLUSION

The properties and efficacy of plant extracts on the health and overall development of the cultured fishes depends upon the type of the plant, it's extraction method and also the concentration of the extract. This review summarises about the various kinds of plant extracts and its effects on the growth parameters, intestinal histology and biochemical parameters such as proteins and carbohydrates content in varieties of fishes. Several plant extracts are rich source of Poly phenolic compounds which act as a promising feed for the weight gain and overall development of the fishes. Extracts also possess various properties such as antimicrobial, antifungal, antiviral etc. These keep the fishes disease resistant and thereby improving the overall health of the fishes.

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Table1: List of plant extracts on fishes and its medicinal properties

| Fish | Plant extracts | Properties | Reference |
|--|---|---|--|
| Nile tilapia (<i>Oreochromis niloticus</i>) | Guava leaf extract (<i>Psidium guajava</i>) | Antimicrobial | Gobi <i>et al.</i> , 2016. |
| | Jerusalem artichoke (<i>Helianthus tuberosus</i>) | Anti-fungistatic, anti-carcinogenic and antioxidant | Boonanuntanasarnet <i>et al.</i> , 2018. |
| | Indian Jujube (<i>Ziziphus mauritiana</i>) leaves extract | Anti-diabetic, anti-inflammatory, anti-plasmodial, and anti-microbial and antioxidant | Amin <i>et al.</i> , 2019. |
| | Milkvetch (<i>Astragalus polysaccharide</i>) | Antiaging, antiviral, antitumor, antifibrosis | Zahran <i>et al.</i> , 2014. |
| Juvenile mirror | Sweetleaf (<i>Steviarebaudian</i>) | Antioxidant, anti- | Wanget <i>al.</i> , 2021. |





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|---|---|---|--|
| carp <i>Cyprinus carpio</i> Rainbow trout (<i>Oncorhynchus mykiss</i>) | a)Leaves extract | inflammatory, antidiabetic, antihyperlipidemic, antitumor, antibacterial, antifungal and anticancer. | |
| Hybrid <i>Catla</i> fish (<i>Labeo catla</i>) | Lasiaextract(<i>Lasia spinosa</i>) Ricepaddyherb (<i>Oryza sativa</i>) | Antioxidant, anti-inflammatory, antimicrobial. Antiseptic. | Munglue et al., 2019, 2020. |
| Golden pompano (<i>Trachinotus blochii</i>) | Dandelion extract (<i>Taraxacum</i>) | Antimicrobial, antiviral, antioxidant, anti-inflammatory, anticancer. | Tan et al., 2017. |
| Hybrid giant tiger grouper (<i>Epinephelus lanceolatus</i> × <i>epinephelus fuscoguttatus</i>) | Ginkgo (<i>Ginkgo biloba</i>)leaf extract Milkvetch (<i>Astragalus membranaceus</i>) extract Groundsel(<i>Senecio scandens</i>)buch ham extract | Antioxidant. Antiaging, antiviral, antitumor, antifibrosis. Anti-inflammatory, antimicrobial, anti-leptospirosis, anti-infusorial, antioxidant, antiviral, antitumoral. | Tan et al., 2018. Sun et al.,2022. Sun et al.,2020 |
| Electric blue hap (<i>Sciaenochromis fryeri</i>) | Wood betony extract (<i>Pedicularis canadensis</i> L.) | Antioxidant. | Mohammadi et al.,2019 |
| Black sharkminnow (<i>Labeo chrysophekadion</i>) | Celery (<i>Apium graveolens</i>) | Anticancer, anti-obesity, anti-hepatotoxic, and antihypertensive agents. | Sutthiet al, 2020. |
| Common carp (<i>Cyprinus carpio</i>) | White goosefoot (<i>Chenopodium album</i> L.) extract Bhumi amla (<i>Phyllanthus niruri</i> L) diet Chinese foxglove (<i>Rehmanniaglutinosa</i>) root extract | Antioxidant, antimicrobial Antioxidant Antioxidant, antisenescence, anti-inflammatory. | Iman DawAmhamed et al,2018. Sunitha et al., 2017. Wang et al., 2015. |



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|---|--|---|---|
| | Aloe vera extract(<i>Aloe barbadensis miller</i>) Oak leaf extract (<i>Quercus leucotrichophorn</i>) | Anticancer, antioxidant, antidiabetic, and antihyperlipidemic. Antimicrobial, anticancer, anti-inflammatory, antinociceptive, antioxidant. | Khanalet <i>al.</i> , 2021. Parayet <i>al.</i> , 2020. |
| Caspian white fish(<i>Rutilus kutum</i>) | Peppermint extract (<i>Mentha piperita</i>) | Antimicrobial, antifatigue, antioxidant. | Adel <i>et al.</i> , 2015. |
| Zebra fish (<i>Danio rerio</i>) | Chaste tree (<i>Vitex agnuscastus</i>) extract | Anti-inflammatory, antimicrobial, anticancer, antiepileptic, antioxidant. | Enayat Gholampouret <i>al.</i> , 2020. |
| Green chromide (<i>Etrophlussuratensis</i>) | Velvet bean (<i>Mucuna pruriens</i>) Ashwagandha (<i>Withaniasomnifera</i>) Drumstick tree (<i>Moringa oleifera</i>) | Anti-inflammatory. Anti-tumor, anti-inflammatory, anti-bacterial, fungicidal, anti-stress. Anticancer, antioxidant. | Smjet <i>al.</i> , 2017. |
| Indian major carp (<i>Catlacatla</i>) | Bermuda grass (<i>Cynodondactylon</i>) | Antiviral and antimicrobial activity. | Kaleeswaranet <i>al.</i> , 2012. |
| Spotted snakehead (<i>Channa punctatus</i>) | Spurge plant extract(<i>Euphorbia tirucalli</i>) | Antiviral, antiherpetic, antioxidant, antimicrobial. | Tiwari and Singh, 2006. |





Phytochemical Screening, HPTLC Finger Printing Profile, FT-IR Analysis and Evaluation of *In vitro* Anticancer Efficacy in the Ethanolic Extract of *Eugenia jambolana* Seeds against Breast Cancer and Lung Cancer Cell Line

Poongothai.V¹ and Siva Ganesh. M^{2*}

¹Research Student, II MSc Biochemistry, RVS College of Arts and Science (Autonomous) Affiliated to Bharathiar University, Coimbatore - 641402, Tamil Nadu, India.

²Assistant Professor of Biochemistry, RVS College of Arts and Science (Autonomous) Affiliated to Bharathiar University, Coimbatore - 641402, Tamil Nadu, India.

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*Address for Correspondence

Siva Ganesh. M

Assistant Professor of Biochemistry,
RVS College of Arts and Science (Autonomous)
Affiliated to Bharathiar University,
Coimbatore - 641402, Tamil Nadu, India.
E.Mail: msgbiochem17@gmail.com



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ABSTRACT

The aim of the present study to evaluate the phytochemical screening, HPTLC, FT-IR analysis and *In-Vitro* anticancer efficacy in the ethanolic extract of *Eugenia jambolana* Seeds against Breast cancer and Lung cancer cell lines. The preliminary phytochemical screening showed that the most of the secondary metabolites mainly found in ethanolic extract of *Eugenia jambolana* seeds. Especially bioactive compounds such as Alkaloids, Flavonoids, Phenols, Saponin and Tannin were highly present in the ethanolic extract when compared to other solvents like Chloroform, Ethyl acetate, Petroleum ether and Aqueous extract based on the solubility. HPTLC analysis study revealed that flavonoids compounds were found as identified fraction in the ethanolic extract of *Eugenia jambolana* seeds. compared with standard Kaempferol. The ethanolic extract of plant *Eugenia jambolana* seeds possess essential organic functional groups were found by FT- IR analysis. Anti-cancer efficacy was done in two cell lines such as MCF-7 and A549 were found effective anti-proliferative agent as it arrested cell proliferation in these cancer cell-lines by MTT assay. This review focus *Eugenia jambolana* extract as medicinal compounds against cancer and hence proved that it can be used alone or in combination with chemotherapeutic drugs in future.

Keywords: *Eugenia jambolana*, Phytochemicals, HPTLC, FT-IR, MTT assay, MCF-7 & A549 cancer cell line.





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INTRODUCTION

Medicinal use of plant and their products was passed down from generation to generation in various parts of the world throughout its history and has significantly contributed to the development of different traditional systems of medicine.[1] The World Health Organization reportedly estimates that 80% of the population in underdeveloped nations relies on traditional medicinal herbs as a source of medication. Since ancient times, therapeutic plants, often known as medicinal herbs, have been identified and employed in traditional medical practices[2]. In Indian plants of therapeutic potential are globally used by all sections of people both as folk medicines in different endemic systems of medicine like Siddha, Ayurveda, and Unani and also as processed product of pharmaceutical industry. According to the World Health Organization (WHO), as many as 80% of the world's people depend on traditional medicine for their primary healthcare needs. The importance of medicinal plants to human health is enormous [3]. Cancer is a group of disease defined as uncontrolled growth and spread of abnormal cells. Cancer is caused by many external factors and some internal factors. Cancer is a genetic disorder. Worldwide about 9.6 million deaths were evaluate in cancer. The cancer is a second leading cause of deaths[4].The most different types of cancer breast cancer, lung cancer, colorectal cancer, prostate cancer, Skin cancer, Liver cancer and Stomach cancer[5]. *Eugenia jambolana* (Myrtaceae) is globally used in India to treat several ailments in the traditional system of medicine. *Eugenia jambolana* seeds were collected during may month. In this plants its dietary use, importantly the seeds are used to treat a range of ailments and being diabetes. Globally about 10 million people per year are identified with cancer and more than 6 million die of the disease and over 22 million people in the world are cancer patients[6].

Eugenia jambolana Lam. Of family (Myrtaceae) is widely broadcast in the plains from sub-Himalayas to South India and is a famous traditional medicinal plant in India. *Eugenia jambolana* is various pharmacological actions are documented in traditional system of medicine particularly in Unani medicine. Preclinical studies range of pharmacological actions, it has been widely used as anti bacterial , anti fungal, anti-viral, anti-ulcerogenic ,cardio protective, anti-allergic, anti-diarrhea, assistance its traditional uses. The fruit is valued in Ayurveda, an ancient Indian medical system, for its therapeutic properties[7]. Many components from dietary or medicinal plants have been identified that they possess substantial chemo preventive properties. Antioxidant also have known to play a vital role in preventing cancers. Therefore ,there is an urgent need to create new therapeutic approaches to combat this fatal illness[8]. Breast cancer as a chronic disease. Breast cancer is a commonly identified cancer in female in both economically developed and developing countries. Recent studies have shown that *Eugenia jambolana* seeds may have potential as an anticancer agent in breast cancer treatment[9]. Strong anti-inflammatory and antioxidant capabilities have been observed in the active chemicals found in *Eugenia jambolana* seeds. These compounds are believed to help prevent the growth and spread of cancer cells by suppressing the formation of new blood vessels that supply nutrients to the tumor cells. This process, known as angiogenesis, is essential for the growth and survival of cancer cells[10].

MATERIALS AND METHODS

Collection of plant materials

The seeds of *Eugenia jambolana* were collected from the local area of village in Tirupur, Tamil Nadu. Seed part of the plants were prepared for different solvent extractions such as Ethanol, Ethyl acetate, Petroleum ether ,Chloroform and Aqueous for sample analysis.

Preparation of plant extract

25 gm of plant seed was dissolved in 250 ml of ethanol , ethyl acetate, chloroform, petroleum ether and aqueous and kept it in a shaker for 24 hours. The extract was filtered through Whatman No 1 filter paper and residue was collected. The filter was concentrated using a rotator vacuum evaporator to get ethanol extract of the dried plant powder.



**Poongothai and Siva Ganesh****Soxhlet Extraction**

Soxhlet extraction process is essentially needed where the desired active compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance.[11]

Phytochemical analysis

Phytochemical studies were performed for observing the secondary metabolites present in the different extracts Using procedures described by Trease and Evan [12]

Detection of Alkaloids**Mayer's test**

Extracts were treated with Potassium Mercuric Iodide .

Detection of Flavonoids**Alkaline reagent test**

Extracted were treated with few of drops of sodium hydroxide solution.

Detection of Tannins**Lead acetate test**

3-5ml test solution with few drops of 1 ml Leadacetate solution.

Detection of Phenols:**Ferric chloride test:**

Extracts were treated with 3-4 drops of ferric chloride solution.

Detection of Saponins**Foam test:**

0.5 ml of extract was dissolved with 2ml of water.

Detection of resins:

A 3 ml of test solution was added with 5 to 10 ml of acetic anhydride solution then tubes were kept in boiling water bath for few minutes. Then tube were cool at room temperature add 5 ml of sulphuric acid.

Detection of steroids

3 ml of test solution and minimum quantity of chloroform was added with 1-4 drops of acetic anhydride and one drop of concentrated Nitric acid .

HPTLC (High Performance Thin Layer Chromatography) Analysis

TLC technique was adopted to separate the flavonoids, to identify and quantify rutin. HPTLC is a perfect analytical technique. This method is rapid, visual, and economical as it utilizes smaller volumes of solvents with minimum sample clean up. Above all, in a short duration, a huge number of samples are evaluated simultaneously.[13]

Preparation of Test Sample

200 mg of ethanolic extract of the sample was diluted with ethanol up to 75 ml, sonicated for 20 min; centrifuged and 10 µL of the test sample was applied as band on plate for quantification.

Preparation of Standard Kaempferol Solution

15 mg of Rutin was prepared with ethanol to make a stock solution of 1200 ppm strength. From this stock solution 10.0 ml was diluted up to 100 ml to obtain the final concentration of 100 ppm i.e. 100 µg/ml.



**Poongothai and Siva Ganesh****Chromatographic Conditions**

Samples of ethanolic extract of *Eugenia jambolana* and standard kaempferol were spotted on a precoated TLC aluminum sheet silica gel 60 F254 (10 x10 cm, 20 x10 cm, 0.2 mm thick). The mobile phase used was ethyl acetate: water: acetic acid: formic acid (8:1:0.5:0.5 v/v) and the plates were kept for saturation in twin trough chamber for 30 min. After development, the plates were dried in air and scanned at 366 nm by using CAMAG TLC-Scanner with UV-Visible spectrum, Linomat 5 samples applicator equipped with a 100 µL syringe. A constant application rate of 5 µL s⁻¹ was used. Automatic scanning was done with CAMAG TLC Scanner in remission absorbance mode controlled by win CATS software resident in the system. The slit dimensions were 6.00 x0.45mm, micro and the scanning speed was 20 mm s⁻¹. The radiation source was deuterium lamp and W emitting continuous UV radiation between 190-500nm. The plates were photographed at 254 nm and 366 nm by using CAMAG Visualizer.

FT-IR ANALYSIS

The Fourier Transform Infrared Spectrophotometer (FTIR) is the most efficient tool for identifying the sorts of chemical bonds and functional groups present in substances. The wavelength of light that is absorbed describes the chemical connection. The infrared absorption spectra can be used to determine a molecule's chemical bonds. Dried powder containing different solvent extracts of each plant material was employed for the FTIR investigation. To create translucent sample discs, 10 mg of the dried extract powder and 100 mg of KBr pellet were combined. Each plant specimen's powdered sample was placed into an FTIR spectroscope, which had a scan range of 400 to 4000 cm⁻¹ and a resolution of 4 cm⁻¹. The light absorption wave length is characteristic of the chemical bond as can be observed in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined [14].

INVITRO CYTOTOXICITY ASSAY**Cell line and culture**

Breast cancer (MCF-7) cell line and Lung Cancer cell line (A549) were obtained from NCCS, Pune, India. The cells were maintained in medium supplemented with fetal bovine serum (FBS), CO₂ at 37°C.

Principle

MTT 3-(4, 5-dimethylthiazol-2-yl)- 2, 5diphenyl tetrazolium bromide enters into the cells and reacts with the mitochondrial enzymes where it reduced into insoluble colored (dark purple) formazan product. The formazan crystal within the cells is then solubilized with an organic solvent DMSO and the released solubilized purple color formazan reagent is measured spectrophotometry at 570nm.

***In vitro* CYTOTOXICITY ASSAY- MTT Assay**

An anticancer test method is performed for the given test samples by selecting a culture flask with 80- 90% confluences. After trypsinization and centrifugation the cancer cells are seeded in the well plate for 24 hrs at 37±1oC incubation to form a monolayer. The culture medium from the cancer cells is replaced with fresh medium Test sample at different concentration in triplicates were added to the cells. After the incubation of sample with cells at 37±1oC for 18-24 h, MTT (1 mg/ml) were added in all the wells and incubated for 4 h. After the time interval DMSO is poured on the wells and read at 572 nm using spectrophotometry method. Cytotoxicity and cell viability were calculated by the formula mentioned below: The percentage of cytotoxicity was calculated using standard formula [15].

Cytotoxicity = [(Control – Treated)/ Control] X100

Cell viability= (Treated / Control) X 100



**Poongothai and Siva Ganesh****RESULTS AND DISCUSSION****Phytochemical screening**

The different solvent extracts like Ethanol, Ethyl acetate, Aqueous, Chloroform, and Petroleum ether extracts of the seed part of *Eugenia jambolana* showed the presence of secondary metabolites such as Alkaloids, Flavonoids, Phenols, Tannins, Saponin and Phytosterols reported that the most of the secondary metabolites found in EEEJ when compared to other solvents.

HPTLC Finger Printing Profile

HPTLC analysis study revealed that flavonoids compounds were found as identified fraction in the ethanolic extract of *Eugenia jambolana* seed. HPTLC finger printing analysis study showed that the identification of flavonoids found in the separated fractions compared with the standard compounds kaempferol. The ethanolic extract of the plant has the excellent pattern of flavonoids identified by using HPTLC technique.

FT-IR Analysis

FT-IR studies revealed that the ethanolic extract of plant possess in essential organic functional groups were identified for analysis. The essential functional group such as Phenols, Alcohols, Alkanes, Aldehydes, Carboxylic Acids and its derivatives, Aromatic alkanes and amine groups were found in EEEJ. It acts as an important active component for binding interaction in a drug preparation for various diseases.

In- vitro Cytotoxicity Assay – MCF-7 & A549 Cancer Cell line - MTT Assay

The Ethanolic extract of *Eugenia Jambollanna* showed Moderate to Severe cytotoxicity in A549 cells after 24hrs. The higher concentration of plant drug has excellent cytotoxic activity in both breast cancer cell line and lung cancer cell line. The increased level of cytotoxicity was found MCF-7 breast cancer cell line when compared to lung cancer cell line. The decreased cancer cell viability based on the dose dependent manner and the life span of cancer cell viability totally decreased due to the higher concentration of secondary metabolites present in EEEJ. The cancer cell viability for both MCF-7 & A549 were completely decreased as 15% and 19% respectively in a maximum concentration of plant drug EEEJ. The increased percentage of cell cytotoxicity found as 85% in MCF-7 and 81% in A549 cell line in a maximum concentration up to 100µg ethanolic extract of *Eugenia jambolana*. In the same observation related to the ethanolic extract of *Nigella sativa* seed possess the increased cytotoxicity in HeLa cell line and A549 cell line in a maximum concentration of 100µg based on dose dependent manner by MTT assay. [16] Results revealed that the ethanolic solvent extract possess the higher solubility and better active constituents for different type of cancer cell line. The result concluded that the ethanolic solvent extract of the plant compounds might be a better drug formulation for various type of cancer alternative to chemotherapy in future aspects.

SUMMARY AND CONCLUSION

Phytochemical screening reported that the most of active compounds were highly found in ethanolic extract of plant seed *Eugenia jambolana* when compared to other solvent extracts based on the solubility. The identified fraction of the active compounds' flavonoids were identified in EEEJ when compared to standard compounds kaempferol by HPTLC technique. It could be better drug for the analysis of cancer studies. The ethanolic extract of the plant has the excellent pattern of flavonoids identified for further analysis. The essential functional group such as Phenols, Alcohols, Alkanes, Aldehydes, Carboxylic Acids and its derivatives, Aromatic alkanes and Amine groups were identified in EEEJ by FT-IR Analysis. The ethanolic extract of *Eugenia jambolana* enhanced the anticancer activity in different cancer cell lines by MTT assay. The increased percentage of cytotoxicity were observed in both Breast cancer and Lung cancer cell lines and also evaluated decreased cancer cell viability in a maximum concentration of plant drug EEEJ by MTT assay. The higher concentration of plant drug has an excellent cytotoxicity activity in both breast cancer cell line and lung cancer cell line. Herbal drugs are derived from natural medicinal plants it does not lead to any adverse effect and any side effect to our human body. Finally, it is hoped that this review would be a



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source of guidance and support for the thirst of researchers to conduct further preclinical and clinical studies through herbal drug for the treatment of cancer.

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Disclosure & Conflicts of interest

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Table 1: Phytochemical screening of *Eugenia jambolana* seeds Extracts

| | ETHANOL | AQUEOUS | PETROLUUM ETHER | CHLOROFORM | ETHYL ACETATE |
|------------|---------|---------|-----------------|------------|---------------|
| ALKALOIDS | ++ | ++ | ++ | ++ | + |
| FLAVONOIDS | ++ | ++ | ++ | ++ | + |
| TANNINS | ++ | + | - | + | + |
| STEROIDS | - | - | - | - | + |
| SAPONINS | ++ | ++ | + | - | + |
| RESINS | - | - | - | - | + |
| PHENOL | ++ | + | - | - | - |

Table 2: HPTLC analysis of the flavonoid fraction peak table of standard

| S.NO | Standards/ sample extract | Retention time (min) | Area [mAU] | Area (%) | CONCENTRATION (mg/ml) | |
|------|------------------------------|---|---------------|-------------|---------------------------|---------|
| 1 | KAEMPFEROL | 0.49 | 2362.6 | 2.05 | 50 | |
| 2 | Plant extra | Flavonoids | 0.49 | 5044.1 | 4.39 | Present |
| | Proximate Quantification | Ethanollic Extract contains kaempferol =106.74 mg/l | | | | |

Table 3: Identified functional group by FT-IR analysis of ethanolic extract of *Eugenia jambolana*

| S.NO | CHARACTERISTIC ABSORPTION (cm-1) | INTENSITY | ASSINGMENT | FUNCTIONAL GROUP |
|------|-------------------------------------|--|---|-----------------------------------|
| 1 | 3340.15 | Stretching Vibration, Strong | O-H (free), usually sharp O-H (H-Bonded), usually broad | Phenols & Alcohols |
| 2 | 2972.73 2927.41 2880.17 | Stretching Vibration, Strong | CH ₃ , CH ₂ , CH 2 or 3 bands | Alkanes |
| 3 | 2348.87 | Stretching Vibration Medium | C-H | Aldehydes |
| 4 | 1715.37 | Stretching Vibration, Strong | C=O (H-bonded) Acids | Carboxylic Acids & Derivatives |
| 5 | 1455.03 1418.39 | Bending Vibration, Medium | CH ₂ & CH ₃ deformation | Aromatic Alkanes compounds |
| 6 | 1378.85 | Bending Vibration, Medium | CH ₃ deformation | Alkanes |
| 7 | 1276.65 | Stretching Vibration Medium-Strong | O-C (sometimes 2-peaks) | Carboxylic Acids & Derivatives |
| 8 | 1086.69 1044.26 | Stretching Vibration, Medium Strong | C-N | Amines |
| 9 | 879.381 803.206 | Bending Vibration, strong- Medium | C-H bending & ring puckering | Arenes |
| 10 | 675.025 | Bending Vibration, strong | C-H Deformation | Alkynes |

FT-IR studies revealed that the ethanolic extract of plant EEEJ possess in essential organic functional groups were identified for analysis .





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Table :4 : Breast Cancer Cell line - MCF -7

| CONCENTRATION (µg) | Liquid: seed extract | | |
|--------------------|----------------------|----------------|------------|
| | % of cytotoxicity | Cell viability | Reactivity |
| 5 | 42 | 58 | Mild |
| 25 | 53 | 47 | Moderate |
| 50 | 61 | 39 | Moderate |
| 75 | 73 | 27 | Severe |
| 100 | 85 | 15 | Severe |

Table :5 : Anticancer activity-Lung Cancer Cell line-A549

| CONCENTRATION (µg) | Liquid: seed extract | | |
|--------------------|----------------------|----------------|------------|
| | % of cytotoxicity | Cell viability | Reactivity |
| 5 | 57 | 43 | Moderate |
| 25 | 62 | 38 | Moderate |
| 50 | 68 | 32 | Moderate |
| 75 | 73 | 27 | Severe |
| 100 | 81 | 19 | Severe |

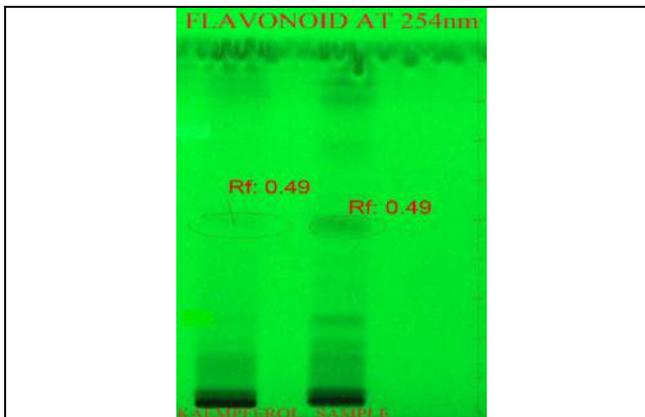


Figure 1:HPTLC Photo documentation of Flavonoid standard Kaempferol and Ethanolic Extract of plant sample at 254nm

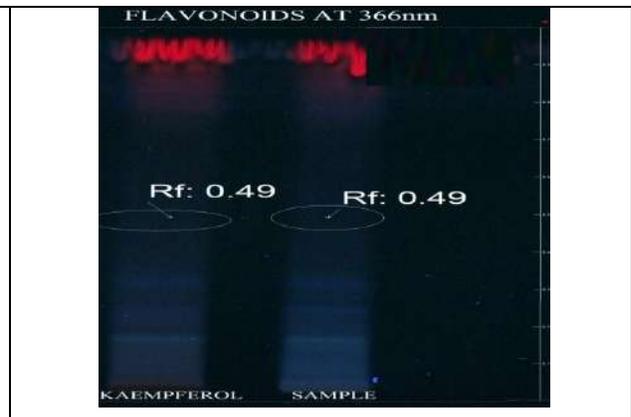


Figure 2 :HPTLC Photo documentation of Flavonoid standard Kaempferol and Ethanolic Extract of plant sample at 366nm

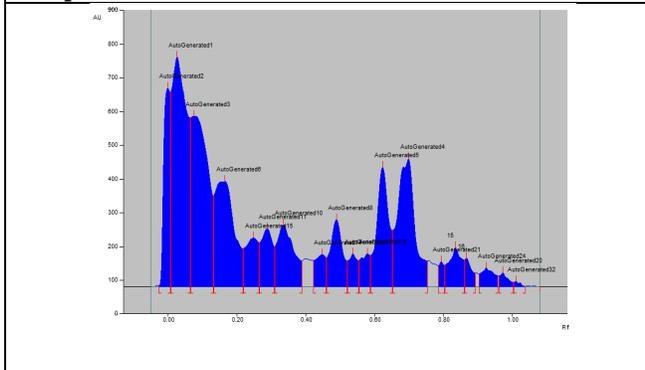


Figure 3: The Chromatogram of Ethanolic Extract of plant sample

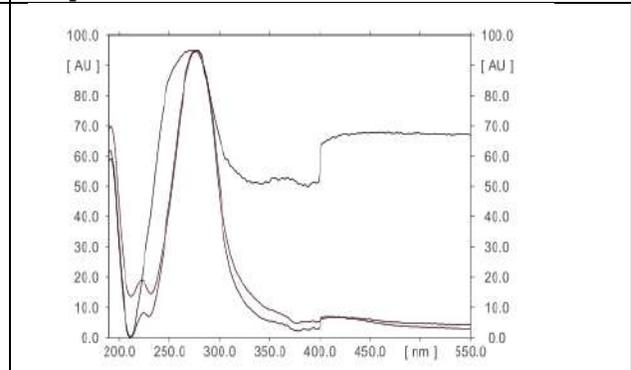


Figure 4: The UV-Vis matched Spectrums of Flavonoid standard and sample





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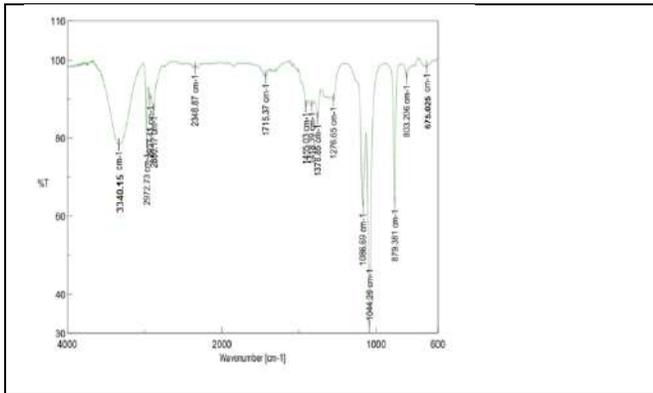


Figure 5: FT-IR spectrum of Ethanolic extract *Eugenia jambolana*

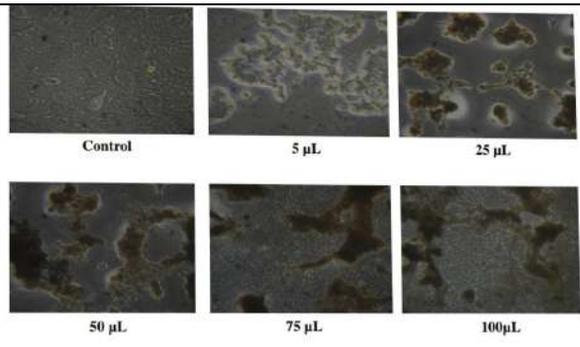


Fig -6 : The ethanolic seed extract of *Eugenia jambolana* showed mild to severe cytotoxicity in MCF-7 Cell line after 24hrs in a maximum concentration of 100µg

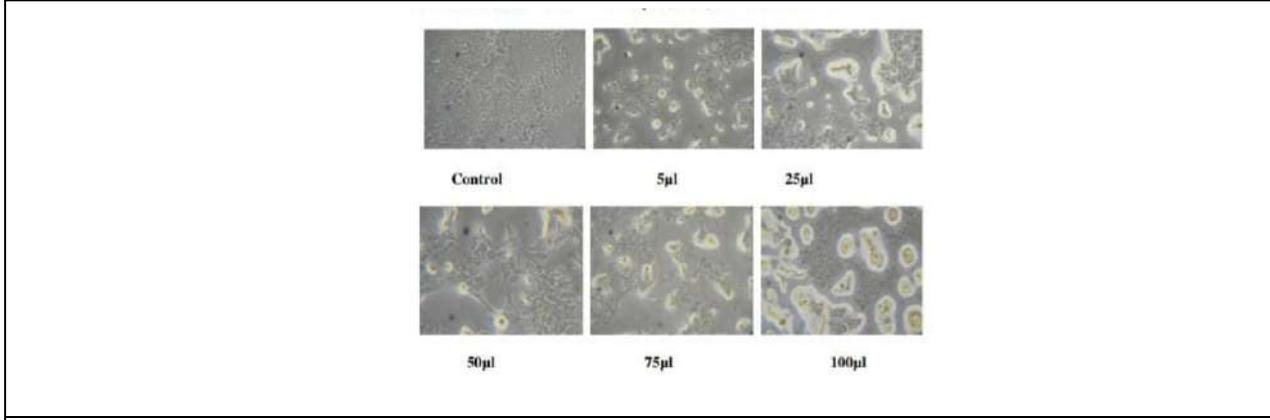


Fig -7 : The ethanolic seed extract of *Eugenia jambolana* showed moderate to severe cytotoxicity in A549 Cell line after 24hrs in a maximum concentration of 100µg.





A Review of the Therapeutic Characteristics and Applications of Betel Leaves

Divya K S¹, Talluri Rameshwari K R² and Sumana K^{3*}

¹Post Graduate, Department of Microbiology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, Mysuru- 570015, Karnataka, India.

²Research Associate, Department of Microbiology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, Mysuru- 570015, Karnataka, India.

³Associate Professor, Department of Microbiology, Faculty of Life Sciences, JSS Academy of Higher Education and Research, Mysuru- 570015, Karnataka, India.

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*Address for Correspondence

Sumana K

Associate Professor,

Department of Microbiology,

Faculty of Life Sciences,

JSS Academy of Higher Education and Research,

Mysuru- 570015, Karnataka, India.

E.Mail: sumana.k@jssuni.edu.in



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ABSTRACT

Betel leaves are consumed by roughly 223.79 million people in India. It is grown in India using conventional methods on approximately 55,000 acres, with an annual production value of over Rs 9000 million for the past few years. The state of West Bengal contributes about 66 per cent of total production, with over 20,000 ha comprising about 4-5 lakh Boroj and employing almost the same number of agricultural families. During storage, transit, and glut season, there is a significant loss of leaves. Furthermore, if the excess leaves are not properly disposed of, they may pollute the environment and pose health risks. Waste can be reduced in a variety of ways, including the extraction of essential oil from leftover betel leaves. This oil can be used as a raw material in the production of pharmaceuticals, perfumes, mouth fresheners, tonics, food additives, medicinal significance, and antimicrobial activity. The leaves are nutritious and contain anti-carcinogens, making them potentially useful in the development of a blood cancer treatment. According to some contradictory claims, chewing betel leaves excessively can lead to mouth cancer. The current review was studied in light of the health benefits of betel leaf and its usefulness as an antimicrobial.

Keywords: Betel leaf, Medicinal Impact, antimicrobials agents and their chemical components, etc.,





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INTRODUCTION

Betel leaves (*Piper betel*) a member of the Piperaceae family, is located in central and eastern Malaysia, Southeast Asia, and has valuable mending spices. In India, about 60-70% of people eat betel leaves regularly which can also be found in Bangladesh, Sri Lanka, Malaysia, Thailand, Taiwan, and other Southeast Asian countries. In India, betel leaf is found in about 85% of households. It is also known as Tamboolavalli, Tamboola, Tamboola vallika, and other names, and it's commonly used as tobacco for biting, therefore it is dubbed "Skillet," which is similar to tea and caffeine in terms of everyday use (80 % of people drink tea and 45 % prefer caffeine). Despite contradictory claims, betel leaf is mostly recognised in India & commonly used [1,2]. The numerous references in the writings, especially the Indian sacred scriptures, demonstrate its importance [3,4,5]. Surprisingly, around roughly 3,500 B.C., Austronesian brokers from Sumatra, Java, and the Malay Peninsula made contact with Dravidian speakers from Sri Lanka and southern India, in which the betel biting was spread [6,7,8]. This is sensible to develop under obscure and climatic conditions in the tropical woods zone, allowing for precipitation of roughly 2250-4750 mm, relative moistures of 40-80%, and temperatures of 15-40°C, respectively. The soil condition for growth and development should be very depleted fertile sandy, with a pH of roughly 15-40 [1]. In the summer, areas with less precipitation (1500-1700 mm) are grown and watched underwater every day, followed by a three-to-four-day interval in the winter [9,10].

For example, adequate trash is required for the widespread creation of the yield [11]. Starting with Pakistan city extended to Papua New Guinea, this piper betel grew wild in the Indo-Chinese peninsula and southern parts. This requires a healthy tree or regular assistance. Betel leaf requires ripe, fertile soil [12,13]. Betel leaf is commonly found in many areas like Assam, Madhya Pradesh, Orissa, Uttar Pradesh, Madras, West Bengal, Bihar, Andhra Pradesh, and other parts of India. Presenting a betel piece (dish supari) to visitors to the Indian subcontinent is a natural act of courtesy. Kapoori tuni in Andhra Pradesh, Desawari, Calcutta pan, Vishnupuri pan in Madhya Pradesh, Banaras in Uttar Pradesh, and saunifia pan are examples of diverse assortment. Varanasi, Prayagraj, Lucknow, Nagpur, Mumbai, and Hyderabad are all major Calcutta Pan consumers. Finally, the Telangana state structure in south India is the primary state from which India obtains pan from West Bengal due to its sweet and spicy flavour [14,15,16]. There are around 90 different types of betel plant, 45 different kinds can be seen in our country remaining 30 are noticed in West Bengal [17]. Those Betel leaves are found in the jungles and subtropics for their evergreen leaves. The Betel (Flute player betel) whose leaves have repairing properties [18]. In some parts of the plant in which some are enlarged near the hubs. Piper betel consists of substitute, flat, sparkling, cardio form which will be followed by piper betel leaf, having a pointed apex. This leaf also comprised a 5-7rib cage emerging at the bottom and tiny blossoms [19].

These leaves are incredibly nutritious, containing significant amounts of numerous nutrients and chemical compounds [20,21]. The leaves also include molecules like arginine and diastase, as well as histidine, catalase and lysine, as well as a slew of other essential amino acids. Many previous Betel leaf investigations focused on the constituents of betel leaf extracts [26]. There are several mixtures in proving a feasible extraction technique that separates the phyto-parts from betel leaves. Solvents such as methanol, ethanol, propanol, and ethyl acetic acid derivatives have been used extensively for phenolic extraction from novel varieties [26,27,28].

National Scenario

Betel leaves are considered one of the essential business harvesting crops developed commonly by minor and small-scale merchants or agriculturists in many parts of the country such as Andhra Pradesh, Madras, West Bengal, Maharashtra, Assam, Karnataka, Bihar in the areas include 53,539 ha (Hectare) and a yearly income of Rs. 9000 million and around 20 million so can individuals be assessed to their jobs or by implication of their part the way or completely from assembling, handling, dealing with, transport, and commercialization. piper betel leaf is valued at 30-40 million dollars which are trafficked to many countries like Nepal, European Countries, Canada, Bahrain, Pakistan, Saudi Arabia and the United Kingdom [21,22,23].





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Karnataka Scenario

It is extremely common for modest and small farmers in Karnataka to develop their major business crops. The usual betel plant farming locations include Mysore, Bijapur, Davanagere, Shimoga, Haveri, Bangalore, Chikmagalur, Tumkur, and Dharwad. Betel pepper is developed in a 6,988 ha (Hectare) area with a yield of 1,31,795 lakh leaves results fruit full of 18.86 lakh leaves/ha during 2011-12. It is a heritage of India and is referenced in Vedic literature [24,25]

Mysore Scenario

Mysore is one of the traditional cities which religiously presented betel leaves as a sign of respect and prosperous well-being. Areca nuts which were kept on top of the pile of betel leaves were given to elders to take blessings and also this is one of the essential things offered at weddings and functions. A Mysuru region that had cultivated betel leaves for ages as Yele Thota would offer title deeds within a short period. Although 150 of them would become the landscapes near the sewage homestead in vidyaryapuram, 95 others would be offered lands and elective parts at Mahanahalli on the edges of the city. The farmers had been mentioning territory, as the yele thota cultivated by the recent Construction Industry Training Board (CITB) meant various devices, along with the unloading of trash [30,31]. Mysuru betel leaf is an assortment of heart-framed betel; (piper betel) leaves occupied all over Mysore. It is eaten as a pan or betel quid with or without tobacco. Mysore is one among the cities which advance its name, as a prefix to numerous eatable things such as Mysore mallige, Mysore silk, Mysore agarbathies, (incense sticks), Mysore sandalwood, oil stand, and importantly Mysore betel leaves which commonly known as Mysore vilyedele and Mysore chigurele which having astounding Geographical Indication (GI) tag all over India. However, some factors have influenced these consumables [28,29]. In terms of its cultivation, the leaves were first cultivated around 5000 years ago in the Mysore Maharaja's nurseries and afterwards expanded to 100 pieces of land between Poorniah Choultry in Old Agrahara and Vidaryaryapuram crossroads, which connects Mysore to Nanjangud Road in Mysore. It gradually expanded to roughly 500 parcels of land in the Mysore area. A kind of heart-shaped betel leaves that covered and encircled Mysore. Mysore betel leaves are distinguished from other betel leaves by their smooth surface and spicy flavour [21]

The Taxonomical Classification of the betel leaf:

| | | |
|----------|---|---------------|
| Kingdom | : | Plantae |
| Division | : | Magnoliophyta |
| Class | : | Magnoliopsida |
| Order | : | Piperales |
| Family | : | Piperaceae |
| Genus | : | Piper |
| Species | : | Betel |

Piper betel leaves have various vernacular name it includes:

| | | | |
|-----|----------|---|---|
| 1. | Hindi | : | Pan |
| 2. | Marathi | : | Nagbael |
| 3. | Gujurati | : | Nagarbael |
| 4. | Arabic | : | Tambool, Tambol |
| 5. | Javanese | : | Bodeh, Suruh, Sirih |
| 6. | Thai | : | Pelu |
| 7. | Bengali | : | Pan |
| 8. | Semang | : | Serasa, Cabe |
| 9. | Tamil | : | Vetrilai |
| 10. | Sanskrit | : | Nagani, Tambool, Nagavallari, Saphashira, Varnalata, Nagavallika, Mukhbhushan |
| 11. | English | : | Betel vine, Betel, Betel pepper |
| 12. | Telugu | : | Tamalapaku, Nagballi |



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The betel leaf has a variety of mending properties. It can also be used in Ayurveda, or medicinal plant, to treat skin sensitivities, ulcers, wounds, rashes, skin removal, skin stripping, and so on. Betel leaf is used to treat a variety of ailments such as hypertension, diabetes, cerebrum poison, bubbles and abscesses, migraine, leucorrhoea, cuts and wounds, ringworm invasion, gum expansion, voice problems, illness, wound healing, stoutness, conjunctivitis, stoppage, scraped area, and so on [52]

Preparation of Wine

Leaves of piper betel leaf is belonging to the Piperaceae family, which comprises kava and pepper. This is one of the emerging creepers, with reflexive heart-moulded leaves and white catkin. Betel requires high land and particularly prolific soil. It was identified that has healthful properties as well as a gentle energizer. leaf comprises gentle energizer 0.8% fat, 6.1% Carbs, 3.1% protein, 2.3% minerals, 85.4 % dampness, nutrients like niacin, thiamine, L-ascorbic acid, carotenes and riboflavin and minerals[51].

Wine was prepared with the live help of herbs, for example, *Moringa oleifera*, *Sesbania sesban*, *Erythrina indica* and *Sesbania grandiflora* which additionally give conceal and add to the expansion in mugginess. The closeby positioning of plants additionally aids in dampness, maintenance and formation of microclimate helpful for development. It is just between one and two metres, as opposed to the training in which the plants are allowed to reach the level of the supporting tree. This adjustment of the plant environment is accomplished by the direct development and advancing plentiful stretching. By suppressing the straight growth and promoting copious elongation, the crop habitat is altered. The plant kinds under-regulated growth are modified to fit the typical situations.

For significant improvement, such air circumstances with enough sunlight (picture of produced dynamic solar radiation around 1200 and 1800 moles metres - 2 S-1) are very useful. With the advancement in nursery/glasshouse developments, it is now extremely simple to take crops out of their usual living situation and grow them under controlled conditions by controlling the moisture occurring in sunlight and temperature. Development that takes place in a constrained environment is practised in semi-tropical regions where the relative humidity is prevalent and temperature swings are frequent (40°C is the midsummer highest temperature and 10°C is the wintertime lowest temperature). Spoilt grape of value was taken and make diluted by involving disinfected saline in a series of test tubes. Solidified YEPDA media (yeast remove, peptone, dextrose and agar) 100µ liter of inoculate substances were spread and incubated at 28°C degree for continuous two to three days. Observe the plates after a certain period for the colony and microscopic characteristics. By picking a suitable colony and streak on a solidified agar plate to get a pure culture. The final acquired colony was stored in a refrigerator for like clockwork [53]

Inoculum preparation

flasks are plugged using cotton in an aseptic condition. The contents were stirred for 2 days and were kept under static at room temperature. After 20 days, the contents of the flasks were filtered, heated at 60°C for 30 minutes in a water bath and then stored for future use. The wine sample was determined by the potassium dichromate method (Outreach College of Science University of Canterbury New Zealand). Total sugar estimation was done by the method of Scot and Melvin [11]. Total polyphenol concentration was determined by this method of Vernon *et.al.*,(2011) [12]. Total protein was estimated by Lowry's method [13]and glucose by DNS method [14]. pH was checked using the digital pH meter. During the fermentation of betel leaf wine, most phytochemicals were tested every 3 days [54].

Inoculum readiness

In 250 ml dried conical flask, 100 ml of YEPD (yeast extract, peptone, and dextrose) stock solution was taken, topped using cotton, sterilized and cooled after some time. Yeast inoculum was taken with an inoculation loop and add one of the conical flasks. Yeast about 0.05g of yeast was added to another flask and incubate at 28°C for one day. Leaves of betel are taken and disinfected with 1% sodium hypo chloride and cleaned with refined water [56]. Piper betel leaves were cut into little pieces, these bites along with refined water with various weakening, 1:20 (into 200ml of refined water add 20g of betel), 1:15(into 100ml of refined water add 10g of betel) 1:10 (into15 ml of water add 15g of



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betel) these weakening were named as given commercial yeast 01:20(CY01:20), commercial yeast 01:15 (CY01:15), commercial yeast 01:10 (CYO1:10); white wild yeast 01:20 (WY01:20), white wild yeast 01:15(WY01:15); white wild yeast 01:10, control (01:20)(01:15) 20g of sugar is added to each conical flask and warmed at 60°degree and cool at room temperature. Then add 10% of commercial and white wild yeast inoculum is added to the conical flask and stopped by cotton by maintaining an aseptic condition. At static conditions all the things were mixed and kept for 2 days at room temperature. followed by 20 days, things were isolated from the jar separately and again warmed at 60° degrees for around half an hour in a water bath and take and kept away for some time. Ethanol focus in wine is determined in the air by the potassium dichromate technique. Complete sugar assessment was determined by the different strategies [11]. Complete polyphenol estimated in the air by the technique spectrophotometer for Vernon et.al., [12] Complete protein was assessed by Lowry's technique [13] and glucose by DNS strategy. pH was taken a look at utilizing the computerized pH meter. During the maturation of betel leaf wine, most phytochemicals were tried at regular intervals [55]

Chewing the leaves of the Betel Nut

Every year 15-20 million Indians and 2 billion foreign shoppers grow betel vine (betel pepper leaf) for its dark green cardio form leaf [32]. The leaves are used for chewing, but they also have cell replenishing, soothing, anti-apoptotic, anti-disease, and anti-microbial characteristics. A look at the current state of biotechnological intervention in betel vine, as well as the potential for the future (Piper beetle leaf) [33,34]. Betel leaves are also used to make wine in the Kodagu region of Karnataka, India. Wild yeast and readily available yeast are used to make betel leaf wine. According to a recent study, the commercially accessible yeast used in the production of betel leaf wine produces preferred phytochemicals to wild yeast [35]

Mysore Veeleyadelae (Biting betel leaves)

The Mysurupiper betel is a kind of cardio form betel (betel pepper) leaves found all over the Mysore land. This can be used as pan or as betel quid along with tobacco or excluded. This can be presented as a token of gratitude and good fortune, which is usually a mound of betel leaf. The Betel leaf has been used in the Indian subcontinent for a long time [36]. Betel leaves are regarded in Indian culture as a basic oil secret from the betel leaves that aids in the treatment of respiratory catarrh and anti-septic improvement in learning and memory [36,37]. In Indian culture, these leaves are said to be useful for increasing learning and memory. An Examination of Betel Leaf (Skillet) in China, betel leaves are used to cure a variety of ailments and are said to have antioxidant and antimutation detoxifying qualities. Daxesh Patel and Rajendra Toprani Returning to the benefits of an ancient Indian spice, betel leaf [38].

Ayurvedic importance of betel leaves

Betel leaf has enormous Ayurvedic importance and it is one of the Vedic plants which has Vedic name as saptasira and it includes different Sanskrit names such as Nagani, Nagavelleri, Tamboo which can be utilized as treatment opposed to different kinds of sickness. The greater part of the piper betel leaves is accompanied by numerous health-giving effects that can be featured. Instances of these leaves occur right from vatsyayana's Kamasutra and Panchatantra down to kalhan's Rajatarngini (that might be the remainder of the perceived old Vedic literature composing of recorded importance) Betel leaves have been mentioned consequently, usually across long period of a time. Essential belongings of piper betel leaves have been listed in the Indian Ayurvedic medicinal framework and such essential properties are; Tikta Vipak (Digestion); katu virya (Intensity); Guna (Quality); laghu, ruksha, Tikshan Rosa (Taste); Ushana Prabhav (Effect). piper betel has separate is often utilized as an additive and blended along with various medicinal properties for improved impacts close to its free use as therapy in Ayurveda. Betel leaves are depicted as sharp, sweet-smelling, hot, and advantageous for diuretic, canapé, and voice, apart from this it has to aggravate and bother pitta and placate vata [2].

Furthermore, the Spanish fly impact of tambool biting is described in ancient texts. skillet likewise accepted to give solidarity to heart and direct blood. Betel leaf usage has calming and hostile to biological is underlined at a few spots. It goes about as Kapha and vata suppressant in Indian Ayurveda. Additionally, it helps in removing the body fluids





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from the organs that are involved in breathing since of its warming effectiveness. Betel leaf is having a great smell and has sharp taste also which initiates desire and taste, it can be tonic to the heart, liver, and cerebrum, make a sound as if to speak, decontaminate the blood and diminishes the thirst

CHEMICAL CONSTITUENTS OF THE BETEL LEAF

Betel leaf has piperol-B, methyl flute player betlol, piperol-A, a fundamental oil made out of eugenol, terpinen-4-ol, allyl pyrocatechol monoacetate, safrole, eugenyl acetic acid derivation, flute player betel leaves, Cadinene carvacrol, eugenol, allyl catechol, hydroxyl chavicol, p-cymene, chavibetol, estragon, caryophyllene, cineole are considered as necessary constituents of flute player betel and betel oil. Betel leaves incorporate tannins, sugar fundamental fuel. The fundamental fuel is a lemon-yellow fluid of odorous scent and distinct in savour [60,65]. Which comprise chavicol one of the phenols likewise an amazing antimicrobial property [37,38].

As Reported the synthetic component existing in the medicinal plant which is known as betel leaves comprises essential oil in their stalks, stems, leaves, roots and fruits and is classified under normal piper betel that can be seen in the agriculture of Nagawalli, Ratadalu, Galdalu, Kudamaneru, and Mahamaneru [13]. Essential oil of normal piper betel leaves contains chavibitol, acetic derivation (12.5%) and safrole (48.7%). Whereas malbulath does not comprise the above two constituents. Interestingly Malbulath oil has allylpyrocatechol diacetate (34.4%), one of the third most significant components as in usual piper betel oil (11.3%) [61]. In addition to this eugenol, 4-terpineol, betacaryophellene, chavibitol acetate, α -cymene and safrole are identified in the normal leaves of oil but in Malbulath were deprived of all these major constituents [63]. The composition of various portions of natural piper betel was demonstrated using Gas Chromatography-Mass Spectroscopy and noticed that the structure of the tail oil was contrary when compared with that of various portions that do not have perceptible measures of allyl pyrocatechol diacetate. The essential oil has a significant component which is extracted from the stalk, the leaf, and the stem was identified as safrole but in the case of fruit oil was different that is α -phellandrene. The compound organization in Ethylene oxide (EO) of betel leaves seems to be nearer to that of agriculturist Deshwari in India [57].

The mixtures present in the Ethylene oxide (EO) differ along with the development of the leaves and determined one of the most significant components chavibitol, acetate and safrole derivations in their leaves which is most extreme at the collecting stage. However, beta-phellandrene and eugenol constituents stayed consistent in later development [59]. The content of allyl pyrocatechol diacetate enhanced rose to the reaping level and persist changeless afterwards [62]. The development stage at which the leaf must be gathered for employment relies upon explicit mixtures that can be easily detected by concentrating on the different arrangements of the EO [64,66]. Also, ayurvedic doctors notice the development of the plant in drug preparation was legitimized [58].

Traditional uses of Betel leaves are

Swelling

It is used regionally, betel pepper is valuable for therapy, for example, for joint pains, and wounds and betel pepper could utilize to patch wounds. The extracted oil from a couple of leaflets should be removed and used in the injury. thereafter, a betel leaf has to be folded around the area. After two or three uses, the air pocket will burst out all the pussy matter [37].

Bubbles

Piper betel is a powerful solution for bubbles. These leaves are delicately warm until they turn out relaxed then, covered by applying a coating with Chinese wound oil. The oiled leaf is spread over the ignited region. This leaf must be replaced, at regular intervals. After a couple of uses, the bubble will burst out all the pussy matter [38].

Headache

Piper betel is the best-known medical result of cerebral pain. The leaf of betel comprises relieving and chilling effects. This will generally use around the affected region to get a profitable outcome to avoid migraine [37]





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Sparse or Hindered Urination

The oil extracted from the leaves of the medical plant has an attributable quality which helps to control fluid balance and is blended along with debilitated milk [37].

Inadequacy of Nerve fibre

Piper betel assumes a critical part in the treatment who are suffering, from anxious fatigue and weakness. The oil extracted from a couple of leaves, together with a teaspoon of nectar is used as a decent stimulant [37].

The discomfort of the throat

This leaflet is unbelievable family medicine for treating the hack and discomfort of the throat. Close use of this medical leaflet has viable for the treatment of discomforters. The squashed natural product or any particular fruit has to be blended along the nectar that helps the calm exacerbating hack [38].

Pulmonary disease

Betel pepper leaf helps the inspiratory relationship between adolescence and advanced people. It absorbed Chinese wood oil and is heated, which might use as an application to the rib cage to aid in reducing trouble and also helps in relaxing [37].

Constipation

This is an example of obstruction in youngsters, a medicine consisting of the tail of a leaflet immersed in Chinese wood oil that could give in to the anus. That immediately eases obstruction [37,38,39].

ISOLATION AND CHARACTERIZATION OF BETEL LEAF COMPOUNDS

Isolation of the biologically active compounds from Extraction of bioactive mixtures from medicative is a convoluted cycle that helps to find out bioactive mixtures that might be pertinent in food, refreshment ventures, drugs and so forth, this operation incorporates different kinds of the path for instance assortment, unit activities, ID materials, determination of plant species, portrayal and extraction [68]. From the available writing, we need to choose plant species by looking at their age, development of the plant, customary, therapeutic properties, utilization and chemotaxonomical examination. Before isolation, a part situation such as drying (freeze-drying, refractive window drying, sun drying, froth mat drying, radiation drying, superheated steam drying, bureau plate drying and so forth) this process relies on the examination work [68].

When you complete with drying process the materials that are obtained by the drying procedure to various processes of extraction that comprise distinguishing and detachment of the bio-dynamic mixtures available in plant materials [69]. By enhancing the handling condition effective extraction can be expected, it includes the time of extraction, strong fluid proportion, dissolvable kinds, temperature and so forth. There are the presence various kinds of extraction processes, for instance, progressed extraction procedures and normal extraction strategies. The normal extraction process includes assimilation, imbue, permeation, and bubbling under reflux, alongside maceration was the easiest extraction process which consumes most of the day for the extraction process only. Whereas progressed extraction process includes sonication (ultrasound-helped extraction) speed up dissolvable extraction, supercritical liquid extraction hydro-refining, Soxhlet extraction and microwave refining are taken to stay away from the disadvantages of normal extraction strategy [69].

Regular isolation strategies like dissolvable extraction (permeation, maceration, mixture extraction, Soxhlet) refining and non-regular techniques comprising sped-up dissolvable and supercritical liquid extraction have been commonly carried in the recuperation of normal cancer prevention agents from different plant life. As they are powerful, high energy utilization and dissolvable energy and delayed extraction time sort them uncomfortable from a conservative point of view [71]. Out of refining strategies (steam refining, microwave-helped hydro-refining, vacuum refining, hydro-refining, ultrasound-assisted hydro-refining and so on), hydro-refining has turned into the





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most famous strategies on in terms of a few benefits' immaculateness, simple fix and upkeep, straightforwardness, the inexpensiveness of isolation of oil [70].

For the extraction of betel leaf EO Clevenger's contraption is utilized, however, it includes a few downsides, for example,unfortunate cooling effectiveness, long extraction time (3-8 hrs) term, slow extraction process etc., To prevent these downsides and to work on the effectiveness of the extraction cycle and the yield of EOother adjusted procedures were embraced like betel leaf oil extractor [97]ultrasound-helped hydro-refining, microwave-helped hydro-refining and so forth [98].

As of late, In the recuperation of wanted intensifies ultrasound utilization has been demonstrated as a proficient and successful extraction procedure as far as earning more yield with decreased dissolvable use and extraction time. For the extraction of mixtures from plant sources, the Ultrasound-helped extraction (UAE) was helpful which depends on the peculiarity of acoustic cavitation and mechanical impacts. The breakdown of the cavitation rises on the plant grid's surface caused by bringing about the higher and quicker entrance of the dissolvable into the plant material and the cell dividers to burst. In the same way, because of the extraction of the ideal mixtures is sped up and improved mass exchange [97]. The bioactive mixtures recognized by the extraction strategies are identified via somechromatographic procedures, for instance, HPFC (high-performance fluid chromatography), slim layer chromatography, gas chromatography-mass spectrometry, and Fourier change infrared spectrometry [99].

Mechanism of Action

Genus Piper (Piperaceae) comprises more than 1000 species around the world. Piper betel alongside some more Piper species (P), incorporates *P. nigrum*, *P. longum*, *P. retrofractum*, *P. sarmentosum*, *P. pendulispicum*, are regularly seen in the tropical jungles and are accounted for the utilized in local functions, flavours as vegetables and also in conventional medication. Usually, piper betel will be present in Thailand, Sri Lanka, Malaysia, Taiwan and India. This is also presents in the area where moderate daylight and high mugginess in clayey, much-depleted soil [3, 4]. It is an enduring climber with a semi-woody stem. It is one of the evergreen plants having green leaves being the inconsistent base with cordate melled [5].P. betel has terpenes and phenolic compounds which are considered its trademarks [4].

Leaves of betel include fat (0.4-1%), water (85-90%), carbs (0.5-6.1%), minerals (2.3-3.3%), fibre (2.3%) and proteins (3-3.5%). From betel leaves, we can isolate numerous substances are chavibetol, eugenol, a-pinene, allylpyrocatechol, Piperbetol, a-terpineol, caryophyllene, quercetin, safrole, myrcene Germacrene-D, Ethylpiperbetol Piperol A, Germacrene-D, Piperol B, Piperol A, camphene and so on [2,5]. Dynamic mixtures of P. betel leaves significantly incorporate ethylpiperbetolchavibetol, hydroxychavicol, Piperbetol, Piperol A, and Piperol B were important in helpful in impacts. In the current story audit, we expected Currently we can expect dynamic mixtures of leaf present in plant and comprehend numerous curative impact which includes antidiabetic, gastroprotective, pain relieving, calming, cancer prevention agent, cardioprotective properties and so forth.

MEDICINAL IMPORTANCE OF BETEL LEAF

The leaves are also intended to be used to solidify the gums, protect the tooth and reduce acid reflux, cough, asthma, and congestion [4]. To bring better dental cleanliness for a tooth hole, this leaves is Asia's second most common daily consumption item. It comprises a broad germicidalactionopposed to widespread tiny living organisms,[23] like *Pseudomonas aeruginosa*, *Escherichia coli*, *Streptococcus pyrogen*, *Staphylococcus aureus* *Proteus Vulgaris* so on. It possesses a strong ability to function as a typical inhibitor. An anti-oxidant quality is connected to various organic operations, such as hepatoprotective, anti-diabetic, In the properties of anti-arthritis, anti-stroke, and anti-cancer even as unbounded revolutionaries are engaged with this large number of sicknesses. These leaves have soothing properties for wounds. Leaves Betel extract boosts slaver, whichbuilds how much spit lysozyme, bacterial-fighting antibodies, peroxidase,and in the oral cavity. Chewing leavesapart speeds up slaveras well as increases digestive fluid and triglycerol pancreatic lipase, which helps during digestion in Malaysia, Taiwan, and Papua New Guinea.





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Botanical Uses of Piper betel Leaf

The leaf juice is used to cure indigestion in children, as well as to combat malaria, antibacterial action, anti-fungal review, insecticidal activity, cell reinforcement, hostile to diabetic action, gastro defensive action, antinociceptive action, cytotoxic action, and platelet inhibition [40,41].

Nutritional fact about betel

Because of some parts that advance good, betel leaves have huge medical advantages. A review was done on the nutritious realities of betel leaf (3.1% of protein, 6.1% of starches 2.3% of minerals, 0.8% of fat, and 2.3% of fibre, 85.4% of dampness per 100 gm are available). Betel is a solid source of calcium, carotene, thiamine, riboflavin, niacin, and nutrients, plus minerals and nutrients. There is also a lot of flavonoid and polyphenolic content in the leaves. The characteristics of the aroma of betel leaf pass on because of the presence of fundamental oils made up of phenols and terpenes [42].

Biological uses

Betel bites cause catecholamine release from the adrenal cortex, which can affect endurance, blood glucose, and pulse neuronal movement, among other things. Piper, a bioactive extract from betel leaves, might delay the platelet build-up of ethyl piper betel [43].

Antioxidants Movement

The cancer-prevention agent ability of allyl pyrocatechol is attributed to concentrates betel leaves containing polyphenol combinations, such as Catechol. The methanolic concentrates of betel leaves have been shown to have lowered DPPH revolutionary and superoxide anion power scavenging and deoxyribose debasement tasks. Watery betel leaf extract was found to be revolutionary in scavenging H₂O₂, extreme superoxide, and hydroxyl [44].

Anti-malignant growth impact

Because of the existence of bioactive elements such as hydroxychavicol and chlorogenic bio-actives in the leaves of betel has been found to have against cancer capabilities in addition to tobacco cancer-causing compounds that can be destroyed [67].

Antimicrobial action

The antimicrobial capacity of betel leaves was demonstrated to be against *Pyogenes Streptococcus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *E. coli* etc. more, leaf concentrates with bactericidal action has additionally been recommended against pathogenic microscopic organisms, for example, *Enterococcus faecalis*, *Citrobacter koseri*, *Klebsiella pneumonia*, *Citrobacter freundii*, and so forth [67].

Habitat and Ecology

Piper betel plant flourishes below sticky timberland situation having a flood of moistness [67]. It inclines towards profound, generally depleted around, clayey soils and friable loamy, well off in natural substances along with a pH of around 7-7.5. It thrives in regions 2250-4750mm yearly precipitation and is developed at levels ahead of 900m [66].

Cultivation and Propagation

Development in a dark, friable, mud topsoil looking like tank earth, which comprises an enormous amount of natural organic substances, however, the very good dish was filled in Bengal on low-density soil blind rosy in variety. This variety of plants is developed from ocean plane to 1000mt, precipitation more compared to 179cm is needed. It flourishes top quality near the tropical timberland condition alongside concealing significant stickiness and a lot of earth dampness. Under regular circumstances and harnessed conditions there are two trimming frameworks extensively. The opened arrangement of development below the normal circumstances is experienced in areas where places having a lot of earth dampness and direct daylight win over time. The sort is fundamentally a climber that needs helping trees like coconut (*Cocos nucifera*) and areca nut (*Areca catechu*) and accomplishes 10-15m level alongside lavish extending at the top and mass of foliation. Another sort of harvesting is to some extent





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pressurized development that is normal in the areas where more stickiness and very fewer daylight conditions that will not dominate over the year and the plant life is to be shielded by three exorbitant daylight [35,38].

The development of the *piper betel* plant within restricted conditions is additionally an instance of making a native arrangement of an "ecological chamber" with the substances accessible in the habitat. Bareja which is the enclosed construction and its design is contained locally accessible substances, for example, bamboo tail, an assortment of grasses as covering material and wooden posts. Bajeras are the part of the perpendicular upright in shape and useful size frequently 50×30meter. where bareja is raised on inclines, west to east inclination favoured. It's obligatory to keep the west and east unit more limited than the south and north unit. The specific shape of the leaf conveys rationale as far as dampness and temperature control. Breezes from the west are dry as well as higher and very low-temperature harnesses. To limit the breeze sway, covering the west-side unit is strongest so the breeze velocity entering (the journal of phytochemistry and pharmacognosy)Bejera is decreased. The enclosing on the north side unit of the bajera is stronger than on the eastern side unit. The side which is pointing to the south kept slender, conceivably to permit some light from that bearing [89].

According to this act of development, bajera might be more established than 600-400 BC. The appropriate water supply and daylight control that cannot be kept up with it receive few adverse consequences in the shape, the taste of its leaves, size and variety. A few points of effects are listed here:

1. Adequate light, however an excessive amount of soil dampness: kicking the bucket plants,spoiled roots more fragile taste, leaf lopsided surfaces stoppage in leaf development [2,6].
2. Too much light, however, satisfactory soil dampness: leaves get a harsh taste and harder, the leaf becomes more obscure green.
3. Adequate light, however less soil dampness: more modest size, harsh taste, early falling leaf, hard leaf, a smaller number of leaves per plant, broken-lopsided edges, turn leaf yellow, powerless plant stem.
4. Too less light, yet satisfactory soil dampness: leaf turns out to be light green, more modest leaf sizes, short enduring as bitten, taste becomes a lighter, more slender leaf.

ECONOMIC STATUS

The betel leaves having very high monetary or economic status in the overall market relies on the actual India final result. Leaves of betel and its items have various structures like fluid, pulverization, capsules, and so forth. These are very much astounding because of their numerous therapeutic utilization and concern. Few portions of leaves are significantly worth added substances that are easily accessible in a wide reach of the market as food and drink items, corrective substances, dietary enhancements, oral considerations, and drugs. The betel leaves have been fabricated in the modern era specifically with betel toothpaste and powders, fragrances, betel leaf oil, mouthwash, pan masala, face cream, moment betel quid and pellets, betel treatment, cleanser, sterile salves, and beauty care products items and different restorative (Guha, 2000; Guha, 2007).

The previously mentioned items of betel acquired a decent commodity effective and procured some unfamiliar trade through sending out. In India, betel leaves trade emerging on to numerous nations like Malaysia, Pakistan, Burma, Bangladesh, Thailand and Indonesia. The cost of the betel leaf relies upon the pressing and transcription costs. Betel leaf's yearly turnover was assessed at Rs. 10,000 million. According to food and agricultural organization, the leaf cultivating yield variety relies upon the different districts, plant life, condition of the season and so on. It is estimated that the overall profit might be Rs 735 for every 150 feet in squares (14 meters) of betel ranch at regular intervals. The financial dependability of agriculturists diminishes because of the vacillation in the cost of betel leaves. To conquer these issues, an all-around controlled advertising framework ought to be created [91].





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Morpho-Anatomy of the Betel leaf

Macroscopic characters

Betel leaves are having very unique characteristics they are having yellowish green to dull green along with reflexive top subsurface and their odour characteristic is very pleasant. Its taste varied with different leaves that depend on the place and with the existence of revivifying oils, flavours might range between pleasant to powerful. Then come to shape and size is very unique and has a heart formed with various size. Size can vary with different cultivators having 7-15cm long and width having 5-14cm. Leaves are straightforward substitutes specify petals having 0.75 to 3.8cm, applaud elongated extensively praise cordate or sideways elliptic whole hairless coriaceous ten to eighteen centimetre long and five to ten-centimetre wide taper slanted and adjusted base [16,17,18].

A cross-section of part of the leaf directed with midvein displays four stratified upper berths and two stratified down cuticles and dainty on the down cuticle. Cells present in the external cuticle layers on the bilateral of the leaf are little, which have oil and tannic acid. The sub-cuticle cells on the upper side are amplified and H₂O is stored. Precious stone and fuel holds are seen in the sub-cuticle cells on the two sides. The surrounding part is very much recognized they are twofold layered brief deep minimized cells and mesophyll tissue is three-four layered and little loop. Tough bounded sporadic secretory tissue is found with thick items in likely a rejuvenating ointment.

Biological activities

Antimicrobial activity

Piper betel leaves have very huge antimicrobial properties by examination few have been identified. The EO extracted from the leaves has solid antimicrobial exercises of piper betel that lesser the adhesiveness of primal spot biological entities. The antifungal movement of *Aspergillus flavus* can be reduced by the movement of antibacterial movement of Eugenol of piper betel. Specific parts of concentrates of the betel leaf (stalk and stem) and EO have been shown a very much powerful antifungal movement at the contraction range of 450mg/dl [78,79,80]. Extracts of leaves which additionally have the watery and *Manikara zapota* L. methanolic *Terminalia catappa* L., also mainly piper betel were chosen to examine the medicinal action in opposition to few chosen microbes, among these much more powerful to hinder the bacterial strains shown by the methanolic concentrate [79]. The medicinal activity of other rough ethanolic concentrates of betel leaves in opposite few animal diseases such as *Microsporium gypseum*, yeast like *Candida Albicans*, *Microsporium canis* and *Trichophyton mentagrophyte* [79].

Betel leaves extricate displayed the more powerful fungicidal attributes holding the malignant tumour hindrance action of approximately IC₅₀ belief which went from 110.44 to 119.00g/ml. Concentrate on bactericide action of essential oil and concentrate that can dissolve of piper betel leaf chosen in the that of yeast like *M. pachydermatis* and *C. Albicans*, chromatography-mass utilizing the Kirby-Bauer test. Finally, the watery concentrate, EO, Methanolic which displayed powerful activeness in that of yeast, examined the fungicidal action of Maggi assortments of piper betel leaves essential oil [90].

EO acquired via betel leaves was viewed as 0.7 microliters per mile-litre in that opposition to *A. flavus* by the method called minimum bactericidal concentration (MBCs) which proved more efficient. Some of the unstable mixtures or components like as chavibetol, chavicol, allyl pyrocatechol diacetate, hydroxychavicol, and propenylphenols, were recognized by the chloroform removed piper betel leaves separate made a critical bactericidal difference were noticed in many studies [81]. Some studies have been designed on the bactericidal actions of piper betel leaf extraction utilizing liquid amount, ethyl acetic acid derivation, ether oil and methanol which act as dissolvable factors in opposition to the chosen pathogenic microorganisms like *staphylococcus aureus*, *Escherichia coli* and *streptococcus pyrogens*. Outcomes of all these found each one of the concentrates showed a remarkable zone of hindrance in opposition to the microbes in light of the great convergence of sterols. As well the rough ethanol concentrates of betel leaf exhibit powerful bactericidal activeness in that of *Staphylococcus aureus* [80].





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In opposition to many pathogenic microorganisms very specifically, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* by utilizing the medicinal activities of piper betel leaf extracts (methanolic and ethanolic) assessed [92]. The zone of hindrance particularly was 17.5mm in that of *P. aeruginosa*, in that of *E. coli* 15 mm and 25mm in that of *S. aureus*. Whereas more powerful properties other than ethanolic were exhibited by the methanolic concentrate in opposition to pathogens. These two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and three gram-positive bacteria (*Staphylococcus aureus*, & *Micrococcus luteus*) were elected for bactericidal actions of ethanol and fluid *P. betel* leaves concentrate [79,80]. The successful and critical medicinal actions compared with fluids as the presence of tannins, glycosides, alkaloids, phenolic substances and so forth was separately shown by the Ethanol. Besides the medicinal activeness of numerous assortments of dried betel leaf were spared and tried in that of various pathogenic microbes like *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa* by utilizing the solid well dissemination technique [57,58].

By the dis-solvable extraction technique, the dried leaf extracts were acquired by methanol (80%), ethyl acid derivation (80%) and ethanol (70%). The assortments of betel leaves of Jhalawar and Bangladeshi were separated and have a compelling and great wellspring of useful medicinal action [80]. To hinder the development of foodborne microbes like *Vibrio cholera*, *Staphylococcus aureus* and *Escherichia coli* betel concentrates of ethanol have shown great potential. *P. betel* and occidental leaves have the bactericidal property that chipped away at microwave treatment in the contrast to *S. cohnii*, *E. coli*, *B. brevis*, *P. aeruginosa*, *S. intestinalis* and *M. luteus* [100,101]. The medicinal movement of activated leaves in that of *E. coli*, *S. intestinalis* and *B. brevis* which was viewed as much more grounded than *P. aeruginosa* and *M. luteus* also stay unaltered in opposition to *S. Nouri*, Nafchi and Cohnii (2014) observed which betel leaves isolated utilizing solvents like methanol, ethyl acetic acid and ethanol shows the powerful property in opposition gram-negative microbes (*Salmonella enteritidis*, *E. coli*, *Klebsiella pneumoniae*, *Salmonella typhimurium*) alongside gram-positive microscopic organisms (*B. subtilis*, *S. epidermis*, *B. cereus*, *L. monocytogenes*, *S. aureus*) that exception of *P. aeruginosa*.

Antifungal Properties

Infections caused by parasitic fungal organisms make tainted body part occurrence various uneasiness like redness, tingling and disturbance. As well bacterial infections leave of betel extricates were utilised in the infections of parasitic fungi. The utilization of medicinal leaves of betel leaves extricates was conducted trial that result in the research by the method dissemination test and solid agar dispersion technique. Has an avenue regarding hydroxychavicol, a molecule isolated from piper betel leaf in the opposite action of two kinds of microbes very specifically *Aspergillus* and *Candida* [17]. The review was centred around tracking the minimum bactericidal concentration (MBCs) and least fungicidal concentration of hydroxychavicol. The medical effect of Minimum inhibitory concentration for fungal species like *Candida* was examined the range went from 15.62 micro/ml to 500 micro/ml and for *Aspergillus* species was 125 micro/ml to 500 micro/ml shown. The trial has shown comparative or twofold overlap results contrasted with minimum inhibitory concentration when concerning minimum bactericidal concentration. This examination affirmed the fundamental outcome of hydroxychavicol to fungal species of *Candida* where hydroxychavicol was viewed as a bio constituent accountable for limiting biofilm organization [79].

The organization of biofilms is identified by the raised take-up of propidium iodide by fungal species exhibited in the layer without a doubt distributed by hydroxychavicol. Though Sarma. explored the ability of piper betel leaves to isolate in that microscopic organisms and parasitic organisms [22] the betel leaves were obtained by the four distinct solutions like hexane, ethanol, acetone and water which helps to decide the most accruable component for removing betel leaves. The examination continued by testing two organisms' strains *Candida* and *Aspergillus niger* with piper betel. All the extracts show hindrance regions in opposition to the microscopic organisms and growths. The survey discovered that contrast with ethanol, water, acetone and hexane extricates gave a bigger area of restraint. To concentrate on three unique species very particularly *C. krusei*, *C. tropicalis* and *C. Albicans*. Kaypetch and Thaweboon et al., (2018) utilized the plate dissemination strategy. All the results revealed piper betel leaves remove all possessed parasitic fungal activities in opposition to tried parasitic species with the presence of a zone of a hindrance with breadth going from 32mm to 35mm [93].





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The extreme inhibitory zone around 26mm, which is higher shown by the extricates of betel leaves was used against the *C. Albicans* when compared with tulsii leaves extrication that yielded more than 13mm. To know the hindrance caused by the piper betel leaves in contrast to all the medicinal leaves which show more hindrance than other compared leaves *Microsporium gypseum* (62 mm), *Trichophyton mentagrophytes* (60 mm), *A. javous* (35 mm), *C. tenomyces serratus* (69 mm), *A. fumigatus* (62 mm), *Aspergillus candidus* (40 mm), *Arthroderma benhamiae* (65 mm) and *A. ochraceous* (51 mm) [81].

Gastroprotective activity

The boiling water removes fundamentally expanded bodily fluid substances sticking to the mass of the stomach mucosal membrane. The body fluids layer is viewed as a significant mucosal safeguard in that of endogenous aggressors for instance acids and also as a specialist in working with the maintained interaction. It is usually accepted that increases in the corrosive emission are the main component for the enlistment of gastric sores. The greater portion of heated water extricates does not contain a huge hindrance in the sharpness or pH of the gastric liquid. Consequently, the gastroprotective impact of flautist betel is unable to intervene (Journal of Pharmacognosy and Phytochemistry) employing restraint of corrosive emission in the gastric mucosa yet by expanding its bodily fluid substance [81].

The greater portion of the heating water extricates gastroprotective properties more prominent than the misoprostol [63]. It has been demonstrated that enemies of oxidants have a broad spectrum of exploration in safeguarding in opposition to gastric mucosal injury, in addition to restraining the movement of digestive ulcers. The perforation movement is brought about by the free revolutionary initiated ring method. Thus, it captures revolutionary factors that help in quicker healing [64,65]. In various in vitro models allylpyrocatacol has been shown a powerful enemy of oxidant potential. Allylpyrocatacol altogether speeds up the ulcer-recuperating process, which builds the mucosal secretion that usually helps in mending a system safeguarding the ulcer cavity in opposite to aggravating stomach discharge (pepsin and HCL) in turn improving the pace of beside mending process [90].

Antioxidant

Antioxidant is one of the specialists that aids in the harm caused by cells Regarding free extremist and synthetic response carried out by the human body in opposition to ecological changes and more potential sources. An antioxidant is called free anxiety chemical agent which got from one or the other synthesized, regular, or a mix of both. Certain of these oxidants can be extracted and used as a chemical agent by the utilization of DPPH (2,2 diphenyl-1-picrylhydrazyl). Chemical activity is available in DPPH because of hydrogen-giving possession that might be seen in a tried component [22]. Because of the presence of reductions, the capacity to give and reduce electrons power [22,46,47,48].

Piper betel leaves extricate and possess antioxidant activities due to the presence of flavonoids and phenol in their leaves. The anti-oxidant and revolutionary rummaging movement is directly proportional to the higher organization of flavonoids and phenol. The free radicals scavenging properties were shown that the greatest in the ethanolic concentrate (89.46%) and least (62.03%) in the refined water removal [94]. Methanolic concentrates of piper betel leaves are introduced in most elevated cell reinforcement activities by purposefully restraining half of DPPH revolutionaries at a low grouping of arranged a trail of deciding cancer prevention agent properties of piper betel leaves [80]. The extricated piper betel leaves have an 80% ethyl acetic acid derivation which displays and promotes dissolvable cancer prevention agent compound extraction. Regarded cancer prevention agent activities of piper betel and leucoskye capitella separately utilizing the DPPH measure reviewed by the studies [51]. Fluctuating convergence of leaves of betel only were blended with 0.5ml of 1mM of DPPH from these studies conveyed that piper betel leaves having more extreme anti-oxidant with that of leucoskye capitella because of more flavonoid tannins and phenolic composition in leaves of betel remove. In the same way, started a relative report on malignant hindrance agent movement betel leaves extricates utilizing DPPH [52].





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Extricates of betel leaves were obtained by water solvents and naturally and noticed was resolved that acetone water-acetic corrosive (AWA) blended along with piper betel extricates, which display the greatest cancer prevention activities however hexane showed lower interference. Additionally, some research experienced that the most elevated DPPH radical scavenging activities were accomplished betel was removed utilizing ethyl acetic acid derivation, examined by hexane, methanol lastly fluid concentrates [53]. In contrast with rutin (IC₅₀=33.7 meu g/ml, and quercetin (IC₅₀ 30 MEU G/ML), the ethyl acetic acid derivation removes had a prevention agent IC₅₀=4 × 40 meu g/ml. Though extricates got utilizing methanol and hexane, they required more than 100meu g/ml fixation. In respect to watery concentrates, interference fail to reach even half, by implication demonstrating the shortcoming of that dissolvable in extricating cancer prevention agent compound from piper betel leaves [72]

Radioprotective

Radiotherapy is the pillar of disease therapy; it means to kill or take away malignant growth cells. Moreover, harming the impact on typical tissue is inescapable [46,47]. Vulnerable to ionized emission increments mitochondria subordinate ROS creation, which increments lipid peroxidation [46,82]. Treatment with P. betel diminishes lipid peroxidation as demonstrated by the decrease in the degree of TBARS, formed diene and lipid hydroperoxide protective impact of Piper betel is contributed by its phenolic components, for instance, allyl pyrocatechol and chavibetol, which were accounted for to have extremist rummaging cancer prevention agent movement while at highest focus, it incited apoptosis, restrained development and cell cycle movement [56].

Anti-inflammatory

The activity of inflammation is an intricate biologic response including water-conducting tissues [18,19]. Cardinal indication of inflammation incorporates hotness, agony, redness, loss of capacities and expanding [18] inflammation fills as a defensive component in opposition to different kinds of destructive boosts. Moreover, an exuberant response promotes the advancement of different incendiary problems [20]. Aggravation was actuated in exploratory creatures by the organization of degrading specialists for example acetic acid and carrageenan to their ears and paws led to the improvement of oedema was estimated [21]. The animals which have got P. betel extracts showed a critical decrease in the amount of the idea created following the organization of harmful agents [22]. For Intense irritation, Nitric Acid (NO) and Prostaglandin E₂(PGE₂) were called to be an enhancer [23,24]. Now different increase or decrease rates of NO and PGE₂ were observed in various models it follows [72,73]. In Freund's supportive actuated theory of joint irritation or inflammation in rodents, NO creation was seen to be diminished in murine in the tissue which lines abdominal muscle and girdle space scavenger cells hatched with piper betel leaf [25]. In the synovial invasion of patients with rheumatic joint pain, that was seen, allyl pyrocatechol diminished the creation of NO in penetrating macrophages [26]. In lipopolysaccharide (LPS)- incited creation of NO and PGE₂ in a murine macrophage cell line, allyl pyrocatechol was displayed to restrain the development of both NO and PGE₂ in a portion subordinate way [27].

Allyl pyrocatechol likewise decreased the incendiary reaction of scavenger cells using restraint of mRNA articulation of p40, COX-2, IL-12 and iNOS direct down guideline of the atomic component kappaB (NF-κB) path, demonstrating that allyl pyrocatechol might possess helpful potential in diminishing aggravation. Allyl pyrocatechol likewise essentially rummaged hydroxylandsuperoxide extremists in penetrated neutrophils [26].

Anticancer action

Reactive oxygen species (ROS) has been ensnared during the time spent in carcinogenesis and which is also known as cytotoxic. Over creation of ROS show a significant character in the commencement and movement of illness via DNA harm brought by macromolecule oxidation [49]. In particular, latent research shows that ROS might include an advantageous portion as a chemo-therapeutic specialist through initiating apoptosis [6,50]. P. betel was characterized to have much more cancer prevention and anti-tumour properties in contrast with other Piperaceae relative families [51,52].





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By utilizing bosom malignant growth cell line in the in-vitro examination, MCF-7 viewed, P. betel alkyl group acid derivation removes 65 $\mu\text{mg/ml}$ hold cytotoxic action, that because of the increase of cancer reduction agent action in that manner helps in decreasing reactive oxygen species development [53]. At 150mg/Kg, body weight Hydroxychavicol has been viewed hindrance to the development and human prostate expansion disease, PC-3 cells [54]. Whereas methanol extricate of Hydroxychavicol showed apoptosis of CML cells utilizing powerhouses of cellular reactive oxygen species relying on endothelial nitric oxide synthase-intervened pathway was explained by the researcher [55]. Piper betel leaves also have gastroprotective, hepatoprotective, and pain improvement, low portion of hydroxychavicol holds the cancer hindrance factor at more focus, it helps to initiate apoptosis, development of restraint, and cycle movement of the cell [56].

Antidiabetic Diabetes Mellitus (Type 1)

Inadequacy of beta-cell in the pancreas to make up enough measure of Humulin to switch glucose over completely to glycogen which therefore increments glucose levels in the blood [54,55] is known as antidiabetic Diabetes mellitus (type 1). Drug for treatment has been accounted to make many secondary effects in patients [54]. Recent studies have viewed that elective remedies which have normal sources treating diabetes are frequently increasing in the modern era to reach needy people. By utilizing the rodents as model organisms Hossain investigated that piper betel leaves have the antidiabetic properties of action [54].

The rodents used as a model organisms are gathered in three kinds of groups along with various centralization of piper betel leaf extricates beginning from 50mg/kgbw for bunch A, 100mg/kgbw for bunch B, 200mg/kgbw for bunch C. Investigators have discovered that the plasma glucose level of the regulated rodents diminished together; 36.1% for bunch A, 16.6%, and 23.76%, concerning bunch B and C. In some of the studies, Santhakumari et al. (2006) have shown Rodents who have diabetes by obliterating beta cells by using Streptozotocin which later rodents with diabetes were supported with piper betel extricates [95]. Usually, general examination in both piper betel extricates (boiling water concentrate and cold ethanolic separate) have sealed antihyperglycemic movement (by the improvement of glucose resistance test and bringing down the glucose level in Streptozotocin-prompted diabetic rodents) and hypoglycaemic action (tried in abstained normoglycemic rodents).

From these discoveries, betel leaf has been recommended as that has the capability to do insulinogenic action. By considering the use case, scientists have shown a few discomforts, in which they need to dully feed the tried rodents with piper betel extricates for intention to continue to work [76]. Aside from that led a concentration on Streptozotocin-instigated creatures explicitly the Wistar rodents for treating the antihyperglycemic action of piper betel extricates [57]. Diminishing body weight showed by STZ acuted rodents since the obliteration of protein brought about by diabetes. The presence of hyperglycemia properties in leaves of betel can be used to treat the Streptozotocin-prompted rodent that showed typical to minimal weight reduction among the rodents [78].

Especially at expanded doses, extricates of betel leaf have shown the solid ability to repress alpha-glucosidase catalysts. To know the antidiabetic property separate Perumaland Kavitha performed both in-vitro and in-vivo tests. The ethanolic concentrate piper betel would wise to rate (%) of restraint contrasted with control build, acarbose (hostile to diabetic medication). Kavitha and Perumal performed both in vitro and in-vivo tests for the antidiabetic properties of Piper beetle separately [59]. The specialist found that the ethanolic concentrate of Piper beetle would be wise to rate (%) of restraint contrasted with control build, acarbose (hostile to diabetic medication) [73,79].

Phyto-constituents

The leaves of betel are very much rich in many phytochemicals, it contains minerals (2.3-3.3%), Essential oil (0.08-0.2%), Vitamin-C (0.005-0.01%), Fiber (2.3%), Alkaloid (araken), Nicotinic corrosive (0.63-0.89mg/100gms), protein (3-3.5%), Fat (0.4-1%), Water (85-90%) as well as it also various kinds of nutrients as like thiamine (10-70 μg /100gms) alongside it has minerals like Potassium (1.1-4.6%), Calcium (0.2-0.5%), Iodine (3.4 μg /100gms) and Phosphorus (0.05-0.6%) there we also can be seen harsh mixtures about (0.7-2.6%). The particular kind of components gave an impressive sharp fragrance in it because of the presence of terpene-like structures [19,72]





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The finite phenol content is fluctuating on sexuality. In contrast with the female plant, the male plant comprises three overlapping very high amounts of complete phenols and two creases higher thiocyanate components, thiocyanate. Actual nature of the leaf relies on the content of phenols i.e, the nature of the leaf will be expected better only when the presence of more phenolic content, as of late many expert work views the betel leaves comprise sugars, starch, rejuvenating and diastases ointment making out of allyl pyrocatechol, eugenol, eugenol acetic acid derivation, terpinene-4-ol, and so forth as a major component [21,22]. The existence of alkaloids, Amino acids, Carbohydrates, steroidal and Tannins by the examination of leave which possesses phytochemicals [23]. A high amount of Tannins can be seen in the centre piece of the primary planta. Also recognized that significant phenols like cadinene, caryophyllene, chavicol, limonene, safrole, camphene, partner pyrocatechol, and terpenoids incorporate 1,8-cineole, pinene, chavibetol and eugenol. Essential oil of leave has eugenol as an anti-fungal component. More amount of natural balm diastase chemicals and sugar in new leaves when compared with old leaves. In contrast carbolic corrosive chavicol is multiple times powerful as a clean specialist [79].

CONCLUSION

Piper betel is a traditional medicinal plant that is well-known all over the world as a potent source of new therapeutic applications. Because betel leaves are high in minerals, antioxidants, and a variety of phytochemicals, they are a good candidate for future use as a treatment for a variety of ailments. Overview of the botanical Uses of Piper betel Leaf, Chemical Composition, Traditional Uses of Betel Leaves, Contents of Betel Leaves, and Chemical Constituents has all been addressed in this review. There is a need to further research into the standardisation and stabilisation of betel leaf extracts, which will open the way for future medicinal applications [46,47]. Several vitamins have been discovered in betel leaves, according to research. Riboflavin provides vitamin A, thiamine, nicotinic acid and vitamin C. The leaf is a natural antioxidant with a wide range of biological functions. The leaf includes an enzyme that aids digestion and has potent antibacterial properties against a wide range of bacteria. Nonetheless, the commonly used betel leaf offers a wide range of medical uses for public health [48,49,50]. However, such applications have the potential to expand their use in a variety due to their notable health benefits as good the probability of extra economically useful addition to possibly be safe in the opposition of bad bacteria.

CONFLICT OF INTEREST STATEMENT

No conflict of interest

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AUTHORS CONTRIBUTION STATEMENT

Dr Sumana K was initiated for this review article and altered and edited the manuscript. Ms Divya K S has finalized the topic and written the original Manuscript, Talluri Rameshwari K R helped in writing the manuscript order wise along with the flow chart and importance of the betel leaf etc., Finally, all authors have discussed the points of review and contributed to the final manuscript.

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| Sl no | Piper betel leaf extract | Isolated bioactive compounds | Reference |
|-------|--|---|---|
| 1 | Dried leaves extract using ethanol by ultrasound-assisted extraction | Hydroxychavicol, eugenol, isoeugenol and allylpyrocatechol 3,4-diacetate | Ali, Chong, Mah, et al. (2018), Ali, Lim, Chong, et al. (2018), Ali, Lim, and Wahida (2018)2 |
| 2 | Ethanol extract of red betel leaves by Soxhlet extraction | Neophytadiene, propionic acid, and elemicin | Anugrahwati, Purwaningsih, Rustina, Mangalarini, Alnavis, Wulandari, and Pranowo (2016) |
| 3 | Betel leaf extract powder using Soxhlet apparatus with 70% deionised water and ethanol | Alkaloids, fatty acids, phenolic compounds, alcoholic compounds, flavonoids compounds, terpenes compounds, coumaran compounds and organic acids | Foo, Salleh, and Mamat (2015) |
| 4 | Leaf extract | Tannins, anthraquinones, flavonoids, alkaloids, terpenoids, saponins, cardiac glycosides, glycosides, phlobatanins, hydroxychavicol acetate, allylpyrocatechol piperbetol, isoeugenol, anethole, stearic acid, methyl eugenol, carvacrol, chavicol, and allylpyrocatechol | Kumari and Rao (2014) and Rekha, Kollipara, Gupta, Bharath and Pulicherla (2014) |
| 5 | Piper betel aqueous and ethanol extract | 4-chromanol, phenol 2 methoxy 4-(2-propenyl) acetate, eugenol, | Deshpande and Kadam (2013) |
| 6 | Crude aqueous extract | Hydroxychavicol, Hexadecanoic acid, Octadecanoic acid, 2,3-bis(hydroxy)propyl ester, Benzeneacetic acid, Eugenol | Lakshmi and Naidu (2010); Thurairajah and Rahim (2007) |
| 7 | Dried leaves extract using ethanol by ultrasound-assisted extraction | Hydroxychavicol (66.55%), eugenol (11.92%), isoeugenol (2.90%) and 4-allyl-1,2-diacetoxybenzene (3.21%). | Ali, Chong, Mah, et al. (2018), Ali, Lim, Chong, et al. (2018), and Ali, Lim, and Wahida (2018) |





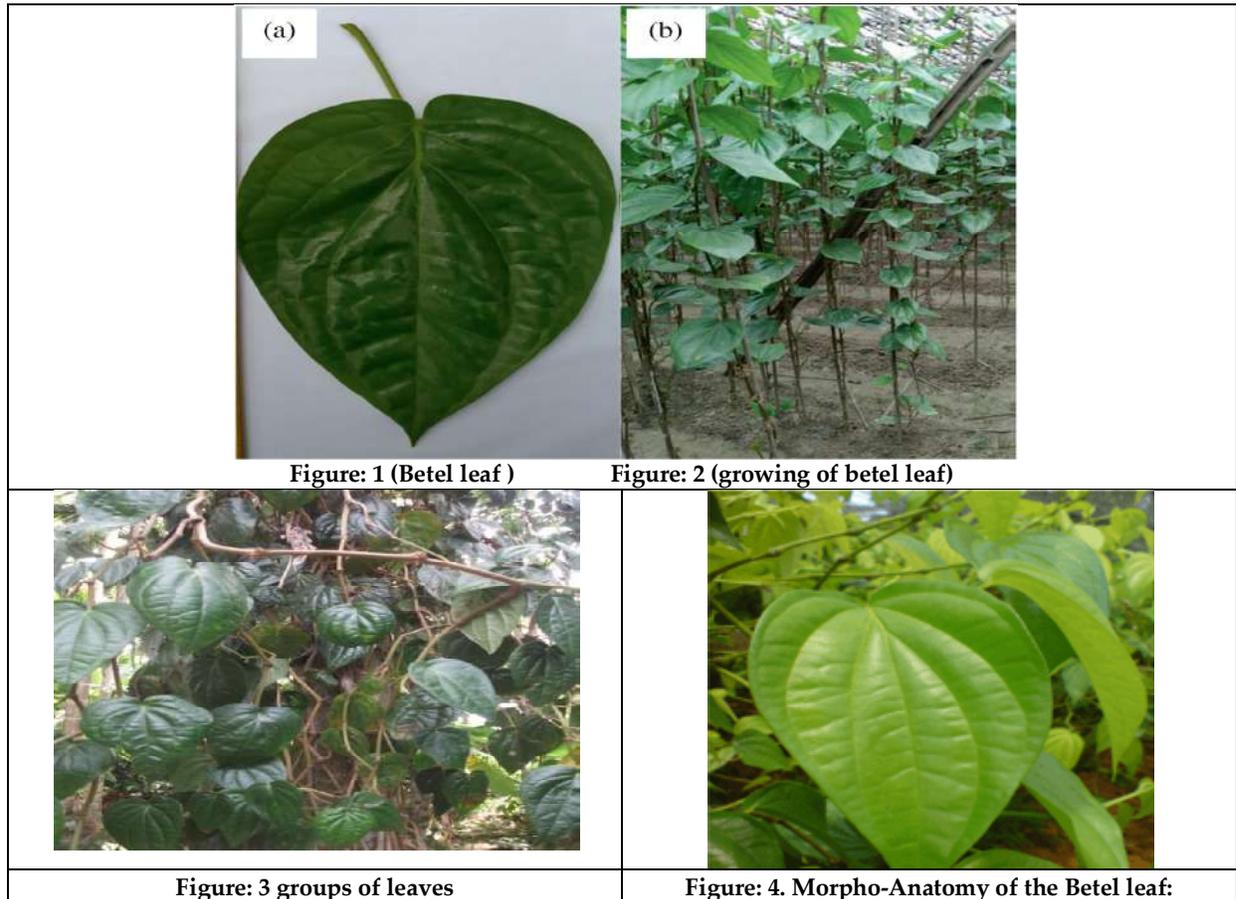
Divya et al.,

| | | | |
|----|--|--|--|
| 8 | Soxhlet extraction | Hydroxy chavicol (69.46%), 4-Chromanol (24%) and Eugenol (4.86%), 1-phenylpropene-3,3-diol diacetate (0.923) and 4-allyl-1,2-diacetoxy benzene | Muruganandam, Krishna, Reddy, and Nimala (2017) |
| 9 | Solvent extraction (methanol) and microwave treatment | TPC, TFC and CQAC | Tan and Chan (2014) |
| 10 | Piper betel aqueous and ethanol extract | 4-chromanol, phenol 2-methoxy 4-(2-propenyl) acetate, eugenol | Deshpande and Kadam (2013) |
| 11 | Leaf extract var. Haldia using chloroform by nuclear magnetic resonance (NMR) analysis | 1-n-dodecanyloxy resorcinol (H1) and desmethylenesqualenyl deoxycepharadione-A (H4) | Atiya, Sinha, & Lal, 2018 |
| 12 | Dried leaf extract var. Meetha and Banarasi paan using distilled water, hexane, acetone and ethanol solvent extraction | Proximate analysis of the highest phytosterol | Sarma et al. (2018) |
| 13 | Ethanol extract of red betel leaves by soxhlet extraction | Neophytadiene, propionic acid, and elemicin | Anugrahwati, Purwaningsih, Rustina, Manggalarni, Alnavis, Wulandari, and Pranowo (2016) |
| 14 | Leaf extract using hexane, ethyl acetate and ethanol | 4-Allyl-1,2-Diacetoxybenzene, chavicol and eugenol | Choopayak et al., 2016, Murata et al. (2009), and Venka deswaran, Thomas, and Geraldine (2016) |
| 15 | Chloroform extraction of the aqueous leaf extract | Hydroxychavicol | Ali et al. (2010) |
| 16 | Solvent extraction and HPLC/ESI-MS-MS | Eight components with major compounds 3-p-coumaroylquinic acid and 4-p-coumaroylquinic acid | Ma et al. (2013) |
| 17 | Spray dried powder of leaf | Phenolic compounds, tannins, flavonoids, steroids, and alkaloids | Arawwawala et al. (2011) |
| 18 | Dry leaf extract maceration extraction, phytochemical screening | TPC, TFC and tannin | Taukoorah et al. (2016) |





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Food and its Impacts on Behavior with Specific Reference to Bhagavad-Gita

R.Bhuvanewari^{1*}, Maloth Nageswar Rao² and K. C. Prakash³

¹Assistant Professor and Research coordinator, Department of Management Studies, Nehru Institute of Information Technology and Management, Coimbatore, Tamil Nadu, India.

²Executive Director Krishnaveni Talent School Diamond point branch, Hyderabad, Telangana, India

³Assistant Professor, Indian Institute of Plantation Management (Agri) (IIP) Bangalore, Karnataka, India.

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*Address for Correspondence

R.Bhuvanewari

Assistant Professor and Research Coordinator,
Nehru Institute of Information Technology and Management,
Coimbatore, (Affiliated to Anna University),
Tamil Nadu, India

E.Mail: niitmrhuvanewari@nehrucolleges.com



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ABSTRACT

Srimad Bhagavad Gita is the part of Mahabhrata with 18 chapters and 700 verses. The conversation between god and arjuna in the battle field of kurukshetra. It brings the Vedanta in universal framework. It is consider as an ancient text book. The aim of this paper is to explore relevance of Bhagvad gita, the views on food safety management presented in it. Methodology is based on subjective interpretation used for understanding the qualitative research based on ancient texts is known as “Hermeneutics”. In this study 16 verses related to food that influence behavior, are explained (gunas) (sattva, rajas, tamas). Sattva guna (behavior) influence is based on ethics and tamas and rajas guna (behavior) is considered unethical. God has given knowledge of different types of food .Three types of food are based on the three guna (behavior).These three types of food based on gunas effect our whole system., Different types of food had different implication on us. It can be concluded that Bhagavad Gita helps in gaining knowledge not only about food but also about problems of our life and provide answers to all our questions.

Keywords: three gunas (behavior of food), types of food, food and digestive system, eat fresh food, energy food increase duration of life





INTRODUCTION

The food its types and the way of eating it, is discussed in Srimad bhagvad Gita. Sativc food is recommended. Tasmic and rajasic food is prohibited. The food which is good for health should be chosen. Not only food but quality and quantity of food also influence our mind. We should also pay attention while purchasing, cooking and consuming food. Bhagavad gita categorized the food into three types -

1. Satviika foods, 2. Rajasic foods and 3. Thamasic foods

Choice of food

Satvika food / goodness:

The food made up of fresh, vegetables, fruits, nuts, seeds, sprouts, honey, and whole grain are known as Stativka food. Food which is prepared with positive energy transmits the energy to the surroundings. If the food is served with love, love and compassion will be generated in the behavior of the person consuming the food. .

Rajas Food / Passion: Food that includes the hot, bitter, sour, dry or salty. The food which gives immediate pleasure and consumed without thinking of consequences such food causes distress, misery and disease.

Tamas Food / Mode of Ignorance / Darkness: we have to take food prepared within three hours otherwise it becomes Tamasic food. Food which is overcooked or over heated become decomposed and considered unclean. Tamasic food results in negative emotions, like anger, jealous. In Bhagavad gita lord Krishna states the Sattivc food increases purity, strength, health and comfort. The food that is fresh and served with pure thoughts is accepted by god. The purity has an impact on mind and body. The person, who consumes unhealthy food, leads the health to adverse effect.

We have to give importance and pay attention to the food

Food and Three Type's Gunas (Behavior)

Lord Krishna explains Arjuna three gunas related to food. The preference is given to the sattvick food. The food which is offered with divine thoughts, purity, austerity and charity by paying attention to god is Sattvick food.

Three Types of Food Related to the Personality Reflect Health, Behavior and Thinking

1. Sattivc means purity, well being – it's a pure vegetarian diet that includes seasonal fresh fruits and vegetables. The people who eat Sattivc food are peaceful, calm energetic and enthusiastic.

2. Rajas food is rich in taste and gives instant energy that the consumer of the food he can be disturbed with digestive system. Rajas food makes ones miserable and sick.

3. Tamas food is processed food, over cooked food, leftovers, deep fried. It creates laziness, overweight, diabetics and obesity.

Sattva – Balance

Rajas – Movement

Thamas –Inertia

Sattva → pure food capacity to build balance diet for deep learning

Raja → action orientation

Thamas →self centered

Types of Food

Bhagavad gita explains how to eat food? What to eat? Lord Krishna shared how to acquire health and happiness and balance in life. Bhgavad gita food activities are balanced and their actions are proper. Those who sleep wakeup early and doing regular meditation this three attributes as is greater impact on once personality. Lord Krishna says about four types of food which are eaten, sucked, licked and drunk





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Food is Consider as Prasadam

Bhagavad gita states that always keep kitchen clean, neat and wash your hands then serve the food neat. While preparing food for god that food not to taste by anyone before serving to god. The food offering process is called Naivedyam after finishing the arti and chanting mantras the food distributed to all. The way of thanking god whatever I have today its divine blessing I received from you. food consider as Prasad (grace of god) the person who eats the food with sacrifice offered to god then than it consider as Prasadam clearly said in chapter 3 verse 13th soloka

यज्ञशिष्टाशिनः सन्तोमुच्यन्तेसर्वकिल्बिषैः |
भुञ्जतेतेत्वर्घपापायेपचन्त्यात्मकारणात् || 13||

yajña-śhiṣṭāśhinaḥ santo muchyante sarva-kilbiṣhaiḥ
bhujjate te tvaghāṇi pāpā ye pachantyātma-kāraṇāt

-Bhagavad Gita, Chapter 3, Shloka13

Not to Eat too Much and Not to Sleep too Much, it Create Risk (Regulations of Diet)

Too much of eating and too much of sleeping is not good for health. There is a need of individual consciousness to do any work it give successes in life. Those who eat too much and sleep for a long a time is not good for health. Eating very little create risk of health issues and sleeping less hours cannot do work properly. There is a need of balance diet and proper rest to mind and body.

नात्यश्रतस्तुयोगोऽस्तिनचैकान्तमनश्रतः |
नचातिस्वप्नशीलस्यजाग्रतो नैव चार्जुन || 16||

nātyaśhnatastu yogo 'sti na chaikāntam anaśhnataḥ
na chāti-svapna-śhīlasya jāgrato naiva chārjuna

-Bhagavad Gita, Chapter 6, Shloka16

Health and Happiness (Happy Life With Good Health)

The person who do work and day today activity on time, live a perfect life. Being tactful in all spheres of life helps him to live a balanced life with good health and happiness. The person who is disciplined with eating habits and doing exercise is eligible to practice yoga. As mentioned below in the sloka –

युक्ताहारविहारस्य युक्तचेष्टस्य कर्मसु |
युक्तस्वप्नावबोधस्य योगो भवति दुःखहा || 17||

yuktāhāra-vihārasya yukta-cheṣṭāsya karmasu
yukta-svapnāvabodhasya yogo bhavati duḥkha-hā

-Bhagavad Gita, Chapt 6, Shloka17

Food with Devotion

Lord Krishna states that if a person offers mea leaf or flower or water with love and devotion I accept it. The person who worship pure mind with pure heart with simple offering of water, leaf or flower in temple (or) in home it accepted by god as mentioned in the 9th Chapter 26thverse





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पत्रंपुष्पफलंतोयंयोमेभक्त्याप्रयच्छति |
तदहंभक्त्युपहृतमश्रामिप्रयतात्मनः || 26||

*patraṃ puṣpaṃ phalaṃ toyāṃ yo me bhaktyā prayachchhati
tadahaṃ bhaktyupahṛitam aśhṇāmi prayatātmanaḥ*

-Bhagavad Gita, Chapter 9, Shloka26

Food and Digestive System

Confidential knowledge information share that loving devotion dedicating everything to lord Krishna states that how to eat and one should concentrate only eating the food with consciousness. The functioning of digestive system depends on consciousness whatever you do, eat, and offer in a sacrifice and whatever you donate to me.

यत्करोषियदश्रासियज्जुहोषिददासियत् |
यत्तपस्यसिकौन्तेयतत्कुरुष्वमदर्पणम् || 27||

*yat karoṣhi yad aśhṇāsi yaj juhoṣhi dadāsi yat
yat tapasyasi kaunteya tat kuruṣhva mad-arpaṇam*

-Bhagavad Gita, Chapter 9, Shloka27

Three Gunas

The entire world is made up of three gunas. Our mind is always under the mind of three gunas. Good action is known as sattvic. Pain action knows as rajasic. Ignorance action is known as thamasic. Sattva guna proceed upwards; rajasic in the middle and Thamasic downwards. Three gunas are responsible for all actions in life. The mind is made of food that eats.

रजस्तमश्चाभिभूयसत्त्वंभवतिभारत |
रजः सत्त्वंतमश्चैवतमः सत्त्वरजस्तथा || 10||

*rajas tamaśh chābhībhūya sattvaṃ bhavati bhārata
rajaḥ sattvaṃ tamaśh chaiva tamaḥ sattvaṃ rajas tathā*

-Bhagavad Gita, Chapter 14, Shloka10

Food Easy to Digest

Lord Krishna states that in all living beings god exists inside "Vaiśhvānaro" means fire of digestion. The digestive fire of human physical body function the activities of breaths of inward and outwards with combination of air prana (exhalation) apna (inhalation) helps for digestion of four types of food eaten by a person. Food is categorized into four types that includes 1. Bhojya food which is possible to chewed such as chapatti 2. Peya the food like liquid or semi-solid such as milk 3. Koshya the food which is sucked like eating sugarcane and the fourth one Lehya this includes like honey/this presented in 15th chapter verse 14th.

अहंवैश्वानरोभूत्वाप्राणिनां देहमाश्रितः |
प्राणापानसमायुक्तः पचाम्यन्नंचतुर्विधम् || 14||

*ahaṃ vaiśhvānaro bhūtvā prāṇināṃ dehaṃ aśhritaḥ
prāṇāpāna-samāyuktaḥ pachāmy annaṃ chatur-vidham*

-Bhagavad Gita, Chapter 15, Shloka14

High Energy Food Promotes the Duration of Life

Food is the mode of goodness increase the life duration. Food gives strength, health, happiness and satisfaction like fresh fruits, fresh vegetables, and milk products increase the life time of a person. Food which taken for passion results distress pain life. The food taken for passion, taste, like food which is too salty, too hot (or) bitter that leads to disease. Any food cooked early than three hours not preferable for eating. The 17th chapter 8th sloka states that food





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increase life span. 'Ayuh' that means life, 'Sattva' means Pure. People who eat good food are physically fit, good food Protect people from diseases. Sattvika food is good for heart, stomach and other organs as well.

1. Sattvic food: hygienic nutrition consider as good foods
2. Rajasic food: not consider as good food
3. Thamasic food: not good for well being (unwholesome foods)

आयुःसत्त्वबलारोग्यसुखप्रीतिविवर्धनाः |
रस्याः स्निग्धाः स्थिराहृद्याआहाराः सात्त्विकप्रियाः || 8||

*āyuh-sattva-balārogya-sukha-prīti-vivardhanāḥ
rasyāḥ snigdḥāḥ sthirā hṛidyā āhārāḥ sāttvika-priyāḥ*

-Bhagavad Gīta, Chapter17, Shloka 8

Eat to Live Not Live to Eat

The solka states that Rajasic foods are bitter, very sour, very salty, hot, dry, and very chill. These kinds of food create ill-health, agitation and despair. The purpose of consuming food is to live and not live for consuming food is eating. Choose a Sattvic food and live a healthy life. Food is to keep the body strong and healthy. Sound health and peaceful mind required to do good work. The food which tastes very bitter, sour, salty, hot create tensions, worries sorrow and it's not good for health is liked by Rajasic people.

कटुम्ललवणात्युष्णतीक्ष्णरूक्षविदाहिनः |
आहाराराजसस्येष्टादुःखशोकामयप्रदाः || 9||

*kaṭv-amla-lavaṇāṭy-uṣhṇa- tīkṣhṇa-rūkṣha-vidāhinaḥ
āhārā rājasasyeṣṭā duḥkha-śhokāmaya-pradāḥ*

-Bhagavad Gīta, Chapter17, Shloka 9

Eat Fresh Food Gita

Gita states that stale food, tasteless putrid, polluted, bad taste of food and Impure are classified in the mode of Thamas food. "Yāmaṁ" means that remains for more than three hours before eating. The sloka clearly teach us to cook and eat fresh food and not to store food for long time. Lord Krishna states about Tamasic food that lost its original taste, kept for long time it become tasteless, it lost natural taste. Putrid means bad smell food "Paryusitam" means the food by lapse of time "Uchchistam" means leftover food, such food are consider unclean food and not fit to offer for worship.1. Overcooked (or) over heated food is not good for health.2. Food should not be polluted and 3. It should be served neatly and cleanly.

यातयामंगतरसंपूतिपर्युषितंचयत् |
उच्छिष्टमपिचामेध्मंभोजनंतामसप्रियम् || 10||

*yāta-yāmaṁ gata-rasaṁ pūti paryuṣhitam cha yat
uchchhiṣṭam api chāmedhyaṁ bhōjanam tāmasa-priyamthe*

-Bhagavad Gīta, Chapter17, Shloka10

Nature of Sattvic Mind

The work done by one whom completes without selfish, motives and without any feelings of lust, hate. Doing work with peaceful mid with satisfaction. It consider a pure and sattvic. saṅga-rahitam means action of work free from egoism not for honor, name and rewards is known as sattva guna





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नियतसङ्गरहितमरागद्वेषतः कृतम् |
अफलप्रेप्सुनाकर्मयतत्सात्त्विकमुच्यते || 23||

*niyataṁ saṅga-rahitam arāga-dveṣhataḥ kṛitam
aphala-prepsunā karma yat tat sāttvikam uchyate*

-Bhagavad Gīta, Chapter18, Shlok 23

Nature of Rajasic Mind

The task completed with selfish the action performed with great effort with a sense of false ego known as rajasic mind of nature. Actions that are executed with expectation desire for recognition is known as rajasic guna.

यत्कामेप्सुनाकर्मसाहङ्कारेणवापुनः |
क्रियतेबहुलायासंतद्राजसमुदाहृतम् || 24||

*yat tu kāmepsunā karma sāhankāreṇa vā punaḥ
kriyate bahulāyāsaṁ tad rājasam udāhṛitam*

-Bhagavad Gīta, Chapter18, Shlok 24

Nature of Tamasic Mind

he work done with confused mind, without any cares about horrible results Tamasic behavior. Doing things ego they are big not consider good to others selfish action known as Tamasic nature of a person. Himsa means doing violence the action harm to others are thamasic guna.

अनुबन्धंक्षयं हिंसामनपेक्षयचपौरुषम् |
मोहादारभ्यतेकर्मयतत्तामसमुच्यते || 25||

*anubandhaṁ kṣhayaṁ hinsām anapekṣhya cha pauruṣam
mohād ārabhyate karma yat tat tāmasam uchyate*

-Bhagavad Gīta, Chapter18, Shlok 25

Perfection of life rejecting unwanted things good for mind and health (Conclusion-The Perfection of Renunciation)
Doing work with proper consciousness and activities of mind and body balanced with light food. Routine activities of life balanced with light food doing things right time makes the person perfect and attain to reach the goal. Eating food on time and sleeping right time and doing yoga meditation maintain good health. One should not eat what requires more than that create illness. The person do work with determination and known to control the tongue and body and rejecting unwanted things in life like ego, taste of food, lust and anger. Understanding of life with real needs and desire is known as realization of absolute.

बुद्ध्याविशुद्धयायुक्तो धृत्यात्माननियम्य च |
शब्दादीन्विषयांस्त्यक्त्वा रागद्वेषौ व्युदस्य च || 51||

विविक्तसेवी लघ्वाशीयतवाक्कायमानसः |
ध्यानयोगपरो नित्यं वैराग्यं समुपाश्रितः || 52||

अहङ्कारं बलं दर्पकामं क्रोधं परिग्रहम् |
विमुच्यन्निर्ममः शान्तो ब्रह्मभूयाय कल्पते || 53||

*buddhyā viśuddhayā yukto dhṛityātmānaṁ niyamy cha
śabdādīn viśhayāns tyaktvā rāga-dveṣhau vyudasya cha*

*vivikta-sevī laghv-āśhī yata-vāk-kāya-mānasaḥ
dhyāna-yoga-paro nityaṁ vairāgyaṁ samupāśhritaḥ*

*aṅkāraṁ balaṁ darpaṁ kāmaṁ krodhaṁ parigrahaṁ
vimuchya nirmamaḥ śhānto brahma-bhūyāya kalpate*

-Bhagavad Gīta, Chapter18, Shloka 51, 52 and 53





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CONCLUSION

Food is pleasant to eat and easy for digest are known as sattvick food. Foods which extremely acidic, salty, and spicy which create burning sensation to mouth throat, and stomach creates trouble to digestive part are considered as rajasic food. Hard and dry food creates illness and creates burning sensation in the body. Foods are half cooked, tasteless, leftover, polluted and contaminated foods are known as tamasic food. bhagavad – gita describes the quality of behavior reflects based on the choice of food one who choose. Perfect choice of nature food is known as sattvick not give harm to digestive part. Rajas and thamasic food give disturbance to digestive part. Now days many people follow western and Chinese food. Salty and spicy chips, fried items, grilled and fried items of food. Processed food, junk food, canned drinks these all create illness and all these foods comes under thamasic and rajaic food categories. The consumption of food and preparation of food clean and neat not harm to health. Bhagavad – gita teach about diet and what to eat and not to eat. Well being and physical health is essential to think well and helps to do our duty in right time at tight place. Food should not harm to health eat well balanced diet; don’t stuff with supplements. God teach us the importance of taking pure food good for health. Food consider as impure due to the reason that has been polluted to proximity with unclean object (not actual contact) due to contamination unclean substance it should prohibited. The choice of food reflects in inner state. Sattvic food gives clear thinking taking pure food that reflects pure to think then mind also become pure. Sattvic food gives clarity of knowledge

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Table 1. Saticv Food

| | |
|--|--|
| <p style="text-align: center;">Sattvick food effects</p> <p>1. Good for digestion 2. Promote life longevity 3. Balance diet 4. Good for physical and mental health 5. Reduce risk from chronic disease</p> | <p>Sattvick food</p> <p>sattvic foods are lead to clarity of mind and well-being of physical health</p> |
| <p style="text-align: center;">Rajasic food effects</p> <p>1. Create nervous disorders 2. Niether beneficial nor harmful 3. Leads to hyper activity 4. Creates the feelings of anger 5. Leads to stress</p> | <p>Rajasic food</p> <p>Rajasic food are stimulating effect of mind and body</p> |





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| | |
|---|--|
| <p>Tamasic food effects</p> <ol style="list-style-type: none"> 1. Reduce life time 2. Create stress to physical organs 3. Harmful to health 4. Intense mood 5. Create negative emotions | <p>Tamasic food</p> <p>Tamasic foods increase weakness and laziness</p> |
|---|--|

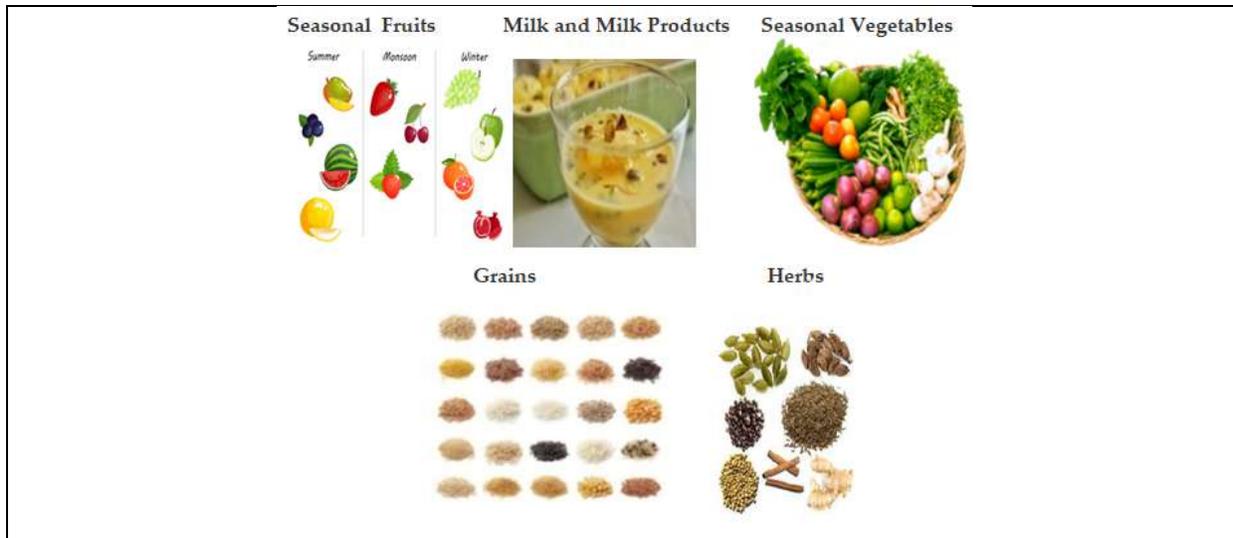


Figure: 1. Saticv food

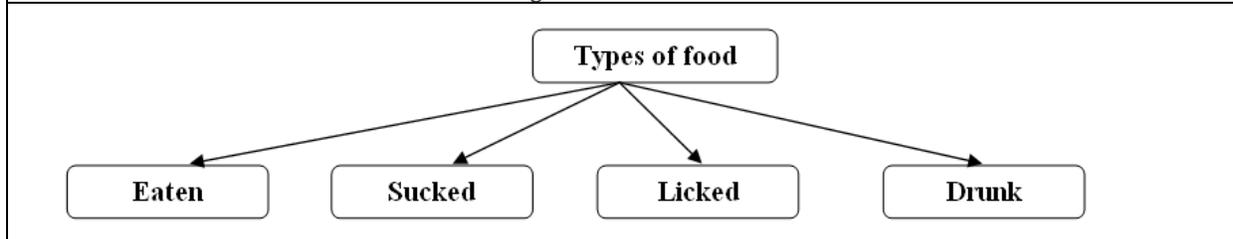


Chart: 1

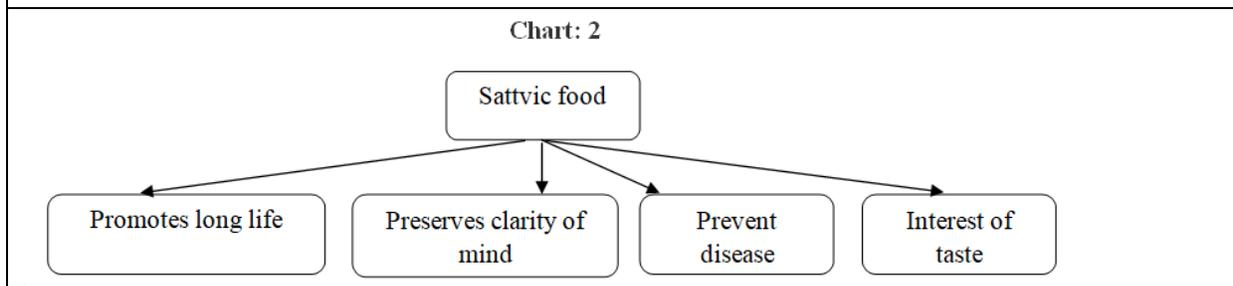


Chart: 2





***Suttigai* Therapy (Thermal Cauterization) in the Management of Plantar Fasciitis – A Case Study**

C. Kalaiarasi^{1*}, R. Keerthika², D. Periyasami³ and N.J. Muthukumar⁴

¹Principal Investigator, Dept. of Varma Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu, India.

²Assistant professor Dept. of Pura Maruthuvam National Institute of Siddha, Chennai, Tamil Nadu, India.

³Associate Professor, Dept. of Pura Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu, India.

⁴Professor and HoD, Department of Varma Maruthuvam, National Institute of Siddha, Chennai, Tamil Nadu, India.

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***Address for Correspondence**

C. Kalaiarasi

Principal Investigator,
Dept. of Varma Maruthuvam,
National Institute of Siddha,
Chennai, Tamil Nadu, India.
E.Mail: sirayalaikacs@gmail.com



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ABSTRACT

Suttigai (cauterization) is also known as heat therapy. *Suttigai* is one of the external treatments in the Siddha system of medicine that is indicated mainly for diseases that are not responsible for internal medicine and other external procedures. A 35-year-old woman reported to the OPD of Ayothidoss Pandithar Hospital, National Institute of Siddha, with a 1-year history of right heel pain that has worsened in 4 months. The pain worsens in the morning when getting up from bed and after a long period of sitting. She was diagnosed with plantar fasciitis and attempted allopathic treatment with ice and oral medication with minimal relief. For further betterment she intended to treat it with alternative medicine and she came to our hospital. The subject was treated with *Suttigai* therapy, and marked relief was found after the therapy. The patient was observed based on the Numeric Pain Rating Scale (NPRS) score before and after the treatment. This study concluded that *Suttigai* treatment can prove to be an effective alternative of plantar fasciitis.

Keywords: Heel pain, Plantar fasciitis, *Suttigai* therapy, Siddha





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INTRODUCTION

In foot and ankle practise, heel pain is a frequent presenting complaint, and plantar fasciitis (PF), which accounts for 11–15% of adult-specific foot complaints requiring medical attention, is the most prevalent cause of persistent pain beneath the heel in adults (1). Plantar fasciitis is an aseptic inflammation of the plantar fascia at its origin at the calcaneum. According to estimates, 1 in 10 people will experience PF at some point in their lives (2). Typically, this pain is worse in the early morning and the patient is unable to bear weight on the foot on getting up from bed. The pain is more while walking and is relieved by rest. Several factors that increase the risk of developing PF include obesity, pes planus, pes cavus and walking on hard surface. There is no effective treatment because its pathogenesis is largely unknown (3). In modern medicine, the first option is non-surgical procedures which include, rest, ice pack, heat, NSAID, orthosis and corticosteroid injection etc. The second option is surgery.

In Siddha, *Kuthikaal Vatham*, is one of the *vatha* diseases, which is referenced in the classic Siddha text *Yugi Vaithiya Kaaviyam*, may be closely associated with plantar fasciitis which is caused by various etiology (4). *Kuthikaal vatham* mainly occurs due to deranged *Vatham*. (4). *Suttigai* (Thermal Cauterization) is a para-surgical procedure that effectively treats muscle, tendon, ligament, bone and joint pain. It is defined as an inflicting micro burn on the surface of the tissue that is causing pain by using a hot instrument that is indicated for *vatham* and *kabam* related diseases depending upon the disease and severity (5). *Suttigai* is indicated mainly for diseases that are not responsible for internal medicine and other external procedures that heal the disease and thereby eliminate the possibility of recurrence. In light of the aforementioned information, this study intended to assess the therapeutic effectiveness of *Suttigai* therapy in the management of plantar fasciitis.

Case presentation

A 35-year-old female patient reported to the OPD of Ayothidoss Pandithar Hospital, National Institute of Siddha, Chennai, with complaints of right heel pain that worsens in the early morning and is unable to wear weight on getting up from the bed for 1 year. Symptoms have increased in the past 4 months. After her physical examination and X-ray were done, she was diagnosed with PF. She consulted an allopathic hospital and took medicines for the same. She attained temporary relief, but the episodes were brief. For further management, she came to Siddha for treatment. The patient is a homemaker, and she belongs to the low socio-economic category.

Clinical findings

The patient was clinically examined. On palpation, severe tenderness in the medial plantar calcaneal region was present. In comparison to the left, the right leg had painful movements of dorsiflexion and tightness of the gastrocnemius muscle. On inspection, there was no swelling, external injury, pes planus, or pes cavus. Vitals were within the normal limit. BP: -130/70 mm Hg HR-80/min; PR-79/min; RR-21/min. She had a normal BMI and had no other associated systemic illnesses. On detailed examination, no associated neurological deficits were seen. Based on Siddha principles, her body constitution was *pitham*, and the *naadi* was *vatham* predominant.

Diagnostic assessment

The patient was a known case of plantar fasciitis as it was confirmed by heel pain that worsens in the early morning and is unable to wear weight on getting up from the bed, which is a classical symptom of PF. Radiological findings also revealed a plantar calcaneal spur at the tuberosity of the calcaneum. On detailed examination, no associated neurological deficits were seen. From the detailed history, clinical examination, and radiological findings, the case was diagnosed as PF.

Therapeutic intervention

Following the diagnosis, to rule out any systemic pathology, routine tests such as CBC, HB, CT, BT, LFT, RFT, CBG, HIV, HBsAg, and VDRL were assessed. All results were within normal ranges. Then, the patients were treated with *Suttigai* therapy as follows:





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Standard operative procedure of Suttigai**Pre-operative procedure**

After taking informed consent from the patient vital signs were recorded then allow the patient to lie in a supine position. The materials required for the procedure like Copper *Suttigai* probe, *aloe vera* leaf pulp, gas stove, *Padikara neer*, and sterile gauze piece were taken.

Operative procedure:

The affected part was sterilised with *Padikara Neer* and then wiped with dry sterilized cotton gauze. Encircle the site with a marking (Tenderness point) for *Suttigai* therapy. Red- hot copper probe was prepared. The strong touch with the probe in the affected area within circle mark.

Post operative procedure:

Aloe vera pulp was applied over the site to reduce the burning sensation. At last dressing with bandage was done. The vital signs were recorded after the treatment. The *Suttigai* site was not be allowed to exposed to water at least for 24 hours to avoid any infection.

Follow up and out come

The patient was advised to follow up on the therapy once a week at every OPD visit for one month. She reported a pain reduction from 8/10 to 2/10 over the following sittings of *Suttigai* therapy and a morning pain reduction from 9/10 to 3/10. The Numeric Pain Rating Scale (NPRS) was used for the assessment (6). The patient got good relief from all the symptoms, and there was no adverse effect noticed during the treatment period. She was able to carry weight painlessly. On palpation, there didn't seem to be any tenderness. She was followed up for 3 months, and the period was uneventful.

DISCUSSION

Despite many medicines dealing with plantar fasciitis, enhancing PF healing it is still a major clinical challenge in orthopedic practice. Most experts agree that early recognition and management of PF leads to a short course of treatment and a great chance of success with conservative therapies. Although modern medicine offers solutions to relieve the symptoms, they also cause severe side effects due to their chronic use. NSAIDs and Steroids may increase pain relief and decrease disability in patients but they confer substantial side effects including gastrointestinal bleeding, gastric pain and renal damage (7).

Suttigai is also known as heat therapy. It is one of the external treatments in the Siddha system of medicine There are five different varieties of *suttigai* described. Metal cauterization (*Uloga Suttigai*) (8) is one of them. A copper metal probe was used in this study. It can be *Uloga Suttigai* can be utilized as a preventive measure, as a curative measure, as a postoperative procedure and as a hemostatic measure. *Suttigai* is a simple, safe and cost-effective OPD procedure that required minimal time.

Plantar fasciitis (*Kuthikaal Vatham*) initially occurred from vitiated *Vatham*, while *Kabam* is the secondary affected humour. *Suttigai* therapy pacifies the *Vatham* and removes the *kabam* blockages from the affected region. In Classical Siddha terms, the analgesic action of *Suttigai* therapy can be explained as follows. Pain is caused anywhere in the body due to the imbalance of *vatham*. Thus, in this condition, *Suttigai* works by giving external heat which helps to alleviate the aggravated *thodam* and hence cures the disease. Depending on the severity of the disease follow-up treatment may be necessary.

As per modern science, therapeutic heat increases blood circulation and leads to the proper nutrition of the tissue. This increases blood circulation and helps to flush away pain-producing substances from the affected site. Heat leads to vasodilatation, the exudation of fluid, and an increase in white blood cells. According to a recent study, heat



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therapy reduces swelling by boosting blood flow to the afflicted area, thereby helping to remove the inflammatory substance (9). The ability to utilise the extremities effectively for carrying out activities is made possible by a rise in body temperature, which induces muscles to relax and increases the efficacy of its action by increasing blood flow and accelerating the contraction and relaxation of muscle fibres (10)

Resulting from this research, *Suttigai* seems to be more effective in providing distinct and instant relief from persistent pain in plantar fasciitis without causing any adverse reactions.

CONCLUSION

Suttigai therapy is a simple, safe and cost-effective OPD procedure that required minimal time. The existing conventional treatment options for plantar fasciitis are unsatisfactory. This study's findings support a step in the direction of establishing the use of *suttigai* therapy in the management of plantar fasciitis. Studies are scarce on plantar fasciitis and *suttigai* therapy management. Scientific validation and clinical evaluation in traditional medical system are much needed to establish its traditional value. This is a preliminary level study, additional clinical studies on a significant number of samples needed to generate evidence.

Information Consent

Written consent was obtained from the patient for publication of this case study.

Source of funding – nil**Conflict of interest – none****REFERENCES**

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Table.1 Outcome assessment

| Suttigai sitting | 1 (1st day) | 2 (7 th day) | 3 (14 th day) | 4 (21 st day) |
|------------------|-------------|-------------------------|--------------------------|--------------------------|
| NRPS Score | 8/10 | 5/10 | 4/10 | 2/10 |



Fig. 1. Copper probe

Fig. 2. Red hot probe

Fig. 3. Tenderness point marking as circle

Fig. 4. During suttigai therapy

Fig. 5. After suttigai therapy

Fig. 6. After bandaging





Management of Uterine Prolapse through Siddha System of Medicine : A Case Report

Divya Jayachandran¹, Gayatri.R², Srinivasan Venkatachalam², Ramamurthy Murugan³, Elansekaran Selladurai³ and Christian Gnanaraj Johnson^{4*}

¹PG Scholar, Department of Noi Naadal, National Institute of Siddha, Tambaram Sanatorium, Chennai-600 047, Tamil Nadu, India.

²Assistant Professor, Department of Noi Naadal, National Institute of Siddha, Tambaram Sanatorium, Chennai -600 047, Tamil Nadu, India.

³Associate Professor, Department of Noi Naadal, National Institute of Siddha, Tambaram Sanatorium, Chennai- 600 047, Tamil Nadu, India.

⁴Professor and Head, Department of Noi Naadal, National Institute of Siddha, Tambaram Sanatorium, Chennai -600 047, Tamil Nadu, India.

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*Address for Correspondence

Christian Gnanaraj Johnson

Professor and Head,

Department of Noi Naadal,

National Institute of Siddha,

Tambaram Sanatorium,

Chennai -600 047, Tamil Nadu, India.

E.Mail: drdj91@gmail.com



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ABSTRACT

Uterine prolapse is one of the health conditions affecting women in the later stage of their life. It is mentioned in siddha texts that for weakness of pelvic muscle, high tannin containing powders like naval (*Zizigiumcumini*), kadukkai (*Terminalia chebula*) etc. can be used as pottanam for its management. In this case study an attempt was made to manage the condition of Uterine prolapse by means of Pottanam (medicated pessaries) made out of Triphala Churanam and some classical internal medicines in Siddha. We present a case report of third degree uterine prolapse managed using medicated pessary and internal medicine. At the end of one month of intervention, uterine prolapse of stage III was reduced to stage I, the outcome of which is measured by using POP-Q scale. The ingredients in this pottanam may have the potential to contract smooth muscle and also may stabilize collagen mechanism and thus might have strengthened the pelvic floor muscles. Siddha medicine is having effective treatment techniques in management of uterine prolapse, but further extensive clinical trials are necessary to generalize the effect.

Keywords: uterine prolapse, pottanam, triphalachuranam, siddha.





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INTRODUCTION

Uterine Prolapse is one of the pelvic organs prolapse condition in which the uterus is herniated through vagina due to the weakening of the supporting structures⁽¹⁾. Even though this condition is not life-threatening, it can lead to sexual dysfunction, poor body image, and lower quality of life due to associated bowel or bladder incontinence. Also, there is chance of prolapse of other pelvic organs like cystocele, enterocele etc. The current available management of uterine prolapse like use of vaginal pessaries and pelvic floor muscle exercise doesn't cure the condition and for later stages of uterine prolapse surgical removal of uterus is opted (1)

Case Details

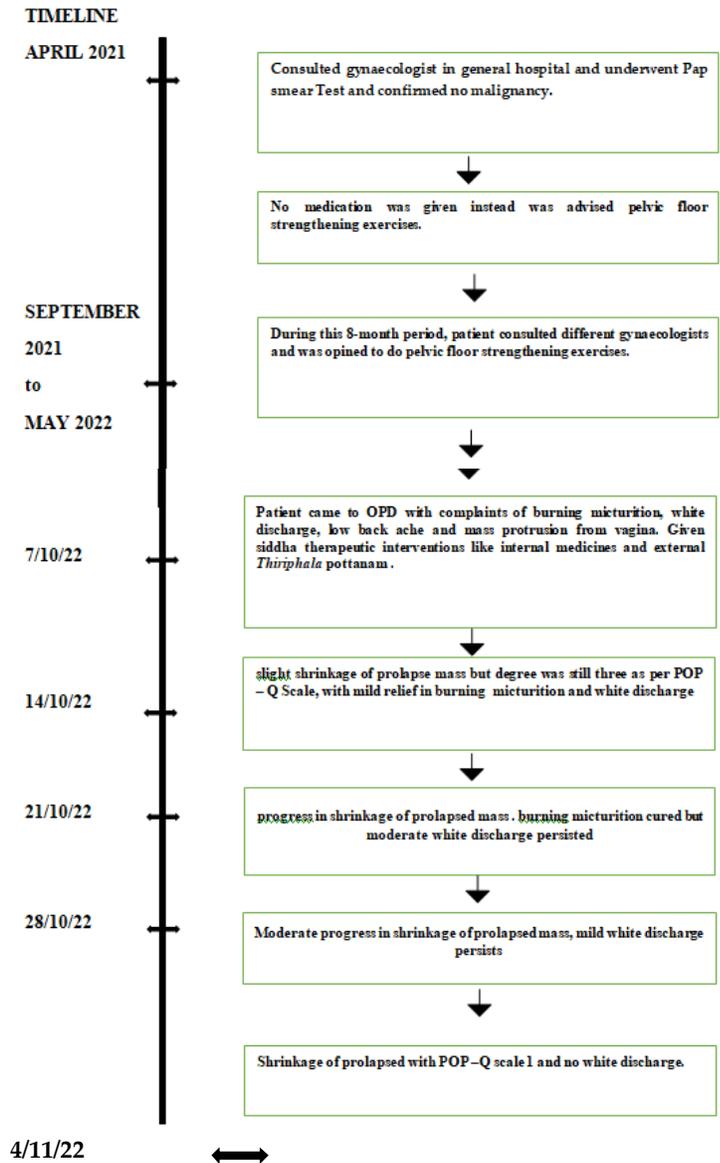
The Pelvic Organ Prolapse Quantification (POP-Q) system helps to determine the degree of uterine prolapse by taking in account the location of the most distal portion of the prolapsed segment in relation to the hymen, that is with measurements proximal to hymen denoted by negative numbers and measurements distal to hymen as positive numbers with the hymen being used as a reference point of "0". Mostly stage 3 and 4 are suggested for surgical correction, which usually manifest as the most distal portion of the prolapsed segment protrudes >1 cm below the hymen but 2 cm less than the total length of the vagina and complete eversion of the vagina respectively⁽²⁾. Pelvic Organ Prolapse condition is amenable to Siddha external therapies. Siddha system of medicine has 32 types of external therapy and 32 types of internal medicines⁽³⁾. Pottanam is one among the 32 types of external therapy in which medicines are kept in a piece of cloth and it is tied together to form a bundle and is usually placed in nose, ears, anus or vagina. It is mentioned in siddha texts that for weakness of pelvic muscle, high tannin containing powders like bark powders of Atti (*Ficus racemose*), Naaval (*Zizigiumcumini*) or Kadukkai (*Terminalia chebula*) can be use as pottanam for its management^(4,5). Triphala Churnam is one among the classical siddha drugs having indication to cure conditions like Uterine prolapse if used orally as well as externally. In this case study an attempt was made to manage the condition of Uterine prolapse by means of Pottanam made out of Triphala Churnam and some classical internal medicines in Siddha.

A female patient aged 59 yrs came to OPD with the complaints of mass protrusion from vagina in standing position and which reduced in sitting position, incontinence of urine, low back pain, white discharge and painful micturition since 2 yrs. On examining the reports, it was confirmed that patient was suffering from same complaints since 2 yr. There is no relevant family history regarding this medical condition. Patient is a known case of Type two Diabetes Mellitus and is under Allopathic as well as Siddha medications. (*T. metformin* 500 mg twice a day and Capsule MadhumeagaChurnam⁽⁶⁾ 1 g thrice a day. In this period of 2 yrs., she had consulted 3 gynaecologists and all of them advised her to do pelvic strengthening exercises. But the condition doesn't seem to have any good prognosis and even worsened the prolapse. Based on the POP-Q scale the stage of uterine prolapse in the patient is Grade III (Picture 1)





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THERAPEUTIC INTERVENTION

Two types of intervention were given, namely internal and external medications enlisted below.

Internal Intervention

| THERAPEUTIC INTERVENTION | EXPLANATION OF INDICATION | DOSAGE, STRENGTH, DURATION | ADJUVANT | MANUFACTURE DETAILS |
|---|---|----------------------------|------------|---------------------|
| Tab Thiriphala (The Siddha Formulatory of India)(7) | Astringent, Anti-inflammatory, anti-cancer, analgesic, anti-bacterial activity(8) | 2 BD | Warm water | IMPCOPS |





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| | | | | |
|---|---|------|--------------------------|---------|
| Cap. Rasagandhi Mezhughu (pulippanimunivarvaidyam-500)(9) | Anti-tumour, anticancer, antioxidant and immuno-modulatory properties.(10,11) | 2 BD | Warm water+ palm jaggery | IMPCOPS |
| T. Silasathu (The Siddha Formulatory Of India)(12) | Astringent, STD's, Neer kaduppu (burning micturation) (12,13) | 2 BD | milk | IMPCOPS |

External Intervention**Thiriphala Churnam- 5g pottanam**

5g of *Thiriphala Churnamis* kept in a piece of clean cloth and it is tied together to form a bundle and is placed inside vagina like a pessary.

Changes in therapeutic intervention

Same medications were continued , without any changes in therapeutic intervention.

FOLLOW-UP AND OUTCOMES

From the patient's perspective, after 1 month of treatment she not only got the prolapsed mass reduced but also got relief in urgency of urination, recurrent burning micturation and white discharge.

Important follow-up diagnostic and other test results:

The patient was examined every week and the prognosis is analysed based on POP-Q Scale(2). Every week the size of the prolapsed part reduced, uterine prolapse of stage III was reduced to stage I on treatment(Picture 2).Patient was followed for another 1 and half month and there was not further prolapse of the uterus.

Intervention adherence and tolerability

Weekly patient was examined and based on compliance form intervention adherence and tolerability was analysed and confirmed.

Adverse and unanticipated events

No adverse events or reaction was observed or experienced by the patient

DISCUSSION

Recurrent uterovaginal prolapse is having untoward effect in the quality of life of women. Genitourinary prolapse affects around 40-50 % of women in their lifetimes with a higher incidence in women of advanced age and parity. The prevalence rises with advanced age and rising parity. The condition is aggravated by hormonal deficiency after menopause, collagen density disorders, and factors contributing to increased intra-abdominal pressure(1,2) Pelvic organ prolapse is the descent of pelvic viscera through the vaginal canal due to the weaker supporting structures of the pelvic floor. Surgery for this condition is one of the major planned gynecological surgeries. The risk of having surgery for pelvic organ prolapse is 11% with at least on third of the surgeries being for recurrence of prolapse(2,5) Almost half of women beyond the age of 50 years have varied degree of prolapse. The Risk of post hysterectomy prolapse is 5.5 times higher if hysterectomy was performed because of prolapsed uterus. There is a need for proper management of prolapsed uterus without any complications. In POP, tissue strength is lost, stiffness is increased(14-16), and ECM quality is compromised(17). Consequently, fibroblasts might be exposed to





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an abnormal matrix. The biomechanical environment may be further compromised if during pelvic reconstructive surgery, stiff, nonresorbable polymeric mesh is used to replace tissue function(18)

Siddha system of Medicine is having an advanced management measures for conditions like Uterine prolapse which may be considered as medicated pessary which are potential collagen stabilizers. The ECM in pelvic floor tissues is kept in balance by the opposing effect of decomposition and synthesis, and its stability mainly depends on balance of matrix metalloproteinases. In this case report patient was managed with a medicated pessary made out of Triphala Chooranam. Triphala Chooranam is having three herbal components namely *Terminalia chebula*, *Phyllanthus emblicus* and *Terminalia bellerica*. *Terminalia chebula* and *Terminalia bellerica* stimulate Fibroblast function which is a biological cell that synthesizes extracellular matrix and collagen(19). *Terminalia chebula* extract facilitate collagen stabilization through collagenase inhibition(20). *Terminalia chebula* control the risk of infection as it has Antimicrobial Activity(21). Moreover, it reduces matrix metalloproteinase (MMP)-9 expression, MMP are high in patients with POP(22) Triphala contains ascorbic acid, which acts as a cofactor for the synthesis of collagen as well as elastin fibres(23). Promotes synthesis of type I collagen and anti-collagenase effects on primary mouse fibroblast cells(24). *Terminalia chebula* and *Terminalia bellerica* stimulate Fibroblast function which is a biological cell that synthesizes extracellular matrix and collagen(19). Tannins stimulate oestrogen and androgen levels which is rich in *Terminalia chebula* and *Terminalia bellerica*(25). Also herbals in RGM also have the capacity to contract uterus and it is also good pain reliever. Likewise, Silassathuparpam is indicated for *Vellei*, *Moothiraerivu*, *neerkattu*, *neerkaduppu* which can be compared with burning micturition, oliguria and other urinary tract infection features. Here in this case patient had complaints of burning micturition, incontinence of urine and white discharge which got reduced after one month of medication. Hence this Silasathu medicine might have played an important role in reducing these clinical features.

Based on POP-Q system, uterine prolapse of stage III was reduced to stage I on treatment (Picture 2). After 1 month of treatment patient not only got the prolapsed mass reduced but also got relief in urgency of urination, recurrent urinary tract infection and white discharge. Applying this treatment in initial stages like 1 and 2 may result in possible management of prolapse without any surgery. In conclusion, a report of uterine prolapse case managed by both internal and external siddha medicine intervention is demonstrated. Siddha medicine has effective treatment techniques in management of uterine prolapse. But further extensive clinical trials are necessary to generalize the effect.

CONCLUSION

In conclusion, a report of uterine prolapse case managed by both internal and external siddha medicine intervention is demonstrated. Siddha medicine has effective treatment techniques in management of uterine prolapse. But further extensive clinical trials are necessary to generalize the effect.

CONSENT

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

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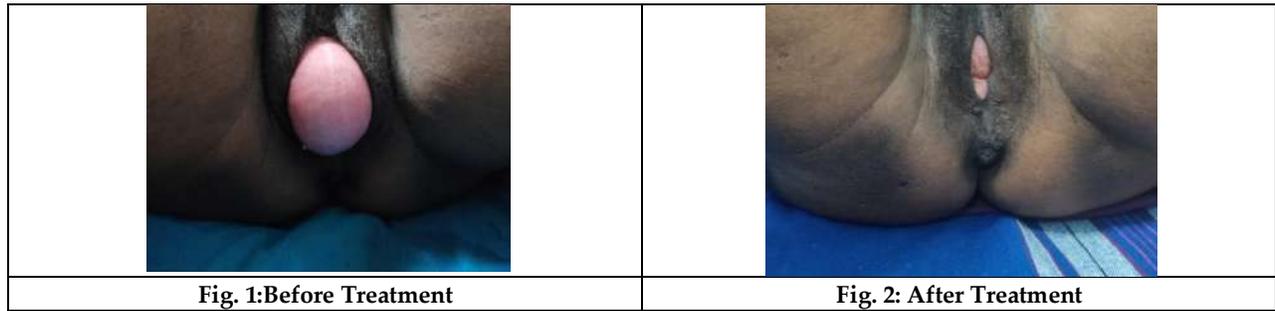
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Collective Memory, History and Rebellion in Naga Community of Nagaland : A Study of Easterine Kire's *A Respectable Woman*

Pinki Sharma¹ and Arpit Kothari^{2*}

¹Ph.D Scholar, Languages, Literatures and Cultural Studies, Manipal University Jaipur, Jaipur, Rajasthan, India.

²Associate Professor, Languages, Literatures and Cultural Studies, Manipal University Jaipur, Jaipur, Rajasthan, India.

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*Address for Correspondence

Arpit Kothari

Associate Professor in English,
Languages, Literatures and Cultural Studies,
Manipal University Jaipur,
Jaipur, Rajasthan, India.
E.Mail: arpit.kothari@jaipur.manipal.edu



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ABSTRACT

This paper attempts to offer study of Easterine Kire's *A Respectable Woman*(2019) which is a portrayal of war memories and transformation of Kohima landscape. The novel is recollection of two generations' memories aim at historical and cultural transformation of Naga community post Kohima war. Kire effortless portrayal of socio-political, cultural, and historical Kohima offers with a view to accentuating Naga community. To study Kire's *A Respectable Woman* from the perspectives of collective memory, history, and rebellion spirit in Naga community of Nagaland. This study closely looks into the historical portrayal of the community and tribal women's ability to overcome conventional and oppressive tribal customs and rituals. It also studies horrendous effect of war on the oppressed section of the Naga society. **Methods:** The study attempts to offer textual analysis of Easterine Kire's *A Respectable Woman* to be able to offer corrective discourse of tribal community during Kohima war. The novel offers ample evidence to study collective memory as a narrative mode, history, culture, and rebellious nature of Naga community. The study finds how Naga women's social status depends on socialisation, social stratification, and gender relations. Alcoholic consumption among men in the Naga community is a largely discussed problem which affects economic growth of a family. Kohima people adversely suffer due to ongoing war. Easterine Kire depicts sensitive issues concerned with patriarchy, domestic violence, corruption, smuggling, political dominance, and war, which are germane to memory, history, and rebellion in Naga community. Men waste all their income on alcohol, whereas women use their learning for family survival.

Keywords: Collective Memory, Culture, History, Naga, Rebellion





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INTRODUCTION

The present paper attempts to study Easterine Kire's *A Respectable Woman*(2019)from the perspectives of history, culture, and rebellious nature of Naga community. The study offers comprehensive social and cultural life of Naga community, ill effects of war, and fragmentation of incidents took place during war. The first section of the novel begins with "After the War" Khonuo's narration, wherein she recollects memory concerned with the Kohima war. The entire narration is lively fragmentation of random incidents of history. Further, the novel's second part begins with Kevinuo's "I Am Born" story. She was raised in an atmosphere of political turbulence, corruption, and military occupation. War and modernity are at the core of Kevinuo's narration. She also talks about the haunting memory of her growing up without a father. The third part of the novel begins with "The Perfect Victim" wherein the idea of *A Respectable Woman* becomes clear. *A Respectable Woman* challenges conventional society, becomes rebel, and steps into the modern world.

OBJECTIVES

To study Easterine Kire's *A Respectable Woman*(2019)from the perspectives of history, culture, and rebellious nature of Naga community. This research aims at evaluatingcollective memory, history, and rebellion spirit in Naga community of Nagaland.

METHODS

The study attempts to offer textual analysis of Easterine Kire's *A Respectable Woman* to be able to offer corrective discourse of tribal community during Kohima war. The novel offers ample evidence to study collective memory as a narrative mode, history, culture, and rebellious nature of Naga community. The paper offers a qualitative study of Kire's *A Respectable Woman*. It uses textual analysis as a central method from the perspective of memory, history, and rebellion.

RESULTS AND DISCUSSION

This can be arguably said that Naga society enjoys classless social stratification; women are considered equal and respectable at social and family level. In Nagaland, women's social status depends on socialisation, social stratification, and gender relations. Naga community is the largest community in the northeast region, having powerful cultural groups. The Japanese-Kohima war creates social unrest and chaos among Naga community. Children and women suffer colonial atrocities. War results into dangerous situation wherein people's survival is at stake. People become alienated within their society. Easterine Kire's *A Respectable Woman*is a narration having collective memory, depicting the socio-cultural landscape and political unrest in contemporary Kohima. Loss of lives and social relationships are part of depiction. The narrator attempts to connect the idea of respectable women with English medium education and modernisation. The novel is a recollection of memories and history of the relationship between Nagas and British.

The term 'collective memory' is "a spurious notion" (Assmann 55) wherein war, culture, socio-political scenario, and family memory are its essential parts. Therefore,visual and verbal signs are inseparable parts of memory. Though there is overlapping of socio-cultural and political scenarios, truthfulness is called into question. There is a pertinent relationship between memory and history—identity and power are essential to recalling incidents. Therefore, memory is an embodiment of selected incidents and deploys forgetting. "The relation of memory as voluntary recollection to the higher activity of the intellect is indicated by Aristotle when he says that recollection is a syllogistic



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process" (Burnham 51). Hence, recollection of historical incidents and memory depends on the cognition and intelligence of the person.

Further, *A Respectable Woman's* story is woven using folktales, oral history, and memories of the most challenging time for the Naga community in Kohima. The comprehensive narrative includes political turmoil, social unrest caused by war, socio-cultural scenarios, and historical landscapes of Kohima. People lost near and dear ones in the Kohima war. The novel consists of three parts, a joint venture of Kire and her mother as a fictional narrator of the story. The novel is a bold step of expression wherein people are made to suffer. The novelist admits, "Two years ago, in Germany, I met a woman whose father had been tortured by the Nazis. He didn't speak about those memories till the end of his life, she told me. Instead, he would simply sit for hours in front of a window staring into nothingness" (Santhanam). Likewise, the novel's opening sentence reflects a scary atmosphere during the war between the Japanese army and Nagas. The novel depicts the revolutionary spirit of Naga women against conservative and traditional tribal customs. The novel begins with Azuo's memory covering almost post world-war forty-four years in Nagaland.

Easterine Kire recollects the days when her mother saved the family from the dominance of the Japanese people. The situation was so grim that Khonuo took more than forty-five years to talk about the incident openly. For instance, Kire portrays, "It took my mother, Khonuo, exactly forty-five years before she could bring herself to talk about the war. She was ten years old when Japanese invaded our hills in 1944" (Kire 03). It took lots of efforts to reach a safer place to escape the Japanese army. They spend more than two months in the Rusoma hills of Kohima. The narrator recollects memories of hardship and survival during the ongoing war. People lived in deprived situation due to ongoing war. People must save their life during the crisis. The story also recollects an uncertain situation wherein people are unknown to their government. They were inclined towards British people and had felt like orphans when they left India.

Furthermore, the story recalls unbearable incidents in the history of war. Everybody used to listen to radio news after dinner. They wait for directions from the government. Kire merges memory and history of the Kohima war. Among the growing political turbulence, the primary worry of the inquiry is, how did people survive during the war? What did they eat, and from where did they get food to eat? The beginning of the novel horrifies with an eye-opening scenario of the war. While recalling the incidents of 1944, Kire depicts a situation wherein groceries sellers left the place. People do not find any food to survive. Kevinuo comes across unbelievable human resilience, loss, and chaos. Nagaland landscapes during the war were so destructive when it pushed human beings into a chaotic situation. The war damaged the entire Kohima village. "Remembering 1944", a section in the book is about recollection of chaos, devastation, food crisis, and lifeless situation caused due to war. As Kire portrays, "The sight of grown women weeping over their lost homes as though mourning their dead was fascinating for us, and we stood at a distance and stared and stared at them" (Kire 10). Thus, the recollection of the memory and history expresses a painful scenario when the writer portrays a historical incident in fiction.

Ongoing war affects the existing social system and puts children and women at the margin vis-à-vis freedom, choice, and education. Christian missionaries are asked to leave Kohima schools. But, some of them managed to hand over schools to local people. Azuo's family life expresses the broken life of people amidst the Japanese-Nagaland war. The novelist paints fragmented memories of post-war Kohima. Kevinuo learns from her mother Azuo about loss, love, and her capacity for resilience. The recollection of memories and historical incidents express how war creates unsettling situations. People do not feel safe in their homes. Kevinuo grows up in a modern family amidst political turbulence. Her father's early death makes her mother face problems concerned with her interaction with men. Kevinuo lost her father at a very early age. Along with her mother, they grew up in the problematic situation. Kire draws complex human characters with profound realist touch. The recollection of war-affected Naga society is a tragic story of the community. The narrator recalls a sense of connectedness with their community people. "It was only people without any relatives who went to eat in the town hotels that sold pork, rice, and boiled mustered leaves" (Kire 47). Post-war people are found cooperating with their relatives and neighbours. The entire community



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live harmoniously with nature and other communities. Their culture and belief system inculcates indigenous values wherein a place of nature remains at the core. However, ill notions like patriarchy and white men's belief stay away from the touch of Naga communities. The entire Naga cultural landscape has emerged as independent and remains unaffected by Hindu society's caste and class discrimination. They are a self-sufficient community. They are very stable in terms of inhabitancy; once settled, they do not move here and there. Thus, it may be said that Naga society is casteless and classless regarding social stratification. For instance, "The Naga society is casteless and classless. It has a marked sense of equality based on community participation irrespective of gender. In social, cultural, and religious matters, all have equal rights. In some communities, women enjoy a higher position than men." (Shimray 374) Thus, the Naga society is the most neutral social organisation in the northeast region. The community has no rigid social practice that undermines women's status and causes subjugation. Though Naga society follows patriarchal norms, women can inherit paternal property only when they do not have a male sibling. Kire skilfully portrays survivors' stories and the Kohima crisis.

However, during the war, people are seen in need of food, shelter, and other necessary items. The existing government helped people in numerous possible ways to overcome the problems caused due to war. For instance, "During the war, our skies were filled with all sorts of planes. Some would drop rations, some dropped leaflets, and some other bombed the areas where the Japanese were reported to be camping" (Kire 54). Naga Society's historical landscapes are profoundly depicted. Both food and bombs are thrown from planes. After the war, people realised there was a huge world outside Kohima. During war, dead bodies of soldiers could be seen falling around the hills of Kohima. Zeno and Npuotsa buried dead bodies of Japanese and Naga soldiers. The ongoing war also brought ecological devastation.

The novel also reveals the complexities of the characters. Azuo and her sister want to resume their schooling. They wait for the war to get over. People seem inclined towards English medium education. People acquire respectable acclaim in their community. Moreover, soldier's life was glorified with a view to participating in the war. This job is associated with glory and the empire's interest. The novelist also recalls her school days when friends empathised with her. Beinuo was the closest friend who provided an excellent companionship when Azuo missed her father. The entire Naga community is considered colonised. They accepted Britishers as their emancipators. They seem quite adamant about their ethnic identity but defied becoming a constituent part of India. Naga community stood against Indian forces to assimilate with the Indian union, but strategic negotiations brought them to India's favour. Initially, it wasn't easy to control Naga revolutionaries. Alongside, Japan was ready to take Kohima under their dominance. India has a major policy shift for taking Kohima in the Indian favour. The spread of modern education and administrative support establishes loyalty between the Naga community and the Government of India. In 1947, when British people left India, a sense of connectedness can be seen between natives and the colonisers. The writer portrays the acceptance of British people as superior: "So, when they left our hills, many people felt orphaned, including grown-up people". Naga villagers repent when English people leave their hills. For instance, "Our parents are leaving us" (Kire 57). The tribal community was essentially backward. The community gets support from Britishers concerned with education, development, etc. Thus, there is an accepted colonial relationship between natives and British people.

During the war, people were highly unsafe inside and outside their houses. Army from both India and Japan caused them to be tortured. Moreover, there was a fight between Naga army and India soldiers. The Japanese military occupied Kohima, and people had to shift villages like Chieswema, Meriema, Rusoma, and Jatsoma to save themselves from the brutal attack of bombs. Injustice and dehumanised treatment were unbearable for people. Most sufferers were marginalised section of the society, such as women and children. Kire depicts increasing crime on women and children with the brutal consequences of the war. As narrator exposes, "We heard that women were raped in these villages. So many men joined the Naga army to fight against the Indian government. So many of those men died; it was like a whole generation of men disappeared because they were all killed one after the other" (Kire 58). Hence, the novel attempts to preserve historical events in a picturesque manner. Kire attempts to portray oral narrative into literary tradition. Naga community was marginalised during the Kohima war. Britishers and Japanese



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forces bombed their region, raped women, and harassed children. During that period, the Naga community was living at the mercy of the British people. It was a sort of livestock of the community. Moreover, Nagas was loyal to the British government, but they lost property, houses, and innocent people. The oral narrative exposes hidden aspects of the war, and Kire truthfully portrays what has been shared with her. There is an instance wherein Noumvüo Khruomo an inhabitant of Kohima states how “the night the war began, they buried their grain and fled to their fields. But soon they learned that the Japanese were eating our grain...killed and ate our domestic animals...British threw firebombs and torched our village” (Chasie and Fecitt 64). Hence, the depiction of Naga community shows marginalisation during the Japanese-Kohima war. The narrator portrays how people hide eatables from Japanese forces intending to save their life. Japanese people had to suffer hunger. As a result, they attacked the Naga community’s grain and domestic animals. Moreover, many people had to work as labourers during the Kohima war.

War affects routine incidents such as schooling, marriage, and managing relationships. The novelist attempts to recall disturbed routine life due to ongoing war. For instance, the narrator states, “we had a small wedding, your father and I...We did not make invitation cards. Our two sets of parents went around and invited all their relatives” (Kire 63-4). Hence, people are made to live with numerous restrictions. The idea of acquiring freedom from the British people disturbed the peace in routine life. People seem not to believe in achieving relaxation by creating chaos in society. Npüo, does not support the idea of acquiring freedom by creating unrest in society. However, Southern Nagaland remains marginalised as any constitutional laws did not administer them. Lushai Hills stay away from the possession of British and Japanese dominance. Most of the North-eastern region was marginalised vis-à-vis administration, education, healthcare, etc. Being an international border state, Nagaland remains deprived of administrative support. Regular chaos and unrest were major factors behind their backwardness.

The Kachins, Mizos, and Nagas had been excluded from whatever opportunities the state could have brought by colonial policies and discourses. And in the same breath, the colonial encounter had undercut their transregional connections and split them apart through a border that negated their presence to produce a neat divide between India and Burma. (Guyot-Réchar 334-35). Thus, everyday people live with fear and insecurity of losing a life. People got married in small gatherings only. The novelist recollects memories of the day when her father and mother got married. The contemporary Naga community was away from technological advancements and was close to nature. The entire region was under military control. Bérénice Guyot-Réchar talks about the contemporary situation in a study, “Fifty-one British army units and 47 US air squadrons took residence in Assam and Manipur, amounting to 340,000 Indian, 100,000 British, 90,000 West African, 65,000 Chinese, and 10,000 American troops” (335). As a result, many Naga villagers have to relocate themselves. Kohima inhabitants migrate from their place of origin to strange landscapes. Further, migration created various problems establishing relationships within their community and other tribal groups. Excessive military occupation created a sense of insecurity among the Naga tribal groups.

The marriage was a formal gathering of a few community people. The novelist attempts to provide autobiographical and biographical touch to the story when she talks about the life of other people around—minute detailing of tribal life as to how tribals converted to Christianity. “You see, spirits are scared of Christians; they cannot approach them because it can end badly for spirits. They prefer to make contact with non-Christians, which is why they frighten us and try to prevent us from going to the Christian side” (Kire 74). Thus, the novelist exposes the coloniser’s strategy to convert people from the Hindu religion to Christianity. Further, the belief can be seen strengthened among people when the author depicts the necessity of the Christian faith. People believed in the Christian religion to overcome the fear of evil spirits. It also reflects coloniser’s strategies for expanding the Christian faith among tribals. Kire exposes how people get attracted to Christianity. The narrator tries to situate the incident in the year 1972 when people keep converting to the Christian religion. In the novel, there is a short description of a man called Lhouvitso who drinks a lot. Christians organised Billy Graham crusade in Kohima. Lhouvitso was asked to become a member of the organising committee. People needed Lhouvitso’s help for the successful completion of the work. It seems to be another strategy to expand the horizon of religion. It becomes clear when Lhouvitso says, “It made me think, what if I give up all this and become a Christian...The church baptised me and I became a member just before the crusade”



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(Kire 92). Hence, the coloniser converted people from Hindu to Christian. Organising a function and seeking support from non-Christian people was key to attracting people. However, Easterine Kire seems a staunch critique of religion. She disagrees that if churches close its door for people, would heaven close its door too for people. Will people stop dying? The novelist critically looks at the issues concerned with the consumption of liquor. Many families' economic situation is ruined due to Naga men's addiction to alcohol. Church does not intervene in such matters because government's tax is involved. For instance, Kire writes, "What right did the church have to excommunicate a man because he drank? Wasn't it the church's duty to be Christ-like and help people who were struggling with alcoholism" (133-34). Hence, Kire interrogates the religious practices and their political intricacies. Church and the British government in India were nowhere different from one another vis-à-vis political ambitions and maintaining authority over natives. Government bans liquor on the demand of women. "After prohibition, the majority of men dying from alcohol abuse were in their thirties and forties" (Kire 136). The fact of the matter is that youth die after adulteration in liquor. Adulteration gets increased to its higher rate when after the ban. The author exposes smugglers. For instance, "A friend of mine, a food inspector, has analysed samples of smuggled alcohol and he discovered a very high content of methanol and other substances" (135). Hence, after prohibition, it became a profitable business for enthusiastic buyers.

There was an ongoing war between the contemporary government and underground armed forces. Native inhabitants were the sufferers of the ongoing war. A minor incident of a bomb blast could disturb routine life for several days. "The underground charged the armed forces with continuing to target Naga villages torturing their inhabitants...The bomb blast destroyed the illusion of peace and progress in the new state of Nagaland" (Kire 98). Thus, common citizens were sufferers of the atrocities. It was a fight between the contemporary government and underground forces to acquire political dominance over Nagaland. Both parties spent massive money in the ongoing battle to create unrest and divergence. The title of the novel, *A Respectable Woman*, can be associated with the women who participate in the growth of family, society, and religion. "The point when drinking becomes a problem is when a man takes the money meant for his family and uses it on drink" (Kire 102). The novelist states that religion does not conflict with the government because tax is involved in it. Therefore, belief comes forward to define a respectable position for women. "Their children went to school, and their mothers paid their fees with the money they earned selling brew" (Kire 101). Further, most men in Kohima were involved in drinking alcohol. Everywhere around Kohima, there was a discussion about earning men, "men were using up their salaries on alcohol and had nothing left to support their families. This was the real problem for affected families" (Kire 102). Hence, women in the Naga community are central to governing their families, whereas men are away from family responsibilities.

Alcoholic addiction among men in the Naga community is a major problem. Easterine Kire depicts sensitive issues concerned with patriarchy, domestic violence, corruption, smuggling, political dominance, war, etc. Men waste all their income on alcohol, whereas women use their learning for family survival. This is what brings the alcohol prohibition act. For instance, Kire portrays, "[t]he churches maintained that alcohol abuse was behind many of the social problems in the state. The families of the alcoholics were starving because of the man of the house was using his salary to pay debts accrued in the drinking houses" (134). The fact of the matter is it could not prohibit the smuggling of liquor. It gave rise to adulteration and smuggling around the state. However, in the case of utilisation of modern facilities, Kohima had few prosperous families. Most of them suffer poverty due to excessive consumption of liquor. It seems that lack of education is the primary factor. The fact of the matter is that limited education facility was the central reason behind the unorganised family economy. But there was a change after the world war- II. As Easterine Kire depicts, education became a priority after the war. Before the war, around 1936, a businessman named John Angami started a school in the Viswema village area so that students in the southern village did not have to travel as far as Kohima to get education" (167). Hence, post-world war Nagaland develops an interest in education. John Angami takes charge of expenses for installing the initial structure. Further, DC Pawsey feels encouraged to convert the school into a high school. DC Pawsey helps people to re-build after massive destruction. World war highly unsettles life of ordinary citizens. DC pays compensation amount of damage to native people. Post-war local administration works very hard to restore normalcy. Houses are modernised after destruction. The entire village-



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based society was transformed into a town. Every sphere of life is modernised, such as agriculture, educational institutes, trading, etc. Post-war Kohima immensely changed from rustic rural life to a modernised urban society.

CONCLUSION

To conclude, the Naga society enjoys classless social stratification; women are in a respectable position both in family and community space. In Nagaland, women's social status depends on socialisation, social stratification, and gender relations. Naga community is considered the largest community in the northeast region, having more than forty sub-groups within their cultural group. As per the government of India, the Naga tribe is known as a scheduled tribe in the constitutional framework. The Japanese-Kohima war created an unsettling situation for the Naga community. Children were harassed, and military personnel raped women. War puts survival at stake. People became alienated from their society. The novel's narration is a collective memory of the narrators, depicting the socio-cultural landscape and political unrest.

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Assessing E-Waste Awareness and Management Practices among Library Professionals : a Case Study in Kerala State

Prakash Abraham¹, Aleena Dominic², Sajan Kuriakose^{3*} and Amritha Thomas⁴

¹Librarian, Mary Matha Arts and Science, Mananthavady, Kerala, India.

²Librarian, Green Valley Public School, Nellikuzhy, Kerala, India.

³Library Assistant, SCMS School of Engineering and Technology, Karukutty, Kerala, India

⁴Assistant Professor, Department of Commerce, St. Joseph's College, Irinjalakuda, Kerala, India.

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*Address for Correspondence

Sajan Kuriakose

Library Assistant,

SCMS School of Engineering and Technology,

Karukutty, Kerala, India.

E.Mail: sajanhuriakose11@gmail.com



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ABSTRACT

Efficient and appropriate management of e-waste is associated with its awareness and knowledge. The digital world necessitates the use of electronic gadgets and library does not have any exception. The transformation of the library into a digital creates it a hub of e-waste. And being a social institution, the library has the foremost role in creating awareness and responsibility for e-waste management. Therefore, the study is conducted among library professionals from Kerala state to evaluate their awareness and knowledge regarding e-waste management and the practices and procedures followed in their libraries. Snowball sampling was selected for the study and a questionnaire was distributed in March and April of 2023 among the 125 library professionals and 101 questionnaires were duly filled. The study found that computers and related devices are the top e-waste materials in the library. Many of the libraries do not have any specific policies regarding e-waste management and selling obsolete devices is the most followed disposal way. Low awareness creates issues more drastic. This investigation revealed a very weak correlation between e-waste awareness and the practices of librarians. The reduction of e-waste must not be enough but an integration of library functions into cloud computing concepts should be appreciated.

Keywords: E-waste, E-waste Management, libraries, Librarians, awareness of e-waste, knowledge of e-waste





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INTRODUCTION

Electronic device is one of the necessities of life after air, food, and shelter. It becomes more difficult to live without any of the electronic devices. The reasons are the industrialization and technological advancements in the field of information and communication. Along with these, the increase in the population, lifestyle changes, and depreciation of the life of electronic devices have increased e-waste significantly though they have transformed the way of working, communicating, and living. E-waste (electronic waste), the fastest growing waste stream, is a waste electronic or electric device that has reached the end of its useful life[1]. It is the discarded equipment. It may include a wide range of products such as computers, smartphones, televisions, printers, and other devices that are unwanted, not working, or reached the end of their useful life.

E-waste is toxic and not biodegradable. Therefore, it is harmful. When it is improperly disposed of, it can be accumulated easily in soil, air, and water. The e-waste should be recycled and disposed of in a scientific, healthy, and formal way. The serious challenge before any country is not only the growth of e-waste but also the lack of awareness and practice to manage e-waste. Not only that, there are technological and infrastructural deficiencies and a lack of trained workforce for the efficient treatment and management of e-waste which create environmental destruction and public health issues. Thus, the idea of e-waste management gets important. It is the process of collecting, recovering, recycling, and disposing of e-waste through proper techniques by which it reduces dangerous impacts on human life and the environment[2–4].

The library recognizes the role of electronic devices in the digital age and provides access to digital resources and technologies; meeting the information needs of the patrons is its primary concern. Library not only makes use of electronic devices, but the library itself is getting transformed into the digital version. The pandemic, COVID-19 demanded the conversion of traditional practices and services of libraries to that of digital. Thus, the library requires many electronic goods. Being part of digital transformation, many electronic devices become discarded or replaced according to the need of time. Thus, it becomes a hub of e-waste. The library being a social institution has a recognizable role in creating awareness among the people on the proper treatment and management of e-waste [5]. The library professionals than the institutional or parental authorities have an immediate role in the proper management of e-waste [6]. In this regard, this study analyses the knowledge and awareness of librarians in Kerala state on e-waste management and their specific role in its management in the days to come.

LITERATURE REVIEW

Kariwala *et al.* [7] conducted a study among the electronic and electric equipment users in Lucknow city to determine their knowledge and disposal practice of e-waste. The majority of the participants noted that e-waste was dangerous to both health and the environment, but their awareness and disposal practices showed that they were not concerned about both. Convenience and requirements were the influencing factors behind the use of electronic and electric equipment. While purchasing electronic goods, proper awareness and guidance on disposal practices were not given. It was eye-opening that 87.3% were unaware of government guidelines. The study recommended that there was an urgent need to give proper guidance and awareness on the disposal of e-waste. The study of Dzah *et al.* [8] among commercial consumers in Ghana was to assess the perception and practices with regard to e-waste. Economic growth, technological advancement, an increase in the population, and the depreciation of electronic products were the reasons to increase the e-waste globally. Participants were aware of the e-waste and health and environmental issues created by them. To them, the disposal of e-waste was challenged due to the unsuitable storage space. The study recommended that e-waste management should be started by arranging suitable infrastructure for recycling and it should be supported by collection facilities.

A survey was carried out by S & Patil [1] to analyze the knowledge and practices of e-waste management among medical students. The majority of the medical students were aware of the health and environmental issues posed by





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e-waste. The study found that coping with the technological advancement and not the dysfunction of the electronic goods was the reason behind purchasing the new electronic goods. It was concluded that a lack of awareness led to improper practices in the disposal of e-waste. Therefore, right from the school, awareness and knowledge of e-waste should be given. Competent [9] surveyed the residents of Naga City to evaluate the attitudes and practices followed regarding e-waste collection and disposal. The result showed that the residents were not capable to identify what e-waste was. Therefore, no proper practice was followed to dispose of the same. The study noted a serious concern as e-waste that ended up in the waste stream should be taken from the civil authorities to strengthen the information campaign. The impractical habits and lack of awareness of e-waste cycling created hazardous effects on human health and environmental matters. In this regard, Azodo et al. [10] tried to analyze the knowledge and awareness of Nigerian Collegiate. The participants of the study used around 17 specified e-gadgets and they tried to fix and re-use the gadgets when they were dysfunctional. But they practiced throwing away the damaged ones without following proper methods. As the study noted of the average awareness and practice of e-waste management, appropriate methods were to be implemented properly to handle the electronic gadgets.

The concern of libraries in the case of e-waste generation was noted by Murugan [6]. Library in the digital environment transformed into a blended model of both print and digital and made use of various digital devices for meeting the information needs of the clients. The study listed ten categories of electronic gadgets used in libraries; computers, networking equipment, products related to power supply, and printers were some of them. The study recommended that storing e-waste in the library should be avoided and certified agencies should be contacted for the proper disposal of e-waste generated in the libraries. The case study of Chibunna et al. [11] identified the challenges of institutions in e-waste management. Only 33.5% of the participants of the study heard or knew of the e-waste. Thus, creating awareness was noted as a great challenge. Repairing was the primary measure to handle the end life of electronic goods, but there was no proper system for the disposal of electronic goods. The dissatisfaction towards e-waste management was an indication to begin proper guidance.

OBJECTIVE OF THE STUDY

The study is conducted to meet the following objectives:

1. To identify the e-waste materials in the library.
2. To analyze the awareness and knowledge of library professionals on e-waste management practices and laws.
3. To assess the attitude, practice, and personal initiatives of libraries and librarians on e-waste management.

METHODOLOGY

The analysis of e-waste management in the context of a library is an evolving topic and very few researches have been conducted on the topic still. Thus, it is considered as a good beginning for further studies and advancements. As the researchers of this study belong to Kerala state, the focus of the data collection was on the library professionals from Kerala. The library becomes a hub of e-waste due to technological advancements and libraries are in the process of transforming to digital. Further, the library is an institution for creating social awareness among the common people. Thereby, the study is significant enough.

The survey was conducted online; the questionnaire was prepared and converted into Google form and distributed to library professionals through email and WhatsApp after collecting the emails and WhatsApp numbers of library professionals. As the researchers do not have the total list of library professionals in Kerala state, snowball sampling was the technique used to select the participants. The data collection was taken place in March and April of 2023. The questionnaire was distributed among 125 librarians and 101 questionnaires were duly filled and the response rate was 80.8%. Informed consent was obtained from the participants of the study. The questionnaire starts with demographical details. The other three parts of the questionnaire were e-waste management and library, personal initiatives in e-waste management, and knowledge and awareness of e-waste management of the librarians. The





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collected data in Google form was converted into Microsoft Excel. After proper coding, the data was entered into Jamovi 2.3.21 software for proper statistical analysis such as percentages and correlation.

DATA ANALYSIS AND INTERPRETATION

Demographical Distribution of Participants

Participants distribution in this study is shown in Table 1. Among 101 participants of the study, 32.7% were male while the remaining 67.3% belonged to the female category. It is observed that the number of female library professionals is high compared to male librarians working in the Kerala context. 46.5% of 101 participants were working in college libraries, 23.8% belonged to school libraries, whereas the remaining 30% of the participants belonged to university libraries, public libraries, special libraries, teaching faculty, and other libraries. The data regarding the working condition indicated that college libraries are the more compared to other working areas. The predominant degree acquired by the participants was MLISc (87.1%). It was followed by BLISc with 7.9%. It is noticed from the collected data that MLISc is the highest degree of the working professionals of the library and it is the minimum requirement to be appointed in college libraries. The top tier experience range of the participants was 0-5 years with 73.3% and 16.8% of participants had 6-10 years of experience in the library profession. This is an indication that the participants are young and not experienced much in library professions.

E-waste Materials in the Library

The data regarding the e-waste materials in the library is picturized in Figure 1. The library is fed up with a lot of electric and electronic materials. According to the E-waste (Management) Rules 2016, there are 21 different types of electronic and electric equipment; computer monitors, motherboards, television sets, and communication devices were some of them [6], [12], [13]. The presented data indicate that computer and computer-related devices like monitors, speakers, keyboards, etc. are 29% of the e-waste materials in the library. Electric goods like bulbs, fans, lamps, batteries, etc. are the necessary materials in the library and 27% of the e-waste materials belong to this category. Printers and fax machines are selected by 18% of the library professionals. Electronic devices like television, DVD and CD players, etc. other than computer-related devices create 17% of e-waste materials in the library. The library makes use of various kinds of communication devices such as telephones, mobile phones, etc. for information dissemination and 9% of the e-waste materials belong to this category. It is true that without these devices and instruments library cannot be functioned effectively and efficiently. But proper policies are to be introduced to minimize the generation of e-waste materials from the library.

Roles of Library and Librarians Concerning E-waste Management

Library as a social institution has the highest duty and responsibility in creating awareness among the common people regarding e-waste management and practices [12], [14]. Before creating awareness, it is necessary that the library itself should be an institution following proper e-waste management and practices. The results of the questions regarding the initiatives, policies, and conducting awareness programmes by the library with regard to e-waste management were eye-opening and it is presented in Figure 2. Among the participants, 71 of them (70.3%) reported that their libraries do not have any e-waste management initiatives. The social responsibility of the library is to be shown in the case of e-waste management by beginning the right practices. 76.2% of the library professionals specified that the libraries in which they are working do not have any e-waste policies or legislation. It is noted that the librarians should initiate the framing of e-waste management policies within the library. The third section was concerned with conducting e-waste awareness programmes and 84.2% disclosed that their libraries do not conduct any of the awareness programmes. The data regarding attending any of the e-waste awareness programme was stunning. The vast majority of the library professionals (80.2%) do not attend any of the awareness programme. The data presented in Figure 2 calls for immediate action regarding the e-waste management process and practices on library premises.





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Disposal Practices of E-waste in Library

In e-waste management, disposal practices have the highest value. The disposal practices show the concern and attitude toward the e-waste management process. If the disposal of e-waste is not in a proper manner, it poses adverse effects on the environment and human health. Due to a lack of awareness, e-waste materials are treated as normal and mixed into normal trash. It is evident in Figure 3, the disposal practice of selling (34%) is selected by close to half of the participants as the best practice. A slight majority (25%) store their e-waste whereas 15% take the e-waste materials to collection points. The least practices are throwing (11%) and donating (5%) the e-waste materials.

Challenges in E-waste Management in the Library

The library faces a lot of challenges in disposing of e-waste materials. The findings related to the challenges are illustrated in Figure 4. Low awareness (23%) is considered as the biggest challenge for library professionals within the library to follow e-waste management practices. It is followed by the challenges of collection and disposal problems (20%) and lack of regulations and policies (18%). The challenges named poor infrastructure for recycling and the absence of a voluntary takeback policy are faced by 16% and 14% of the participants respectively. Inefficient equipment classification is the least challenging and it is posed by 9% only. The lack of proper procedures for e-waste management is considered as the reason behind the challenges [13]. Inadequate funding, lack of policies, and neglecting e-waste are listed as the challenges in managing e-waste materials in libraries [14].

Approach to E-waste Management Practices in the Library

The librarians are asked to report their level of approach to various e-waste management practices followed in their libraries. They are asked to mark their approach at three levels namely high, moderate, and low. The data is presented in Table 2 and it is quite interesting that the majority of the participants marked moderate levels for all e-waste management practices within the library. It is an indication that the participants take a secure state in responding to these questions; it may be due to the lack of proper knowledge about the e-waste management practices in the library or the majority of the participants belonging to the category of 0-5 years of experience. In the case of purchasing and disposing of e-goods, the parental institutions are the sole authority and the librarians working may have the least role in decision-making. Therefore, most of the time, the attitude and level of approach of librarians toward e-waste management practices would be moderate. Purchase of e-gadgets from reputable brands for durability (28.7%) is the only practice that has a high level of approach after the moderate levels. All other practices have low levels of approach after the moderate. The table reminds that there is a need to enhance the duties and responsibilities of librarians with respect to the purchase and disposal of electronic assets.

Knowledge and Awareness of E-waste Management

Playing the biggest role in e-waste management is possible only when people have the right knowledge and awareness of e-waste. The level of knowledge and awareness of e-waste may be different from person to person. But the professionals working in certain institutions with high educational qualifications and working experience do not have any excuse. Eight statements are given to the library professionals to evaluate their understanding. The data related to these statements are reflected in Table 3. Among the eight statements, six statements have noted more than 50% of positive responses. The statements are the following: knowing the meaning of e-waste (87.1%), hazardous materials in e-waste (50.5%), health risks associated with e-waste (66.3%), environmental threats (82.2%), recycling of e-waste (55.4%), and benefits of e-waste management (70.3%). The librarians do not have proper knowledge of the local and international laws related to e-waste management (44.6%) and it may be the reason why they face a lot of challenges to follow the proper e-waste management practices in their working premises. They are well aware of the volume of e-waste (48.5%) that their libraries are generating. The table shows a promising attitude of the library professionals that they would follow proper practices in the case of e-waste management in the future. The findings of the previous studies are supporting to this data [12], [14].

Correlation between E-waste awareness and practices among the library professionals

Analysis of the aggregate awareness and practices among the library professionals regarding e-waste was presented in Table 4. The participants are asked to report on their awareness and practices on e-waste management from





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different angles. The analysis of the correlation between awareness and practices is important for the proper practices followed are the result of the right awareness and knowledge. The aggregate mean for e-waste awareness is 2.3676 whereas the aggregate mean for e-waste practices among library professionals is 1.8119. The study points out that there is a very weak correlation between awareness and practices. Therefore, it is necessary that the practices followed in the library should be continually supported by creating proper awareness and knowledge regarding e-waste management.

CONCLUSION

Electronic waste generated from electrical and electronic devices is highly present at all levels of life and the library is not an exception. For the smooth and easy function of all levels of life, these devices play a central role. E-waste becomes a real challenge to the environment and public health due to the inadequate practices and technologies used to dispose of it. There are a number of laws at the national and international levels for electronic waste management but their focus should be more on the reduction of e-waste generation [13]. The majority of the participants recommended that it is high time to conduct various awareness programmes regarding e-waste management by all sorts of libraries. The participants of this study have raised a serious concern related to government grants and policies as they sometimes create difficulties. Because many of the electronic goods and devices in the library are purchased through various projects and grants. When the time comes to dispose of those electronic materials, the procedures mentioned in those projects and grants are to be followed which would take more time. This pressurizes the librarians to dump e-waste materials in-store or wait for the completion of the procedure.

Reducing the e-waste generation in the technologically advanced world may not be considered as the apt solution. But concepts like cloud computing is acceptable in a library environment in which the e-waste generation would be minimal and many library functions will be potentially shared. But, from a budgetary point of view, its implementation may be difficult. It is urgent to ignite awareness and knowledge among the people for the proper management of e-waste by which ecologically and physically healthy communities would be built. The library being the social institution should be the perfect instrument for creating awareness and knowledge by providing suitable information through discussions, exhibitions, information literacy tutorials, implementing policies, and other ways [5], [14]. Thus, a sense of responsibility would be created among the citizens which would result in the proper treatment of e-waste.

CONFLICT OF INTEREST

Authors declare that there is no conflict of interests.

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Table 1 Demographical Distribution of Participants

| Characteristics | Variables | Frequency | Percent |
|-----------------|---------------------|-----------|---------|
| Gender | Male | 33 | 32.7% |
| | Female | 68 | 67.3% |
| Working in | School Library | 24 | 23.8% |
| | College Library | 47 | 46.5% |
| | University Library | 6 | 5.9% |
| | Public Library | 7 | 6.9% |
| | Special Library | 9 | 8.9% |
| | Teaching Faculty | 2 | 2.0% |
| | Any Other Libraries | 6 | 5.9% |
| Highest Degree | Ph.D. | 2 | 2.0% |
| | MPhil. | 2 | 2.0% |
| | MLISc | 88 | 87.1% |
| | BLISc | 8 | 7.9% |
| | Diploma in LIS | 1 | 1.0% |
| Experience | 0-5 Years | 74 | 73.3% |
| | 6-10 Years | 17 | 16.8% |
| | 11-15 Years | 5 | 5.0% |
| | More than 15 Years | 5 | 5.0% |





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Table 2 Approach to E-waste Management Practices in the Library

| Characteristics | High | Moderate | Low |
|--|------------|------------|------------|
| Proper waste segregation practices | 16 (15.8%) | 59 (58.4%) | 26 (25.7%) |
| Purchase new e-gadgets evenif older ones are working | 12 (9%) | 48 (47.5%) | 41 (40.6%) |
| Purchase reputable brands for durability | 29 (28.7%) | 59 (58.4%) | 13 (12.9%) |
| Purchase used e-gadgets so far they will work | 11 (10.9%) | 43 (42.6%) | 47 (46.5%) |
| Giving damaged e-gadgets freely to waste collectors | 7 (6.9%) | 59 (58.4%) | 35 (34.7%) |
| The rate at which e-gadgets damage usually | 8 (7.9%) | 58 (57.4%) | 35 (34.7%) |

Table 3 Knowledge and Awareness of E-waste Management

| Characteristics | Yes | No | Not Sure |
|--|------------|------------|------------|
| Meaning of E-waste | 88 (87.1%) | 03 (3 %) | 10 (9.9%) |
| Hazardous Materials in E-waste | 51 (50.5%) | 21 (20.8%) | 29 (28.7%) |
| Health Risks Associated with E-waste | 67 (66.3%) | 14 (13.9%) | 20 (19.8%) |
| E-waste a Serious Threat to the Environment | 83 (82.2%) | 10 (9.9%) | 08 (7.9%) |
| E-waste Recycling | 56 (55.4%) | 16 (15.8%) | 29 (28.7%) |
| Knowledge of Local and International Laws | 20 (19.8%) | 45 (44.6%) | 36 (35.6%) |
| Awareness of the Volume of E-waste Generated | 49 (48.5%) | 18 (17.8%) | 34 (33.7%) |
| Benefits of E-waste Management | 71 (70.3%) | 8 (7.9%) | 22 (21.8%) |

Table 4 Correlation between E-waste awareness and practices among the library professionals

| No. of Participants | E-waste awareness mean | E-waste practices mean | Pearson Correlation |
|---------------------|------------------------|------------------------|---------------------|
| 101 | 2.3676 | 1.8119 | .180 |

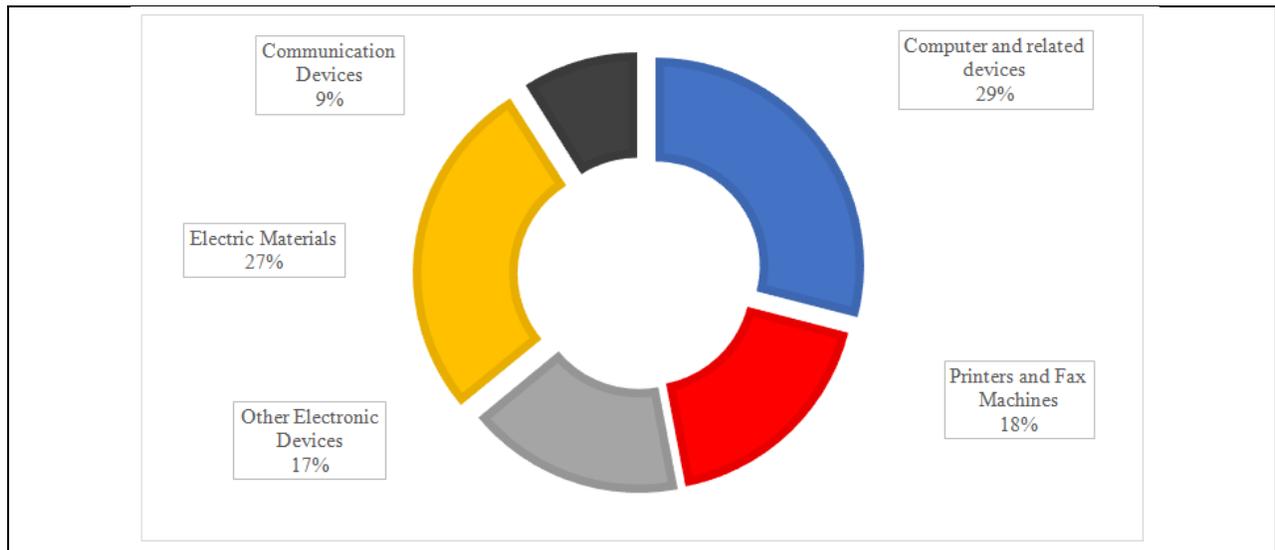


Figure 1 E-Waste Materials in the Library





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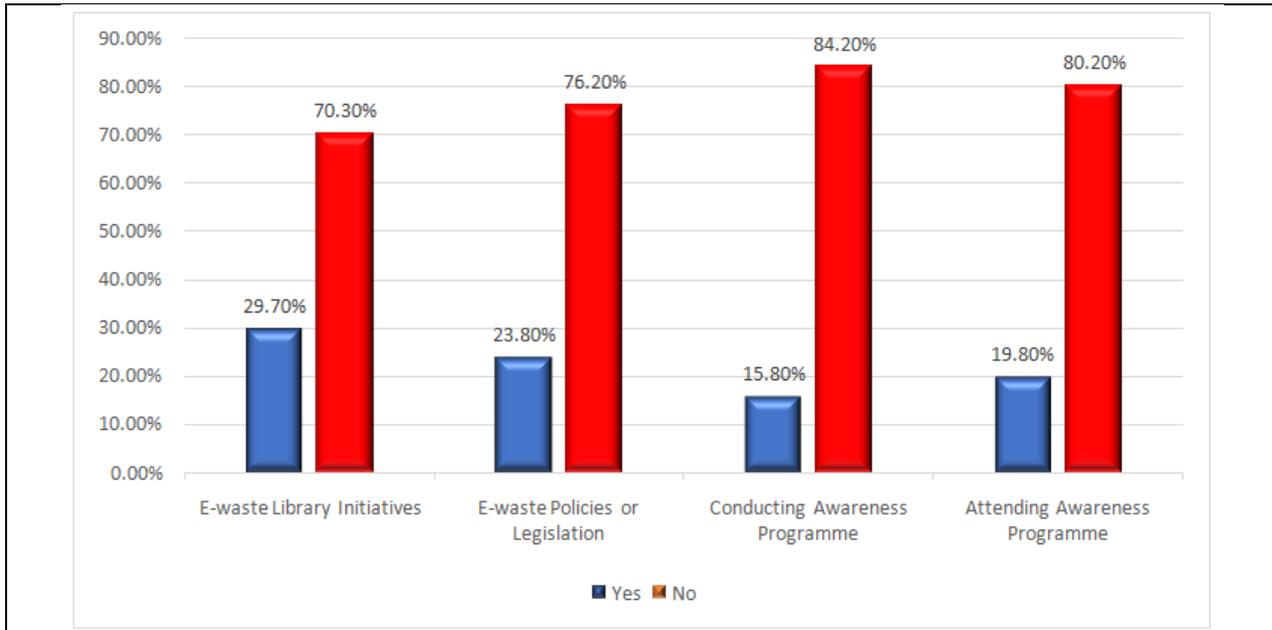


Figure 2 Roles of Library and Librarians Concerning E-waste Management

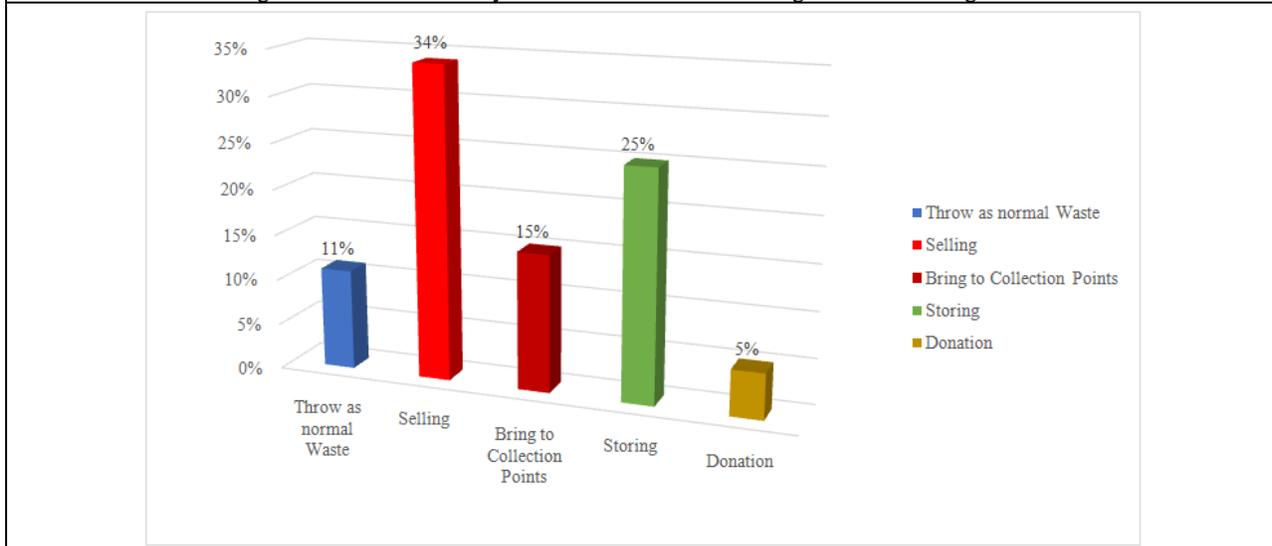


Figure 3 Disposal Practices of E-waste in Library





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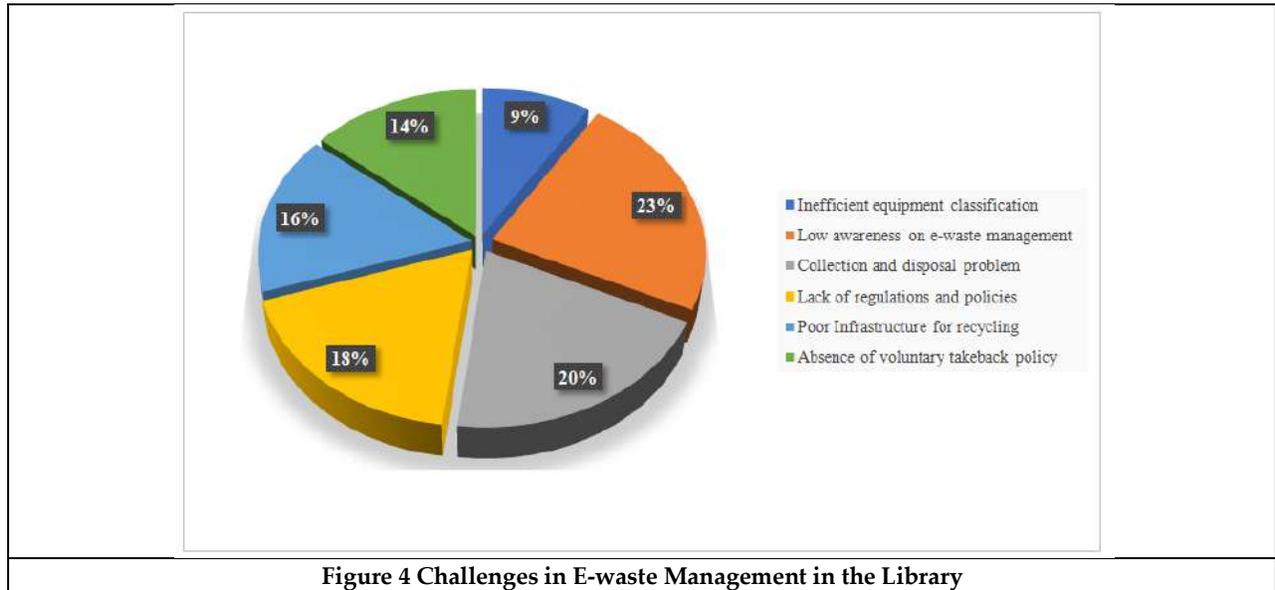


Figure 4 Challenges in E-waste Management in the Library





To Study Different Learning Styles and Academic Achievement of Higher Secondary School Students in Relation to their Gender and Home

Sheetal Khajuria¹, Mohamad Hdyitulah^{2*} and Aman³

¹M.Ed. Student, Department of Educational Studies, Central University of Jammu, Jammu and Kashmir, India

²Research Scholar, Department of Educational Studies, Central University of Jammu, Jammu and Kashmir, India

³Assistant Professor, Department of Educational Studies, Central University of Jammu, Jammu and Kashmir, India

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*Address for Correspondence

Mohamad Hdyitulah

Research Scholar,

Department of Educational Studies,

Central University of Jammu,

Jammu and Kashmir, India

E.Mail: mhdyitulah@gmail.com



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ABSTRACT

The first and foremost purpose of education is to evolve a person into a responsible, sensitive and a good human being and secondly it enables him to face the real life challenges to be a successful in personal and professional life. The purpose of this study is to evaluate the impact of home environment and gender on student learning styles and student academic achievement. The research was carried out in District Kathua of J & K on a sample of 354 IX standard students. Learning styles of students was measured by Learning Style Inventory by Dr.Uzma Siddiqui. Home Environment was measured by the Inventory prepared by Dr. Karuna Shankar Misra. t-test was used for the hypothesis testing. The research finding revealed that the auditory learning style of Higher Secondary School students belonging to favourable home environment is significantly better than the students belonging to unfavourable home environment and in case of others viz. Visual and Kinesthetic learning styles the difference was not significant. Also, in case of male and female students belonging to favourable and unfavourable home environment there was no significant difference in case of all three styles of learning. Further, it can also be highlighted that there was no significant difference in the academic achievement of students belonging to favourable and unfavourable home environment in totality and in case of males and females separately.

Keywords: Learning Style, Home Environment and Academic Achievement.



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INTRODUCTION

“Education is the process of developing the capacities and potentials of the individuals as to prepare that individual to be successful in a specific society or culture”. (Aremu, 2000). The acquisition of information, values, beliefs habits, skills or the process of facilitating learning can be termed as education. As the food is necessary for a healthy body, similarly the education is very vital for a happy and successful life. The education constructs our future as its intrinsic nature is constructive. It not only helps a individual to develop his status of mind but also his body. It is the sole and significant path of growth and success. The purpose of this study is to evaluate the impact of home environment and gender on student learning styles and on student academic achievement. It has been established that what impact student learning style and home environment and improve learner’s academic performance is no longer based on talk, chalk and every other learning material, but how the student learn in the process of learning (Albert, et al., 2016). There are numerous factors affecting students’ academic achievement. These factors may be home, school and peers oriented. Mangle (2007) studied learning from multi-dimensioned perspective and found students’ aptitude, interest, home environment, peer’s interactions and nature of learning materials has significant influences on students learning. Parveen (2007), Codjoe (2007) and Muola (2010) unanimously reached to the conclusion that home environment is the most influential factor among all the mentioned factors. It is the first institution where a child starts to learn, and mother is the first teacher for the baby, while the role of peers is performed by the other members of the family.

Kumawat (2016) conducted a study on “Impact of Home and School Environment on Learning Style of Secondary School Students.” There is significance difference of learning Style’s Dimension’s, Figural Reproducing, Figural Constructive, Verbal Reproducing, Verbal constructive, and sum all of learning style’s dimensions between good and poor school Environment students and also found that there is no significance correlation among learning style and Home Environment students. Khan and Unnisa (2017) conducted a study on “The effect of learning style, home environment and school environment on academic achievement of ninth standard students of English medium schools of Raipur city.” The results of the study showed that there is little to no correlation between family environment and academic success. Additionally, a favourable but weak association between academic success and the school environment has been established by investigators. The academic achievement of pupils who gained information with their right and left hemispheres differs significantly, while there is little variation in the academic achievement of students who learned with their right or left hemisphere.

Shukla (2021) conducted a study on “A study of academic anxiety and home environment in relation to the academic performance.” The results revealed that Academic Anxiety is a hindrance factor of Academic Achievement. High academic anxiety and an unfavourable home environment reduce the efforts and motivation of the students in case of their academic achievement. But a little amount of academic anxiety is possible to exist in students. The study also indicates that academic anxiety and academic achievement is negatively correlated. The correlation indicates that the negative correlation is statistically significant. So, it can be summed up that excess academic anxiety is always harmful and impacts and badly affects student’s academic achievement.

Jayalakshmi (2023) conducted a study on ‘Achievement motivation of secondary school students in relation to their learning styles. The findings shows that the Secondary school students have moderate level of achievement motivation and it was found there exist no gender difference in achievement motivation, learning style of Secondary School Students. Further, it was observed that there is significant relationship between achievement motivations, learning style of Secondary School students. Masutimath (2023) conducted a study on “A study influence of learning style and academic achievement of secondary school students”. Findings of the study reveal that, kinesthetic learning style was found to be more prevalent than visual and auditory learning styles among secondary school students. There exist positive high correlation between kinesthetic learning style and academic achievement. The main effects of the three variables - visual, auditory and kinesthetic are significant on academic achievement.



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In accordance with the review above, a number of researchers have investigated the different aspects which affect different learning styles and academic achievement of the students. A detailed, brief study paper concentrating on such factors, such as gender and home environment context, however, was not located. So, from these reviews it is very difficult to draw common conclusion because of their unexpected findings and use of diverse tools and techniques. It is under this background that a separate study needs to be planned for studying different learning styles and academic achievement of Higher Secondary School students in relation to gender and home environment with respect to the District Kathua of J&K.

SIGNIFICANCE OF THE STUDY

From the review of related literature it is evident that numbers of studies were conducted while taking the different variables and also revealed that there are many important variables related to home and learning styles which still remained unproved. Roashani (2021) conducted a co relational study on learning style and academic achievement of z learners. The major findings of this study revealed that learning styles have no significant effect on academic achievement and students with different learning styles do not statistically significantly different in their academic performance based on the form of their study. Besides these the personal characters, vocational aspirations, creativity, intelligence attitude, values, etc. also influence it. But home environment and learning style plays a major role. Younas, Javaid, Liu, Khalid and Bakar (2020) conducted a study on Effect of home environment on student's academic achievements at higher level. The results of the study indicated a weak positive correlation between home environment and student's academic achievement. It also revealed that there were no differences existed between the opinions of male and female status perception about home environment and academic achievement in relation to gender. The researcher has already been explored the studies related to different learning styles and home environment in relation to academic achievement separately, but not in the way that the researcher planned research work.

Considering this in view it is planned to study different learning styles and academic achievement of Higher Secondary School students of District Kathua in relation to their gender and home environment. The study will be helpful to the teacher as it will make them aware about the styles of student in learning and thinking process and what effects these style have on children's performance in school and that attention should be given to children's performance to assess their levels of ability. The finding of this study will be useful to the government, curriculum planner and examination bodies in policy making and in planning educational strategies for school students. It is also hoped that this work will be a guideline to the future researchers who will carry out their researches on home environment and learning styles. Thus, the purpose of this study is to find out the influences of various dimensions of home environment and gender on the different learning styles and academic achievement of the school going children.

STATEMENT OF THE PROBLEM

The statement of the problem is " To Study Different Learning Styles and Academic Achievement of Higher Secondary School Students in Relation to their Gender and Home Environment."

OBJECTIVES OF THE STUDY

The following objectives were framed for the present research study:

1. To study the appropriateness of home environment of Higher Secondary School students.
2. To study different learning styles of Higher Secondary School students belonging to favourable and unfavourable home environment as below:
 - (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
3. To study different learning styles of female Higher Secondary School students belonging to favourable and unfavorable home environment as below:





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- (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
4. To study different learning styles of male Higher Secondary School students belonging to favourable and unfavourable home environment as below:
 - (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
 5. To study the academic achievement of Higher Secondary School students belonging to favourable and unfavourable home environment.
 6. To study the academic achievement of female Higher Secondary School students belonging to favourable and unfavourable home environment.
 7. To study the academic achievement of male Higher Secondary School students belonging to favourable and unfavourable home environment.

HYPOTHESES OF THE STUDY

On the basis of above objectives, following hypotheses were formulated:

1. There will be no significant difference in the learning styles of Higher Secondary School students belonging to favourable and unfavourable home environment as below:
 - (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
2. There will be no significant difference in the learning styles of female Higher Secondary School students belonging to favourable and unfavourable home environment as below:
 - (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
3. There will be no significant difference in the learning styles of male Higher Secondary School students belonging to favourable and unfavourable home environment as below:
 - (i) Visual learning style
 - (ii) Auditory learning style
 - (iii) Kinesthetic learning style
4. There will be no significant difference in the academic achievement of Higher Secondary School students belonging to favourable and unfavourable home environment.
5. There will be no significant difference in the academic achievement of female Higher Secondary School students belonging to favourable and unfavourable home environment.
6. There will be no significant difference in the academic achievement of male Higher Secondary School students belonging to favourable and unfavourable home environment.

METHOD USED

- Survey method under descriptive method of research was used for the investigation.

Population of the Study

- The students studying in class 11th of Government Higher Secondary Schools of District Kathua constituted the target population for the present study.





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Sample of the Study

- A sample of about 354 students (11th Standard) studying in Government Higher Secondary Schools of District Kathua was collected through simple random sampling technique (lottery method) under probability sampling technique.

STATISTICAL TECHNIQUES TO BE USED

Following statistical techniques was used for analysis and interpretation of data:

- Graphical Techniques
- Percentage
- Mean
- Standard Deviation
- Critical Ratio

ANALYSIS AND INTERPRETATION OF DATA

1. To study the appropriateness of home environment of Higher Secondary School students.

TOOLS USED

For the present study, the following tools have been employed for the collection of data:

| S.No. | Name of the tool | Developed by | Year |
|-------|----------------------------|------------------|------|
| 1. | Home Environment Inventory | Dr. K.S. Misra | 2020 |
| 2. | Learning Style Inventory | Dr.Uzma Siddiqui | 2021 |

STATISTICAL TECHNIQUES TO BE USED

Following statistical techniques was used for analysis and interpretation of data:

- Graphical Techniques
- Percentage
- Mean
- Standard Deviation
- Critical Ratio

ANALYSIS AND INTERPRETATION OF DATA

- To study the appropriateness of home environment of Higher Secondary School students.

Testing of Hypotheses

1. There will be no significant difference in the learning styles of Higher Secondary School students belonging to favourable and unfavourable home environment
2. There will be no significant difference in the learning styles of female Higher Secondary School students belonging to favourable and unfavourable home environment
3. There will be no significant difference in the learning styles of male Higher Secondary School students belonging to favourable and unfavourable home environment.
4. There will be no significant difference in the academic achievement of Higher Secondary School students belonging to favourable and unfavourable home environment.
5. There will be no significant difference in the academic achievement of female Higher Secondary School students belonging to favourable and unfavourable home environment.
6. There will be no significant difference in the academic achievement of male Higher Secondary School students belonging to favourable and unfavourable home environment.

FINDINGS

From the analysis and interpretation of the data following findings and conclusions may be drawn:



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1. Majority (50%) of Higher Secondary School Students are having favourable Home Environment, a good percent (39.80%) are having average Home Environment. However, a few percent (10.17%) are having unfavourable Home Environment.
2. There is no significant difference in the Visual learning style of Higher Secondary School students belonging to favourable and unfavourable home environment.
3. There is significant difference in the auditory learning style of Higher Secondary School students belonging to favourable and unfavourable home environment. Also, the auditory learning style of Higher Secondary School students belonging to favourable home environment is significant better than the students belonging to unfavourable home environment.
4. There is no significant difference in the kinesthetic learning style of Higher Secondary School students belonging to favourable and unfavourable home environment.
5. There is no significant difference in the Visual learning style of female Higher Secondary School students belonging to favourable and unfavourable home environment.
6. There is no significant difference in the auditory learning style of female Higher Secondary School students belonging to favourable and unfavourable home environment.
7. There is no significant difference in the kinesthetic learning style of female Higher Secondary School students belonging to favourable and unfavourable home environment.
8. There is no significant difference in the Visual learning style of male Higher Secondary School students belonging to favourable and unfavourable home environment.
9. There is no significant difference in the auditory learning style of male Higher Secondary School students belonging to favourable and unfavourable home environment.
10. There is no significant difference in the kinesthetic learning style of male Higher Secondary School students belonging to favourable and unfavourable home environment.
11. There is no significant difference in the academic achievement of Higher Secondary School students belonging to favourable and unfavourable home environment.
12. There is no significant difference in the academic achievement of female Higher Secondary School students belonging to favourable and unfavourable home environment.
13. There is no significant difference in the academic achievement of male Higher Secondary School students belonging to favourable and unfavourable home environment.

CONCLUSION

Hence, from the above findings, we can conclude that the auditory learning style of Higher Secondary School students belonging to favourable home environment is significantly better than the students belonging to unfavourable home environment and in case of others viz. Visual and kinesthetic learning styles the difference was not significant. Also, in case of male and female students belonging to favourable and unfavourable home environment there was no significant difference in case of all three styles of learning. Further, it can also be highlighted that there was no significant difference in the academic achievement of students belonging to favourable and unfavourable home environment in totality and in case of males and females separately.

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Table1. Sample of Students Drawn from Different Government Higher Secondary Schools of District Kathua

| S.No. | Name of the school | Total No. of Students (11 th standard) | 20% of total no. of students |
|--------------|---------------------|---|------------------------------|
| 1. | GMHSS, HIRANAGAR | 175 | 35 |
| 2. | GHSS, SALLAN | 144 | 29 |
| 3. | GGHSS, KATHUA | 314 | 63 |
| 4. | GBHSS, KATHUA | 302 | 60 |
| 5. | GHSS, KOOTAH | 154 | 31 |
| 6. | GHSS, CHANN ARORIAN | 127 | 26 |
| 7. | GHSS, BUDHI | 141 | 28 |
| 8. | GHSS, MARHEEN | 192 | 40 |
| 9. | GHSS, CHANDWAN | 75 | 15 |
| 10. | GHSS, FORELAIN | 134 | 27 |
| TOTAL | | 1758 | 354 |





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Table 2. Home Environment of Higher Secondary School Students

| Home Environment | No. of Students | Percentage |
|--|-----------------|------------|
| Favourable (P ₅₀ & above) | 177 | 50.00% |
| Average (Between P ₅₀ - P ₁₀) | 141 | 39.80% |
| Unfavourable(P ₁₀ & below) | 36 | 10.17% |
| Total → | 354 | |

Table 3. Different Learning Styles of Students Belonging to Favourable and Unfavourable Home Environment

| Learning style Dimension | Type of Home Environment | N | Mean | SD | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|--------------------------|--------------------------|-----|------|------|------|-------------------------------|---------------------------------------|
| Visual | Favourable | 177 | 7.57 | 2.01 | 0.70 | Not Significant at 0.05 level | Accepted (CR< 1.96) |
| | Unfavourable | 36 | 7.83 | 2.00 | | | |
| Auditory | Favourable | 177 | 6.68 | 1.68 | 2.72 | Significant at 0.01 level | Rejected (CR> 2.58) |
| | Unfavourable | 36 | 5.89 | 1.56 | | | |
| Kinesthetic | Favourable | 177 | 7.92 | 1.70 | 0.88 | Not Significant at 0.05 level | Accepted (CR< 1.96) |
| | Unfavourable | 36 | 7 | 1.98 | | | |

Table 4. Different Learning Styles of Female Belonging to Favourable and Unfavourable Home Environment

| Learning style Dimension (Female) | Type of Home Environment (Female) | N | Mean | SD | df | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|-----------------------------------|-----------------------------------|----|------|------|-----|-------|-------------------------------|---------------------------------------|
| Visual | Favourable | 87 | 8.31 | 1.74 | 102 | 0.019 | Not Significant at 0.05 level | Accepted (CR<1.98) |
| | Unfavourable | 17 | 7.94 | 2.22 | | | | |
| Auditory | Favourable | 87 | 6.99 | 1.58 | 102 | 0.039 | Not Significant at 0.05 level | Accepted (CR< 1.98) |
| | Unfavourable | 17 | 6.35 | 1.93 | | | | |
| Kinesthetic | Favourable | 87 | 8.23 | 1.53 | 102 | 0.052 | Not Significant at 0.05 level | Accepted (CR< 1.98) |
| | Unfavourable | 17 | 7.23 | 2.11 | | | | |





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Table 5. Different Learning Styles of male Belonging to Favourable and Unfavourable Home Environment

| Learning style Dimension (Male) | Type of Home Environment (Male) | N | Mean | SD | df | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|---------------------------------|---------------------------------|----|------|------|-----|-------|-------------------------------|---------------------------------------|
| Visual | Favourable | 90 | 6.99 | 2.04 | 106 | 0.044 | Not Significant at 0.05 level | Accepted (CR<1.98) |
| | Unfavourable | 18 | 7.72 | 1.90 | | | | |
| Auditory | Favourable | 90 | 6.51 | 1.72 | 106 | 0.066 | Not Significant at 0.05 level | Accepted (CR<1.98) |
| | Unfavourable | 18 | 5.5 | 1.04 | | | | |
| Kinesthetic | Favourable | 90 | 7.75 | 1.75 | 106 | 0.014 | Not Significant at 0.05 level | Accepted (CR<1.98) |
| | Unfavourable | 18 | 6.78 | 1.93 | | | | |

Table 6. Academic Achievement of Student’s Belonging to Favourable and Unfavourable Home Environment

| Academic Achievement | Type of Home Environment | N | Mean | SD | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|----------------------|--------------------------|-----|-------|-------|------|-------------------------------|---------------------------------------|
| A.A | Favourable | 177 | 73.30 | 13.24 | 1.02 | Not Significant at 0.05 level | Accepted (CR<1.96) |
| | Unfavourable | 36 | 75.87 | 13.95 | | | |

Table 7. Academic Achievement of Female Belonging to Favourable and Unfavourable Home Environment

| Academic Achievement | Type of Home Environment | N | Mean | SD | df | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|----------------------|--------------------------|----|-------|-------|-----|------|-------------------------------|---------------------------------------|
| Female | Favourable | 87 | 77.18 | 10.41 | 102 | 0.01 | Not Significant at 0.05 level | Accepted (CR<1.96) |
| | Unfavourable | 17 | 75.22 | 16.50 | | | | |

Table 8. Academic Achievement of Female Belonging to Favourable and Unfavourable Home Environment

| Academic Achievement | Type of Home Environment | N | Mean | SD | df | CR | Level of Significance | Acceptance or Rejection of Hypotheses |
|----------------------|--------------------------|----|-------|-------|-----|-------|-------------------------------|---------------------------------------|
| Male | Favourable | 90 | 70.01 | 13.86 | 106 | 0.039 | Not Significant at 0.05 level | Accepted (CR<1.96) |
| | Unfavourable | 18 | 76.60 | 10.54 | | | | |





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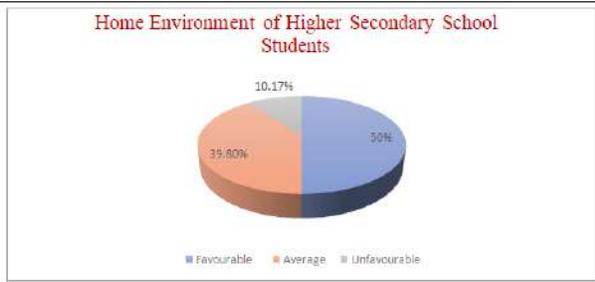


Fig. 1. Home Environment of Higher Secondary School Students

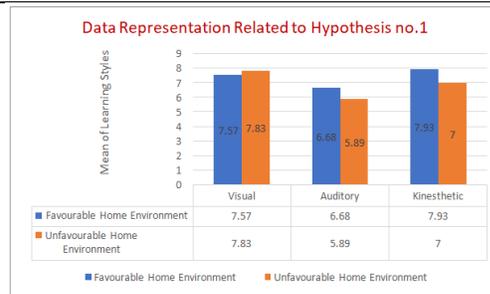


Fig. 2. Data Representation Related to Hypothesis no.1

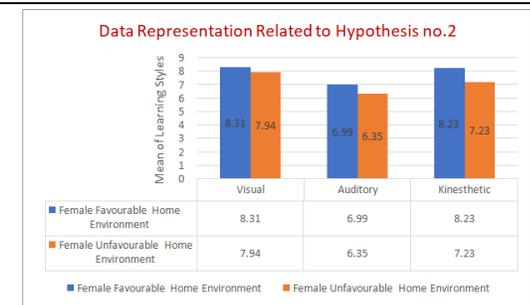


Fig. 3. Data Representation Related to Hypothesis no.2

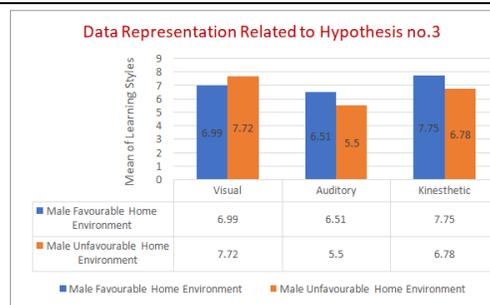


Fig. 4. Data Representation Related to Hypothesis no.3

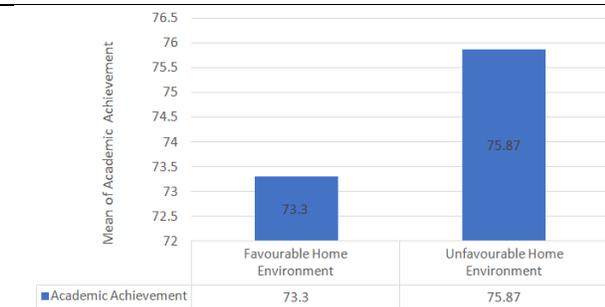


Fig. 5. Academic Achievement of Student's Belonging to Favourable and Unfavourable Home Environment

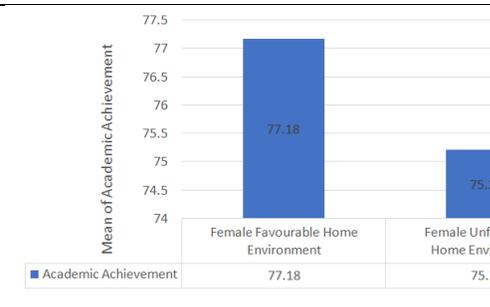


Fig. 6. Academic Achievement of Female Belonging to Favourable and Unfavourable Home Environment

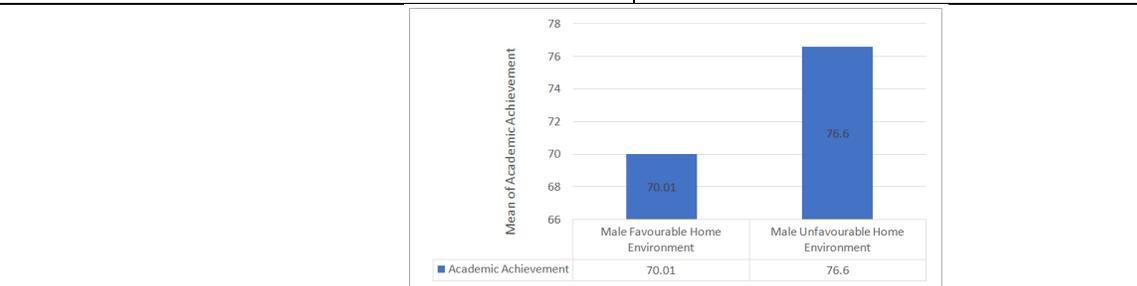


Fig.7. Academic Achievement of Male Belonging to Favourable and Unfavourable Home Environment





A Comparative Study on Academic Stress in Private and Government School Children in Bangalore South

Manimugdha Medhi*

Assistant Professor, Faculty of Humanities and Social Sciences, Assam down town University, Guwahati, Assam, India.

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*Address for Correspondence

Manimugdha Medhi

Assistant Professor,
Faculty of Humanities and Social Sciences,
Assam down town University,
Guwahati, Assam, India.
E. Mail: manimedhi60@gmail.com



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ABSTRACT

The research was undertaken to find out the academic stress in school going children (Age group- 13 to 18) in Southern parts of Bengaluru, Karnataka, its factors and consequences. The research outlined its focus on the fact that majority of the students have stress or pressure of one thing or another that leads to mild or moderate level of stress in them. The research concentrated mainly in understanding the prevalence of stress in high school students studying in private and government schools and the results point out that majority of the private school students are suffering from moderate to high levels of stress that can lead to many problems in present and future

Keywords: Stress in children, academic pressure, tension, Suicide, Expectations

INTRODUCTION

Stress is an interesting word. Most people have no trouble identifying their level of stress and attributing all of their problems to it. However, due to the concept's oversimplification and difficulty in definition, psychologists and educators have tended to steer clear of stress. Stress is the result of the interaction between external stimuli, life events, and a variety of personal reactions to those stimuli, such as anxiety or depression.

Stress is defined as any physical, chemical, or emotional factor that causes physical or mental unrest and may contribute to the development of disease. Stress can be brought on by physical and chemical factors such as trauma, infections, toxins, diseases, and injuries of any kind. Stress and tension have a wide range of emotional causes. Is all





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stress bad if it disturbs the body's equilibrium and functionality? No, not always. Sometimes a little bit of stress and strain is good. For instance, when working on a project or assignment, a little bit of stress often motivates us to do a good job, pay attention, and work diligently. Similar to how exercising may temporarily stress out some bodily processes, its positive effects on health are undeniable. It is frequently referred to as Eustress or Positive Stress (Hans Selye, 1956). The negative effects of stress only manifest when it is excessive or poorly managed. We can learn to control our stress levels and its negative effects on our physical and mental health by developing stress management skills. Negative stress is mostly referred to as Distress (Hans Selye, 1956). Young children's health, wellbeing, development, and learning are supported by the creation of enabling outdoor environments.

Stress is a condition of mental or emotional tension or strain brought on by difficult or demanding circumstances. Stress is characterized as a negative relationship between people and unreasonably high demands or other forms of pressure. (Parry, 2005) Stressors are things that make people anxious and they can be internal or external. Hunger, pain, noise, temperature changes, crowds, fatigue, are some of the examples of internal stressors whereas separation from loved ones, friends, and coworkers, interpersonal conflicts, peer group influences, academic pressure, violence, aggression, loss, and accidents are a few examples of external stressors.

Stress affects kids just like it does adults, but the effects and coping mechanisms aren't always the same. Theorists contend that children's behaviour reflects their difficulties in controlling and responding to stressful situations. A child is at a high risk of illness when normal stress turns into a prolonged one or when there is too much stress (distress) that causes both psychological and biological changes. Children's stress levels are dangerously rising today as a result of the dual demands of co-curricular activities and academic pressure. Additionally, a significant contributor to children's increased stress is the grading system. Typically, school-related stressors like the constant pressure to study, lack of time, writing term papers, taking tests, career plans, and cumbersome instructors were the most annoying daily hassles. (Schaffer, 1996)

Not all children can cope up with such high levels of expectations and competitions. Parents do not seem to realize or accept that their children are under tremendous pressure. Symptoms like tiredness, anxiety, stomach ache, headache, panic, frustration, depression, stammering, stuttering, and many more psychological problems arises, that are triggered by different day to day situations in children's life such as school demands, higher fees, homework, submissions, records, reports, time management, projects, compulsory physical and co-curricular activities, bullying, ragging, eve teasing etc. The study will analyze various sources of stress, their consequences, parental expectations , suicide rates etc.

MATERIALS AND METHODS

Aim of the Study

The aim of the study is to understand various sources of stress in school going children.

Objectives of the Study

- 1) To analyze the stressors in school going children and its consequences.
- 2) To analyze the expectation levels of parents from their children.
- 3) To get a glimpse of increasing suicide rates in school children due to the rise of academic pressure.
- 4) To compare the stress levels in Private and Government school children.

Scope of the Study

The study was being undertaken to find out the awareness about stress, level of stress and its impact on academic performance and improve their knowledge on coping strategies to overcome stress.





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Research Design selected for the Study

The researcher in order to achieve the goal of the research has chosen Descriptive research design in order to carry out the study and fulfill the objectives of the research.

Universe of the Study

The researcher decided to conduct the study in Bangalore South (Lok Sabha Constituency) area entitling the research as "A comparative study on Academic stress In private And government school children in Bangalore South". The children will fall under the Age Group of 13 to 18. i.e. Class 9 to 12.

Sampling method selected for the Study

The researcher has chosen "Simple random sampling" and "Accidental sampling" for the purpose of this research study. Sample size for the study consist of 40 students out of more than 2 Lakh students studying in various Private and Government schools in southern part of Bengaluru. Out of 40 students, 20 students are from Private schools and 20 students are from Government schools, selected at random for statistical analysis.

Sources of Data Collection

In the present study the data or information is collected from the respondents through Questionnaire method. The researcher has also collected data from secondary sources like Newspaper articles, References from books , Journals and magazines , Browsing or web site, Research studies

Limitation of the Study:

- 1) Sample size of the questionnaire is limited to only 40 respondents (students) of various private schools and government schools.
- 2) Research was conducted in a small range.
- 3) There were time constraints while conducting the research.
- 4) Very small fraction of the population was included in the study.
- 5) There was a problem of language barrier for the researcher while conducting the study in government schools.

RESULTS

Table 1: School wise distribution

| Sl no. | School | Male | Female | No.of respondents |
|--------|-------------------|------|--------|-------------------|
| 1 | Government School | 12 | 8 | 20 |
| 2 | Private School | 12 | 8 | 20 |
| | Total | 24 | 16 | 40 |

Table 2: Respondents who feel their syllabus is lengthy and vast

| Sl no. | School | Feels | Donot feel | Can'tsay | Total |
|--------|-------------------|-------|------------|----------|-------|
| 1 | Government School | 5 | 1 | 14 | 20 |
| 2 | Private School | 18 | 1 | 1 | 20 |
| | Total | 23 | 2 | 15 | 40 |

Table 3 : Respondent's schools have counselors, career advisors, psychologists to address their stress related issues

| Slno. | School | Yes | No | Total |
|-------|-------------------|-----|----|-------|
| 1 | Government School | 3 | 17 | 20 |
| 2 | Private School | 6 | 14 | 20 |
| | Total | 9 | 31 | 40 |





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Table 4: Respondents who goes to tuitions or coaching classes for extra help

| Sl no. | School | Go | Don't go | Total |
|--------|-------------------|----|----------|-------|
| 1 | Government School | 11 | 9 | 20 |
| 2 | Private School | 15 | 5 | 20 |
| | Total | 26 | 14 | 40 |

Table 5: Respondents who feel there is high competition in class 10 (HSLC) and class 12 (PUC)

| Sl no. | School | Feels | Don't feel | Can't Say | Total |
|--------|-------------------|-------|------------|-----------|-------|
| 1 | Government School | 9 | 2 | 9 | 20 |
| 2 | Private School | 16 | 2 | 2 | 20 |
| | Total | 25 | 4 | 11 | 40 |

Table 6: Respondents who feel their parents have high expectations from them

| Sl no. | School | Feels | Don't feel | Can't Say | Total |
|--------|-------------------|-------|------------|-----------|-------|
| 1 | Government School | 10 | 8 | 2 | 20 |
| 2 | Private School | 17 | 2 | 1 | 20 |
| | Total | 27 | 10 | 3 | 40 |

Table 7: Respondents who goes to tuitions or coaching classes for extra help

| Sl no. | School | Go | Donot go | Total |
|--------|-------------------|----|----------|-------|
| 1 | Government School | 11 | 9 | 20 |
| 2 | Private School | 15 | 5 | 20 |
| | Total | 26 | 14 | 40 |

Table 8 : Respondents who feel teachers are biased or partial

| Sl no. | School | Feels | Don't feel | Can't Say | Total |
|--------|-------------------|-------|------------|-----------|-------|
| 1 | Government School | 8 | 8 | 4 | 20 |
| 2 | Private School | 17 | 2 | 1 | 20 |
| | Total | 25 | 10 | 5 | 40 |

Table 9 : Respondents who feel teachers rush to finish their syllabus

| Sl no. | School | Feels | Don't feel | Mixed Feeling | Total |
|--------|-------------------|-------|------------|---------------|-------|
| 1 | Government School | 6 | 12 | 2 | 20 |
| 2 | Private School | 13 | 4 | 3 | 20 |
| | Total | 19 | 16 | 5 | 40 |

Table 10 : Respondents who have witnessed suicide or attempted suicide in the past

| Sl no. | School | Awareness on suicide | Attempted suicide | Not attempted suicide |
|--------|-------------------|----------------------|-------------------|-----------------------|
| 1 | Government School | 20 | 4 | 16 |
| 2 | Private School | 20 | 8 | 12 |





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Table 11: Respondents are forced to take alcohol, cigarettes, drugs, guthka by their friends or peer groups.

| Sl no. | School | Agree | Disagree | Neither agree nor disagree | Total |
|--------|-------------------|-----------|-----------|----------------------------|-----------|
| 1 | Government School | 7 | 9 | 4 | 20 |
| 2 | Private School | 12 | 7 | 1 | 20 |
| | Total | 19 | 16 | 5 | 40 |

DISCUSSIONS

From Table 1, it is seen that 40 students are taken out of which 20 students are taken from government school (12 boys and 8 girls) and 20 students were taken from private schools where (12 boys, 8 girls). So in total there were 24 male respondents and 16 female respondents selected at random for the study. From the table 2, it is seen that only 5 students feel that their syllabus is lengthy and vast in government schools out of 20 and 14 students were not sure how they feel about their syllabus. But in private schools 18 students feel that their syllabus is very lengthy and vast out of 20 students. Overall, more than 50% of the students feel their syllabus is lengthy and vast. The data clearly shows that majority of the private school students feel stressed about their course syllabus which can hinder their academic performance.

According to the table 3, only 3 students from government schools and 6 students from private schools reported to be having counselors or career advisors in their schools while the others denied of any involvement of counselors in their respective schools. The overall data shows that 77.5% of total students have never discussed their stressful issues with a counselor, career advisor or a psychologist in their schools. The ones who said yes also mentioned that they have consulting child counselors who visits once every 1-2 months. Students have a lot of study related stress issues in their academic life which they feel uncomfortable to share with their parents or teachers. They need an unbiased opinion on their issues and a proper guidance mechanism to resolve their problems. It should be made compulsory for every schools (government or private) to have a counselor or a school social worker or a career advisor or a child psychologist to address students issues stress levels.

From the table 4, it is seen that 11 students from government school goes to tuitions and coaching classes on a regular basis and 9 of them studies by themselves. Whereas 15 students goes for tuitions and coaching classes on a regular basis and only 5 students do self-study. The Table shows that 65% of the total respondents goes to tuitions and extra coaching classes after their school hours which suggest that there is heavy workload on students to excel in their studies. 75% of private school students attend coaching classes after school hours which makes them vulnerable to depression and burnout.

From the table 5, it is seen that 9 students feel there is high competition in class 10 and 12 in government schools, 9 of them are not sure about it. But in private schools 16 students believe that there is high level of competition in class 10 and 12 and only 2 of them feels otherwise. The Overall data shows that 62.5% of the students feel there is high level of competition in class 10 and 12. The data clearly indicates that majority of the students are well aware of the competition levels of HSLC and PUC and feels the pressure of studies which affect their academic performance both positively and negatively.

From the table 6, it is seen that 10 students from government schools feel that their parents have high expectations from them, 8 students don't feel the same way. Whereas in private schools 17 students said that their parents have high expectations from them and only 3 students who don't feel the same way. The overall data depicts that nearly 68% of the total respondents says that their parents have high expectations from them which can induce a high amount of stress in their academic life. The data also clearly shows that 85% of private school students feel pressured due to over expectations from their parents in their academics, leaving them over-burdened and anxious.



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From the table 7, it is seen that 11 students from government school goes to tuitions and coaching classes on a regular basis and 9 of them studies by themselves. Whereas 15 students goes for tuitions and coaching classes on a regular basis and only 5 students do self-study. The Table shows that 65% of the total respondents goes to tuitions and extra coaching classes after their school hours which suggest that there is heavy workload on students to excel in their studies. 75% of private school students attend coaching classes after school hours which makes them vulnerable to depression and burnout.

According to the table 8, it is seen that 8 students from government schools feel their teachers are biased, 12 of them feels otherwise while 17 of the private school students feel their teachers show bias and partiality and only 3 students feel otherwise. The overall data shows that 62.5% students feel that their teachers are biased and partial. The data also highlights the fact that 85% of the private school students feel their teachers discriminate them from others and show unfairness in giving marks, assignments and punishments which makes the student aggressive, violent, rebellious and withdrawn, which influence their stress levels and behavior.

According to the table 9, it is seen that only 6 students from government school says that the teachers hasten to finish the syllabus and 12 of them says they go slow, 2 of them have mixed feelings. But on the contrary 13 students from private school feels that teachers scramble to finish their syllabus soon. only 4 of them speaks otherwise. The overall data shows that 48% students feel the syllabus is finished early by the teachers and 40% students deny it. But the highlight of the study is that 65% of the private school students feel their syllabus is rushed and 62.5 % students from government school feels the teachers take their time to finish the syllabus. The table gives an idea that Private school teachers try to finish the syllabus as fast as they can than the government school teachers which makes the students confused, perplexed and disoriented and puts them under tremendous pressure to keep up with the class which is not a good sign.

According to the table 10, 16 of the government students said no to previous attempts of suicide or knowing anyone who have committed or attempted suicide, and so did the 12 private school students. But 8 of the private school students and 4 of the government school students have seen someone attempting suicide. Out of the 8 students of private school, one of them did attempt to suicide after her HSLC results due to her poor academic grades and constant scolding from her parents. This becomes the responsibility of both parents and teachers to sensitize the students about suicide and suggest strategies of coping up with stress to minimize suicidal thoughts. Overall 87.5% of the students know about suicide which is a good sign. Knowing about suicide and its consequences reduces the possibility of its occurrences among students and more and more knowledge and sensitization should be given regarding this topic and its relations to student's academic stress.

From the table 11, we can see that 7 students from government schools agrees to taking alcohol, cigarettes, gutka in the influence of peer groups, 9 of them disagrees to it and 4 of them have mixed feeling. 12 students from private feels they are forced to take alcohol, cigarettes, gutka by their peer groups, 7 students disagree to it and 1 of them has mixed feelings. The Overall data depicts that 45% of the total respondents feel they are forced by their peer groups and 30% of them disagrees it. 60% of the private school students take alcohol, cigarettes, guthka, etc in the influence of their peers. Some of them said that they use it to lessen stress, some said that they want to do what adults do. The parents should keep a track on what their children are doing and improve their lifestyles.

CONCLUSION

The study in general, points out that majority of the children studied have stress or pressure of one thing or another that leads to mild or moderate level of stress in them. The study results emphasizes the fact that contradictory to the common belief that only adults suffer from stress and stress related problems, children from a very young age itself suffer from tension and stress of different types at varying levels. Stress is normal part of life that can either help us learn and grow or can cause us significant problems but severe stress releases powerful neuro-chemicals and



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hormones that prepare us for action (to fight or flee). If we don't take action, the stress response can lead to health problems as we have seen in the study. Stress that is prolonged, uninterrupted, unforeseen, and uncontrollable is the most harmful kind.

The study concentrated mainly in understanding the prevalence of stress in high school students studying in private and government schools and the results point out that majority of the private school students are suffering from moderate to high levels of stress that can lead to many problems in present and future. Here further research on the causes leading to this state in children needs immediate attention. Also the measures to overcome this condition needs further probe. Since most students suffer from stress, it is important to understand the factor that is giving the pressure and tension to children. As the stressors vary with age, culture and society the causes have to be identified first. Our increasing knowledge about the importance and impact of stress on young adolescence should be put to good use in reducing stress factors for them and in assisting them to increase coping strategies and healthy responses to manage the unavoidable stresses in their lives.

Every child's mental health is important, many children have mental health problems, and these problems are real and painful and can be severe. The more we understand the challenges of the young people we serve, the more effective and life changing our services become. Awareness- raising is needed for parents, teachers and professionals and the authority (Government) to take joint action to relieve the suffering caused by stress in many of these respondents. It is proved that stable family and happy school, where there is love, care and concern with less mental pressure is of paramount importance in the alleviation of child stress and that should be the goal of parents and teachers so as to help them to face the challenges of life confidently and positively. However, the findings point to the need for an urgent, more detailed research on large sample for a better understanding of childhood stress and its causes. There is also a growing need of school counsellors, social workers and career advisors who can reduce stress in students by developing learning activities, extracurricular activities, group activities outside classroom, individual student planning and appraisal, family counseling, play therapy, consultation and referral services. Growth, promise, excitement, frustration, disappointment, and hope are all abundant during the high school years. Students are starting to learn what their futures hold at this time. Both public and private schools should work to improve the educational process, support students' academic careers, and foster their social and emotional growth.

Recommendations

- Parents should make every effort to be with their children whenever possible.
- Workloads from private schools should be reduced a notch.
- Syllabus should be cut short specially for students studying in CBSE, ICSE mediums in private schools.
- Parental pressure should be reduced.
- Teachers should support, motivate, encourage and guide the students to help achieve them their true potentials.
- Special classes, use of academic aids and jovial nature of teachers should be encouraged to teach subjects like Math, Physics, Chemistry, Accounts etc. so that students gain interest in studying them and hence uplift their stress.
- Teachers should not discriminate students on the basis of marks or performance. They should not be biased or prejudiced. They should treat all students as equal and unique.
- Knowledge on Values, morals, sex education and stress coping strategies must be given in both Government and Private schools. Teachers must inculcate these values and information in all the schools.
- Schools should ensure that their campus is Bully-free, Ragging-free, and harassment-free.
- Eating habits and nutrition levels of students should be monitored well by the parents and teachers at all times.
- Peer influence should be checked by parents
- Parents and teachers should educate their children about bad habits, addictions, and the effects of alcohol, drugs, smoking, etc.
- Suicide awareness and prevention should be a part of curriculum in every schools.





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- More importance should be given on students behavior outside classroom. They should be encouraged to indulge themselves in forms of music, arts and other cultural activities or games and sports for overall growth and psycho-social development.
- Use of Mobile phones, social media applications, television, etc should be limited to a certain extent.
- Yoga, meditation, exercises should become a part of the academic curriculum to make the students physically fit and mentally sound.
- Role of school counselors, social workers, psychologists or career advisors should be accommodated in every schools as their programs can help students to achieve optimal personal growth, acquire positive social skills and values, set informed career goals and realize their full academic potential to become productive, contributing members of the community overall.

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Bounds for Fractional Domination Number of Some Graphs and their Dual Graphs

Mahesh Sarada^{1*}, Rekha Jain² and Ganesh Mundhe³

¹Research Scholar, Department of Mathematics, Medi-Caps University, Rau, Indore-453331, Madhya Pradesh, India and Assistant Professor, Pimpri Chinchwad College of Engineering and Research, Ravet, Pune - 412101, Maharashtra, India.

²Head of Department of Mathematics, Medi-Caps University, Rau, Indore-453331, Madhya Pradesh, India

³Assistant Professor, Army Institute of Technology, Dighi, Pune, Maharashtra, India.

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*Address for Correspondence

Mahesh Sarada

Research Scholar,

Department of Mathematics,

Medi-Caps University, Rau, Indore-453331,

Madhya Pradesh, India and

Assistant Professor,

Pimpri Chinchwad College of Engineering and Research,

Ravet, Pune - 412101, Maharashtra, India.

E.Mail: mahesh.sarada@gmail.com



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ABSTRACT

The graph with notation $\gamma_f(G)$ and $\gamma_f(G')$ be the fractional domination number of graph G and their dual graph G' . In the given study, we have presented the bounds for fractional domination number of some graphs and their dual graphs. The specific graphs with Cycle graph, Wheel graph, Complete graph, Star graph, Bi-Star graph, Sunlet graph, and Cartesian product of graphs. We have presented some results on the union and join of $\gamma_f(G)$ and $\gamma_f(G')$ so that programmes and algorithms can leverage the specified relation.

Keywords: Dominating Set, Fractional Domination Function, Fractional Domination Number, Dual graph.

2010 Mathematics Subject Classification: 05C69.





INTRODUCTION

Graph domination sets have been studied mathematically since around 1960, the challenge of calculating the absolute minimal number of queens necessary to cover or control a chessboard was studied by De Jaenisch in 1862. Figuring out the bare minimum of queens that can be used for a chessboard to ensure that every square is either under attack or covered by a queen. The study of graphs, which are mathematical representations of the pair wise relationships between objects, is known as graph theory. In a graph theory there will be a number of vertices and edges. Graph theory is a broad field with many practical applications, which gives academics more ideas for how to solve problems in practical settings. The concept graph domination was first made public by Ore in the 1960, and since then, it has become an important area of study in graph theory. The study of fractional domination was introduced by Johnson, Robertson, Seymour and Thomas in the late 1980 as a generalization of the classical notion of domination. Further fractional domination is studied by M. Farber [5] though it was first studied by Hedetniemi, Cockayne, Fricke, Mynhardt [6]. The survey paper of S.T. Hedetniemi, E.J. Cockayne has communicated lot of interest in study of domination in graphs [7]. It was also investigated by Teresa, Haynes, Hedetniemi and P. Slater, E. R. Scheinerman, D. H. Ullman [8]. Since then, number of Graph theorists are increasing like G. Domke, Hedetniemi, Laskar, A. Majumdar, Fricke, S. Arumugam, K. Sampathkumar and many more. So, In the given study we found the bounds for fractional domination number of some graphs and their dual graphs. Here, we have taken a few specific graphs and used them to illustrate various results to support our findings.

In the second section we are going to give basic definitions of dominating set, fractional domination function, fractional domination number, and dual graph which are useful in further sections. Third section contains bounds for domination and fractional domination number of various graphs. Fourth section contains applications which are given in terms of union and join.

BASIC DEFINITIONS

The basic definitions of *Dominating Set*, *Fractional domination function*, *Fractional domination number*, and *Dual graph* is given in [1, 13] as follows:

Definition 2.1 Dominating set

The graph $G = (V, E)$ is consisting V as vertices and E be the edges. A set $D \subseteq V$ is said to be dominating set of G if every vertex in $V \setminus D$ is adjacent to one or more than one vertex in D . A dominating set D is called a minimal dominating set if no proper subset of D is a dominating set of G . The domination number of G is the minimum cardinality taken over all minimal dominating sets in G and it is denoted by $\gamma(G)$. The upper domination number of G is the maximum cardinality taken over all minimal dominating sets in G and it is denoted by $\Gamma(G)$.

Definition 2.2 Fractional Domination functions

let the function $f: V(G) \rightarrow [0, 1]$ is called dominating function of graph G which assigns values to each and every vertex $v \in V(G)$ in the unit interval $[0, 1]$. The function f is known as fractional domination function if each vertex $v \in V(G)$, $f(N[v]) = \sum_{u \in N[v]} f(u) \geq 1$ for all $v \in V(G)$. The sum of values $f(u)$ considering each vertex. $u \in N[v]$ is at least 1. A fractional dominating function f of graph G is minimal if for all functions $g: V(G) \rightarrow [0, 1]$ such that $g \leq f$ and $g(v) \neq f(v)$ for at least one $v \in V(G)$, g is not a dominating function of G .

Definition 2.3 Fractional Domination Number

The minimum cardinality of minimal fractional domination function f on graph G is known as fractional domination number of G and it is denoted by $\gamma_f(G)$. Whereas the maximum cardinality of minimal fractional dominating function f on graph G is known as upper fractional domination number of G and it is denoted by $\Gamma_f(G)$.

$\gamma_f(G) = \min \{ |f| : f \text{ is the minimal fractional domination function of given graph } G \}$, and

$\Gamma_f(G) = \max \{ |f| : f \text{ is the minimal fractional domination function of given graph } G \}$,

Where $|f| = f(V) = \sum_{v \in V} f(v)$.



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Some preliminary known results:

Theorem 2.4. [1] For any graph G we have $\left\lfloor \frac{n}{1+\Delta(G)} \right\rfloor \leq \gamma(G) \leq n - \Delta(G)$.

Theorem 2.5. [1] For any graph G we have $\frac{n}{1+\Delta(G)} \leq \gamma_f(G) \leq \frac{n}{1+\delta(G)}$,

Where $\Delta(G)$ is maximum degree of graph and $\delta(G)$ is minimum degree of graph.

It is known that every minimal dominating set induces a minimal dominating function, and every minimal dominating function is itself a minimal fractional dominating function.

This implies that $\gamma_f(G) \leq \gamma(G) \leq \Gamma(G) \leq \Gamma_f(G)$.

Definition 2.6. Dual Graph: A planer graph G is having its dual graph G' with vertex for each faces or regions of G . There is one edge for each pair of regions or faces in the dual graph G' that a border separates from one another by an edge and self-loop created when same face or region appears on both side of that edge. Therefore, every edge for the given graph G is having corresponding dual edge in its dual graph G' whose end points are dual vertices which corresponding for the region or face on other side of an edge. In the graph theory aspect, planer graph is considered to be embedded in plane. It also drawn on the plane such way that their edges intersect only at their common end points. Planer graph is graph such that no two edges cross each other. The initial instance of dual graph is to discovered with the pairing platonic solids into the dual polyhedral structures. Concept of rectangular dual is used in area planning for VLSI integrated circuits.

BOUNDS FOR FRACTIONAL DOMINATION NUMBER OF SOME GRAPHS AND THEIR DUAL GRAPHS

Definition 3.1 Cycle graph (C_n)

A simple graph with n vertices and $(n \geq 3)$ where edges counted as n is called a cycle graph. If each and every vertex in graph having degree two, then each edge forms a cycle of length n .

Theorem 3.1.1 If G is the cycle graph of order n and G' its dual graph, then

$$\gamma_f(G) + \gamma_f(G') = 1 + n/3 \text{ and } \gamma_f(G) * \gamma_f(G') = n/3.$$

Proof. For cycle graph (C_n) where $n = 3, 4, 5, \dots, n$ it is known that $\gamma_f(C_n) = n/3$. If we locate the cycle graph's dual in accordance with the definition, we have its dual graph as connected graph with two vertices only. Then each vertex is of degree at least 3. Cycles and cuts are dual in our three edge-connected graphs; hence the dual graph's fractional domination number is always 1. The lower bound for cycle graph is 1 and upper bound is $(n/3)$ while the lower and upper bound for its dual graph is always 1. For the sum we have $\gamma_f(G) + \gamma_f(G') = 1 + n/3$ and $\gamma_f(G) * \gamma_f(G') = n/3$. See the FIGURE-1 (Blue graph is planer graph and red graph is its dual graph).

Illustration-1: For cycle graph C_n , we have $\gamma_f(C_n) \geq \gamma_f(C_n')$ in graph C_4 , $\gamma_f(C_4) = 4/3$, and $\gamma_f(C_4') = 1$ in generalized way if G is the cycle graph of order n and G' its dual graph then $\gamma_f(G) + \gamma_f(G') = 1 + n/3$ and $\gamma_f(G) * \gamma_f(G') = n/3$.

Definition 3.2 Wheel Graph (W_n)

A cycle graph C_{n-1} , is used to create a wheel graph, in addition with a new vertex. This new vertex, known as a hub, is joined to every other vertex of C_{n-1}

Theorem 3.2.1 If G be the wheel graph of order $n \geq 4$ and G' its dual graph, then

$$\gamma_f(G) + \gamma_f(G') = 2 \text{ and } \gamma_f(G) * \gamma_f(G') = 1.$$

Proof. Self-dual graphs include wheel graphs by [12]. According to Euler's formula, every self-dual graph of n number of vertices has exactly $2n-2$ edges. A wheel graph consists of central vertex connected to $n-1$ vertices that form a cycle so for wheel graph (W_n) we have $\gamma_f(W_n) = 1$. The domination number of (W_n) is minimum vertices needed to dominate all other vertices in graph. This is because any set of $n-1$ vertices that includes the hub vertex will





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dominate all other vertices in graph. So $\gamma_f(G) + \gamma_f(G') = 2$ and $\gamma_f(G) * \gamma_f(G') = 1$. See the FIGURE-2 (Blue graph is planer graph and red graph is its dual graph)

Illustration-1 For wheel graph (W_4) we have $\gamma_f(W_4) = 1 = \gamma_f(W_4')$

Definition 3.3 Complete graph: In a graph if every pair of vertices is going to be adjacent, then the graph is complete. A simple undirected graph is referred to as a complete graph if every pair of different vertices is connected by a single edge. The Complete graph known as (K_n) is planer if and only if ($n < 5$).

Theorem 3.3.1 If G is the complete graph with vertices $n < 5$ and G' be its dual graph then

$$\gamma_f(G) + \gamma_f(G') = 2 \text{ and } \gamma_f(G) * \gamma_f(G') = 1.$$

Proof. If G is the complete graph of vertices ($n < 5$) we get $\gamma_f(G) = 1$ because one vertex is adjacent to all other vertices. For its dual graph we get $\gamma_f(G') = 1$. See the figure 3, complete graph K_4 we have $\gamma_f(K_4) = 1$ and $\gamma_f(K_4') = 1$ because in its dual graph we have connected four vertices graph where one vertex is adjacent to all other vertices. It is known that the graphs K_n are non planer for $n \geq 5$. Complete graph with all 4 vertices known as K_4 is planer graph and this graph is dual to 3-edge connected graphs. Therefore if G is the complete graph with ($n < 5$) vertices and G' be its dual graph then $\gamma_f(G) + \gamma_f(G') = 2$ and $\gamma_f(G) * \gamma_f(G') = 1$. See the FIGURE-3. (Blue graph is planer graph K_4 and red graph is its dual graph).

Definition 3.4 Star graph (S_k): Star graph is the complete bipartite graph $K_{1,k}$ a tree with one internal node and k leaves.

Theorem 3.4.1 If G is the star graph of order n and G' its dual graph, then

$$\gamma_f(G) + \gamma_f(G') = 2 \text{ and } \gamma_f(G) * \gamma_f(G') = 1.$$

Proof. A single vertex in star graph belonging to one set, while every other vertex in the graph belonging to the other set, making it a complete bipartite graph known as star graph. So $\gamma_f(G)$ for star graph is always one and its dual graph is only one vertex graph with self-loops equal to number of pendent vertices so $\gamma_f(G')$ is also one. Here if G is the star graph of order n and (G') its dual graph then, $\gamma_f(G) + \gamma_f(G') = 2$ and $\gamma_f(G) * \gamma_f(G') = 1$. See the FIGURE-4.

Illustration 1: For star graph $K_{1,3}$ we have $\gamma_f(K_{1,3}) = 1 = \gamma_f(K_{1,3}')$

Definition 3.5 Bi-Star graph: The apex or center vertices of two copies of the star graph used to create the Bi-Star graph.

Theorem 3.5.1 If G be the Bi-Star graph of order n and G' its dual graph, then

$$\gamma_f(G) + \gamma_f(G') = 3 \text{ and } \gamma_f(G) * \gamma_f(G') = 2.$$

Proof. Bi-Star graph is two copies of Star graph obtained by joining the apex vertices. The vertex set of $B_{n,n}$ is $V(B_{n,n}) = \{u, v, u_i, v_i \mid 1 \leq i \leq n\}$, such that u, v are apex or centre vertices and u_i, v_i are pendent vertices. The edge set of $B_{n,n}$ is $E(B_{n,n}) = \{uv, uui, vvi \mid 1 \leq i \leq n\}$. So, $|V(B_{n,n})| = 2n+2$ and $|E(B_{n,n})| = 2n+1$. And for Bi-Star graph and its dual graph we have $\gamma_f(G) = 2$ and $\gamma_f(G') = 1$. The dual graph is only one vertex graph with self-loops equal to number of pendent vertices are connected so $\gamma_f(G') = 1$. Here if G be the Bi-Star graph of order n and G' its dual graph, then $\gamma_f(G) + \gamma_f(G') = 3$ and $\gamma_f(G) * \gamma_f(G') = 2$. See the FIGURE-5.

Definition 3.6 Sunlet graph: Sunlet graph is the graph with $2n$ vertices obtained by attaching n pendent edges to a cycle graph C_n .

Theorem 3.6.1 If G be the n -Sunlet graph and G' its dual graph, then

$$\gamma_f(G) + \gamma_f(G') = (n + 1) \text{ and } \gamma_f(G) * \gamma_f(G') = n.$$





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Proof. Sunlet graphs are a simple unit distance graph that also graceful. Unit distance graph have graph dimension of 2 or less in which vertices are distinct point and all edges are of length one. A graph on $2n$ vertices obtained by attaching n pendent edges to a cycle graph C_n with the use of [3] we have taken Sunlet graph with 8 vertices obtained by attaching 4 pendent edges to a cycle C_4 then its fractional domination number is 4. See the Figure-6. Now for $n = 3, 4, 5, \dots, n$ we observe that there will be n pendent edges for its generalized graph of $2n$ vertices so Sunlet graph has $\gamma_f(G) = n$ for $n = 3, 4, 5, \dots, n$. And its dual graphs contain only two vertices with self-loops equal to number of pendent vertices and parallel edges so $\gamma_f(G')$ is always one. Therefore if G is n -Sunlet graph and G' its dual graph, then $\gamma_f(G) + \gamma_f(G') = (n + 1)$ and $\gamma_f(G) * \gamma_f(G') = n$.

Definition 3.7 Cartesian product of graph $(K_2 \times P_n)$: Complete graph K_2 and path graph P_n . The Cartesian product denoted by $(K_2 \times P_n)$. For the Complete graph with two vertices K_2 vertex set is $V(K_2) = \{k_1, k_2\}$ and the vertex set of path graph P_n is $\{p_1, p_2, \dots, p_n\}$ respectively. The vertex set for Cartesian product of $K_2 \times P_n$ is $V(K_2 \times P_n) = V(K_2) \times V(P_n)$ and $V(K_2) \times V(P_n) = \{(k_i, p_j) \mid k_i \in V(K_2), p_j \in V(P_n)\}$ and 'e' be the edge for cartesian product $(K_2 \times P_n)$ iff $e = \langle (k_i, p_j), (k_r, p_s) \rangle$ where the condition holds as following,

- 1) $i=r$ and $p_j, p_s \in$ Edge set of (P_n)
- 2) $j=s$ and $p_j, p_s \in$ Edge set of (K_2)

We have the known result involving fractional domination of graph products. Clearly $\gamma_f(G + H) \leq \min \{\gamma_f(G), \gamma_f(H)\}$ Laskar, Mujumdar Domke and Fricke found the result as for the join of graphs G and H , as $(G+H)$, the upper fractional domination number $\Gamma_f(G + H) = \max\{\Gamma_f(G), \Gamma_f(H)\}$. The Vizing's conjectured that the domination number of the Cartesian product of graph satisfies $\gamma(G \times H) \geq \gamma(G) \gamma(H)$. Further they also proved the corollary as for the Cartesian product $(G \times H)$, $\gamma_f(G)\gamma_f(H) \leq \gamma_f(G \times H)$.

We need the following theorem on $\gamma_f(K_2 \times P_n)$,

Theorem 3.7.1 [14] If G be the graph of Cartesian product $(K_2 \times P_n)$ with $n > 1$ then

$$\gamma_f(K_2 \times P_n) = \begin{cases} \frac{n+1}{2}, & \text{if } n \text{ is odd} \\ \frac{(n^2+2n)}{2(n+1)}, & \text{if } n \text{ is even} \end{cases}$$

Proof. In the graph of Cartesian product $(K_2 \times P_n)$ for $n > 1$, we observe that K_2 and P_n both are distance one graph and Cartesian product of both unit distance graph is also a unit distance graph. According to [9] and Domke et al. Domination in graphs advanced topics. If $K_2 = G$ and $P_n = H$ then $G \times H$ has $|G| |H|$ nodes with $[(a, b), (c, d)]$ belongs to Edge set of $(G \times H)$. If either $a = c$, and $[b, d]$ belongs to edge set of (H) , or $b = d$ and $[a, c]$ belongs to edge set of (G) . Let $G_1 = K_2 \times P_2$ be the cartesian product of K_2 and P_2 we can see that there will be four vertices with degree two. With the properties of fractional domination, where sum of values $f(w)$ for each vertex $w \in N[v]$ is one or more. Where w is the weights for vertices of G_1 . $\gamma_f(K_2 \times P_2) = 4/3$. Let $G_2 = K_2 \times P_3$ be the cartesian product of K_2 and P_3 we can see that there will be four vertices with degree two and two vertices with degree three so $\gamma_f(K_2 \times P_3) = 2$ for each vertex $v \in V$ with the properties of fractional domination where sum of values $f(w)$ for each vertex $w \in N[v]$ is one or more. Where w is the weights for vertices of G_2 . Similarly, if $G_3 = K_2 \times P_4$ be the cartesian product of K_2 and P_4 then $\gamma_f(K_2 \times P_4) = 12/5$, as a result of this in general If G be the graph of Cartesian product $(K_2 \times P_n)$

with $n > 1$ then $\gamma_f(K_2 \times P_n) = \begin{cases} \frac{n+1}{2}, & \text{if } n \text{ is odd} \\ \frac{(n^2+2n)}{2(n+1)}, & \text{if } n \text{ is even} \end{cases}$

Theorem 3.7.2 If G be the graph of Cartesian product $(K_2 \times P_n)$ for $n > 1$ and G' be its dual graph then $\gamma_f(G) +$

$$\gamma_f(G') = \begin{cases} 1 + \frac{n+1}{2}, & \text{if } n \text{ is odd} \\ 1 + \frac{(n^2+2n)}{2(n+1)}, & \text{if } n \text{ is even} \end{cases}$$





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Proof. From the theorem 3.7.1 we have $\gamma_f(K_2 \times P_n) = (n + 1)/2$ if n is odd and $\gamma_f(K_2 \times P_n) = (n^2 + 2n)/2(n + 1)$ if n is even. Now it is easier to check that the dual graph of $(K_2 \times P_n)$ is a graph in which fractional domination number is always one. Let $G_1 = (K_2 \times P_2)$ be the cartesian product of K_2 and P_2 then from FIGURE-7 we have, $\gamma_f(K_2 \times P_2) = 4/3$. Let G_1' be its dual graph then $\gamma_f(G_1') = 1$.

Let $G_2 = K_2 \times P_3$ be the cartesian product of K_2 and P_3 then from FIGURE-8 we have, $\gamma_f(K_2 \times P_3) = 2$ as $n = 3$. Let G_2' be its dual graph then $\gamma_f(G_2') = 1$. Similarly, If $G_3 = K_2 \times P_4$ be the cartesian product of K_2 and P_4 then from earlier deduction we have, $\gamma_f(K_2 \times P_4) = 12/5$. Let G_3' be its dual graph then $\gamma_f(G_3') = 1$. With the observations for $G_2 = K_2 \times P_3$, its dual graph may be a fan graph with two blades by removing its parallel edges. By continuing this process, we get generalized result for Cartesian product $(K_2 \times P_n)$ as its dual graph is fan graph with $n-2$ blades after removing the parallel edges for $n > 2$. One can check that fractional domination for any fan graph F_n is one. So, we have the result if given graph G is the graph with Cartesian product $(K_2 \times P_n)$ for $n > 1$ and G' be its dual graph then

$$\gamma_f(G) + \gamma_f(G') = \begin{cases} 1 + \frac{n+1}{2}, & \text{if } n \text{ is odd} \\ 1 + \frac{(n^2+2n)}{2(n+1)}, & \text{if } n \text{ is even} \end{cases}$$

Theorem 3.7.3 If G is the graph of Cartesian product $(K_2 \times C_n)$ with $2n$ vertices $n > 2$ then,

$$\gamma_f(K_2 \times C_n) = 2n/4$$

Proof. If G be the Cartesian product of graph $(K_2 \times C_n)$, with $n = 3, 4, \dots, n$ then we have all the graphs are 3-regular graphs and by theorem that if G is the r -regular graph on n vertices then $\gamma_f(G) = n/(r + 1)$. So if G is the graph of Cartesian product $(K_2 \times C_n)$, with $2n$ vertices $n > 2$, then, $\gamma_f(K_2 \times C_n) = 2n/4$.

Theorem 3.7.4 If G be the graph of Cartesian product $(K_2 \times C_n)$ with $2n$ vertices $n > 2$ and (G') be its dual graph with $2n-m$ vertices where $m = 1, 2, \dots, m$ then

$$\gamma_f(G) + \gamma_f(G') = \begin{cases} \frac{2n}{4} + \frac{2n-m}{4+m}, & \text{if } n = 3 \text{ and } m = 1 \\ \frac{2n}{4} + \frac{(2n-2)}{(4+m-1)} & \text{if } n = 4,5,6 \dots \text{ and } m = 2,3,4 \dots \end{cases}$$

Proof. If G be the Cartesian product of graph $(K_2 \times C_3)$, with six vertices or 3-prism graph with its dual graph see the Figure-9 then $\gamma_f(G) = 6/4$. By definition as for each vertex $v \in V(G)$, $f(N[v]) \geq 1$. Whereas the sum of values $f(w)$ for each vertex $w \in N[v]$ is at least 1. Here $w = 1/4$ be the weights for all the vertices of $(K_2 \times C_3)$. In this graph we observed that $\gamma_f(G) = 6/4$ and in its dual graph we have 5 regions or five vertices where vertex $d4$ is adjacent to all other vertices so $\gamma_f(G') = 1$. Hence if G is the graph of Cartesian product $(K_2 \times C_3)$ with $2n = 6$ vertices and (G') be its dual graph with $2n-m = 5$ vertices where $m=1$ then, $\gamma_f(G) + \gamma_f(G') = \frac{2n}{4} + \frac{2n-m}{4+m} = 5/2$ for $n = 3$ and $m = 1$

Illustration 1: Here $\gamma(G) = 2$, $\gamma_f(G) = 6/4$ & $\gamma(G') = 1 = \gamma_f(G')$

Similarly, if G be the Cartesian product of graph $(K_2 \times C_4)$, with eight vertices or 4-prism graph then $\gamma_f(G) = 8/4$. By definition as for each vertex $v \in V(G)$, $f(N[v]) \geq 1$. Whereas the sum of values $f(w)$ for each vertex $w \in N[v]$ is at least 1. Here $w = 1/4$ be the weights for all the vertices of $(K_2 \times C_4)$. In this graph we observe that $\gamma_f(G) = 8/4$ and in its dual graph we have 6 regions or six vertices with weights $w = 1/5$ for all vertices so that $\gamma_f(G') = 6/5$. Hence if G be the graph of Cartesian product $(K_2 \times C_4)$, with $2n = 8$ vertices and (G') its dual graph with $2n - m = 6$ vertices where $m = 2$ then, $\gamma_f(G) + \gamma_f(G') = \frac{2n}{4} + \frac{(2n-2)}{(4+m-1)} = \frac{16}{5}$ for $n = 4$ and $m = 2$.

So in generalized form if G be the graph of Cartesian product $(K_2 \times C_n)$ with $2n$ vertices $n > 2$ and (G') be its dual graph with $2n-m$ vertices where $m = 1, 2, \dots, m$ then,





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$$\gamma_f(G) + \gamma_f(G') = \begin{cases} \frac{2n}{4} + \frac{2n-m}{4+m}, & \text{if } n = 3 \text{ and } m = 1 \\ \frac{2n}{4} + \frac{(2n-2)}{(4+m-1)} & \text{if } n = 4,5,6 \dots \text{ and } m = 2,3,4 \dots \end{cases}$$

APPLICATIONS

Definition 4.1 Union and Join: The Graphs G and H have disjoint set of vertices V_1 and V_2 and the edge sets X_1 and X_2 respectively. The union $(G \cup H)$ is the graph with components G and H such that $V = V_1 \cup V_2$ and $X = X_1 \cup X_2$. So it is known that $\gamma_f(G \cup H) = \gamma_f(G) + \gamma_f(H)$ and $\Gamma_f(G \cup H) = \Gamma_f(G) + \Gamma_f(H)$. Here we have taken $(C_3 \cup P_3)$ so immediately we have $\gamma_f(C_3 \cup P_3) = \gamma_f(C_3) + \gamma_f(P_3)$ and $\Gamma_f(C_3 \cup P_3) = \Gamma_f(C_3) + \Gamma_f(P_3)$ see the Figure 10(A). The Join $(G + H)$ consists of G and H with each vertex of G adjacent to every vertex of H . Here we have taken $(C_3 + P_3)$ see the Figure 10(B).

We need the following theorem based on $\gamma_f(G + H)$ and $\Gamma_f(G + H)$.

Theorem 4.2 [10]. For the graph G and H we have

$$\gamma_f(G + H) = \begin{cases} 1, & \text{if } \gamma_f(G) = 1 \text{ or } \gamma_f(H) = 1 \\ 2 - \frac{\gamma_f(G) + \gamma_f(H) - 2}{\gamma_f(G) * \gamma_f(H) - 1}, & \text{otherwise} \end{cases}$$

Theorem 4.3 [10] For the graph G and H we have using $1/\infty = 0$,

$$\Gamma_f(G + H) = 2 - \begin{cases} 1/\Gamma_f(H), & \text{if } \Gamma_f(G) = \infty \\ 1/\Gamma_f(G), & \text{if } \Gamma_f(H) = \infty \\ \frac{\Gamma_f(G) + \Gamma_f(H) - 2}{\Gamma_f(G) * \Gamma_f(H) - 1}, & \text{otherwise} \end{cases}$$

In Figure 10 for a 3-cycle and path on 3 vertices these are the (A) Union and (B) Join. Since $\gamma_f(C_3) = 1 = \Gamma_f(C_3)$ and $\gamma_f(P_3) = 1 = \Gamma_f(P_3)$, whereas if we take a 5-cycle and path on 4 vertices then as per the (A) Union and (B) Join we get $\gamma_f(C_5) = \frac{5}{3}$, $\Gamma_f(C_5) = \frac{5}{2}$ and $\gamma_f(P_4) = 2 = \Gamma_f(P_4)$. Therefore theorem on Union gives $\gamma_f(C_5 \cup P_4) = 5/3 + 2 = 11/3$ and $\Gamma_f(C_5 \cup P_4) = (5/2) + 2 = 9/2$. Theorem on Join gives $\gamma_f(C_5 + P_4) = 2 - \frac{(\frac{5}{3})+2-2}{(\frac{5}{3})*2-1} = \frac{9}{7}$ and $\Gamma_f(C_5 + P_4) = 2 - \frac{(5/2)+2-2}{(5/2)*2-1} = 11/8$

Theorem 4.4 For the graph G is cycle graph C_n and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 2 - \frac{(n/2)+1-2}{(n/2)*1-1} = 1$

Proof. If G is the cycle graph then by theorem 3.1.1 the lower bound for cycle graph is 1 and upper bound is $n / 3$ while the lower and upper bound for its dual is always 1. Therefore by theorem 4.2, we have $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$. Upper fractional domination number is the maximum cardinality of minimal fractional dominating function so for cycle graph we have $\Gamma_f(C_5) = n/2$ by theorem 4.3, we get $\Gamma_f(G + G') = 2 - \frac{(n/2)+1-2}{(n/2)*1-1} = 1$.

Theorem 4.5 For the graph G is complete graph K_n with $n < 5$ and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 1$.

Proof. By the proof of theorem 3.3.1, if G is the complete graph with $(n < 5)$ we get $\gamma_f(G) = 1$ because one vertex is adjacent to all other vertices and for its dual graph we get $\gamma_f(G') = 1$ so by theorem 4.2, we have $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$. According to [15] for upper fractional domination number of complete graph it is obvious that any minimal fractional dominating function on a complete graph K_n must have weight 1 and so $\Gamma_f(K_n) = 1$ By theorem 4.3 we get $\Gamma_f(G + G') = 1$.





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Theorem 4.6 For the graph G is wheel graph W_n and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 1$.

Proof. By theorem 3.2.1, if G is the wheel graph we get $\gamma_f(G) = 1$ because central one vertex is adjacent to all other vertices and wheel graphs are self dual so $\gamma_f(G') = 1$. Hence proved by theorem 4.2. For upper fractional domination number of wheel graph it is obvious that any minimal fractional dominating function on a wheel graph W_n , we must have weight 1 and so $\Gamma_f(W_n) = 1$, By theorem 4.3 we get $\Gamma_f(G + G') = 1$.

Theorem 4.7 For the graph G is star graph S_n and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 1$.

Proof. By the theorem 3.4.1, if G is the star graph we get $\gamma_f(G) = 1$ and its dual graph is only one vertex graph with self-loops equal to number of pendent vertices so $\gamma_f(G') = 1$. Hence proved by theorem 4.2. For upper fractional domination number of wheel graph it is obvious that any minimal fractional dominating function on a wheel graph S_n , we must have weight 1 and so $\Gamma_f(S_n) = 1$, By theorem 4.3 we get $\Gamma_f(G + G') = 1$.

Theorem 4.8 For the graph G is Bi-star graph $K_{1,n}$ and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 1$.

Proof. With theorem 3.5.1, for Bi-star graph and its dual graph we have $\gamma_f(G) = 2$ and $\gamma_f(G') = 1$ The dual graph is only one vertex graph with self-loops equal to number of pendent vertices are connected so $\gamma_f(G') = 1$. Hence proved by theorem 4.2. For upper fractional domination number of Bi-star graph we must have $\Gamma_f(K_{1,n}) = 2$ by theorem 4.3, we have $\Gamma_f(G + G') = 1$.

Theorem 4.9 For the graph G is n -Sunlet graph and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$ and $\Gamma_f(G + G') = 1$.

Proof. Theorem 3.6.1, for n -Sunlet graph we observe that there will be n pendent edges for its generalized graph of $2n$ vertices so $\gamma_f(G) = n$, for $n = 3, 4, 5, \dots, n$. And its dual graphs contain only two vertices with self-loops equal to number of pendent vertices and parallel edges so $\gamma_f(G')$ is always one. Hence proved by theorem 4.2. For upper fractional domination number of n -Sunlet graph G we have $\Gamma_f(G) = n$ because there will be n pendent edges. By theorem 4.3, we have we have $\Gamma_f(G + G') = 1$.

Theorem 4.10 If G be the graph of Cartesian product $(K_2 \times P_n)$ and G' its dual graph then, $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$.

Proof. By theorem 3.7.2, If G be the graph of Cartesian product $(K_2 \times P_n)$ and G' be its dual graph then, $\gamma_f(G) + \gamma_f(G') = 1 + \frac{n+1}{2}$, if n is odd and $\gamma_f(G) + \gamma_f(G') = 1 + \frac{(n^2+2n)}{2(n+1)}$, if n is even

By the theorem 4.2, we have $\gamma_f(G + G') = 1$, if $\gamma_f(G) = 1$ or $\gamma_f(G') = 1$.

Main Result of Paper

The relation between fractional domination number of the graph and its dual graph. We have studied the bounds for fractional domination number of some graphs and their dual graphs in terms of $\gamma_f(G) + \gamma_f(G')$ and $\gamma_f(G) * \gamma_f(G')$ the specific graphs with Cycle graph, Wheel graph, complete graph, Star graph, Bi-Star graph, Sunlet graph, and Cartesian product of graphs. We have presented some result on the union and join of $\gamma_f(G)$ and $\gamma_f(G')$.

CONCLUSION

Here, we may state that from a practical standpoint [11] like designing Electronic Circuits where two networks are dual to each other with current and voltage are interchangeable. A dual linear graph representation is important for





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space filling location problems of the floor plan. Graph duality used in Road and Railway maps. In the Computer vision, Computer Communication networks, and Electrical network graph duality is very important. The structure of mazes and drainage basins can be better understood by this which provides minimum domination number.

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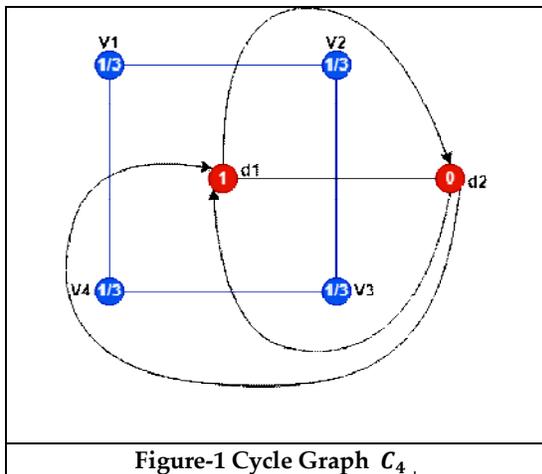


Figure-1 Cycle Graph C_4 ,

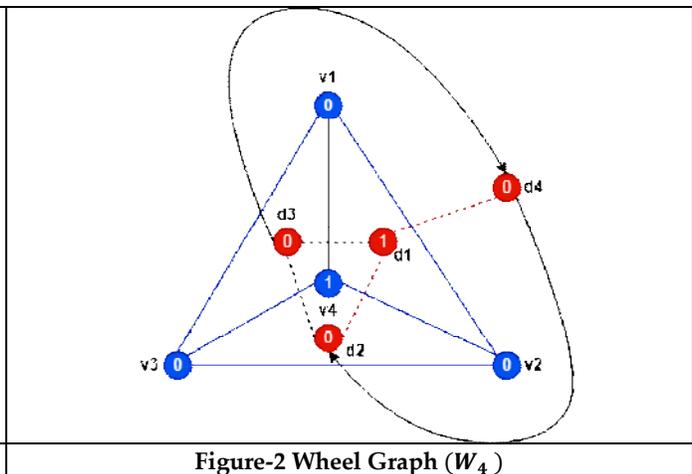
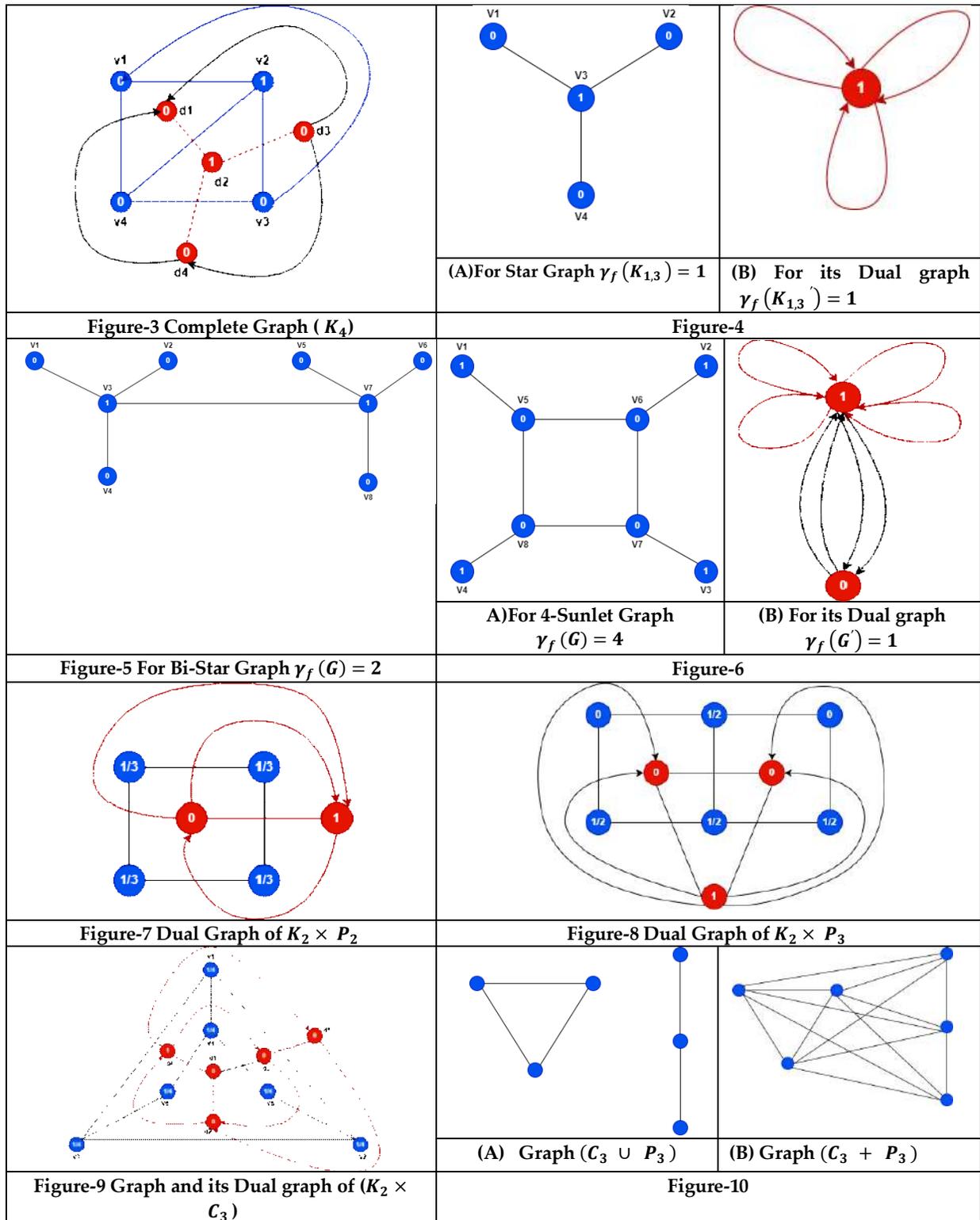


Figure-2 Wheel Graph (W_4)





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Cryptanalysis using extended ASCII Code with the Aid of Laplace Transform of Error Function

Pranjali Kekre^{1*}, Keerti Acharya¹, Rinku Verma¹ and Laxmi Rathour²

¹Assistant Professor, Department of Mathematics, Medi-Caps University, Pigdamber, Rau-453331, Madhya Pradesh, India.

²Department of Mathematics, National Institute of Technology, Chaltlang, Aizawal, Mizoram, India.

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*Address for Correspondence

Pranjali Kekre

Assistant Professor,

Department of Mathematics,

Medi-Caps University, Pigdamber,

Rau-453331, Madhya Pradesh, India.

E.Mail: pranjali.kekre@medicaps.ac.in



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ABSTRACT

In this paper to deliver data safely a new tool is introduced for encryption and decryption using extended ASCII code with the aid of Laplace transform of error function. Here we have used the concept of 2's complement technique to find symmetric key, cipher text and decryption process. Results are verified using Matlab software.

2020 Mathematical Science Classification: 11A07,44A10, 94A60, 11T71

Keywords and Phrases: Laplace transform, Network security, error function, cipher text, symmetric key, congruence relation, extended ASCII code.

INTRODUCTION

Secured data transfer is always a matter of discussion, several techniques are already developed and a number of them are still in pipeline. A new approach of cryptanalysis is introduced in this paper using extended ASCII code with the aid of Laplace transform on error function. Main motto of Cryptography is information security such as data integrity, entity authentication and data origin authentication. Cryptography is a set of techniques to provide information security. While designing a cryptography scheme, cryptographer always kept in mind that the designed algorithm is not very trivial to understand, replicate and therefore easily cracked. To secure the data from hackers it needs to be encrypted with high level of security. It helps to store sensitive information, transmit it across insecure networks like internet so that it can't be read by anyone except the intended receiver.





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Analysis of cryptographic security leads to using theoretical computer science especially complexity theory. The actual implementation of crypto systems and the hard work of carrying out security analysis for specific cryptosystems falls into engineering and practical computer science and computing. The persons or systems performing cryptanalysis in order to break a crypto system are called attackers. The process of such type of attacking is called hacking. Some cryptographic algorithms are very trivial to understand, replicate and therefore easily cracked. To secure the data from hackers it needs to be encrypted with high level of security. Encryption and Decryption are carried forward using mathematical algorithms in cryptography. Initially A. Stanoyevitch [13] Introduced cryptography with mathematical foundations and computer implementations. J.Overbey et al. [9] used technique of Hill cipher keyspace consist of all matrices that are invertible. Use of matrices for encryption and decryption were found by G.A. Dhanorkar and A.Hiwarekar[3]. After that [5], [7], [6]and [8] used Laplace transform techniques for the cryptographic purpose by combining infinite series of various functions . S. Dhingra et al. [4] presented new scheme for the cryptography by combining infinite series and Laplace transform using ASCII code. M. Tuncay Gençoglu [15] used a new method of cryptography using Lapalce transform of Hyperbolic function. Uma Pujeri, Ramachandra Pujeri [10] used Symmetric Encryption Algorithm using ASCII Values. S. Tayal et al.[14] reviewed paper on Network Security and Cryptography. For terminology and notation follow [2] and [12] Cryptography, Information Theory, and Error-Correction. M. Baykara et al. [1] implemented a Novel Symmetric Encryption Algorithm. A. Vijayan et al. found some interesting results on ASCII Value Based Encryption System. S. R. Shinge , R.Patil [11] developed an Encryption Algorithm Based on ASCII Value of Data. S.Verma et al. [16] developed an efficient new symmetric key cryptography algorithm for information security. A. Vijayan et al. [17] ASCII Value Based Encryption System,

In this paper, the process of encryption is expanded using series of error function and taking its Laplace transform. On the basis of literature survey we found while using the cryptography on the basis of Laplace transform only functions with positive terms were considered so far, but in error function we have an alternating series, so we used the concept of congruence relation to change the sign of transformed series terms coefficients , and use modular arithmetic to find symmetric key , cipher text and Decryption process.

PRELIMINARIES

Plain text: It signifies a message that can be understood by the sender, the recipient and also by anyone else who gets access to that message.

Cipher text: When a plain text message is codified using any suitable scheme, the resulting message is called as cipher text.

Encryption and Decryption: Encryption transforms a plain text message into cipher text, whereas decryption transforms a cipher text message back into plain text.

Symmetric and Asymmetric Key

Cryptography algorithms classified mainly into two major types: Symmetric-key cryptography and public key (Asymmetric) cryptography[10]. In Symmetric-key cryptography, each sender and receiver shared the same key used to encrypt and decrypt data with disadvantage of key management required to keep the key secure. The Data Encryption Standard (DES) and the Advanced Encryption Standard (AES) are examples of Symmetric-key cryptography methods. In public-key cryptography, each sender and receiver use two different keys to encrypt and decrypt data – public key and private key-, the public key can be freely distributed, while its paired private key must remain secret. In public-key cryptography, we overcome the key management distribution issue of Symmetric-key cryptography, but at the expense of performance speed

Laplace transform

Laplace transform is useful out of many transformations that are used for security purposed and as per the requirement which is a useful factor for changing key where algorithm plays an important role. That's why it will be





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difficult for an hacker to trace the key by any mode. For any function $f(t), t \geq 0$ Laplace transform $L\{f(t)\}$ is defined as

$$L\{f(t)\} = \int_0^{\infty} e^{-st} f(t) dt = f(s), \tag{2.4.1}$$

Where t is known as time domain parameter and s (may be real or complex) is known as frequency domain parameter.

Error Function

$$erf(\sqrt{t}) = \frac{2}{\sqrt{\pi}} \int_0^{\sqrt{t}} e^{-u^2} du = \frac{2}{\sqrt{\pi}} \sum_{i=0}^{\infty} (-1)^i \frac{t^{(2i+1)/2}}{i! (2i+1)} \tag{2.5.1}$$

ENCRYPTION ALGORITHM

The proposed algorithm uses the Laplace transform of error function to generate the cipher text and a sender key as symmetric encryption. In the beginning this secret key between sender and receiver is determined and shared on the basis quotient remainder theorem and congruence relation.

Step 1: Sender and receiver agree on secret key.

Step 2: Select the message to be sent and find the length of message, including space in between, let the length of the given message be " m ".

Step 3: Convert each plain text alphabet as a number corresponding to the extended ASCII Code assign these values to the coefficients i.e.

$$C_i = \begin{cases} \text{Ascii code} & i = 0, 1, 2, \dots, m-1 \\ 0 & i \geq m \end{cases}$$

Step 4: Now writing these numbers as a coefficients of $t^{2n} erf(\sqrt{t})$ consider $f(t) = C t^{2n} erf(\sqrt{t})$ where $n \in I^+$ set of positive integers, where C is array of C_i .

i.e.

$$\begin{aligned} f(t) &= \frac{2}{\sqrt{\pi}} \left\{ C_0 t^n - \frac{C_1 t^{3n}}{3} + \frac{C_2 t^{5n}}{2! 5} - \frac{C_3 t^{7n}}{3! 7} + \frac{C_4 t^{9n}}{4! 9} - \frac{C_5 t^{11n}}{5! 11} + \frac{C_6 t^{13n}}{6! 13} + \dots \right\} \\ &= \frac{2}{\sqrt{\pi}} \sum_{i=0}^{m-1} (-1)^i \frac{t^{(2i+1)n}}{i! (2i+1)} C_i \end{aligned} \tag{3.1}$$

Step 5: now taking Laplace transform of (3.1), we get





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$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} L\left\{C_0 t^n - \frac{C_1 t^{3n}}{3} + \frac{C_2 t^{5n}}{2! 5} - \frac{C_3 t^{7n}}{3! 7} + \frac{C_4 t^{9n}}{4! 9} - \frac{C_5 t^{11n}}{5! 11} + \frac{C_6 t^{13n}}{6! 13} + \dots\right\}$$

using $L\{t^n\} = \frac{\sqrt{n+1}}{s^{n+1}}, n+1 \geq 0$

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \left[\frac{C_0 \sqrt{n+1}}{s^{n+1}} - \frac{C_1 \sqrt{3n+1}}{3s^{3n+1}} + \frac{\sqrt{5n+1}C_2}{2! 5s^{5n+1}} - \frac{\sqrt{7n+1}C_3}{3! 5s^{7n+1}} + \frac{\sqrt{9n+1}C_4}{4! 9s^{9n+1}} - \dots \right]$$

As n is an integer, we get

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \left[\frac{C_0 n!}{s^{n+1}} - \frac{C_1 (3n)!}{3s^{3n+1}} + \frac{C_2 (5n)!}{2! 5s^{5n+1}} - \frac{C_3 (7n)!}{3! 5s^{7n+1}} + \frac{C_4 (9n)!}{4! 9s^{9n+1}} - \dots \right]$$

i.e.

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \sum_{i=0}^m (-1)^i \frac{((2i+1)n)!}{(2i+1)! s^{((2i+1)n+1)}} C_i$$

Step 5: Input n, simplify the terms and then convert the series terms into binary equivalent using 2's complement technique for alternating terms having negative sign. Convert into decimal again. Let the obtained series is

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \sum_{i=0}^m a_i \frac{1}{s^{((2i+1)n+1)}} C_i$$

Step 6: Put the values of coefficients C_i in extended ASCII form, simplify it and let the new coefficients are $D_i, i = 0, 1, 2, \dots, m-1$.

Step 7: Now apply quotient remainder theorem on $D_i, i = 0, 1, 2, \dots, m-1$ to obtain corresponding r_i and q_i using $r_i = D_i - 257q_i$ (257 is used being the total number of extended Ascii code)

These $r_i, i = 0, 1, 2, \dots, m-1$ gives the code for cipher text and $[q_i, n], i = 0, 1, 2, \dots, m-1$ gives secret symmetric key.

Input : Plain Text Message : "A KING" and $n = 1$

Length $m = 6$

Coefficients $C_0 = 65, C_1 = 32, C_2 = 75, C_3 = 73, C_4 = 78, C_5 = 71$

$$f(t) = \frac{2}{\sqrt{\pi}} \left\{ C_0 t - \frac{C_1 t^3}{3} + \frac{C_2 t^5}{2! 5} - \frac{C_3 t^7}{3! 7} + \frac{C_4 t^9}{4! 9} - \frac{C_5 t^{11}}{5! 11} \right\}$$

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \sum_{i=0}^{m-1} (-1)^i \frac{(2i)!}{i! s^{(2i+2)}} C_i$$

$$L\{f(t)\} = \frac{2}{\sqrt{\pi}} \left[\frac{C_0}{s^2} - \frac{2C_1}{s^4} + \frac{12C_2}{s^6} - \frac{120C_3}{s^8} + \frac{1680C_4}{s^{10}} - \frac{30240C_5}{s^{12}} \right]$$

Applying the steps of Algorithm we get





| I | a_i | C_i | D_i | r_i | q_i |
|-----|-------|-------|---------|-------|-------|
| 0 | 1 | 65 | 65 | 65 | 0 |
| 1 | 6 | 32 | 192 | 192 | 0 |
| 2 | 12 | 75 | 900 | 129 | 3 |
| 3 | 142 | 73 | 10366 | 86 | 40 |
| 4 | 1680 | 78 | 131040 | 227 | 509 |
| 5 | 37822 | 71 | 2685362 | 226 | 10448 |

Obtain Cipher Text “AÀ Vââ” as per the extended ascii value chart.

With Secret key as [0 0 3 40 509 10448 1]

DECRYPTION ALGORITHM

Steps involved in Decryption are as follows:

Step 1. Input the cipher text and key received $[q_i, n]$ received from the sender.

Step 2. Find the length (m) of the obtained cipher text and convert it into to a corresponding finite sequence of numbers using extended Ascii Code, named them as Cr_i .

Step 4

Calculate $a_i = \left((-1)^i \frac{((2i+1)n)!}{(2i+1) i!} \right) i = 0, 1, \dots, m-1$, convert values into binary equivalent using the technique of 2’s compliment to make negative values as positive and convert again into decimals.

Step 5

We get the decoded sequence as

$$S_i = \frac{(q_i * 257 + Cr_i)}{a_i} \text{ for } i = 0, 1, \dots, m-1.$$

Step 6. Generate the equivalent character corresponding to extended ASCII code obtained as S_i for $i = 0, 1, \dots, m-1$, to get the message text as array of S_i .

Input :

Cipher Text “AÀ Vââ”

Secret key

[0 0 3 40 509 10448 ,1]

Applying the steps of Algorithm we get

| i | r_i | q_i | a_i | S_i |
|-----|-------|-------|-------|-------|
| 0 | 65 | 0 | 1 | 65 |
| 1 | 192 | 0 | 6 | 32 |
| 2 | 129 | 3 | 12 | 75 |
| 3 | 86 | 40 | 142 | 73 |
| 4 | 227 | 509 | 1680 | 78 |
| 5 | 226 | 10448 | 37822 | 71 |





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Output : Plain text Message 'A KING'

Runtime summary for the algorithms

Profile Summary

Generated 06-Feb-2023 10:22:06 using cpu time.

| Function Name | Calls | Total Time | Self Time* | Total Time Plot (dark band = self time) |
|-------------------------|-------|------------|------------|--|
| gencode | 1 | 11.187 s | 11.007 s | |

Profile Summary

Generated 06-Feb-2023 10:31:05 using cpu time.

| Function Name | Calls | Total Time | Self Time* | Total Time Plot (dark band = self time) |
|-------------------------|-------|------------|------------|--|
| decoded | 1 | 61.237 s | 61.077 s | |

Self time is the time in seconds that is spent in a function excluding the time spent in its child functions. Self time also includes overhead resulting from the process of profiling.

CONCLUSION

In the proposed work we expand an innovative cryptographic scheme using Laplace transforms of error function and modular arithmetic functions used in coordination of binary digits with 2's complement techniques.

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Optical, Structural, Morphology and Biological Properties of Rhodamine-B Doped Magnesium Oxide Nanoparticles Prepared by Green Method

A.Suba^{1,4*}, P.Selvarajan^{2,4} and J. Jebaraj Devadasan^{3,4}

¹Research Scholar, Reg.No. 19132152132001, Department of Physics, Pope's College, Sawyerpuram, Thoothukudi-628251, Tamil Nadu, India.

²Associate Professor, Department of Physics, Aditanar College of Arts and Science, Tiruchendur, Thoothukudi-628216, Tamil Nadu, India

³PG and Research Department of Physics, Pope's College, Sawyerpuram, Thoothukudi-628251, Tamil Nadu, India.

⁴Manonmaniam Sundaranar University, Abishekapatti, Tirunelveli-627012, Tamil Nadu, India

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*Address for Correspondence

A.Suba

Research Scholar, Reg.No. 19132152132001,

Department of Physics, Pope's College,

Sawyerpuram, Thoothukudi-628251,

(Affiliated to Manonmaniam Sundaranar University,

Abishekapatti, Tirunelveli-627012)

Tamil Nadu, India.

E.Mail: suba.aldrin@gmail.com



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ABSTRACT

Dye-doped nanomaterial is an attractive sample in the researchers' area because it is widely applicable in electrical, optical and biomedical fields. In this work, a dye-doped magnesium oxide nanomaterial has been synthesised by green method with the help of betal leaf extract. Magnesium nitrate is considered as the precursor chemical and rhodamine-B dye is used as the dopant. By green synthesis and calcination method, dark-pink coloured rhodamine-B doped magnesium oxide (RBMO) nanomaterial has been prepared. The synthesised nanoparticles were characterized by powder XRD, UV visible spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), SEM – EDX. XRD patterns are well matched with the JCPDS file. The presence of magnesium, oxygen and dye constituents is investigated by using EDX spectrum. AFM and SEM studies are used to identify the shape and size of the RBMO nanoparticles.

Keywords: Rhodamine-B dye; powder XRD; SEM-EDX; FTIR; doping; AFM; optical spectrum; MgO





INTRODUCTION

Physics is the mother of natural sciences. In principle, physics can be used to explain everything that goes on at the nano scale. There is active physics research going on in nanomechanics, quantum computation, quantum teleportation, artificial atoms etc. At nanometer scale physics is different. Properties not seen on a macroscopic scale now become important - such as quantum mechanical and thermodynamic properties. Rather than working with bulk materials, one works with individual atoms and molecules and by learning about an individual molecule's properties, we can put them together in very well-defined ways to produce new materials with new and amazing characteristics. The technology of twenty first century requires the miniaturization of the electronic devices into nanometer scale while their ultimate performance is significantly improved. This miniaturization of the electronic devices raises many concerns regarding the advanced materials for realizing precise functionality and selectivity [1].

Green synthesis is a very promising scenario in the free competition to obtain nano structured materials in relation to other methods. When we see the expression "green synthesis," we know that it implicitly represents an economical and simple procedural method where expensive or sophisticated equipment is not considered. These aspects have allowed green synthesis to excel in emerging and growing methods for metallic nanostructures. Soon, a greater number of new extracts, fruits, bacteria, and other green components are expected to be used to obtain, stabilize, and synthesize metallic nanoparticles [2]. Synthesis of nanoparticles (NPs) through biological things is preferred over chemical and physical methods since the design process is simple, quick, cost-effective and eliminates the generation and use of hazardous substances [3, 4].

In this investigation, betel leaf extract is used in the green synthesis. The scientific name of betel leaf is *Piper betel* L. belongs to the family Piperaceae. This is the Black Pepper family [5]. From the literature survey, it is found that the researchers have used many biological agents except synthesizing of Rhodamine-B dye doped MgO nanoparticles. The rhodamine-B is a basic dye that belongs to xanthene which has been widely used in many fields owing to their high quantum yield, high absorption coefficient and excellent light stability [6, 7]. It has been used as dye due to the bright colour at the early stage. In the recent years, the use of rhodamine-B for fluorescent probes, especially in single-molecule imaging [8, 9] and aging in living cells [10-12] has also been reported.

Praveen kumar *et al.* have synthesised MgO NPs by using neem leaves and papaya leaves [13]. Khan *et al.* have prepared MgO NPs by using *Dalbergia sissoo* leaf extract [14]. Sharmila *et al.*, adopted the green synthesis of MgO NPs using *Pisonia alba* leaf extract [15]. Raveesha *et al.*, have prepared MgO NPs by using the *Withania somnifera* leaf extract through the low temperature green combustion method [16]. Suresh *et al.*, have used the sustainable green synthetic strategy to synthesize magnesium oxide nanoparticles by employing *Costus pictus* plant leaf extract as a reducing agent [17]. Mohana Srinivasan *et al.* have reported a low-cost, eco-friendly, and reproducible microbes *Lactobacillus* sp. mediated biosynthesis magnesium oxide nanoparticles [18]. Jhansi *et al.* have used the green, environmental friendly, and cost-effective technique for the preparation of MgO nanoparticles via white button mushroom aqueous extract [19]. Balakrishnan *et al.* have studied microstructure, optical and photocatalytic properties of MgO nanoparticles and this paper explains the photocatalytic degradation of methylene blue dye is evaluated using the as-prepared MgO nanoparticles under UV light [20]. Patil *et al.* have explained vanadium-doped magnesium oxide nanoparticles formation in presence of ionic liquids and their use in photocatalytic degradation of methylene blue [21]. Ikram *et al.* have prepared graphene oxide-doped MgO nanostructures for highly efficient dye degradation and bactericidal Action [22]. The aim of the work is to prepare the rhodamine-B dye doped MgO (RBMO) nanomaterial by green synthesis and to report the results obtained from the various studies used for characterization of the synthesized nanomaterial.





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MATERIALS AND METHODS

Magnesium nitrate and Rhodamine-B were purchased from Merck and Sigma Aldrich Company. Betel leaves were purchased from the local market in Tamilnadu. Fresh betel leaves were separately added to deionized water and this is heated at 80°C and stirred by using a hot-plate magnetic stirrer for 30 min. The extract was allowed to cool down to room temperature and it was filtered by using a Whatman filter paper to remove solid particulates. The extract was added to aqueous magnesium nitrate solution and 1 mol% of Rhodamine-B was added as the doping agent and stirred well for 3 hours at 30°C. Precipitate of dark pink colour was formed and it was filtered out. Calcination was performed with a muffle furnace at 400°C for 12 hours. The flow chart for synthesizing rhodamine-B doped magnesium oxide (RBMO) nanoparticles is shown in figure 1 and the prepared nanomaterial is presented in the figure 2.

RESULT AND DISCUSSION

Optical Characterization

The UV-visible transmission spectrum of Rhodamine-B dye doped magnesium oxide (RBMO) nanoparticles was recorded on a SHIMADZU UV-240 IPC spectrophotometer in the range of 200-900 nm and recorded the spectrum is shown in the figure 3. From the result, the lower cut-off wavelength for the sample is observed to be at 560 nm and the percentage of transmission is noticed to be about 80% in the visible region. The creation of nanosized MgO particles has been confirmed by the peak at 268 nm, which is seen to be very high lower cut-off wavelength in rhodamine-B doped MgO nanoparticles. The optical absorption coefficient (α) of RBMO nanoparticles was determined using the relation.

$$\alpha = [2.303 * \log_{10} (1/T)] / d \quad (1)$$

Where, T is the transmittance and d is the sample holder's path length. Figure 4 shows the wavelength versus absorption coefficient plot for RBMO nanoparticles. The absorption edge for the doped MgO nanoparticle is found to be at 278 nm, and it is observed that the linear absorption coefficient is low in the visible region. The sample's related electronic transitions cause absorption in the near ultraviolet range. Optical band gap (E_g) was evaluated from the spectrum and optical absorption coefficient (α) near the absorption edge using the relation proposed by Tauc.

$$(\alpha h\nu)^n = A(h\nu - E_g) \quad (2)$$

Where, A is a proportional constant, E_g is the optical band gap and h is the Planck's constant and ν is the frequency of incident photons [23]. Since MgO sample is a direct band gap material, the value of n equal to 2. Tauc's plot was drawn between $(\alpha h\nu)^2$ versus $h\nu$ as shown in figure 5. The obtained value of optical band gap for the doped MgO nanoparticles is 3.3 eV and this is observed to be low compared to the band gap of MgO nanoparticles. It is mentioned here that band gap of MgO nanoparticle is 4.94 eV [24]. The lower band gap energy is certainly attributed to nano regime of RBMO nanomaterial and it is due to the presence of 4-coordinated surface anions at the edges in the MgO nanoparticles whereas the bulk material comprises 6-coordinated surface anions.

The extinction coefficient of the sample was calculated using the relation

$$K = \alpha\lambda/4\pi \quad (3)$$

Where, α is the linear absorption coefficient and λ is the wavelength of the light. The plot of photon energy dependence of extinction coefficient for sample is shown in the figure 6. The result indicates that the extinction coefficient is low in the visible region. Since the extinction coefficient of the sample is low of the order of 10^{-5} , the MgO nanoparticles can be used in optical applications. It is noticed that the extinction coefficient of the sample is





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high at the fundamental absorption in the UV region [25, 26]. The values of linear refractive index (n) can be computed by using the following relation [27].

$$n = \left(\frac{1}{T} + \left(\frac{1}{T} - 1 \right) \right) \quad (4)$$

Where, T is the transmittance. The plot of refractive index against the wavelength is displayed in the figure 7 and it is seen that as wavelength increases, the refractive index is decreases near the cut-off wavelength region. Utilizing the relationship shown below, the optical conductivity of RBMO nanoparticles in SI units was calculated [28].

$$\sigma_{op} = \epsilon_0 c n \alpha \quad (5)$$

Where, C is the velocity of light in free space, α is the linear absorption coefficient, ϵ_0 is the permittivity of free space or vacuum and n is the refractive index. Since optical conductivity is a function of refractive index, the plots of optical conductivity versus refractive index for RBMO nanoparticle is presented in the figure 8. The results reveal that the values of optical conductivity linearly increase with increase of refractive index.

FT-IR Analysis

FTIR stands for Fourier Transform Infrared Spectroscopy. FTIR Analysis measures the infrared region of the electromagnetic radiation spectrum, which has a longer wavelength and a lower frequency than visible light. This spectrum is measurable in a sample when submitted to infrared radiation (IR). The basic theory at work is that the bonds between different elements absorb light at different frequencies. FTIR analysis measures the range of wavelengths in the infrared region that are absorbed by a material. This is accomplished through the application of infrared radiation (IR) to samples of a material. The sample's ability to absorb the infrared light's energy at various wavelengths is measured to determine the material's molecular composition and structure. FTIR Analysis can be used to identify unknown materials, additives within polymers, surface contamination on a material, and more. The results of the tests can pinpoint a sample's molecular composition and structure. Figure 9 displays the sample's recorded FTIR spectrum. The OH stretching modes correspond to the absorption maxima at 3696 and 3155 cm^{-1} . Since, water acted as our synthesis's solvent, the OH stretching can be observed in the FTIR spectrum. The peak at 2360 cm^{-1} is responsible to CH stretching mode. The absorption peak at 1590 cm^{-1} is due to CC Stretching. The absorption peak at 1400 cm^{-1} due to the metal-oxygen group was indicated. Other absorption peaks are due to presence of unwanted impurities. The infrared absorption peaks and their FTIR assignments are given in the table 1.

Powder X-Ray diffraction Analysis

X-ray diffraction (XRD) is useful for finding the crystal structure of the material. The XRD data is analyzed for the reflection angle to calculate the inter-atomic spacing or d spacing. The prepared sample of rhodamine-B dye doped MgO nanoparticles has been subjected to powder X-ray diffraction (PXRD) analysis to confirm the lattice constants of the sample and to confirm the reflection planes. The sample was scanned over the required range for 2θ values (10° – 80°). X-ray diffraction data gives the angle of scattering (2θ) and the corresponding intensities of diffracted beam for each reflection [29]. The recorded PXRD pattern of the sample is depicted in figure 10. The d -spacings corresponding to different peak positions were calculated using the Bragg's law, $2d \sin\theta = n \lambda$ where d is the interplanar spacing, θ is the Bragg's angle, n is the order of diffraction and λ is the wavelength of X-rays. The Bragg's diffraction peaks were indexed for the cubic crystal system. The broad peaks of the XRD pattern indicate that the prepared sample is nano sample. The diffraction peaks of sample are indexed as (111), (200), (220), (311) and (222), which is matched with JCPDS No. (No.04-005-4664). There are several contaminants that are detected during the preparation. Hence, the contaminated element is represented by additional peaks. The sample exhibits the reflection corresponding to the cubic MgO phase. The obtained value of lattice parameter for MgO nanoparticle is $a = 4.220 \text{ \AA}$. With the help of Debye Sherrer formula, the crystallite size of the sample is calculated. The value of crystallite size is 23nm.





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Scanning Electron Microscope with EDX

Scanning Electron Microscope is used to analysing the morphology of the sample. In this microscope, the secondary electron detector to detect the secondary electron in which the incident electron is incident on the sample. The sample is emitted the secondary electron. This electron is detected the secondary electron detector. This is useful to analysing the morphology of the sample. Energy Dispersive Spectrum detector is used to analyse phases of the sample. In this detector is used to provide the information of element of the sample. Figure 11 shows, the shape of the nanoparticles are spherical structure. Here the dye is doping agent; extra contaminants are present in the sample. Figure 12 shows the EDX spectrum of the sample. In this analysis, magnesium, oxygen and chlorine are present in the sample. Because of the rhodamine-B dye contains chlorine. Thus in EDX spectrum chlorine also presents.

Antibacterial Activity

The antibacterial effects of green synthesized rhodamine-B doped magnesium oxide nanoparticles are evaluated against four bacterial strains, of which two were gram-positive (*S.aureus*, *Streptococcus*) and two were gram-negative (*P.aeruginosa*, *Klebsiella*). The bacterial cultures were treated with 20 μ l doses of RBMO nanoparticles dissolved in water. The zones of inhibition of RBMO material against all bacteria are shown in figure 13. The values of zone of inhibition (ZOI) are listed in Table 3. The results demonstrate that, in the presence of RBMO nanoparticles, the growth is significantly inhibited against some of the tested species. The maximum response against three tested bacterias (*streptococcus*, *P.aeruginosa*, *Klebsiella*) is noticed. Thus, RBMO sample has highest antibacterial activity against the bacterial specimens like *streptococcus*, *P.aeruginosa* and *Klebsiella*.

Atomic Force Microscopic Analysis (AFM)

By measuring forces at extremely close ranges between a sharp probe (less than 10 nm) and the surface, the atomic force microscope (AFM) creates a 3D profile of the surface on a nanoscale (0.2-10 nm probe-sample separation). The cantilever that holds up the probe is flexible. When the AFM tip lightly contacts a surface, it measures the minimal force that is applied [30]. The morphology of the material is examined in this work using a non-contact method. The shift in resonance frequency brought on by the interaction force between the tip and sample is detected in the non-contact mode (dynamic mode), which is then utilised to map the surface topography. If the driving frequency is maintained constant, the cantilever's shift in resonance frequency results in the change in amplitude. AFM analysis is used to determine the surface shape and roughness of the synthetic rhodamine-B doped magnesium oxide (RBMO) nanoparticles made from betel leaf extract. Figure 14 shows the AFM images of synthesized RBMO nanoparticle using plant extract with a scanning area to 0 m to 6.25 μ m, and the synthesized nanoparticles have sticky like structure.

CONCLUSION

The rhodamine-B is an interesting dye which has high quantum yield, high absorption coefficient and excellent light stability. Due to this reason, this dye is acting as a doping agent for synthesis of MgO nanomaterial. Rhodamine-B doped magnesium oxide (RBMO) nanoparticles are widely used in the bio-medical and agricultural fields. From the XRD and AFM analysis of the sample, the crystallite size of the nanoparticle is observed to be less than 100 nm. FTIR spectrum of the sample has been analyzed. UV visible spectrum of RBMO nanoparticle is recorded and linear optical parameters have been determined. Scanning electron microscope analysis confirmed the spherical structure of the nanoparticle. The Energy Dispersive X-ray spectrum of the sample has been analysed and the presence of elements like magnesium, oxygen and chlorine is confirmed. The antibacterial activity of RBMO nanoparticle is analysed. In the presence of RBMO nanoparticles, the growth is significantly inhibited against the tested bacterial species. The shape of the nanoparticle is sticky liked structure is obtained by Atomic Force Microscopic analysis.





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Table 1. FT-IR assignments for RBMO nanomaterial

| Absorption peak number | Wave number (cm ⁻¹) | FTIR assignments |
|------------------------|---------------------------------|--------------------------|
| 1 | 3696.97 | OH asymmetric stretching |
| 2 | 3155.62 | OH symmetric stretching |
| 3 | 2360.72 | CH stretching |
| 4 | 1590.31 | CC stretching |
| 5 | 1400.61 | Metal Oxygen Bond |
| 6 | 1180.93 | CO Stretching |
| 9 | 1125.50 | CO stretching |
| 10 | 686.03 | CC Bending |

Table 2. Powder XRD data for RBMO nanoparticles

| Two-theta (degrees) | d-spacing (Å) | Relative Intensity (%) | hkl |
|---------------------|---------------|------------------------|-----|
| 38.01 | 2.36 | 23.64 | 111 |
| 42.89 | 2.10 | 100 | 200 |
| 62.23 | 1.49 | 50.06 | 220 |
| 74.54 | 1.27 | 4.56 | 311 |
| 78.54 | 1.21 | 9.56 | 222 |

Table 3: Antibacterial activity data for RBMO nanoparticles

| Bacteria name | Zone of Inhibition (mm) | |
|---------------|-------------------------|----------------|
| | 20 µl | Standard value |
| S.aureus | 15 | 20 |
| Streptococcus | 20 | 15 |
| P.aeruginosa | 20 | 18 |
| Klebsiella | 20 | 20 |





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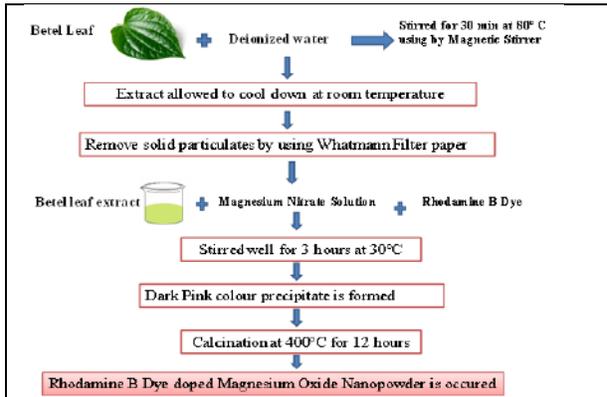


Figure 1. The flow chart for preparing Rhodamine B Dye doped MgO nanoparticles

Figure 2. Synthesized nanomaterial of rhodamine-B doped MgO (RBMO)

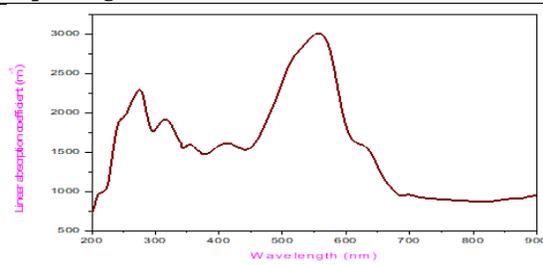
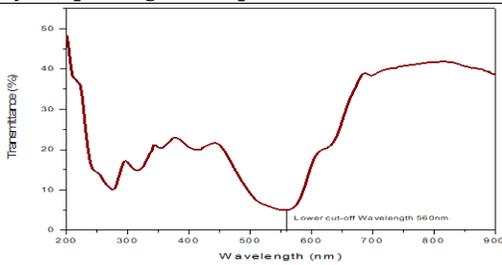


Figure 3. Transmittance spectrum of rhodamine-B doped MgO nanomaterial

Figure 4. Variation of absorption coefficient with wavelength for rhodamine-B MgO nanoparticle

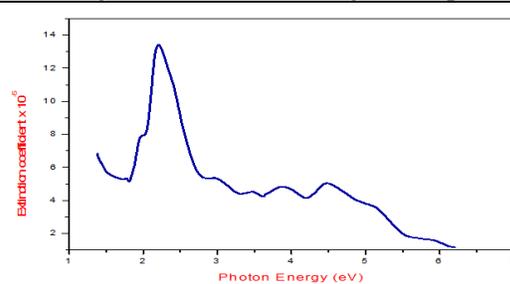
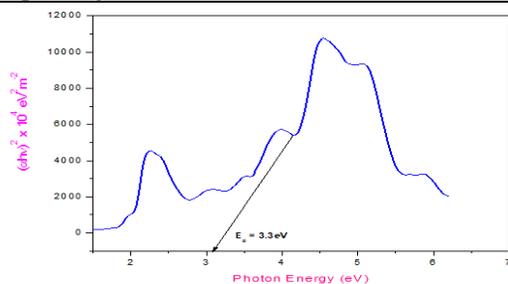


Figure 5. Tauc's plot for rhodamine_doped MgO (RBMO) nanoparticles

Figure 6. Variation of extinction coefficient with photon energy for RBMO nanoparticles

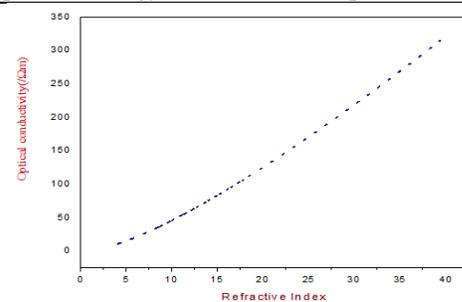
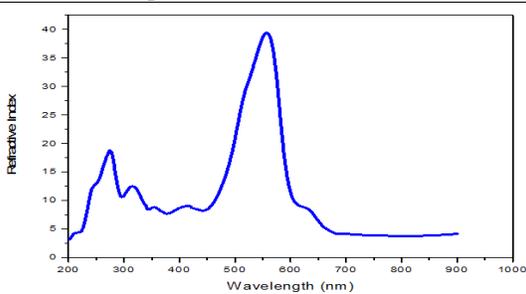


Figure 7. Variation of linear refractive index with wavelength for RBMO nanoparticles

Figure 8. Plot of optical conductivity (in SI units) versus refractive index of rhodamine-B doped MgO nanoparticles





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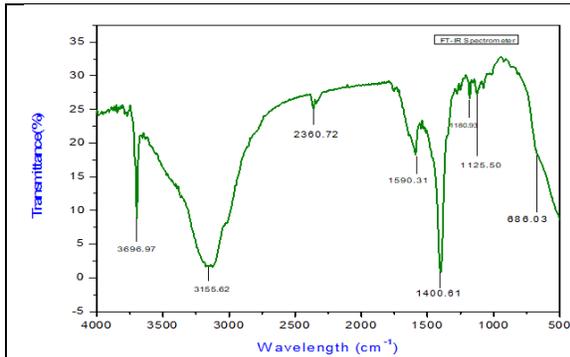


Figure 9. FTIR spectrum of RhB doped MgO nanomaterial

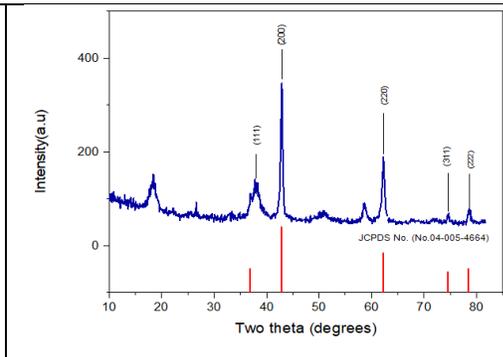


Figure 10. Powder XRD pattern of RBMO nanoparticles

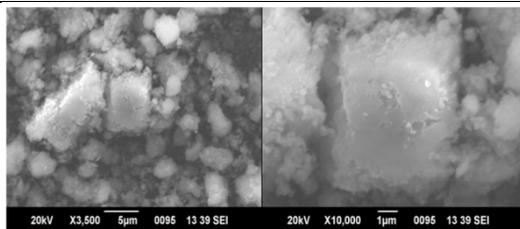


Figure 11. SEM Images of RBMO nanoparticles

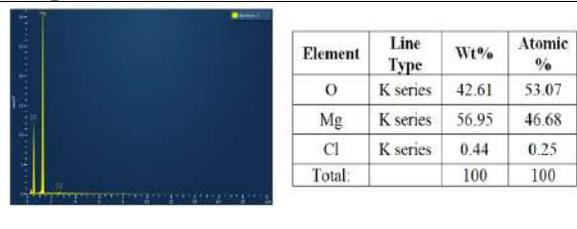


Figure 12. EDX spectrum of RBMO nanoparticles



Figure 13. The inhibition area diameter of RBMO nanoparticles

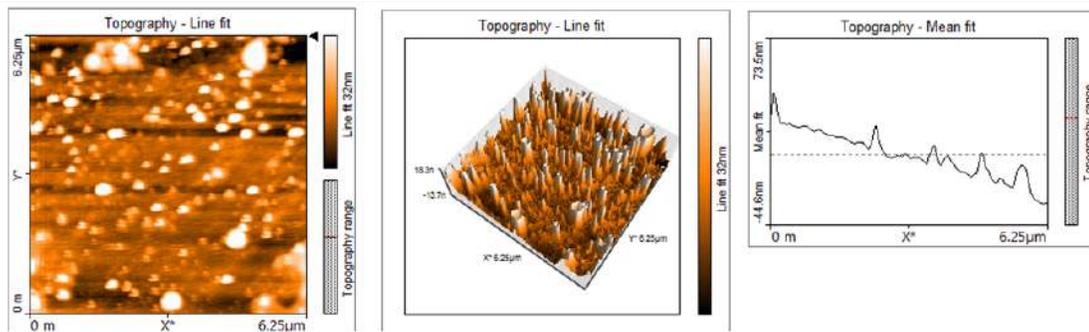


Figure 14. AFM picture of RBMO nanoparticles





Solid Waste Management; a Brief Study on Namphakey Village of Dibrugarh District, Assam

Abhinandan Konwar^{1*}, Parinita Devi Nath² and Mousumi Rai³

¹Research Scholar, Department of Education, Dibrugarh University, Dibrugarh, Assam, India.

²Assistant Professor, Department of Botany, Tingkhong College, Dibrugarh, Assam, India.

³Research Scholar, Department of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India.

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*Address for Correspondence

Abhinandan Konwar

Research Scholar,
Department of Education,
Dibrugarh University,
Dibrugarh, Assam, India.



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ABSTRACT

Increasing urbanization and industrialization recently results in generation of solid waste. Health risks and urban environment damage result from improper garbage management. With rising urbanisation, shifting lifestyles, and rising consumption, solid waste management already a monumental task in India will become even more challenging. The moment has come to consider a method that may be quickly put into use to permanently lessen the impact of the trash problem without harming the environment or biodiversity. The objective of the present study is to identify the different forms and sources of solid waste in Namphakey Village, Dibrugarh. The investigators select 30 households as the sample of the present study in order to collect data.

Keywords: Solid Waste, Solid Waste Management

INTRODUCTION

From the ancient period, waste is seen as an integral part of society. The growing population increases the volume of garbage. Urbanization and industrialization will result in the generation of solid waste [1]. The increasing solid waste is the topmost reason for health hazards and environmental disasters. In addition to ruining the aesthetics, improper disposal causes diseases to proliferate and hygienic conditions to worsen [2]. Solid waste generation is not a recent phenomenon. Municipal waste is the waste that results from human activity and includes things like waste from the home, waste from businesses, waste from sweeping the streets, waste from construction, etc. The phrase “Solid Waste” refers to waste (including food waste, paper, plastic, wood, metal, and rags), demolition products





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(including bricks, masonry, and pipes), sewage treatment byproducts, dead animals' manure, and other waste materials. An essential aspect of solid waste management is the prediction of municipal solid waste generation [3]. Solid wastes are generally composed of biodegradable and non biodegradable materials. Such wastes as these can and do disintegrate due to natural forces are known as biodegradable wastes. Food materials, kitchen wastes, and other natural wastes are some examples of biodegradable waste. The risks and problems that biodegradable wastes provide to the environment are minimal. On the other hand, non-biodegradable wastes are those that cannot be decomposed or dissolved by natural agents. They endure on earth for tens of thousands of years without degrading. The commonly used plastic materials are a notable example of non-biodegradable waste. This non-biodegradable waste cannot be broken down by natural organisms and acts as a source of pollution.

These solid wastes are controllable. The negative effects on the environment and human health can be minimized by solid waste management. The management of solid wastes involves a number of procedures, including monitoring, collection, transport, processing, recycling, and disposal. Through it, we can protect both our environment and ourselves from hazard.

REVIEW OF RELATED LITERATURE

Thakuria (2009) suggested that no scientific procedure is used to dispose of solid garbage and additionally there is lack of dustbins in municipality areas. He also stated that a hidden garbage delivery system could lead to road spills and air pollution[4]. Vij (2012) suggested that solving the issue of solid waste management is made very difficult by civic entities' poor social status and general public apathy toward solid waste management activity. The proper disposal and handling of garbage should be governed by strict laws. Prior to having a suitable location for waste disposal and treatment, no new plans for residential or commercial areas should be approved [5].

Dutta (2013) from her study suggested that regarding solid waste management, scientific technology should be developed and there is need to raise the public awareness. Dustbins or storage spaces should be simple to use and disposal must be legal and based on science [6]. Choudhury and Dutta (2017) from their work suggested that urban overpopulation and rapid urbanization have widened the rural-urban gap and exacerbated a range of urban problems. It is imperative that accountable government agencies, working closely with general people, take immediate action to address these problems so they don't get worse [7]. Neog (2020) revealed that most individuals simply dump wastes in the drains; very few people, however discard the solid wastes in trash cans. He also suggested that the municipality corporation must develop educational campaigns to inform the public about the minimizing, reusing, recycling the tangible waste products [8].

SIGNIFICANCE OF THE STUDY

India doesn't employ modern, eco-friendly methods for managing solid waste. Old methods are employed instead of cutting-edge scientific approaches, which results in the generation of several pollutants that have an adverse impact on the environment (Dutta 2013).Solid waste is a major concerning issue in our society today. Solid waste causes a number of different ailments. The study of solid waste management is crucial for preserving a healthy ecosystem. There are several solid wastes in our environment that are harming both the ecosystem's quality and living things.In order to learn more about the sources of solid waste, types of solid waste as well as awareness of the people towards solid waste, the researcher chooses the topic of "solid waste management" for the study. The investigators will be able to determine the population's attitudes regarding solid waste as well as their understanding of solid waste management during the course of the study.

OBJECTIVE OF THE STUDY

1. To study about the types and sources of solid waste of the locality.
2. To study about the methods and steps they are taken for disposing the solid waste.
3. To study about the awareness of people on solid waste management.



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METHODOLOGY

Method

The investigators used Descriptive Survey Study Method to conduct the present study.

Tools

In order to know about the various types and sources of solid waste in their area, the investigators prepared an interview schedule. The interview schedule also takes into account the methods and steps used for disposing of solid waste. To study about awareness of people regarding solid waste management, a questionnaire consisted of 10 items on the Awareness of Solid Waste Management prepared by the investigators based on literature review. Each correct answer receives 1 point, while a wrong answer gets 0 points.

Data Collection

To carry out the study the investigators purposively select the Namphakey Village as the area of the study. The Namphakey village is located at a distance of about 37 km away from the Dibrugarh district of Assam. There are 150 households. Investigators selected 30 household using incidental sampling technique to collect data. The primary data was collected from the locals when visiting the area. Secondary data about the place from magazines, books and internet helped to understand about the place and to have a better view on the people.

ANALYSIS OF DATA

The data after collection has to be processed and analyzed in accordance with the objectives of the present study. The primary data collected through the survey were processed by using statistical technique. With the computation of data, interpretation and analysis is been made.

Objective No. 1

The majority of families in the Namphakey village produce many kinds of solid waste including vegetable waste, plastic bags and materials, paper, iron, glass, bamboo, and wooden materials, among others. A variety of sources create these solid wastes. From the analysed data it has been seen that in all the household solid wastes are generated from kitchen and shopping waste. 56.66% of the respondent said that they have animal wastes like, cow dung and other animal's dung in their households.

Objective No. 2

Methods and steps are taken for disposing of solid waste. Most of the people are concerned about their solid waste. They compost, incinerate and dump the solid waste as required to dispose of the solid waste. They used to sell unused plastics and iron waste. From the analyzed data it has been clearly seen that 76.67% of households compost solid waste materials, and 73.33% of households incinerate solid waste materials. 86.67% of the respondents dump solid waste materials, whereas 66.67% of households use biodegradable waste for agricultural use. Few respondents (23.33%) sell reusable solid waste.

Objective No. 3

The mean score (7.33) of the total score was determined after analysing the collected data, which indicates 77.33% of the respondents are aware of the management of solid waste in their households.

MAJOR FINDINGS

From the analysed data and observation some major findings are formulated, these are as follows-

1. There are many different types of solid waste, including plastics, bottles, papers, bamboo, wood, some wasted vegetables, and various household wastes.
2. There are many different sources of solid waste, including kitchens, shops, animals, and other types of home items.



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3. Throughout data analysis, the researcher discovers about several methods for managing solid waste, including compost incineration and disposal. Additionally, they also sold iron and plastics.
4. While interacting with communities, it was discovered that most of them were conscious of the consequences of solid waste. They occasionally had minor issues like blocked drains and an unsanitary atmosphere brought on by solid waste.
5. While observing the village, it has been seen that the roadsides in the village are generally kept clean.
6. To handle the solid waste, there are various coordinating bodies. An NGO called TANGNI exists. The TANGNI group puts forth an effort to maintain the village tidy and hygienic.
7. They are all cognizant of the need to keep the village clean and appealing. As a result, Namphakey has received a Swachh Bharat Abhiyan award. In every nook and corner of the village, there are bamboo dustbins.
8. The villagers are conscious of their surroundings and their own community. They acknowledged some responsibilities to keep the environment better. They organized public meetings or general awareness campaigns.

SUGGESTIONS

The researcher came up with several suggestions that may assist to ensure a better environment in the area after analysing and discovering the data. They are as follows –

1. The biodegradable waste should be processed by composting, vermin composting, anaerobic digestion, or any other appropriate biological process.
2. Disposal should be done properly and scientifically. Before disposal, solid waste must be separated so that recyclable items may be transported for recycling. Some sort of incentive should be provided to encourage material recycling.
3. Only non-biodegradable and other wastes that are not suitable for recycling or biological processing shall be utilised for land filling. Before land filling, waste should be treated to make it safe for the soil and water in the environment.
4. Adequate storage facilities should be available in proportion to the amount of garbage generated.
5. To prevent residents from piling up waste or throwing it around, door-to-door pickup should be promoted as frequently as possible. For the transfer of waste to dump sites, appropriate trucks that were created specifically for the job should be assigned to ensure that no waste was spread out before arriving at the landfills.
6. For each form of collection, small pits should be dug behind each home separately. Biodegradable waste should be processed at the source level itself by being turned into manure.
7. Since people are ultimately the ones who produce waste, no waste management programme can be successful without their support. To educate and raise awareness among the populace about waste management, appropriate assistance from the area's capable residents, such as Gram Pradhan, RWAs, and NGOs, should be sought out.

CONCLUSION

Solid wastes are any items that have been thrown or left behind in the household, in industry, in agriculture, etc. These wastes are mostly produced by the many sources in our environment. The primary sources of solid waste are industry, residences, agriculture, and municipalities. The investigators saw a variety of solid waste types and sources throughout the study. Solid waste items cause deterioration of environmental and human health conditions. These solid wastes pollute the ecosystem by mixing with it. Therefore, solid waste management is greatly valued. The municipal authorities must arrange awareness campaigns through regular gatherings of representatives of neighborhood residents, welfare associations, and non-governmental organisations in order to ensure community engagement in garbage segregation. Laws alone won't be able to fix the issue. It requires the right mechanisms, the government's commitment, and a shift in public opinion. Both social control and social change are seen as possible outcomes of education. Education is important in preventing and resolving societal issues. At all governmental levels (international, regional, national, and local) environmental pollution is a severe issue. Man and the environment are





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currently thought to be interconnected and dependent on one another. Due to the negative impacts that the earth's dust, light, and sky's air have on people, nature and environs can be both a source of sadness and joy.

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Table 1: Methods and steps are taken for disposing of solid waste.

| METHOD AND STEPS FOR DISPOSING OF SOLID WASTE | N (NUMBER OF HOUSEHOLD) | % |
|---|----------------------------|--------|
| Composting of solid waste | 23 | 76.67% |
| Incinerate (burn) the solid waste | 22 | 73.33% |
| Dumping of solid waste | 26 | 86.67% |
| Used in agriculture | 20 | 66.67% |
| Sell reusable solid waste | 7 | 23.33% |

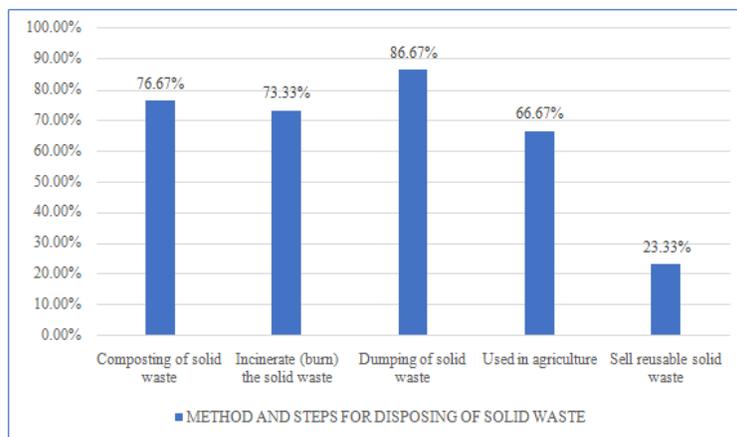


Figure 1: Graphical representation of methods and steps are taken for disposing of solid waste.





Comparative Analysis of Physico-Chemical, Bacteriological Parameters and Water Quality of Lakes in North Bangalore

Sonia Angeline M^{1*}, Priya Josson Akkara¹, Aasriitha², Nehaa R², Abhishek Sunil² and Avanthika Kurup²

¹Assistant Professor, Kristu Jayanti College Autonomous, Bangalore-560077, Karnataka, India.

²UG Student, Kristu Jayanti College Autonomous, Bangalore - 560077, Karnataka, India.

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*Address for Correspondence

Sonia Angeline M

Assistant Professor,

Kristu Jayanti College Autonomous,

Bangalore-560077, Karnataka, India.

E.Mail: sonia.m@kristujayanti.com



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ABSTRACT

Water quality can be defined as the chemical, physical and biological characteristics of water, usually in respect to its suitability for a designated use. As we all know, water has many uses, such as for recreation, drinking, fisheries, agriculture and industry. Each of these designated uses has different defined chemical, physical and biological standards necessary to support that use. We expect higher standards for water we drink and swim in comparison to that used in agriculture and industry. Water quality standards are put in place to ensure the efficient use of water for a designated purpose. Water quality analysis is to measure the required parameters of water, following standard methods, to check whether they are in accordance with the standard. Water quality analysis is required mainly for monitoring purpose. Some importance of such assessment includes to check whether the water quality is in compliance with the standards, and hence, suitable or not for the designated use. The study aims to analyse the quality of water from lakes in North Bangalore. The contaminants in the water may have an impact on the behaviour of organisms and pollute food sources. Analysis of the physiochemical properties of water from the lakes in North Bangalore will provide information on the quality of water. The purpose of the analysis is to be aware of the water quality and the anticipated use of the water.

Key words: Water, Quality, Lake Water, BOD, COD, MPN



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INTRODUCTION

After air, water is arguably the most valuable natural resource. Although the majority of the earth's surface is made up of water, relatively little of it is really exploitable, making it a very scarce resource. Therefore, care must be taken when using this valuable and finite resource. Water must be suitable before usage because it is used for a variety of uses. Additionally, water sources must be routinely checked to see if they are sound or not. Water bodies in poor condition pose a threat to the ecology as well as being a sign of environmental degradation [1,2]. In industries, poor water quality can result in risks and significant financial loss. Water quality is therefore crucial for both environmental and economic reasons. Analysis of the water's purity is therefore necessary before using it for any purpose. After many years of study, there are already certain established techniques for water quality analysis. There are rules for sample collection, storage, and analysis [3]. Here, the typical chain of events is briefly outlined for the benefit of researchers and analysts.

The chemical, physical, and biological properties of water can be referred to as its quality; usually in relation to how well suited it is for a certain purpose. Water can be utilised for drinking, fishing, farming, industry, and enjoyment. Different defined chemical, physical, and biological standards are required for each of these authorised uses in order to achieve the intended objective [4]. There are stringent standards for water to be used for drinking or swimming compared to that used in agriculture or industry [5]. After extensive study, guidelines for water quality are established to guarantee that water is used effectively for the intended purpose. Analysing the water's quality involves taking the necessary measurements and comparing them to the appropriate standards using accepted techniques [6, 7]. Water quality analysis is often the groundwork of studies for monitoring the potability. Analytical methods routinely used can detect and measure all the natural elements and their inorganic compounds and a very wide range of organic chemical species using methods such as BOD, COD, MPN, TDS etc [8, 9]. The study focuses on water quality analysis using different lake water samples from North Bangalore. Proper analysis of the physico-chemical parameters and water quality using standard techniques will provide details on the level of contamination in the water and the effects of various pollutants.

MATERIALS AND METHODS

Study area

Four lakes were selected from North Bangalore and water samples were collected from the same. Cauvery water was used as control. Totally five different samples were collected for the study. - (13.0874° N, 77.6099° E) - (13.0360° N, 77.6586° E) - (13.0093° N, 77.5741° E) - (13.0466° N, 77.5856° E).

Determination of pH

The collected lake water samples were tested for pH using a pH meter. The water samples were taken in five different beakers, the pH probe was inserted in the water samples and the values were recorded [10].

Determination of total hardness of water

To determine the total hardness of water, titration was performed; EDTA was taken in a burette. 50ml water sample and 10 ml ammonium buffer were added in a conical flask. 2-3 drops of indicator was added to water sample in the conical flask. It was titrated until the end point of wine red to blue colour was obtained [11].

Determination of chloride

To determine the chloride content, 25ml of water sample was taken in the test tube. 5 drops of Chloride Reagent-1 were added and mixed well until a distinct yellow colour developed. Chloride Reagent-2 was added and the contents were mixed well after each drop until the colour changes from yellow to red. The number of drops of reagent 2 required for colour change was counted. The Chloride content was calculated using the formula [12].

Chloride mg/L/ppm= No. of drops X 10



**Sonia Angeline et al.,****Estimation of total dissolved solids**

The evaporating dish was cleaned with ethanol and air dried. The empty evaporating dish was weighed and the value was recorded. 20 ml of water sample was added to the evaporating dish and placed over the Bunsen burner. The water was allowed to evaporate. The evaporating dish along with the total dissolved solids was weighed. The total dissolved salts in water samples were calculated by subtracting the weight of empty evaporating dish from the weight of evaporation dish with total dissolved solids [13].

Determination of Biological Oxygen Demand (BOD):

The water sample was collected from lakes. It was carefully filled in respective BOD bottles without allowing air bubbles. Following which 2ml of manganese sulphate was added to the BOD bottle carefully by inserting the pipette just below the surface of water. This will avoid the formation of air bubbles. About 2 ml of alkaline Potassium Iodide reagent was added in the same manner. The bottle was closed and the contents were mixed by inverting many times. A brownish cloud would appear in the solution as an indicator of the presence of Oxygen. The brown precipitate was allowed to settle to the bottom of the bottle. About 2ml of conc. H_2SO_4 was carefully added without forming air bubbles. The bottle was closed and the contents were mixed well to dissolve the precipitate. The bottle was incubated in BOD incubator for 7 days of incubation. After incubation, 50 ml of sample water was titrated with 0.025N sodium thiosulphate to a pale-yellow colour, followed by 2ml of starch solution. So, the sample turns blue in colour. The titration was continued till the sample becomes colourless; the concordant values were recorded. The concentration of dissolved oxygen in the sample is equivalent to the number of millilitres of titrant used [14].

Determination of Chemical Oxygen Demand (COD)

COD was performed by using 5 conical flasks; 10ml of water sample was poured in each. 5-10 ml distilled water was added in conical flask marked as B which served as blank [15]. 1 ml of $K_2Cr_2O_7$ solution was in each of the conical flasks. All the flasks were kept in water bath at 100°C (boiling temperature). Samples were allowed to cool for 10 minutes. 1 ml of potassium iodide was in each flask. 2 ml of H_2SO_4 was added in each flask. Contents of each flask with 0.1 M sodium was titrated with thiosulfate until the appearance of pale-yellow colour. 1 ml of starch solution to was added in each flask (solution turns blue). It is again titrated with 0.1 M sodium thiosulfate until the blue colour disappears completely.

Determination of Most Probable Number (MPN)

Water samples were collected from the lakes. 5 double-strength lactose broth (LB-2X) tubes was prepared were labelled as 10ml and 5 single- strength broth (LB-1X) tubes as 1ml and another 5 tubes were labelled as 0.1ml, a Durham's tube in inverted position was added in all the tubes. The water samples were mixed thoroughly by proper shaking. [16, 17, 18] Each 10ml tube (LB-2X) was inoculated with 10ml of water sample using 10 ml sterile pipette. Using a 1ml pipette, the five tubes (LB-1X) were inoculated with 1 ml of water sample. Using a 0.1 ml pipette, the five tubes (LB-1X) were inoculated with 0.1ml of water sample. All the 15 tubes were incubated aerobically at 37°C for 48 hours. Compare the number of tubes giving positive reaction to a standard chart and the number of tubes with gas formation was recorded.

Statistical analysis

All the water quality tests were performed in triplicates and the data was analysed using one way ANOVA. The data are represented as mean value \pm S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

RESULTS AND DISCUSSION**Sample collection**

Water samples were collected from four lakes in North Bengaluru, Jakkur Lake, Hormavu Lake, Sankey tank Lake, Hebbal Lake and Cauvery water was used as control sample. Five different samples were used for the study.





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Determination of pH

The pH level in a lake or stream is crucial for the survival of fish and aquatic plants. Changes in pH can cause an overload of available plant nutrients, resulting in excessive plant growth and depleted oxygen levels for fish. The pH of the lake water samples was determined using the pH meter and the pH range was found to be 7- 7.55 (Table 1). The highest pH was found to be 7.53 in the Jakkur lake water (Figure 1).

Determination of total hardness of water

The hardness of water is determined primarily by the amount of calcium and magnesium it contains. Higher levels of these and other minerals make water hard. It was observed that the lake water of Sankey tank had the highest hardness of 150 mg/L (Table 2). All the other water samples were of the moderate hardness range of 60- 120 mg/L. While Cauvery water was 55 mg/L indicating soft water (Figure 2). The data are represented as mean value \pm S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

Determination of chloride

Environmental impacts of chlorides are not usually harmful to human health. Chlorides can contaminate fresh water streams and lakes. Fish and aquatic communities cannot survive in high level of chlorides. The maximum secondary contaminant level in drinking water for chloride is 250 mg/L. Based on the number of drops of chloride reagent used for colour change, the chloride content was determined (Table 3).

- Cauvery water-6 drops was used for colour change which corresponds to 60 mg/L/ppm
- Sankey Tank-8 drops was used for colour change which corresponds to 80mg/L/ppm.
- Hormavu Lake – 7 drops was used for colour change which corresponds to 70mg/L/ppm.
- Jakkur Lake– 23 drops was used for colour change which corresponds to 230mg/L/ppm.
- Hebbal Lake– 8 drops was used for colour change which corresponds to 80mg/L/ppm.

From the values obtained it was determined that Jakkur lake had the highest chloride content of 230mg/L/ppm but lesser than the maximum secondary contaminant level (Figure 3). The data are represented as mean value \pm S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

Determination of TDS

Total Dissolved Solids (TDS) is defined as the substances remaining after evaporation and drying of water samples. The remaining fraction is approximately equivalent to the total content of the dissolved and suspended matter in the water sample. The amount of dissolved solids (salts, minerals, metals, anions, cations) per 20ml of the water sample was estimated using evaporation technique (Table 4). The TDS of Hebbal lake was found to be 3 mg/L (Figure 4). The data are represented as mean value \pm S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

Determination of BOD

The presence of a sufficient concentration of dissolved oxygen is critical to maintaining the aquatic life and aesthetic quality of streams and lakes. Determining how organic matter affects the concentration of dissolved oxygen (DO) in a stream or lake is integral to water quality management. The decay of organic matter in water is measured as biochemical or chemical oxygen demand. Oxygen demand is a measure of the amount of oxidizable substances in a water sample that can lower DO concentrations (Table 5). Hence by comparing BOD level of water bodies with the standard BOD levels it could be inferred that Jakkur lake water has the highest BOD level of 6 mg/L which is poor quality. BOD range of 3-5 is considered to be fair quality of water (Figure 5). The data are represented as mean value \pm S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

Determination of COD

The increase of pollution by discharging large amounts of various chemically oxidizable organic substances of different nature entering the aquatic systems requires determination of Chemical oxygen demand (COD) as BOD alone does not give a clear picture of the organic matter content of the water sample. COD is a better estimate of the





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organic matter; from the results it was observed that Sankey Tank had the highest COD of 0.95 mg/L and Jakkur Lake showed the lowest of 0.8 mg /L (Table 6, Figure 6). The data are represented as mean value±S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

Determination of MPN

Most Probable Number (MPN) Test is often used technique for the sanitary analysis of water. It involves three sets of dilutions containing fermentative broth and the water sample. The formation of acid and gas indicates a positive result. The quantitative analysis of coliform is determined by counting the number of tubes giving a positive result and comparing the pattern of positive results with the statistical data. MPN test detects the presence or absence of faecal coliforms, which can cause faecal contamination in water and making it unsafe for human consumption. Therefore, the quality testing of water becomes necessary to know the concentration of microorganisms or water quality, whether water is potable or not (Table 7). On a comparative basis the water sample from Sankey Tank, Hormavu Lake and Jakkur Lake had a MPN/ 100 mL of 8 at 95% confidence limits (Figure 7). The data are represented as mean value ± S.D, *p value between 0.05 and 0.01, significant at 5% ANOVA.

DISCUSSION

Water quality is crucial for all living things as well as for agriculture. Optimal pH levels are an essential factor in maintaining water quality and healthy ecosystems. One of the most important indicators for water quality is its pH level. Freshwater lakes and streams typically have pH levels between 6.0 and 8.0. Deeper lakes usually have a higher pH near the surface. Aquatic organisms are sensitive to pH changes [10, 19]. This condition, known eutrophication, threatens the aquatic life. In the present study the pH range of lakes in North Bangalore was found to be 7- 7.55. Hard water forms solid deposits comprised of mainly calcium and magnesium salts and can damage equipment, while soft water may be corrosive and therefore, it is important measuring and knowing levels of hardness in water to maintain the delicate balance between scaling and corrosivity [11]. The results from this study showed that Sankey tank water had the highest hardness of 150 mg/L and the other water samples were of the moderate hardness range of 60- 120 mg/L. Elevated levels of chloride in the environment raise concerns about negative ecological impacts to aquatic and terrestrial life.[12, 20] Thus it is an important measure that need to be taken into account during a water analysis report, our results indicate that Jakkur lake had the highest chloride content of 230mg/L/ppm but lesser than the maximum secondary contaminant level. BOD and COD analysis helps in determining the oxygen demand in water bodies. Failure to reduce the organic content can result in eutrophication of rivers, lakes and other water bodies [14, 21,22,23].

The present study indicates that Jakkur lake water has the highest BOD level of 6 mg/L which is poor quality and Sankey Tank had the highest COD of 0.95 mg/L. MPN is most commonly applied for quality testing of water, that is, to ensure whether the water is safe or not in terms of bacteria present in it [18, 24, 25]. Results from the study show that water samples from Sankey Tank, Hormavu Lake and Jakkur Lake had MPN/ 100 mL of 8 at 95% confidence limits. In order to reduce contaminants in the water bodies for the safety of aquatic organisms and the environment, we must take the appropriate steps to compare the levels of pollution found in the water samples taken from lakes in North Bangalore. The findings highlight the need of encouraging excellent cleanliness and sanitation habits, which may eventually help to maintain a higher standard of lake water.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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Table 1- pH of lake water

The pH of the different lake waters ranged from 7- 7.55

| SL.NO. | WATER SAMPLE | pH |
|--------|---------------|------|
| 1. | Cauvery water | 7.33 |
| 2. | Sankey tank | 7.09 |
| 3. | Hormavu lake | 7.49 |
| 4. | Jakkur lake | 7.53 |
| 5. | Hebbal lake | 7.22 |

Table 2- Total hardness of water

Lake water of Sankey tank had the highest hardness of 150 mg/L

| SL.NO. | WATER SAMPLE | TOTAL HARDNESS mg/L |
|--------|---------------|---------------------|
| 1. | Cauvery water | 50 |
| 2. | Sankey tank | 150 |
| 3. | Hormavu lake | 80 |
| 4. | Jakkur lake | 100 |
| 5. | Hebbal lake | 100 |

Table 3: Determination of Chloride

Jakkurlake had the highest chloride content of 230mg/L/ppm

| SL. NO. | WATER SAMPLE | CHLORIDE mg/L/ppm |
|---------|---------------|-------------------|
| 1. | Cauvery water | 60 |
| 2. | Sankey tank | 80 |
| 3. | Hormavu lake | 70 |
| 4. | Jakkur lake | 230 |
| 5. | Hebbal lake | 80 |

Table 4 - Determination of TDS

The amount of dissolved solids (salts, minerals, metals, anions, cations) per 20 ml of the water sample was estimated using evaporation technique

| SL. NO. | WATER SAMPLE | TDS mg/L |
|---------|---------------|----------|
| 1. | Cauvery water | 0.5 |
| 2. | Sankey tank | 1 |
| 3. | Hormavu lake | 0.5 |
| 4. | Jakkur lake | 2.5 |
| 5. | Hebbal lake | 3 |





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Table 5: Determination of Biological Oxygen Demand mg/ L

Oxygen demand, a measure of the amount of oxidizable substances in a water sample that can lower DO concentration was estimated.

| SL. NO. | WATER SAMPLE | BOD mg/L |
|---------|---------------|----------|
| 1. | Cauvery water | 3.5 |
| 2. | Sankey tank | 4.4 |
| 3. | Hormavu lake | 5.2 |
| 4. | Jakkur lake | 6 |
| 5. | Hebbal lake | 4.8 |

Table 6 – Determination of COD

Chemical Oxygen Demand was estimated to assess the organic matter in the water sample

| SL. NO. | WATER SAMPLE | COD mg/L |
|---------|---------------|----------|
| 1. | Cauvery water | 0.5 |
| 2. | Sankey tank | 0.95 |
| 3. | Hormavu lake | 0.82 |
| 4. | Jakkur lake | 0.8 |
| 5. | Hebbal lake | 0.87 |

Table 7- Most Probable Number

Most Probable Number (MPN) test was used for the sanitary analysis of water

| Volume of Broth (ml) | | 10 ml | | | 5 ml | | | MPN Index per 100ml | | | |
|------------------------|-------------------|-------|---|---|------|---|---|---------------------|---|---|---|
| Concentration of Broth | | 2X | | | 1X | | | | | | |
| Sl. no | Water Sample (ml) | 10ml | | | 1ml | | | 0.1ml | | | |
| 1. | Cauvery Water | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 |
| 2. | Sankey Tank | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| 3. | Hormavu Lake | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| 4. | Jakkur Lake | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 8 |
| 5. | Hebbal Lake | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |

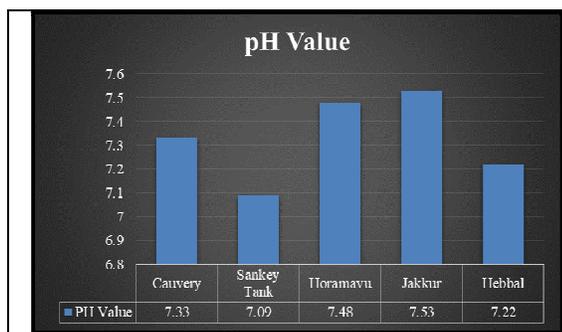


Figure 1: pH of the lake water
The highest pH was found to be 7.53 in the Jakkur lake water



Figure 2: Total hardness of water
Hardness of Cauvery water was found to be 55 mg/L indicating soft water





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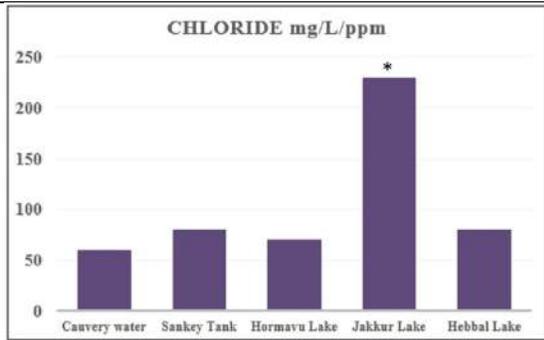


Figure 3: Determination of Chloride
The Chloride content in all lakes under study was found to be lesser than the maximum secondary contaminant level

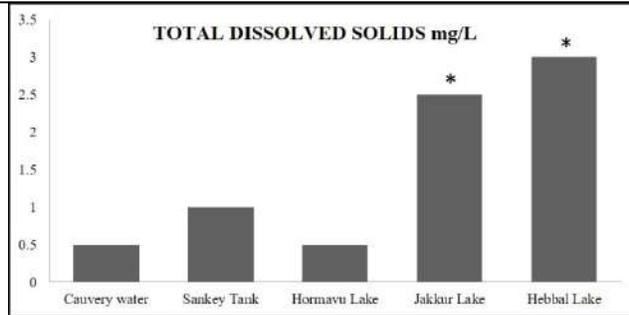


Figure 4 – Determination of TDS
The TDS of Hebbal Lake was found to be 3 mg/L and found to be the highest

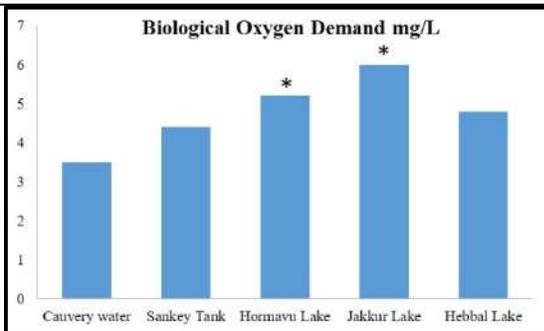


Figure 5: Biological Oxygen Demand mg/L
Jakkur lake water has the highest BOD level of 6 mg/L which is poor quality

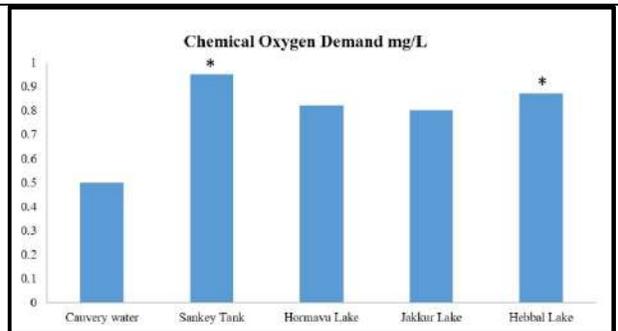


Figure 6 - Determination of COD
Sankey Tank had the highest COD of 0.95 mg/L and Jakkur Lake showed the lowest of 0.8 mg/L

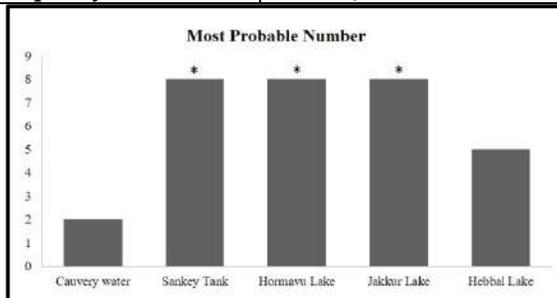


Figure 7: Most Probable Number
On a comparative basis the water sample from Sankey Tank, Hormavu Lake and Jakkur Lake had a MPN/ 100 mL of 8 at 95% confidence limits





Enhancement of Retinal Layer in OCT Images with Choroidal Neovascularization

Miriam Zipporah B^{1*} and D Francis Xavier Christopher²

¹Research Scholar, Department of Computer Science, Rathnavel Subramaniam College of Arts and Science, Coimbatore, Tamil Nadu, India

²Principal and Professor, Department of Computer Science, SRM Trichy Arts and Science College, Trichy, Tamil Nadu, India

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*Address for Correspondence

Miriam Zipporah B

Research Scholar,

Department of Computer Science,

Rathnavel Subramaniam College of Arts and Science,

Coimbatore, Tamil Nadu, India

E.Mail: miriamzipporah19@gmail.com



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ABSTRACT

One of the leading causes of near blindness is an eye illness called age-related macular degeneration. AMD is distinguished by the occurrence of drusen, which alters the physiological structure of the retinal pigment epithelium (RPE) and the edges of Bruch's membrane layer (BM). Hereditary and environmental factors influence/ contribute to age-related macular degeneration (AMD) etiology. The retinal pigment epithelium (RPE) is necessary for retinal photoreceptor cells to function adequately. OCT (Optical Coherence Tomography) is the first quantitative ocular test for diagnosing eye disease based on morphological changes caused by Drusen. Using CAD (Computer-Aided Detection) systems augments accurate observation and understanding of the condition. This work aimed to create a method for segmenting Bruch's membrane, the retinal pigment epithelium, and the inner limiting membrane (ILM) in OCT images of healthy individuals and individuals with intermediate AMD.

Keywords: Age-related macular degeneration, Bruch's membrane, Retinal pigment epithelium, Computer-Aided Detection, Optical Coherence Tomography.

INTRODUCTION

Age-related macular degeneration (AMD) is indeed an illness of the eyes that progresses with time. Millions of people worldwide, particularly those over 50[1]. It is a significant component of vision loss in older people and can significantly impact a person's quality of life. In this article, we'll look closer at AMD, its causes, symptoms, and what





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you can do to identify this condition. AMD is a degenerative macula. The retina region responsible for core vision is damaged by a disorder. The macula is found at the back of the eye and is responsible for helping us see fine detail, colors, and straight lines. AMD causes damage to the cells in the macula, leading to a loss of central vision. Two types of AMD have been identified: dry and wet. Dry AMD is the most common form, accounting for around 85-90% of all cases. It is caused by a buildup of waste products, called drusen, in the macula[2]. Over time, this buildup can cause the cells in the macula to die, leading to a loss of vision. Wet AMD, on the other hand, is less common but more severe. Under the retina, it develops when defective blood vessels grow, leaking blood and fluid and causing damage to the macula. The symptoms of AMD can vary depending on the type and stage of the condition. In its early stages, AMD may not cause any noticeable symptoms. However, as the condition progresses, the following symptoms may develop.

- Blurred or distorted central vision
- Difficulty reading or recognizing faces
- Reduced color perception
- At the center of your field of vision, there is a blind spot
- Straight lines appearing wavy or crooked

The most significant risk factor for AMD is age. The condition is much more common in people over 50, and the risk increases as you age. Other risk factors include

Genetics - AMD tends to run in families, so if you have a close relative with the condition, you may be more likely to develop it yourself.

Smoking - Smoking adds a significant risk factor for AMD and can greatly increase your chances of developing the condition.

Obesity - Being obese or overweight can increase your risk of developing AMD.

Cardiovascular disease - Conditions like high blood pressure and high cholesterol can increase your risk of AMD.

Sun exposure - Long-term exposure to sunlight can increase your risk of developing AMD.

AMD has no cure, but specific treatments can help slow its progression and improve vision. The treatment options for AMD depend on the type and stage of the condition. For dry AMD, there is currently no specific treatment. However, your eye doctor may recommend taking specific vitamins and minerals. Specifically vitamins C and E, zinc, and copper. It has slowed the progression of the condition in some cases. By examining digital retinal images, image processing techniques can help discover and diagnose AMD. Optical coherence tomography (OCT), a widely used technique, employs light waves to produce sharp retina images. Specific characteristics linked to AMD, such as drusen (yellow deposits) and pigment alterations, can be identified by processing OCT images. These traits can be automatically detected and quantified by algorithms, which can help diagnose and monitor AMD[3].

LITERATURE REVIEW

Using image processing and segmenting the retina's boundary layers in OCT images is an effortful task since it is intricate to handle noisy and misshaping images originating from pathologies (drusen, atrophy) that can modify the properties of the layer edges. Multiple studies have been produced based on the methodologies of segmentation of the retinal layers. Several studies have demonstrated methods for segmenting the edges of retinal layers. Below are some of the reviews we came across during the study. In [1], DR. ALFONSO DUPINET SÁNCHEZ investigates AMD disease & vision loss and types of Macular degeneration and how it affects eyesight. What are the symptoms and the identification of the problem in the eye? In [2] "An Efficient Automated System for AMD Detection from Fundus Images" by S. M. Zahid *et al.* This study proposes an automated system for AMD detection using preprocessing techniques such as CLAHE and segmentation techniques such as thresholding and edge detection. In [3] "Automated Segmentation of Macular Layers in OCT Images and Its Application for Early Diagnosis of AMD" by Y. Wu *et al.* This study proposes an automated segmentation method for macular layers using preprocessing techniques such as filtering and segmentation techniques such as graph cut and active contours.





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In [4] "Preprocessing and Feature Extraction for Diagnosis of Age-Related Macular Degeneration" by P. Chen *et al.* This study proposes a preprocessing and feature extraction method for AMD diagnosis using CLAHE and wavelet transform techniques. In [5] "AMD Diagnosis Using an Automated Multi-Objective Preprocessing Framework" by H. Rashwan *et al.* This study proposes an automated preprocessing framework for AMD diagnosis using CLAHE, filtering, and segmentation techniques. In [6] "Automated Diagnosis of Age-Related Macular Degeneration using OCT Imaging and Machine Learning Techniques" by A. Jain *et al.* This study proposes an automated diagnosis method for AMD using OCT images and preprocessing techniques such as filtering and segmentation techniques such as active contours and random forests.

In [8], present an approach to segment OCT images automatically. Proposing a pipeline is contrived of a preprocessing step that uses the BM3D algorithm to reduce the speckle noise. In [9], Image preprocessing and normalization by modified adaptive histogram equalization were used for image standardization to refine effective deep learning. A U-Net-based deep learning algorithm was developed and trained. In [13] gives an inference that delimits the volume of the image. As a result, regions that are very far from the retina and serve as the image's "background" are not processed unnecessarily.

In [21] exhibits the basic operations of morphological image processing, namely erosion, and dilation, showing a hopeful channel in digital image processing. Depending on the structuring elements, the erosion operation shrinks the foreground. In contrast, the dilation operation enlarges the foreground of the image. Thus, this process can eliminate the noises in an image. In [22], the author presents a clear idea and the importance of segmentation of blood vessels on retinal images because this method effectively removes noise and makes blood vessels on images more comprehensible. In [23], pivots on the two-dimensional OTSU algorithm then apply the finest features of other algorithms to the OTSU, proposes its improvement scheme, and authenticate the algorithm's effectiveness.

MATERIALS AND METHODS

Pre-processing, defining the area of interest, early segmentation, and final segmentation are the four steps of the proposed methodology for fragmenting the retinal layers (ILM, RPE, and BM) in OCT images.

Pre-Processing

Preprocessing refers to the techniques used to prepare data for analysis or machine learning models[5]. It involves a series of steps to transform raw data into a format easily analyzed or used by machine learning algorithms. Some standard preprocessing techniques include Data cleaning, Data normalization, Feature extraction, Data transformation, and Dimensionality reduction. In this work, we have proposed the following preprocessing methods.

Histogram Equalization

A notable contrast enhancement technique due to its performance on almost all types of images. In this work, the advanced form of (AHE) Adaptive Histogram Equalization (CLAHE) Contrast Limiting Adaptive Histogram Equalization is used, proposed in[5].CLAHE is a technique used for enhancing the contrast of images. It works by dividing the image into small blocks, computing a histogram for each block, and then redistributing the pixel values based on the histogram. It prevents the over-amplification of noise, which results in the AHE technique. CLAHE uses a contrast amplification limiting procedure for each neighboring pixel, forming a transformation function to reduce the noise problem(1). Here pixels will be distributed uniformly as small tiles(8*8). The thresholding value has been set with CLAHE implementation,fig.2 shows the Histogram equalization based on the CLAHE.

The normalized histogram,

$$P(n) = \text{several pixels with intensity } n / \text{total number of pixels}$$

(1)





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When applying CLAHE, the parameter that has to be paid attention to is clip limit – This parameter sets the threshold for contrast limiting. The default value is 40. tile grid size – This sets the number of tiles in the row and column. The clip limit is between 0 and 1, where 0 indicates no contrast enhancement, and 1 indicates maximum contrast enhancement. For each block of pixels in the image, Using equation 1, CLAHE computes a histogram of the pixel values and redistributes them based on the histogram. The clip limit determines the number of pixels allocated to each histogram bin. If the number of pixels in a bin exceeds the clip limit, the excess pixels are redistributed to other bins.

Image Filtering

Filtering is a fundamental operation in image processing that modifies spatial characteristics. The basic idea behind filtering is to apply a mathematical function or kernel to each pixel in the image, which changes the pixel value based on its neighbors. Many such filter types are used in image processing; we have applied BM3D filtering[8]. The BM3D algorithm is based on a two-stage process. In the first stage, similar image blocks are identified and grouped. This is done using a block-matching algorithm, where each block is compared to other blocks in the image to find the most similar ones. The similar blocks are then averaged together to create a set of reference blocks. In the second stage, each noisy block in the image is denoised using a collaborative filtering approach. The denoising process is based on the similarity between the noisy and reference blocks. The collaborative filtering is done in a 3D transform domain. The block-wise 2D discrete cosine transform (DCT) and a transform in the third dimension are applied to capture correlations across the reference blocks. BM3D is very effective at denoising images, particularly in cases where the noise is random and uncorrelated[8]. It has also been extended to handle other image restoration tasks such as super-resolution, deblurring, and compression artifact reduction. There are several metrics commonly used to evaluate the performance of image-denoising algorithms. The below-mentioned metric(2) was applied in the proposed method, and fig.3 shows Non-preprocessed and preprocessed images.

PSNR was calculated using the formula:

$$PSNR = 20 * \log_{10}(\max \text{ pixel value} / \sqrt{MSE})$$

Here, the logarithm is base 10

(2)

Image Segmentation

MedGA(Medical Genetic Algorithm) can be applied to AMD images for various tasks such as feature selection, parameter tuning, and model selection. In this research, we have used image classification; MedGA was used to select the most informative features from the AMD images and tune the classification model's hyperparameters to achieve better performance. In this case, the input to MedGA was a set of features extracted from the AMD images, along with the corresponding labels indicating the AMD severity level. This parameter is the selection method to select the best individuals from the population for the next generation. In MedGA, two types of selection methods are commonly used. The kind of selection applied in our work was the proportionate fitness selection, also known as roulette wheel selection. It is a widely used method in genetic algorithms for selecting individuals for the next generation based on their fitness values. The probability of choosing an individual i from a population of N individuals is given by the following equation(3):

$$P(i) = f(i) / \sum(f(j)) \text{ for } j = 1 \text{ to } N$$
(3)

where $P(i)$ is the probability of selecting individual i , $f(i)$ is the fitness value of individual i , and $\sum(f(j))$ is the sum of the fitness values of all individuals in the population.

Finally, the region of interest in the image was extracted using this algorithm.

Otsu's method is a popular image segmentation algorithm for thresholding grayscale images. It works by finding an optimal threshold value that separates the image's foreground and background pixels based on their intensity values. However, in some cases[23], the results of Otsu's method may need to be more satisfactory due to uneven illumination, noise, or other image artifacts. The 2D Otsu's approach, which expands Otsu's method to two-



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dimensional images, was proposed as a solution to this problem. The key idea behind 2D Otsu's method is to use a histogram of local gradient magnitudes instead of intensity values to find the optimal threshold value. This is because the edges and contours in the image are generally more critical for segmentation tasks than the overall intensity values, and the local gradient magnitudes offer more reliable information about these features.

An algorithmic description of the 2DOtsu method:

Input: Grayscale image I, block size b

Output: Binary image B

- Compute the gradient magnitude image G by convolving I with a Sobel filter or other edge detection filter.
- Divide G into non-overlapping blocks of size $b \times b$.
- For each block, compute the gradient magnitude histogram H.
- Apply Otsu's method to H to find the optimal threshold value, T.
- Create a binary image B by thresholding G with the local threshold values T.
- Apply morphological operations such as erosion and dilation to remove noise and smooth the segmented regions.
- Output B.

RESULTS AND DISCUSSION

Age-related macular degeneration is an eye problem that can lead to permanent blindness and loss of vision, rendering early warning of the condition necessary. The disease's key symptom is the development of druses, which result in displacements between the Bruch's membrane and the layers of the retinal pigment epithelium. In this paper, we bring out a method for segmenting the borders of the lower limiting membrane, retinal pigment epithelium, and Bruch's membrane, three layers of the human retina that are essential for diagnosing AMD. Pre-processing, which removes noise and highlights edges, has two steps to specify the target area, and final segmentation makes up the proposed method's four steps. This study's findings demonstrate our approach's durability in healthy retinas and AMD. The statistics for mean absolute for the layers of the Bruch's membrane and the retinal pigment epithelium are superior to those discovered in state-of-the-art. Our approach differs from previous research in presenting a model emphasizing segmenting the ILM, RPE, and BM edges.

CONCLUSION

The proposed method for retinal segmentation presented in this study appears to be effective in accurately segmenting the layers of Bruch's membrane and the retinal pigment epithelium. The approach involves pre-processing steps to remove noise and highlight edges, followed by two steps to specify the target area and a final segmentation step. The study results show that the mean absolute statistics for the layers of Bruch's membrane and the retinal pigment epithelium are superior to those achieved in state-of-the-art methods. Furthermore, the proposed approach emphasizes segmenting the ILM, RPE, and BM edges, which departs from previous research. The findings suggest that the proposed method is robust and effective in healthy retinas and those with AMD. However, further research may be necessary to assess its generalizability to other populations and conditions. Nonetheless, the method's performance and emphasis on specific edge segments make it a promising approach for retinal segmentation tasks.

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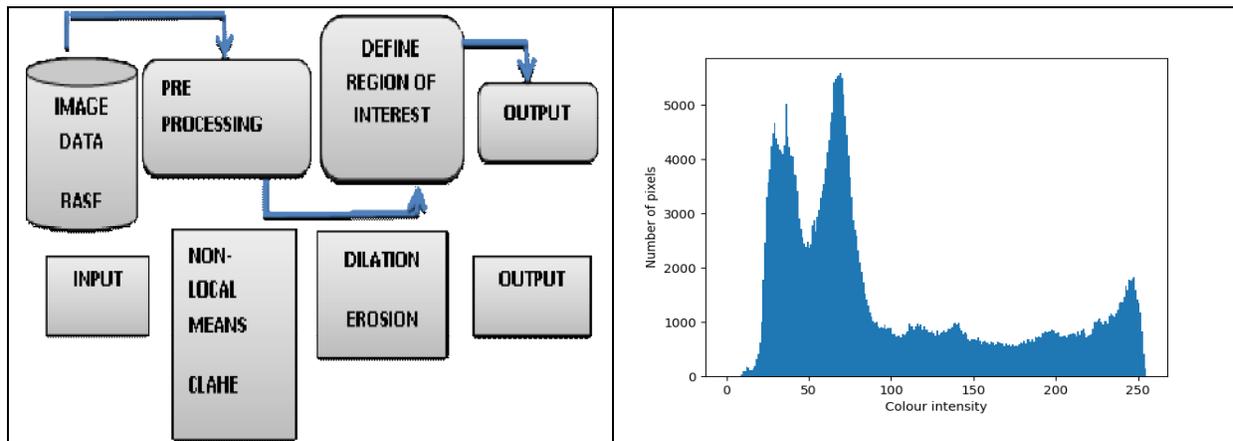


Fig.1 Pre processing Flowchart

Fig.2. Histogram Equalization

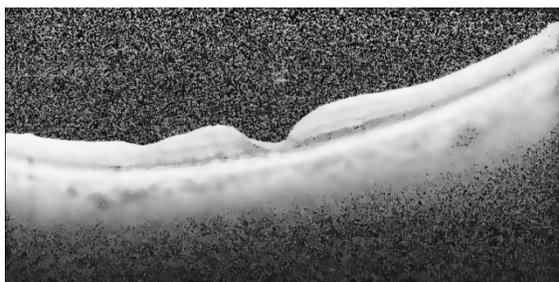


Fig.3.(a) Non-Preprocessed

(b) Preprocessed





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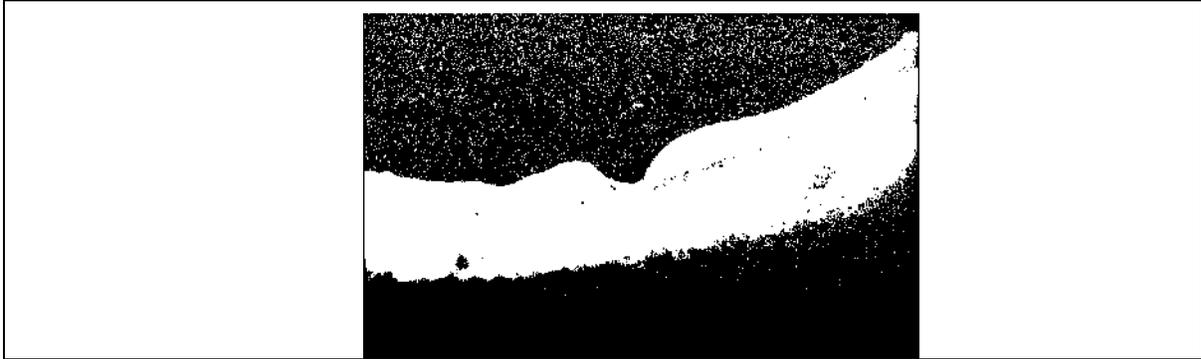


Fig.4. Region of Interest extraction from the AMD images





Effectiveness of Janda's Approach Versus Core Stability Exercise using Swiss Ball on Pain in Parul University Students with Subacute Non-Specific Low Back Pain. A Comparative Study

Garima Shah^{1*}, Madhavi Sontakkey¹ and Bhavana Gadhavi²

¹Assistant Professor, MPT Community Rehabilitation, Parul Institute of Physiotherapy, Limda, Waghodia, Vadodara, Gujarat- 391760, India

²Dean, Principal, Parul Institute of Physiotherapy, Limda, Waghodia, Vadodara, Gujarat- 391760, India.

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*Address for Correspondence

Garima Shah

Assistant Professor,
MPT Community Rehabilitation,
Parul Institute of Physiotherapy,
Limda, Waghodia,
Vadodara, Gujarat- 391760, India.



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ABSTRACT

Non-Specific Low Back Pain(NSLBP) is defined as pain that is not caused by a specific pathology e.g., infection, structural deformity, osteoporosis, fracture, inflammatory disorders such as ankylosing spondylosis, radicular symptom or cauda equina syndrome. Janda's approach and core-stability exercises(CSE) can be used to treat NSLBP but there is deaths of studies performed evaluating which is better to reduce it in 2 weeks. To compare the effect of Janda's approach and CSE on intensity of pain in NSLBP in 2 weeks. A total number of 20 subjects of age between 18-25 years were recruited and divided into 2 groups and were analysed by NPRS to assess intensity of pain in NSLBP before the start of treatment and at the end of 2 weeks. Wilcoxon signed rank test and Mann Whitney U test was used to evaluate the data. There was significant improvement in pain in both the groups but there is more improvement in group A with p value 0.001. Hence Janda's approach is more beneficial to improve pain in subjects with NSLBP.

Keywords: Janda's approach, Core stability exercise, NPRS.

INTRODUCTION

Low back pain is recorded as most frequent cause of disability among young college going females. LBP is defined as pain or discomfort located at 12th rib and above inferior gluteal fold with or without leg pain. It is the most common musculoskeletal disorder which affects the adult population.





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An internationally accepted practical classification of low back pain has divided it into 3 categories which is also used as a diagnostic triage:

- Specific spinal pathology
- Nerve root/radicular pain
- Non-specific low back pain

Non-specific low back pain is defined as pain that is not caused by a specific pathology e.g. Infection, structural deformity, osteoporosis, fracture, inflammatory disorders such as ankylosing spondylosis etc.(1) According to recent review of national guidelines NSLBP is described as a diagnostic exclusion where pain caused by a suspected or confined serious pathology or presenting other radicular syndrome have been ruled out. Prevalence of LBP in females in the age group of 18-25 is 70-80%. Exercise for low back pain for maintaining the spinal stability, core stabilisation exercises are improving neuro muscular control, endurance and strength of muscle to dynamic spinal stability.

Activities like prolong sitting while taking lectures, prolong standing in during clinical hours, patient handling, dysmenorrhea, psychological and emotional factors, use of heavy backpack, physical fitness, prolonged sitting time, bad postural habits, short sleep hours, discomfort in bed and college furniture are considered to be the most significant risk factors for LBP among the female college going students. CSE include a range of exercise programs with different approaches having common role of improving lumbo pelvic and abdominal control. These exercises are designed to enhance the ability of neuromuscular and motor control systems.

Janda identified 2 group of muscles based on their phylogenic development but functionally muscles can be classified as tonic or flexors and phasic or extensors.(2,3) It was noted that the tonic muscles were more prone to tightness and phasic muscles were more prone to weakness/ inhibition. Lower crossed syndrome is characterised by facilitation of thoraco-lumbar extensors, rectus femoris, and iliopsoas as well as inhibition of transverse abdominus and gluteal muscles.(3) Janda's approach works on correcting the biomechanics of a peripheral joint, restoring the muscle balance and restoring the normal tone of the muscle of surrounding joints. Numeric pain rating scale is used to measure the intensity of pain. Exercise on swiss ball increases core muscle activity and Janda's approach includes the treatment of muscle imbalance and movement. Previously, a few studies have been done to see the effect of Janda's approach and CSE using swiss ball on NSLBP. Also no study shows 2 weeks' treatment effect of respective exercises on intensity of pain. Hence this study aims to find the effect of Janda's approach and CSE using swiss ball on intensity of pain in 2 weeks in college going female students.

METHODOLOGY

- Study design: Comparative study
- Sampling: convenient sampling
- Duration: 2 weeks
- Outcome measure: Numeric pain rating scale(NPRS)
- Inclusion criteria: Medical and para-medical female students of age group 18-28 years, with low back pain >4weeks, normal BMI, give consent for study.
- Exclusion criteria: BMI <or > 18- 22.9kg/m², underwent any recent abdominal surgeries, PIVD, and trauma of back, congenital conditions of lumbar spine, pregnancy, any pathological back pain.
- Procedure: total 20 subjects were taken fulfilling the inclusion and exclusion criteria. All participants were informed about the objectives of study and consent was taken. Participants were then randomly divided into 2 groups. Group A Janda's approach group (n=10) and Group B Core stability exercises group using swiss ball (n=10). Subjects were explained about the protocol in both the groups, assessment was done before the start of treatment.





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Group A underwent

- Stretching exercise for:
 1. erector spinae and back extensors muscles
 2. iliopsoas muscle and rectus femoris muscle
 Stretching exercise performed actively 30 second hold 3 repetitions.
- Strengthening exercise for:
 1. abdominal muscle
 2. gluteal maximus muscle

Strengthening exercise performed by 10 second hold 10 repetitions.

Group B

- Week 1:
 - 1- lie on your back with knees bent, hold drawing in manoeuvre for 10 seconds. Rest. Repeat for 10 times.
 - 2- Stay on your hands and knees. Hold drawing in manoeuvre for 10 seconds. Loosen your muscle. Repeat 10 times.
 - 3- Sit on swissball. try to maintain balance. Hold drawing in manoeuvre for 10 seconds while sitting straight. Rest. Repeat for 10 times.
 - 4- Sit on swiss ball and maintain balance. Arms away from side of the body. Bring one leg up and hold for 10 seconds. repeat with other leg for 10 times.
- Week 2:
 - 1- Stand, perform drawing in manoeuvre and walk for 20 minutes per day.
 - 2- Try to hold drawing in manoeuvre and contract lumbar Para spinal muscles in all daily activities.
 - 3- Sit on swiss ball. Hold drawing-in manoeuvre and lumbar Para spinal muscles contraction for 10 seconds while keeping your back straight. Then lift one of your arms. Hold it for 10 second. Loosen your muscles. Repeat for 10 times also with other hand.
 - 4- Sit on swiss ball. Hold drawing-in manoeuvre and lumbar Para spinal muscles contraction for 10 seconds while keeping your back straight. Then lift both of your arms. Hold it for 10 second. Loosen your muscles and then repeat for 10 times.

RESULT

The results were analysed by Wilcoxon Signed Rank Test for comparison within group and Mann Whitney U test for comparison between the groups. There was significant difference within both the groups for NPRS but the results showed that stretching and strengthening is more effective than core stability exercises with swiss ball on reducing pain(NPRS). ($P < 0.005$). Within group analysis of pre and post NPRS in group A and B was done using Wilcoxon Signed Rank Test. Analysis showed significant difference between pre and post treatment NPRS scores in group A and B

Graph 1: shows intragroup mean and SD difference in Group A

Graph 2: shows intragroup mean and SD difference in Group B

Graph 3: shows intergroup mean and SD difference between Group A and B

Mann Whitney U test was applied to analyse the comparative effectiveness on NPRS score of both the groups. Analysis showed there was significant difference between group A and group B. ($p = 0.001$)

DISCUSSION

The present study was intended to provide new information on effect of janda's approach versus core stability exercise on treating pain in Non-specific low back pain. This study was conducted on 20 subjects with non-specific





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low back pain with 18-28 years and according to other inclusion and exclusion criteria, who were divided into 2 groups. Group A underwent Janda's approach; stretching and strengthening exercise and group B underwent core stability exercise using swiss ball. Both the groups were assessed before and after the treatment to determine the extent of reduction in level of pain by using Numeric Pain Rating Scale. At the end of 2 weeks, the subjects in both the groups showed reduction in pain but group A showed statistically significant improvement than group B ($p < 0.05$). Thus, this study result shows that stretching and strengthening (Janda's approach) is more effective than CSE using swiss ball on outcome of NPRS. The finding of this study are similar to Sapnanandlal Tank. Who did study on effect of Janda's approach on pain and function in patients with non-specific low back pain- an interventional study. In that study 34 patients were divided into two groups group 1 was Janda's approach with conventional physiotherapy group and group 2 was only conventional physiotherapy group. Pain intensity and function was measured using NPRS and modified Oswestry disability index. Result concluded that Janda's approach along with conventional physiotherapy was more beneficial in relieving pain and function.

Also a study done by Vijay kageand B.B. putti had similar findings who did study on effectiveness of stretching and strengthening on subjects with postural backache a randomised controlled trial. In that study 40 participants were divided into two groups group A was controlled group and group B was Janda's approach group, Group A was given SWD with core stability exercises and Group B was given SWD with CSE and Janda's approach. Pain intensity and severity of malalignment and muscle strength and flexibility were measured using Visual analogue scale and index of lumbar lordosis. The results concluded that stretching and strengthening exercises are beneficial in reducing pain, normalising the lumbar lordosis curvature, increasing strength of abdominals and gluteals and increasing flexibility of rectus femoris, iliopsoas and erector spinae.

Ui-CheolJeong, MS et.al., did studied The effects of gluteus muscle strengthening exercise and lumbar stabilization exercise on lumbar muscle strength and balance in chronic low back pain patients. This study randomly and equally assigned 40 participants who divided into to a lumbar segmental stabilization exercise plus exercise to strengthen the muscles of the gluteus group (SMG + LES group) and a lumbar segmental stabilization exercise group.

Outcome measures of this study were: strengthen the muscles of the gluteus; lumbar stabilization exercise; Oswestry Disability Index; isometric lumbar flexion strength; isometric lumbar extension strength; stability index; and weight distribution index.

Results shows that both SMG+LSE and LSE were effective for improving the low back pain disability index, lumbar muscle strength, and balance in chronic low back pain patients. Comparison of the changes in the two groups revealed that there was a more significant effect on low back pain index, lumbar muscle strength, and balance in the SMG+LSE group than in the LSE group. It is considered that the lumbar segmental stabilization exercise strengthened deep muscles, increasing lumbar segmental stability, and that hip muscle strengthening exercise increased the stability of the pelvis and hip joint, thereby positively affecting an increase in lumbar segmental stability. (5) A study done by Monika et al presents use of strength exercises in rehabilitation process of persons with low back pain syndrome and concludes that increase of muscle strength also positive influence on range of motion of trunk and lower limbs and decrease of pain in persons with low back pain syndrome. Mc Ceary proposed that in the erect position, weakness of abdominals permits an anterior pelvic tilt and a lordotic posture which lead to muscle imbalance hence cause low back pain. In this study decrease in pain and decrease in anterior pelvic tilt that could have been caused by strengthening of abdominals. (4) Khwairakpam Zhimina Devi et.,al studied effectiveness of muscle stretching on chronic low back pain, according to them stretching of erector spinae, tensor fascia lata and hamstring improves flexibility, reduces pain and improves function. (6)

CONCLUSION

The results of this study suggest that stretching and strengthening exercises (Janda's Approach) compared to Core stability exercises using swiss ball has more significant level of improvement in pain intensity of NSLBP within 2 weeks.





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LIMITATIONS OF STUDY

- Sample size was too small
- Long term follow-up was not there to check the consistency and long term effect of treatment.

FUTHER RECOMMENDATIONS

- Study can be done for more duration to rule out the long term effect of the treatment.
- Multi center trials can also be done to check carry over effect.

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Table1: Descriptive analysis of AGE

| AGE | | Group A | Group B |
|-----|------|---------|---------|
| | Mean | 20.6 | 22.3 |
| | SD | 1.837 | 2.584 |

Table 2: Pre and Post Mean of NPRS Group A

| Group A | | | | Test Name | |
|---------|------|------|------|-----------|---------|
| Mean | | SD | | Z value | P-Value |
| Pre | Post | Pre | Post | | |
| 5.5 | 2.3 | 1.08 | 0.48 | -2.825 | 0.005 |

Wilcoxon Signed Rank Test

Table 3: Pre and Post Mean of NPRS Group B

| Group B | | | | Test Name | |
|---------|------|------|------|-----------|---------|
| Mean | | SD | | Z value | P-Value |
| Pre | Post | Pre | Post | | |
| 5.7 | 4.2 | 1.41 | 1.31 | -2.87 | 0.004 |

Wilcoxon Signed Rank Test

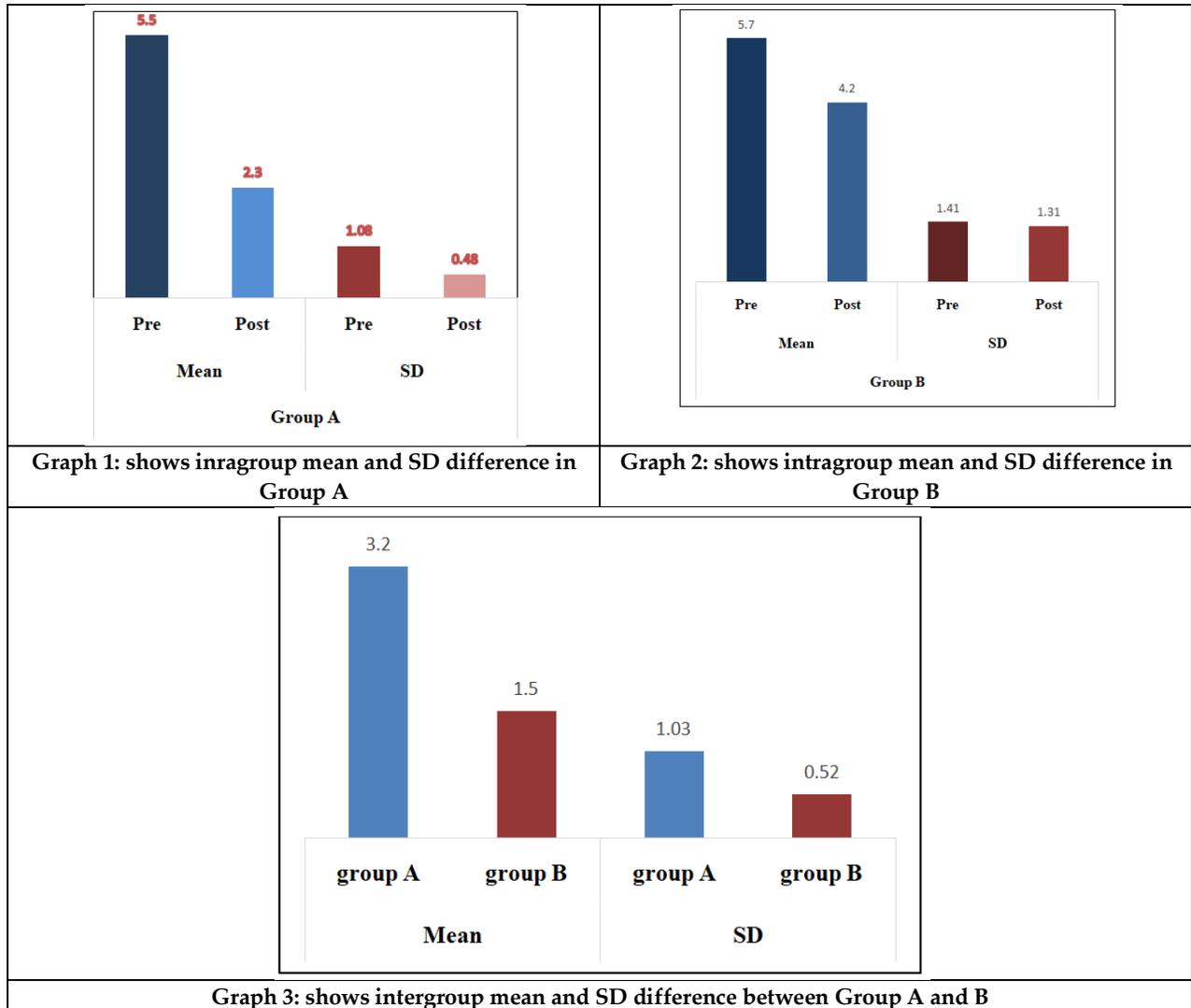




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Table 4: Comparison of group A and B mean of NPRS

| Mean | | SD | | Z-Value | P- Value | Test Name |
|---------|---------|---------|---------|---------|----------|-------------------|
| group A | group B | group A | group B | | | Mann-Whitney Test |
| 3.2 | 1.5 | 1.03 | 0.52 | -3.357 | 0.001 | |





Unlocking the Land Classification Puzzle Through Geospatial Approach for Thirumakudalu Narasipura Taluk, Karnataka, India

Manjunatha M.C^{1*}, Abrar Ahmed² and Basavarajappa H.T³

¹Assistant Professor, DBT-BUILDER, JSS AHER, Mysuru-570 015, Karnataka, India.

²Assistant Professor, Dept. of Geology, SEA College of Science, Commerce and Arts, Bengaluru-560 049, Karnataka, India

³DoS in Earth Science, University of Mysore, Mysuru-570 006, Karnataka, India.

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*Address for Correspondence

Manjunatha M.C

Assistant Professor,

DBT-BUILDER, JSS AHER,

Mysuru-570 015, Karnataka, India.

E. Mail : mcmanju1@gmail.com



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ABSTRACT

Land classification mapping serves a major part in the interpretation of national assets and their specific utilization for the economic growth of the present taluk. Thirumakudalu (T.) Narasipura taluk of Karnataka state was taken up for land classification mapping through RS, GIS, and field observations. Mapping land patterns from any suitable satellite images in a GIS environment is an innovative task that effectively extracts meaningful information from larger areas like a bird's view. A correct method of interpretation for land suitability accomplishes greater knowledge of economic functions and national growth.

Keywords: Land Classification, LISS-III satellite Image, GIS, T. Narasipura taluk.

INTRODUCTION

Land patterns are the end-product of both socio-economic and natural components utilized by humans over space-time [32]. An overall investigation of these lands for specific utilization and growing of economic trees, medicinal plants, and crop types is very much necessary in achieving standard schemes of land classification and improving socio-economic conditions [12,15]. Catchment area, interception, infiltration, evaporation, and surface run-off are the hydrologic factors that control the land classes [17,29] and impact soil fertility and erosion. Larger proportions of agricultural land had modified into wastelands mainly due to the growing demands of human forces, and adverse bio-environmental circumstances [3] that leads to ecological imbalance [5]. Our land had already resulted as a scarce



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commodity by the immense pressure of industrialization and improper agricultural practices caused severe environmental problems and negatively affected climatic conditions, deforestation, global warming, biodiversity loss, an increase in disasters, and socio-economic mobility from local to worldwide[20,28]. Adapting to the changing socio-economic and climatic circumstances are vital strategies that can be achieved through advanced tools and technologies.

Standard schemes of land classification at appropriate scales through geospatial way matches the requirement of society and protects the natural environment required for all organisms on the earth through feasible agricultural activities, increase in the yield of food systems, and water resources[12,32]. The universally accepted land classification scheme is enumerated by Anderson which includes multi-levels of classes modified to be suitable for the details of different levels [19,30]. Identification of changes over time, comparing between different places over seasons, and reducing the duplication efforts can be fulfilled with a standardized classification system. Remote Sensing (RS) approach with its synoptic view will be an effective tool for the reclamation of degraded land in a proper way is being taken up by many organizations worldwide [13]. IRS LISS-III satellite images have been extensively utilized for evaluating the accurate land classes of T.Narasipura[22] through the powerful tools of GIS software [7]. Thus RS and GIS reveal proper scientific planning and implementation strategies in achieving the growing necessities of all the organisms on earth[26].

METHODOLOGY

Study Area

It is observed between 76°47' to 77°09' E longitude and 12°06' to 12°27' N latitude with 640 mts elevation above MSL and cover 598.83 km²(Fig.1a). Both perennial Rivers Cauvery and Kapila joins at this taluk and flows towards the south easterly direction. It is endowed with a semi-arid climate zone with 916mm of annual rainfall during 2020 [8].The Cauvery River drains major regions in the taluk and supplies water sources for growing major crops of paddy, sugarcane, maize, ragi, tur, pulses, tobacco, fruits, and vegetables [8].

METHODS AND MATERIALS

Toposheets of 57D/15, 16, 57H/3, 4 had acquired from the SoI (Survey of India) office, Bengaluru; where PAN+LISS-III satellite images (Nov-2001 and Jan-2002) of 5.8mts spatial resolution were acquired from National Remote Sensing Centre, Hyderabad[19].Land-classified maps were prepared using LISS-III satellite images by adding permanent features of village boundaries, settlements, coordinates, power lines, and major roads from the Soltopo sheet[18]. A supervised classification tool was applied on multispectral PAN+LISS-III merged data (Fig.1a) with medium scale through ArcGIS v10 and cross-verified during limited field visits using handheld GPS (Garmin-12) and field photo collections (Fig.3a to 3j).

RESULTS AND ANALYSIS

Level-I Classification

Agricultural lands cover farming, irrigation, natural & artificial plantations, commercial/ horticultural crops, fallow lands, and other food production activities for a long duration [24]. *Built-up lands* are human-occupied areas for development & transportation purposes such as buildings, rail/roadways, utilities in connection with vacant lands, vegetation, and water[1,19,24]. *Forest lands* are the areas of notified boundary that are associated with tall, thick trees of canopy shelter beyond 10% in higher than 0.5 ha. These areas also include timber, fodder, and any other vegetation types that attain a minimum height of 5 mts[10]. *Waterbodies* are the are as of ponds, channels, lakes, canals, streams, rivers, and reservoirs [10]. *Wastelands* are degraded lands that can occur due to the lacking of water, and soil management associated with natural effects[24] that act differently in different regions, environments, and physicochemical qualities of the management restrictions[25]. *Others* serve as diversified land by their physical



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appearance, natural causes, and other characteristic features observed from satellite images. These level-I classes of T. Narasipura taluk are shown in Figure.1b.

Level-II Classification

Agricultural plantations are the types of commercial farming exhibiting a dispersed or contiguous pattern adopted on a larger scale by determining the zones of soil and climatic conditions [24]. Coconut and other plantations were widely scattered around Makaanahalli village(Fig.2a, 3a; Table.1). *Barren rocky* is the notice able outcrops of diverse rock types, soil types, and vegetation cover that frequently exposed as barren and devoid lands[24]that are directly exposed to sun and wind actions[10]. These are differentiated effortlessly from other types of deteriorated lands by their specific spectral region. These rocky lands were identified as elevated land with a lack of greenery in the villages of Gavipura, and Bettahalli(Fig.2a,3b; Table.1). *Croplands* are dominantly distributed types of double (Kharif + Rabi) and Kharif crops in the present study (Fig.2a, 3c; Table.1) that also includes un-irrigated, fallow land and plantations[23] and digitized using respective season-wise acquired LISS-III satellite images based on the date of standing crops. *Degraded forests* are damaged or reduced the wealth of greenery within the notified forest boundary covering less than 10% that occurred due to frequent forest fires, overgrazing and constant felling of trees during extreme summer conditions[18]. These were frequently recorded around the temples of the Talakadu area (Fig.2a; Table.1).

Fallow lands were kept at rest in one of the seasons in the same year [24]. The irregular shape of fallow land associated with varying in size where prominently seen in the villages of Vaddara Koppalu, T.Narasipura town, Hyakanuru, Danayakanapura, Bannuru, Gavipura, Kolathuru, Doddebagilu, and Talakadu (Fig.2a, 3d; Table.1). *Forest plantations* are the lands of growing and managing theeconomical inferior species within the notified forest areas that are observed around the temples of Talakadu area, and Mudukuthore (Fig.2a; Table.1). *Gullied lands* are the narrow and deep channels formed by the weaving away of soil by running water and rainfall impacts. These are easily identifiable near Bolegowdanahundi, Mudalahundi, Hyakanuru and around the hills of Arie Mallappa betta, Bajamma betta in the southern parts of the taluk (Fig.2a; Table.1). A distillery company occupying *industrial areas* located in the village of Vaddara Koppalu with an aerial extent of 0.13 km² (Table.1). Discharge of chemical effluents from large-scale industries caused deterioration of most of the nearby lands and mixes with the earth's surface (Fig.2a, 3e; Table.1). 6 major and 33 minor lakes were recorded in northern and central parts of the taluk and Bannuru Village *Lake* is the largest among all lakes that store greater amounts and receives water sources along the definite channels of Perennial River Cauvery(Fig.2a, 3f; Table.1).

Land with scrubs is observed all along the linear ridges and steep slope areas interrelated with bad land vegetation type[4]that are dominantly identified near the villages of Kethupura, Megalakopplu, Gavipura, Kallipura, Shivapura, and around Hyakanuru (Fig.2a, 3g; Table.1). Perennial River Cauvery (Fig.3h; Table.1) and Kapila join together in the study taluk and provide water sources for agricultural lands. Excess *salts* were recorded in association with specific soil types and patches of grass growth were identified near the villages of Thottavadi Mole, Vattalu, and Sujjaluru. These negatively impact the growth of most viable plants, herbs, and shrubs (Fig.2a; Table.1). Well-exposed *bars* were observed along Cauvery and Kapila rivers and meandering regions in the villages of Hosa Hemmige, Tadimalangi, Malingi Kukuru, Hampapura and around the temples of Talakadu (Fig.2a, 3i; Table.1). *Scrub Forestlands* are the forest blanks dominated by shrubs and canopy density of less than 10% identified around Mudukuthore temple during extreme summer seasons (Fig.2a; Table.1) [12]. T.Narasipura was the *town* identified in the taluk that supports mixed built-up land, semi-public utilities, transportation, communications, commercial areas, recreational places, and others (Fig.2a; Table.1) [24]. A clump of *tree groves* was located extensively in the villages of Kukuru, Mudukuthore, Hyakanuru, Kannayakanahalli, central region of the T.Narasipura and doesn't show much undergrowth such as fruit cultivation(Fig.2a; Table.1) [5]. Nearly 130 *villages* were identified in T.Narasipura taluk that showed close association with agricultural patterns and non-commercial activities [24]. The area coverage of level-II land classes were given in Table.2.





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Level-III Classification

Double crops were extensively concentrated adjacent to Cauvery and Kapila Rivers with very high cropping intensity due to horizontal topography, irrigated, and fertile soil [4]. Cauvery Canal was a system of shallow groundwater levels were recorded due to the Cauvery Canal system that helps in the growth of natural vegetation. Rigorous agricultural practices are observed in NW, central, and SE parts growing two types of crops in a year on the same land (Fig.2b; Table.2). *Kharif* are the rainfed crops grown between June to September under dry land farming and restricted irrigational activities. The study taluk has a moderately deep to deep soil system on horizontal topography to very gently sloping areas of good to moderate groundwater level potentials that are put into intensive cropping patterns [20] (Fig.2b; Table.2).

DISCUSSION

The major changes in land use patterns for many decades are mainly due to the surface and sub-surface hydrologic factors since River Cauvery drains major parts of T.Narasipura taluk [27]. RSimages have made it achievable to analyze different levels of landclasses and their ground verification through field photos (Fig.3a to 3j) in a more meaningful way at inexpensive and greater precision[2,16].The present study interpreted 6 major classes in Level-I (Fig.1b; Table.1) 17sub-classes in Level-II (Fig.2a; Table.1) and 2 sub-classes in Level-III classified maps[20](Fig.2b; Table.1). The output classified maps illustrate a greater understanding on the spatial distribution of cropping pattern, fallow lands, forest boundaries encroachment, specific reclamation strategies for wastelands in association with surface water bodies for suitable developmental planning [26].*Kharif* is grown during the rainfall season that holdsan aerial coverage of 174.89 km² (29.20%). Double crops are widely recorded adjacent to Cauvery and Kapila Rivers and their drainage system produces a well-developed canal system for irrigation purposes [19]. The major crops spread here are paddy, jowar, ragi, tur, maize, pulses (tur, avare, Bengal gram, cowpea, green gram, black gram), tobacco, fruits, vegetables, oil seeds (groundnuts, sunflower, castor, sesame), commercial crops (cotton, sugarcane, tobacco) [8].Built-up land covers an area of 20.28 km² (3.38%) during the year Jan-2002 and a further increase in population and demand for natural resources can negatively impact our bio-diversity, increase the soil erosion into streams & lakes, and affects natural land resources[18]. The wastelands of T. Narasipura taluk covers an area of 9.64 km²(1.6%) and these can be converted into useful lands with proper scientific management.

CONCLUSION

The rapid rise in human demands and change in time had transformed huge land cover from one level to another level of classification such as agricultural area20 years ago, is probably an urban land today and forest cover is most likely an encroached agricultural lands and plantations right now. Dragging the historic references of these lands helps in effective utilization and can achieve major policy decisions through recent advanced technologies in the present era. RS and GIS play a promising role in the effective manipulation of land cover with little period as compared to manual studies. Thus this study ensures the planning and development of proper scientific utilization of land and restoration of degraded, deforestation, and overgrazed lands using historic information.

Conflicts of Interest

The authors declare no conflicts of interest.

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Table.1. Key elements for various Land categories interpretation as seen in False Color Composite of LISS-III Satellite image [9,14,19]

| Land category | Tone/Color | Size | Shape | Texture | Pattern |
|--------------------------|-------------------------------------|-----------------|----------------------------|------------------|------------------------------------|
| Agricultural plantations | Dark red to red | Small to large | Regular to irregular | Coarse to medium | Dispersed contiguous |
| Barren rocky | Greenish blue to yellow to brownish | Varying in size | Irregular, discontinuous | Coarse to medium | Linear to contiguous and dispersed |
| Croplands | Bright red to red | Varying in size | Regular to irregular | Medium to smooth | Contiguous to non-contiguous |
| Degraded forest | Red | Varying in size | Irregular to discontinuous | Smooth to medium | Contiguous to non-contiguous |
| Fallow land | Yellow to greenish blue | Small to big | Regular to irregular | Medium to smooth | Contiguous to non-contiguous |





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|--------------------|---------------------------------|----------------------|----------------------------|-----------------------|--------------------------------------|
| Forest plantations | Light red to red | Varying in size | Regular to irregular | Smooth to medium | Contiguous to non-contiguous |
| Gullied land | Light yellow to bluish green | Varying in size | Regular to broken | Very coarse to coarse | Dendritic to sub-dendritic |
| Industrial land | Light bluish to black dark gray | Small to medium | Irregular in shape | Mottled texture | Contiguous dispersed |
| Lakes | Light blue to dark blue | Small, medium, large | Regular to irregular | Smooth to mottled | Non-contiguous dispersed |
| Land with scrub | Light yellow to greenish blue | Varying in size | Irregular to discontinuous | Coarse to mottled | Contiguous dispersed |
| River | Light blue to dark blue | Long narrow and wide | Irregular, sinuous | Smooth to medium | Contiguous, dendritic/ sub-dendritic |
| Salt-affected land | White to light blue | Small to medium | Irregular, discontinuous | Smooth to mottled | Dispersed, non-contiguous |
| Sandy area | White to light yellow | Varying in size | Irregular, convex | Coarse to mottled | Dispersed, contiguous |
| Scrub forest | Light red to brown | Varying in size | Irregular, discontinuous | Coarse to mottled | Contiguous to non-contiguous |
| Town/ Villages | Dark bluish green | Small to big | Irregular | Coarse | Clustered to scattered |
| Tree groves | Dark red to red | Small to big | Regular to irregular | Coarse to medium | Dispersed contiguous |

Table.2. Area coverage of Level-I, Level-II, and Level-III classification analysis

| Level-I categories | Area (km ²) | Area (%) | Level-II categories | Area (km ²) | Area (%) | Level-III categories | Area (km ²) | Area (%) |
|--------------------|-------------------------|--------------|---------------------------|-------------------------|--------------|----------------------|-------------------------|--------------|
| Agricultural land | 528.19 | 88.20 | Agricultural Plantation | 10.20 | 1.70 | Double Crop | 340.07 | 56.78 |
| | | | Barren rocky / Sheet area | 0.41 | 0.06 | | | |
| | | | Cropland | 514.96 | 85.99 | | | |
| Built-up land | 20.28 | 3.38 | Degraded forest | 0.53 | 0.08 | | | |
| | | | Fallow land | 3.02 | 0.50 | | | |
| | | | Forest plantations | 1.99 | 0.33 | | | |
| Forest | 2.83 | 0.47 | Gullied/ Ravenous land | 1.88 | 0.31 | Kharif Crop | 174.89 | 29.20 |
| | | | Industrial area | 0.13 | 0.02 | | | |
| | | | Lake/ Tanks | 8.68 | 1.44 | | | |
| Waterbodies | 35.87 | 5.99 | Land with scrub | 4.84 | 0.80 | | | |
| | | | River/ Stream | 27.18 | 4.53 | | | |
| | | | Salt affected land | 0.73 | 0.12 | | | |
| Wastelands | 9.64 | 1.60 | Sandy area | 1.76 | 0.29 | | | |
| | | | Scrub forest | 0.31 | 0.05 | | | |
| | | | Town | 2.42 | 0.40 | | | |
| Others | 1.80 | 0.30 | Tree groves | 1.80 | 0.30 | | | |
| | | | Village | 17.72 | 2.95 | | | |
| Total | 598.61 | 99.94 | Total | 598.56 | 99.87 | Total | 514.96 | 85.98 |
| TGA | 598.83 | | TGA | 598.83 | | TGA | 598.83 | |

Note: TGA= Total Geographic Area





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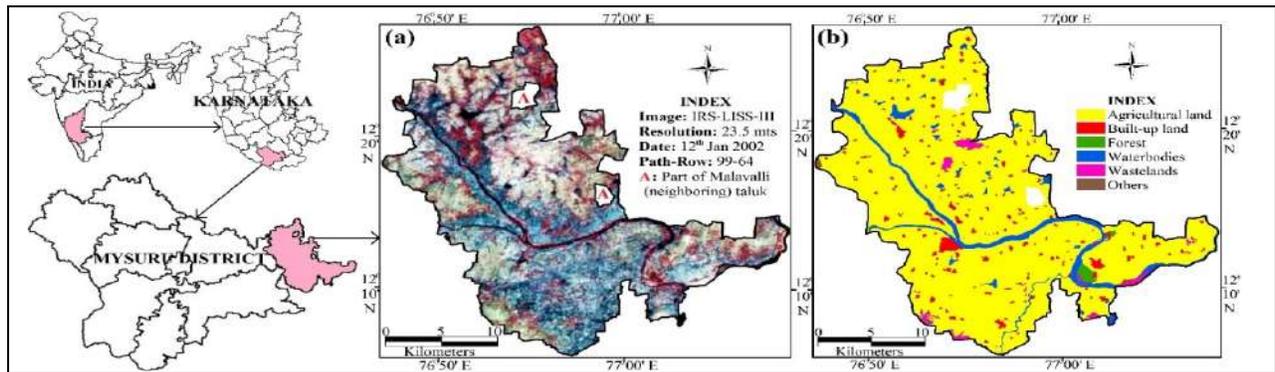


Fig.1.(a) Location and LISS-III data; (b) Level-I Land Classification map of T.Narasipura taluk

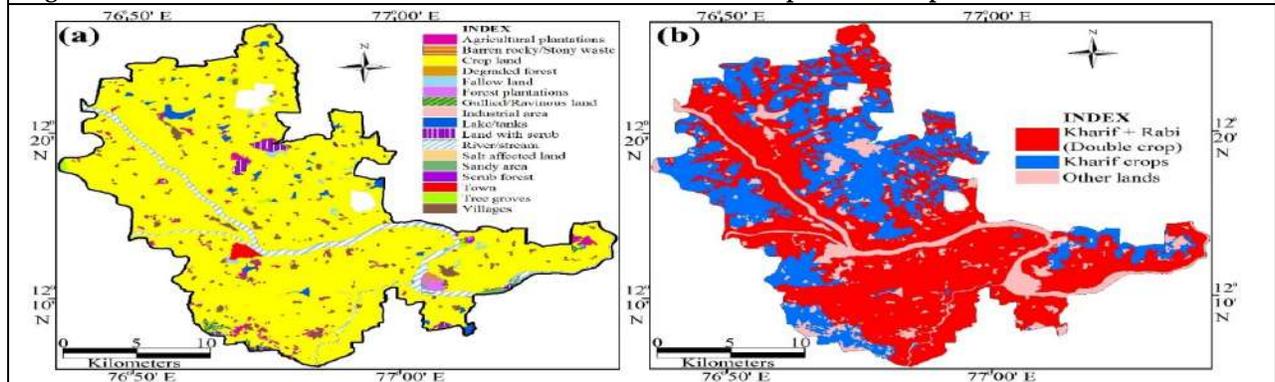


Fig.2. (a) Level-II and (b) Level-III Land Classified map of T. Narasipura taluk

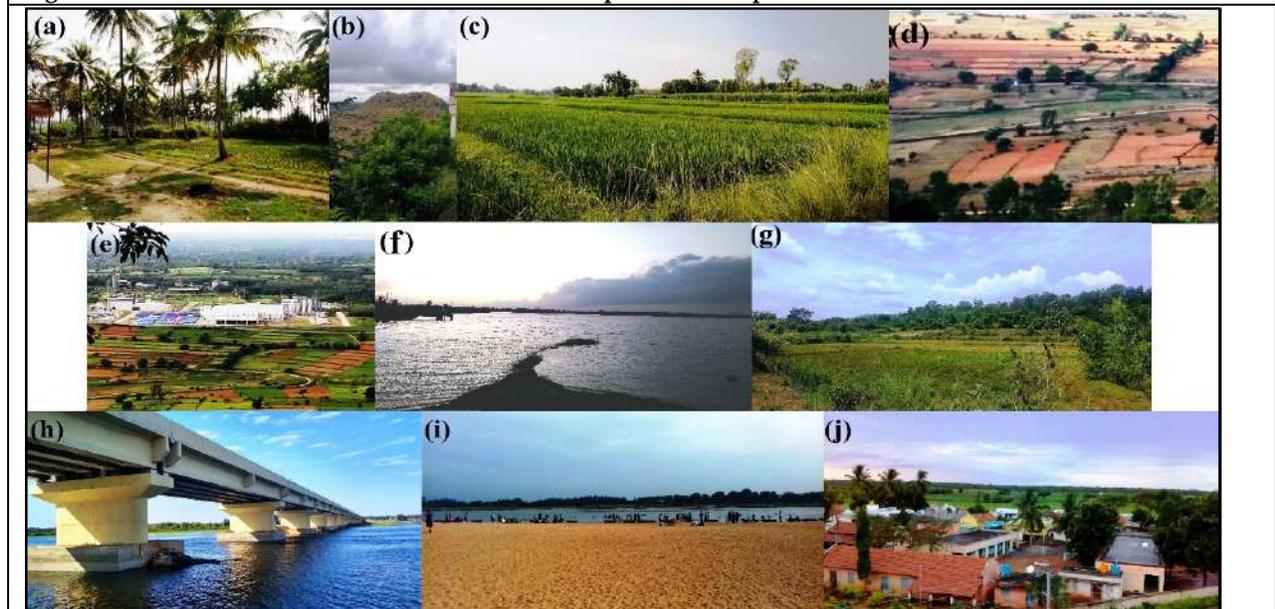


Fig.3. (a) Coconut plantation of Makanahalli village; (b) Barren rocky type in Gavipura village; (c) Cropping pattern of Kadukothanahally village; (d) Fallow land of Vaddara Koppalu village; (e) Industrial area of Vaddara Koppalu village; (f) Bannuru village Lake; (g) Land with scrub at Shivapura village; (h) Perennial River Cauvery near Dasanapura village; (i) Sandy area near Talakadu village; (j) Aerial view of Vattalu village





Impact of Organic Manure and Mineral Nutrient on Growth and Yield of *Spinacia oleracea* (Linn.)

Vaishnavi. V.V¹, T. Mythili² and S. Uma Gowrie^{3*}

¹Research Scholar, Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women (Autonomous), Affiliated to the University of Madras, Chennai - 600 008, Tamil Nadu, India.

²Associate Professor and Head (Self Supporting), Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women (Autonomous), Affiliated to the University of Madras, Chennai – 600 008, Tamil Nadu, India.

³ Research Supervisor, Department of Plant Biology and Plant Biotechnology, Principal and Secretary, Ethiraj College for Women (Autonomous), Affiliated to the University of Madras, Chennai – 600 008, Tamil Nadu, India.

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*Address for Correspondence

S. Uma Gowrie

Research Supervisor,

Department of Plant Biology and Plant Biotechnology,

Principal and Secretary, Ethiraj College for Women (Autonomous),

Affiliated to the University of Madras,

Chennai – 600 008, Tamil Nadu, India.

E. Mail: umasezhian@gmail.com



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ABSTRACT

Green leafy vegetables are highly nutritious and have medicinal benefits due to the presence of phytochemicals they contain. Spinach, which is native to central and western Asia is a good source of vitamin A, C, K and minerals such as magnesium, manganese, folate and iron. These phytochemicals scavenge reactive oxygen and prevent oxidative damage, cancer, obesity and ulcer. However, the cultivation of green leafy vegetables is limited to specific abiotic and edaphic factors. To enhance the growth and yield of *Spinacia oleracea*, a study was conducted using different concentrations (5%, 10%, 20% w/v) of aqueous extracts of Neem cake and Micro, Macro nutrients on different day intervals (15th/30th/45th). The Per cent germination, growth parameters such as length, biomass of shoot, root and yield were calculated. Qualitative and Quantitative phytochemical analysis was carried out. The results indicated that 20% concentration of neem cake had the maximum rate of germination, seedling growth and yield compared to other concentrations of neem cake and micro, macro nutrients. The spinach leaves treated with the aqueous extract of neem cake at 20% concentration also had higher protein content, total chlorophyll and flavonoid content compared to spinach grown in micro, macro nutrients. Therefore, it was concluded that neem cake at 20% concentration can be used as bio-fertilizer to enhance the growth





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and yield of *Spinacia oleracea*. This strategy can also be taken as a part of Integrated Pest Management due to the potential Phytoconstituents present in Neem cake.

Keywords: *Spinacia oleracea*, Phyto-constituents, Neem cake, Micro nutrients, Macro nutrients, Growth and yield.

INTRODUCTION

The Phytochemical and other constituents present in fruits and vegetables are believed to be responsible for their medicinal properties. These constituents have been found to lower the risk of certain ailments including cardiovascular diseases like heart attack and stroke, by working together with vitamins and other nutrients [1]. Local communities rely on medicinal plants for treating infections caused by a range of micro-organisms. The use of plants for medicinal purposes has been practiced since ancient times and different parts of plants have been utilized in the treatment and prevention of various diseases [2]. Due to the emergence of multi-drug resistant pathogens and increasing antibiotic resistance, there is a need to search for alternative treatments, leading to the screening of various medicinal plants. Spinach, a green leafy vegetable that has been used as a food source since ancient times is highly nutritious and beneficial for human health specifically in developing countries where nutrition and health are major concerns. *Spinacia oleracea* belongs to the family Amaranthaceae, is an edible flowering plant that can grow upto 30 cm height and survive over winter in temperate regions. The leaves vary in size and shape with larger leaves at the base of plant and smaller leaves higher up on the flowering stem. The inconspicuous flowers mature into small, hard, dry, lumpy fruit clusters containing several seeds [3]. Neem cake is a residue that is obtained after cold pressing of fruits and kernels of the neem tree and solvent extraction process of neem oil cake. It is a valuable source of organic manure that can be used to enrich the soil. Additionally, neem cake can produce organic acids when mixed with soil, it can reduce soil alkalinity. Micronutrients are minerals that are required in very small quantities and are essential for plant growth and metabolism [4]. Some of the examples are boron, iron, chlorine, manganese, copper, zinc, molybdenum, and nickel. Macro nutrients are nutrients that plants require in larger quantities to support their growth and development. These nutrients provide energy to the plant and some examples include nitrogen, phosphorous, potassium, calcium and sulphur. Hence, the objective of the present study is to evaluate the effect of neem cake and micro, macro nutrients on the growth and yield of *Spinacia oleracea*.

MATERIALS AND METHODS

Collection of Seeds, Neem Cake and Micro, Macro Nutrients

The seeds of *Spinacia oleracea* were obtained from a local seed shop PKR green in Chennai and grown in sterilized soil. Organic manure in the form of neem cake (Go Garden) and micro, macro nutrients (Nutrimax) were purchased from local garden shop in Chennai and added in the soil at varying concentrations of 5%, 10%, 20%. Plant samples were collected on 15th, 30th and 45th day after sowing the seeds. Per cent germination, Growth and yield parameters were calculated. Various concentrations (i.e) 5%, 10% and 20% of neem cake and micro, macro nutrients were amended in soil and distilled water were used as a control treatment. The concentrations of 5%, 10% and 20% with 6 replicates at 15 days intervals was collected.

Bioassay of Germination

Twenty seeds were sown in each replicate at different concentrations (5%, 10% and 20%) of neem cake and micro, macro nutrients. Percent germination was calculated.





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Percent Germination

The average percentage of maximum seeds germinated after treatments were calculated
Percent Germination = No. of seeds germinated/ Total No. of seeds × 100

Growth Parameters**Shoot and Root Length (Growth)**

Both shoot and root length was periodically assessed with a measuring scale and readings are recorded for both control and various concentrations (5%, 10% and 20%) in order to track their development over time [5].

Fresh and Dry Weight of Shoot and Root (Biomass)

The weight of shoot and root of newly harvested seedlings of 15th, 30th and 45th day were measured and the readings were recorded. Additionally, the yield was calculated after harvesting. The seedlings were then dried in the shade for one week and their dry weight was measured after being ground into a powder using a mortar & pestle [6]. The readings were recorded.

Collection of Plant Materials

The Plant material, *Spinacia oleracea* leaf samples was harvested from the house garden in Chennai, Tamil Nadu on 45th day. The harvested sample was washed with distilled water to remove any soil particles and was then stored in plastic zip lock bags for extraction and subsequent analysis [7].

Preparation of Plant Extracts

The freshly collected leaf samples were crushed using mortar & pestle. A portion 0.5g of the plant sample was immersed in 80% acetone to extract chlorophyll while the remaining sample was mixed with water to obtain an aqueous extract for quantifying protein and flavonoids. The mixture was left to rest for 30 minutes, after which it was filtered and used for further analysis. 5 grams of plant sample were ground using acetone as an extraction solvent. The extract was used to estimate the amount of chlorophyll present in the sample and an aqueous extract was used to estimate the amount of protein and flavonoids [8].

Phytochemical Analysis

The aqueous leaf extract were analysed for the presence of phytoconstituents.

Qualitative Analysis of Flavonoids

5ml of dilute ammonia solution were added to a plant extract followed by the addition of conc.H₂SO₄. A yellow colouration absorbed in the extract indicates the presence of flavonoids. The yellow colouration disappeared on standing [9].

Qualitative Analysis of Proteins

Millon's reagent usually yields a white precipitate on addition to a protein solution which turns red on heating [10].

Quantitative Analysis**Quantitative Analysis of Chlorophyll**

About 0.5g of fresh leaves were ground with 80% acetone using mortar & pestle. After this, centrifugation was carried out at 5000 rpm for 5 minutes. The supernatant was collected and optical density was taken in the spectrophotometer at 645nm and 663nm with 80% acetone as the blank solution [11].

Quantitative Analysis of Proteins

Estimation of protein was carried out using Lowry's method. The aqueous extracts of fresh plant material (1gm ground in 10 ml of solvent) were placed in 1ml of 1N Sodium hydroxide at 100° for 5 minutes and 5ml of alkaline copper reagent was added, the mixture was allowed to stand at room temperature for 10 minutes. 0.5ml of Folin-Ciocalteu reagent was added and mixed immediately. After 30 minutes, the absorbance was measured at 750nm





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using spectrophotometer [12]. The amount of protein in the samples were calculated with the standard curve prepared using Bovine Serum Albumin (BSA) or Casein.

Quantitative Analysis of Flavonoids

The flavonoids were estimated using Aluminium chloride method. 1ml of aqueous extract of fresh *Spinacia oleracea* leaves were taken in the test tube to which 0.3ml of 5% of sodium nitrite solution was added. After 5 minutes, 0.3ml of 10% aluminium chloride solution and 2ml of 1 M Sodium hydroxide solution were added. The absorbance of the yellowish coloured solution was measured at 510nm using spectrophotometer(13). The amount of flavonoids was calculated using quercetin as standard.

Experimental Design And Statistical Analysis

The experiment was carried out in a Randomised Block Design (RBD). Six replicates of different concentrations such as 5%, 10% and 20% were employed for the pot culture experiments.

RESULTS AND DISCUSSION

Various concentrations of neem cake and micro, macro nutrients had revealed both stimulatory and inhibitory effect on percent germination, shoot and root length, shoot and root weight(fresh and dry). The highest per cent germination was observed at 20% concentration of neem cake. The germination stage is vital for crop plants and the findings had indicated that higher concentration of neem cake and micro, macro nutrients have an stimulatory effect on the growth of *Spinacia oleracea* (14). The effects of neem cake and micro, macro nutrients on the germination and growth parameters of *Spinacia oleracea* were evaluated (Figure 1-3).

Shoot And Root Length (Growth)

The results indicated that at 20% (27.02 ± 2.94 , 6.09 ± 0.62) concentration of neem cake extract has enhanced the shoot and root length (cm),when compared to the control (22.03 ± 2.32 , 4.72 ± 0.45) and other concentrations. When spinach plants are grown in the soil amended with neem cake, it improves the organic matter of the soil, helps to improve the soil texture, water holding capacity and soil aeration. However, concentrations of 5% and 10% resulted only in moderate growth. On the other hand, the growth was significantly reduced in all the concentrations when micro, macro nutrients were used. From the study it was found that the neem cake extract has promoted effective growth on spinach. These results were consistent with the dry and fresh weight of shoot and root. Therefore, it can be concluded that the neem cake extract can promote the maximum growth of spinach at 20% concentration. The Shoot and root length of *Spinacia oleracea* is depicted in Table 1.

Fresh And Dry Weight Of Shoot And Root (Biomass)

The Fresh and dry weight of shoot and Root of *Spinacia oleracea* were evaluated. The result has indicated that at 20% concentration of Neem cake, the fresh and dry weight of shoot and root was found to be higher when compared to other concentrations. When spinach plants are grown in the soil amended with neem cake, it improves the organic matter of the soil, helps to improve the soil texture, water holding capacity and soil aeration. However, the presence of micro and macro nutrients resulted in a significant reduction in growth across all concentrations. The fresh and dry weight of shoot and root of *Spinacia oleracea* is depicted in Table 2. The rate of mineralisation of different types of organic manure in the soil was reflected by crop growth rate and net assimilation rate which were in fact, the gain in dry biomass of the plant when compared with control.

Quantitative Phytochemical Analysis

The phytochemical analysis was carried out for flavonoids and proteins which are the most important phytoconstituents in *Spinacia oleracea*. The results had indicated the presence of both proteins and flavonoids in all concentrations of the aqueous extract of the plant sample. The phytochemicals are essential for human health, particularly proteins which are very much required for growth, repair and defense of cells and tissues [15,16].





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Quantitative Analysis Of Chlorophyll

The chlorophyll content of both neem cake and micro, macro nutrients treated spinach leaves were estimated. Results indicated that there is an increase in chlorophyll content when neem cake was used in the growth process. Analytical determination of total chlorophyll varies with respect to neem cake and micro, macro nutrients. The highest content of total chlorophyll was found in 20% concentration of neem cake when compared to mineral nutrients. (Figure 4&5). Chlorophyll concentration is an important parameter that is frequently assessed to determine photosynthetic efficiency and mechanism in plant metabolism. It is an antioxidant compound that is found and stored in the chloroplasts of green leafy plants. It serves as an indicator of the health and productivity of the plant [17]. In cancer therapy, chlorophyll or chlorophyll derivatives can be utilized as a photodynamic agent.

Quantitative Analysis Of Proteins

The protein content of *Spinacia oleracea* for the various concentrations (5%, 10%, 20%) of Neem cake used was found to be (195.63± 1.23, 196.58 ± 1.32, 201.63 ± 0.70) when compared to the control (163.69± 1.21). The protein content of *Spinacia oleracea* for the various concentrations (5%, 10%, 20%) of Micro, Macro nutrients used was found to be (188.36± 0.83, 192.54 ± 0.97, 190.71 ± 0.11) when compared to the control (187.45± 0.69) was determined using BSA as standard (Figure 6). The estimation of protein in spinach leaves indicated that in neem cake treated soil at 20% concentration, the plant growth was found to be significant when compared to plants treated with micro and macro nutrients. Protein is an essential nutrient that is necessary as building block for the growth and development. Other than carbohydrates and lipids it is considered as one of the major sources of energy. Higher protein content in *Spinacia oleracea* performs various substitute functions, such as facilitating enzymatic activity, transporting nutrients, and other biochemical processes [18].

Quantitative Analysis of Flavonoids

The total flavonoid content of spinach was determined by calculating the quercetin equivalent using a calibration curve. At a concentration of 20%, the flavonoid content was found to be 601.75± 0.21 mg/g which was higher than the control value of 302.71± 0.61 mg/g and the values obtained at concentrations of 5% (518.66 ± 0.72), 10% (528.91± 0.25 mg/g) when neem cake was used (Figure 7). The flavonoid content of *Spinacia oleracea* leaves for the various concentrations (5%, 10%, 20%) of Micro, Macro nutrients used was found to be (201.69± 0.31, 358.96 ± 0.61, 346.21± 0.72) when compared to the control (352.37 ± 0.53 mg/g) were found in micro, macro nutrients (Figure 6 & 7). The total content of flavonoids indicated that there is a high amount of flavonoid content in neem cake amended plants at 20% concentration when compared to mineral nutrients which has revealed minimum content of flavonoids. Flavonoids are secondary metabolites found in plants, meant for regulation of plant growth, pigmentation, and protection against UV radiation. Higher amount of flavonoid content plays a significant role in Human defense system. Due to their presence in various foods consumed by humans, flavonoids seems to be an essential component used to control inflammation which may prevent cancer [19].

CONCLUSION

From the study, it can be concluded that percent germination, growth parameters, flavonoids and protein content of spinach were enhanced by using 20% concentration of neem cake. The stimulatory effect of neem cake extracts is prominent at higher concentration when compared to the effect of lower concentration. The green leafy vegetable, spinach designated as “ Nature’s Anti-aging wonder” is a rich source of many nutrients and are with potential medicinal values. Application of organic manure improves soil fertility which further enhances crop productivity. Thus, the rate of growth and accumulation of phytochemicals such as chlorophyll, flavonoid and protein content of *Spinacia oleracea* is found to be increased when the soil is supplemented with neem cake as organic manures when compared to plants raised with micro and macro nutrients. The presence of effective phytoconstituents in neem cake have the potential to combat pests in addition to enhance its growth thereby increasing the economic and nutrient value of *Spinacia oleracea*. Hence, amendment of Neem cake in soil can be considered as a component of Integrated Pest Management in cultivation of *Spinacia oleracea*.





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Conflicts Of Interests

The authors declared that they had no conflicts of interests.

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Table 1: Effect Of Neem Cake And Micro, Macro Nutrients On Per Cent Germination Of *Spinacia oleracea*

| Concentration of Neem cake(%) | Shoot Length (cm) | Root length (cm) |
|-------------------------------|-------------------|------------------|
| Control | 22.03±2.32 | 4.72±0.45 |
| 5 | 23.14±1.72 | 5.02±0.25 |
| 10 | 24.41±1.87 | 5.04±0.27 |
| 20 | 27.02±2.94 | 6.09±0.62 |

*Mean±SE

| Concentration of Micro and Macro nutrients(%) | Shoot Length (cm) | Root length (cm) |
|---|-------------------|------------------|
| Control | 20.17±1.63 | 3.12±0.19 |
| 5 | 20.92±1.51 | 3.51±0.13 |
| 10 | 21.07±1.33 | 3.95±0.28 |
| 20 | 22.72±2.39 | 3.07±0.11 |

*Mean±SE

Table 2: Effect Of Neem Cake And Micro, Macro Nutrients On Fresh And Dry Weight Of *Spinacia oleracea*

| Concentration of Neem cake (%) | Fresh Weight Shoot (g) | Fresh Weight Root (g) | Dry Weight Shoot (g) | Dry Weight Root (g) |
|--------------------------------|------------------------|-----------------------|----------------------|---------------------|
| Control | 4.64±0.82 | 0.16±0.01 | 1.10±0.11 | 0.11±0.02 |
| 5 | 7.16±1.01 | 0.3±0.02 | 1.22±0.09 | 0.11±0.02 |
| 10 | 6.86±0.97 | 0.3±0.04 | 1.23±0.04 | 0.11±0.01 |
| 20 | 8.61±1.99 | 0.61±0.17 | 1.79±0.21 | 0.11±0.07 |

*Mean±SE

| Concentration of Micro, Macro Nutrients (%) | Fresh Weight Shoot (g) | Fresh Weight Root (g) | Dry Weight Shoot (g) | Dry Weight Root (g) |
|---|------------------------|-----------------------|----------------------|---------------------|
| Control | 4.61±0.77 | 0.15±0.02 | 1.12±0.14 | 0.9±0.03 |
| 5 | 3.93±0.63 | 0.3±0.03 | 1.23±0.18 | 0.9±0.03 |
| 10 | 3.91±0.72 | 0.3±0.05 | 1.21±0.17 | 0.9±0.01 |
| 20 | 3.27±0.48 | 0.37±0.05 | 1.22±0.16 | 0.9±0.04 |

*Mean±SE





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Figure 1: Growth of *Spinacia oleracea* at 15th Day



Figure 2: Growth of *Spinacia oleracea* at 30th Day



Figure 3: Growth of *Spinacia oleracea* at 45th Day

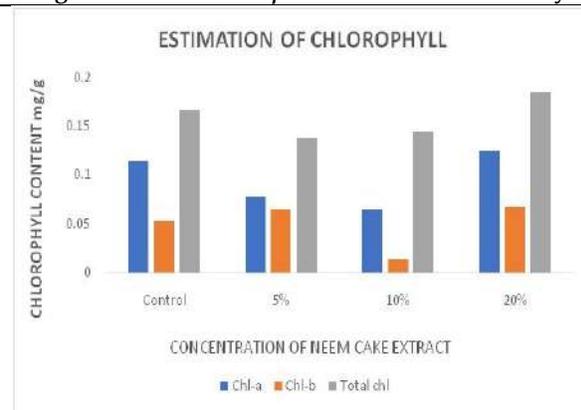


Figure 4: Effect of Neem cake extract on chlorophyll content of *Spinacia oleracea*

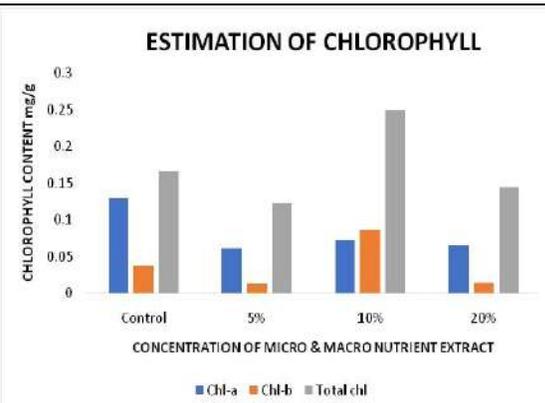


Figure 5: Effect of Micro, Macro nutrients extract on chlorophyll content of *Spinacia oleracea*

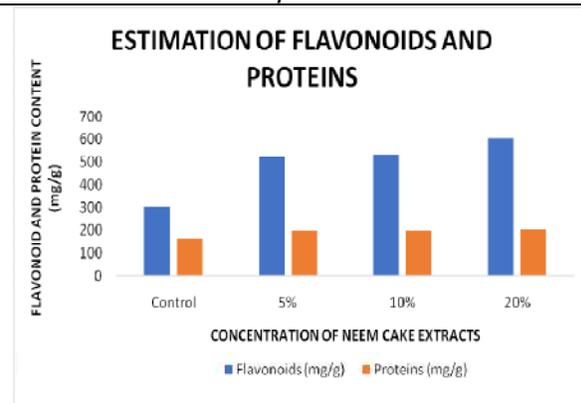


Figure 6: Effect of Neem cake extract on Flavonoid and protein content of *Spinacia oleracea*





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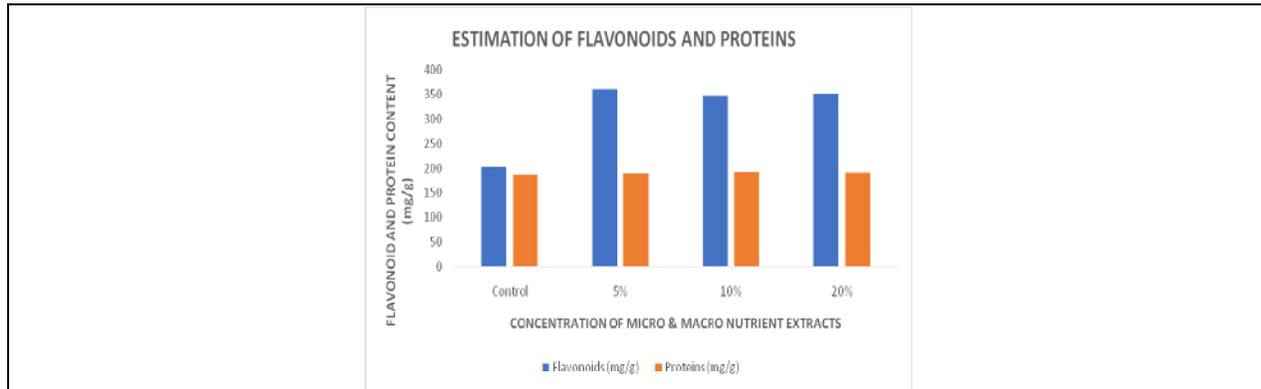


Figure 7: Effect of Micro, Macro nutrients extract on Flavonoid and protein content of *Spinacia oleracea*





A Balanced Software Package Impairment Finding Method in Cloud Situation Overriding Bayes Algorithm

N.Zackariah^{1*} and S.Lavanya²

¹Assistant Professor, PG and Research Department of Computer Science, Presidency College (Autonomous), Chennai, Tamil Nadu, India

²Associate Professor and Head, Department of Software Applications, Thiruthangal Nadar College, Chennai, Tamil Nadu, India

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*Address for Correspondence

N.Zackariah

Assistant Professor,

PG and Research Department of Computer Science,

Presidency College (Autonomous),

Chennai, Tamil Nadu, India

E. Mail: zackariah71@gmail.com



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ABSTRACT

Cloud computing assumes a fundamental part in the IT undertaking. Statistical Process Control cloud outlines sense routine changes and their main drivers are recognized dependent on the differential profiling technique. The majority of the manual overhead brought about in identifying the product changes and the examination time are decreased to a bigger degree gritty investigation of profiling information are not acted in a large portion of the cases. Simultaneously, Trusted Computing Base (TCB) of a figuring hub doesn't accomplish the versatility measure. This work, a Practical Bayes approach concentrates on the issue of identifying programming differences and guarantees adaptability by contrasting data at the current time with authentic information. GenProg utilizes a broad construction of hereditary programming to foster a program variation that holds fundamental usefulness however it isn't powerless against a known inadequacy in cloud. The current programming testing suite distinguishes program absconds in cloud climate. Delta investigating and Structural differencing calculations limit the divergence among variation and the first program as far as least fix. Therefore, Defect Localization dependent on Band (DLB) instrument is acquainted with beat the deformities and rank the diverse OK fixes.

Keywords: Cloud Environment, Variances, Practical Bayes approach, Gaussian blend, Trusted Computing Base, Genprog





INTRODUCTION

Distributed computing is being adjusted and misshaped to another model comprising of administrations that are commoditized and conveyed in a manner similar to ordinary utilities. In a particularly model, clients access administrations dependent on their necessities without knowing from where the administrations are facilitated or how they are appropriated. Distributed computing signifies the framework as a Cloud from which business and customers are capable and capable to get to applications from anyplace on the planet utilizing on request strategies. All IT capacities with applications, organizing, security, extra room and programming are produced for clients to work in assistance, in light of the customer server model. An imaginative far off verification structure called DRAFT as shown in [7] for productive estimating of target framework dependent on a data stream based respectability model. The high uprightness cycles of a framework are first estimated and set up, and these cycles are then bound from gets to start by low trustworthiness measures. A productive cryptographic convention as displayed in [12] that authorizes keystroke respectability by using on-chip Trusted Computing Platform (TCP) forestalls the fake of phony key occasions by malware under sensible suppositions. A sensible supposition that is troublesome in getting to a host's piece and the office to construct application level fine-grained identification arrangements.

Collective provable information ownership plot as displayed in [3] utilizes the methods of Homomorphic evident reactions and hash record chain of importance. Collective neglects to extend more viable and pragmatic CPDP developments. First execution of CPDP plot, particularly for huge records, is genuinely influenced by the bilinear planning activities as a result of high intricacy. Helpful PDP (CPDP) plot demonstrates the security dependent on multi demonstrating zero-information evidence framework in [10], which guarantee solidarity digit however it is influenced by the bilinear planning tasks because of its high intricacy. Also, articulate execution improvement components for CPDP plot present a proficient strategy for recognizing the boundary esteems to decrease the expense required during calculation of customers and capacity specialist organizations. Progressive Attribute Set Based Encryption (HASBE) broadened figure text-strategy Attribute-Set-Based Encryption (ASBE) with a various leveled design of clients. The ASBE conspire as displayed in [15] not just accomplishes adaptability because of its progressive plan, yet in addition acquires versatility and fine-grained gets to oversee in supporting compound ascribes of ASBE. ASBE productively share private information on cloud servers utilizing Hierarchical Identity Based Encryption (HIBE) framework and the Cipher Text-Policy Attribute-Based Encryption (CP-ABE) framework, lastly giving execution expressive compromise as depicted in [12].

The Secure distributed storage framework as portrayed in [10] upholds protection safeguarding public evaluating which performs reviews for a very long time simultaneously and capably. Public auditability for distributed storage is of genuine result with the goal that clients resort to a Third Party Auditor (TPA) as displayed in [13] actually look at the respectability of re-appropriated information. To safely build up an effective TPA, the evaluating system acquires novel weaknesses towards client information security. As portrayed in [5] bread and butter of information legal sciences and post examination in distributed computing is described by giving the data security on touchy archives. Nefeli, a virtual foundation passage as shown in [8] gives organization hints on the likely planning of VM to actual hubs. The presence of conceivable execution bottlenecks, and the presence of hidden equipment highlights. Nefeli research of elective limitation fulfillment ways to deal with address versatility issues present in huge foundations and neglected to offer organization implies that proficiently handle the arrangement of virtual frameworks behind the scenes of genuine huge cloud establishments. SBSE for the cloud as planned in [13] challenges via tending to look through based computer programming. Cloud suppliers share undifferentiated from objectives in decreasing asset utilization; however they are less centered around maintaining their administration level concurrences on interruptions.

A successful shortcoming restriction system would return a main driver utilizing the troubled rundown program components. Albeit existing strategy with issue restriction is successful just on a portion of the cases, unfortunately, for some, different cases, issue limitation techniques are not strong adequate. GenProg has displayed in [15] is a





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computerized technique for fixing deserts in off-the-rack, heritage programs without true condition, program comments, or specific coding rehearses. Primary differentiating calculations and delta investigating decline the contrast between this variation and the exceptional program to a least fix. Underlying drivers are regularly recorded low in the record of most skeptical program components. The lack of quality of issue restriction apparatuses possibly intention numerous designers to doubt issue limitation strategies.

In Practical Bayes (PB) approach, two part Gaussian combinations are utilized to perform deviations. PB screens the monstrous number of cells which is valuable in streaming situations with Bayes per area blunder rate method. A clever component of PB approach is the ability to control deviations that work on the outcome of sharp changes in the peripheral appropriations. The commitment of Practical Bayes approach is to introduce a PB system to recognize programming fluctuations in imbalanced grouped information streams with possibly enormous number of cells. PB system plays out numerous testing utilizing a various leveled Bayesian model and smothers excess cautions caused because of changes in the peripheral appropriations. The construction of paper is as per the following. Segment 1, portrays the Practical Bayes Theoretical Framework. Segment 2, portrays the Defect Localization Mechanism. Segment 3 portrays the PB Experimental Approach with Parametric Factors. Segment 4 portrays the Illustration examination the outcome through table and diagram esteems and area 5 depicts Conclusion.

Applied Bayes Theoretical Framework

Applied Bayes expects to distinguish the product peculiar conduct by looking at information in the current square dependent on notable information. Notwithstanding, PB approach is keen on identifying programming irregular examples as opposed to distinguishing unusual programming records. The plan of PB approach is fixated on the idea of observing factual measures which are registered for blends of distinct characteristics in the data set. Unmistakable trait mixes lead to multi programming's trying at every stretch. To accomplish multi programming testing, Bayes per segment blunder rate is assessed on every cell, where each measurement compares to the levels of an absolute factor.

For simplicity of assessment, the PB approach continues with the presumptions that the multidimensional programming tests comprise of two downright factors with 'A' and 'B' levels individually. 'A' and 'B' levels note the speculation of higher measurements programming differences. Practically speaking, PB takes the addition of the 'A' and 'B' levels for the first and second straight out factors individually at time 't'. Allow X_{ABT} to signify the noticed worth to follow a Gaussian dispersion. Regularly, a specific degree of change is needed for the first information to guarantee estimation of genuine worth. To guarantee surmised programming ordinariness, the counts are seen with the assistance of a square root change. As a rule, PB square root change is indicated as

$$((x + l)^q - 1)/q \quad (1)$$

In (1), 'l' and 'q' are picked to 'balance out' the change and relies upon the mean suggested in programming inconsistency identification. Besides, 'q' is obliged to lie somewhere in the range of 0 and 1, and $q \neq 0$ infers a log change while distinguishing the product differences. Truth be told, the upsides of these boundaries are picked with a sensible worth with the assistance of the underlying preparing information. For time stretch's' PB approach identifies the product fluctuations subsequent to managing the progressions with negligible means. The course of PB approach begins with the conventional meaning of programming peculiarity followed by the component Practical Bayes approach clarified with the assistance of a calculation.

Detailing of PB Software Anomaly

Consider a 2*2 table, the levels of the line include being I, J and the levels of the section highlight being I, j individually. PB signifies the 4 cell passages relating to (Xx, Xy, Yx, Yy) by a vector of length 4.

$$\begin{matrix} Xx & Xy \\ Yx & Yy \end{matrix} \quad (2)$$





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Two qualities are estimated in PB way to deal with investigate the versatility issue, in particular, the normal qualities and the noticed qualities. The deviations subsequent to changing the progressions in the line and segment implies are (0, 0, 0, 0), guaranteeing that it creates no product fluctuations in cloud climate. The huge qualities in the PB approach and the non-changed changes are depicted to be a drop in the primary column mean and an ascent in the subsequent line mean. Henceforth, non-changed cell alteration contains repetitive data which brings about a circumstance where the adapting to edges is desirable.

Let C_{t-1} mean the current verifiable data up to time $t-1$. Deviations at time t are recognized by contrasting the noticed qualities X_{ABT} and the comparing back scientific Practical Bayes conveyances. The PB expected dispersion of information at time t depends on current notable information until $t-1$. Gaussian combination with Practical Bayes mean and fluctuations is registered as given beneath:

$$\mu_{ABT} = P(X_{ABT}|C_{t-1}) \quad (3)$$

$$\sigma_{ABT}^2 = Var(X_{ABT}|C_{t-1}) \quad (4)$$

μ_{ABT} represent the mean of PB approach and σ_{ABT}^2 represent the variance factor of the PB approach. X_{ABT} denote the observed value which is assumed to follow a Gaussian distribution mixture. The Gaussian means current chronicled data up to time $t-1$ for itemized investigation of profiling information in cloud climate.

The focal thought of the Hierarchical Bayesian model is to characterize the imbalanced information streams. $H_{mixture}$ assumes Δ_{ABT} which are arbitrary examples from Gaussian blend dispersion at time ' t ', GM_t . The form of GM_t is known yet relies upon the thickness rate boundary. A Bayesian various leveled model is one that is composed with programming particularity. It is frequently valuable to think about the investigation of information streams utilizing PB inside-unit examination, and one more model for the across-unit investigation. Within unit model portrays the conduct of individual respondents throughout run time, while the across-unit examination depicts the thickness and intricacy of the units. The two models, inside-unit and across-unit b consolidate to frame the various leveled model, and Practical Bayes calculation is utilized to incorporate the pieces commonly and report for all the susceptibility and changes.

A Practical Bayes approach causes derivation about Δ_{ABT} by utilizing estimation of the hyper boundaries to play out the deviations. The product derivation is acquired by mathematically incorporating regarding the back of Δ_{ABT} using a versatile Gaussian combination quadrature. PB Gaussian back appropriation of Δ_{ABT} depends straightforwardly on δ and in a roundabout way on the other δ 's through the back of the hyper boundaries to diminish the intricacy issue. For the most part, such transformation of solidarity makes the back method for Δ_{ABT} regress toward one another and naturally assembles punishment for directing different programming tests. The essential model of PB approach is dissected lastly thickness rate and programming peculiarity recognition proportions are assessed. The cell punishment increments with programming prescient fluctuation. Additionally, the overall punishment of the method at time ' t ' relies upon the hyper boundaries which are unsurprising from information.

Info: Cloud servers with programming's Yield: Detect Software changes in cloud climate Start Stage 1: Produce μ_i for Gaussian appropriation 'G'

Stage 2: Each information from preparing information and fitted with a typical Gaussian circulation

Stage 3: Mean, Median and difference of cells in numerous product testing is created

Stage 4: For every I , recreate $H(\mu_{ABT}, \sigma_{ABT}^2)$

Stage 5: At time ' t ', PB chooses the information streams, and afterward programming fluctuations are created.

Stage 6: Find the product fluctuations at time ' t '

Stage 7: Hierarchical Bayesian Model arranges the imbalanced information streams till the last line and section.





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End On a fundamental level, PB model gives a gauge of back Gaussian prescient means and changes to obtain μ_{ABT} , and σ_{ABT}^2 . Nonetheless, explaining on fitting Gaussian combination on PB approach has been picked and the product is prepared logically by the client. Likewise, to be valuable in information streaming situations, the PB model is handily adjusted to new information. Gaussian combination viably catches C_t and identifies the product fluctuations in cloud climate. Then, at that point, the back prescient mean μ_{ABT} is the example mean and the back prescient variance σ_{ABT}^2 is supplanted by its assessor s_{ABT}^2 for compelling change on each X_{ABT} . To change impacts, a different Gaussian combination is kept up with for each circling.

PB Experimental Approach with Parametric Factors

Execution metric for assessment of PB approach is estimated as far as runtime, programming inconsistency recognition proportion, CPU usage and thickness rate. Runtime factor is characterized as the measure of time burned-through to play out the product differences location, estimated as far as seconds. Versatility factor estimates the nature of administrations gave utilizing the Practical Bayes approach, estimated as far as rate. The quality of a PB framework is that it depicts its ability to adapt and play out an expanded recognition administration. Programming abnormality identification proportion in PB is estimated as the measure of time burned-through to play out the procedure on cloud utilizing Gaussian combination to recognize the differences while the CPU usage is measure of CPU cycles gone through to play out the changes discovery activity, estimated as far as Kilobits each second. At last, the thickness rate is the normal speed of recognizing the fluctuations, estimated as far as rate.

RESULT AND DISCUSSION

Practical Bayes (PB) approach in cloud climate is looked at against the current Statistical Process Control (SPC) system and Trusted Computing Base (TCB) with Open Stack model on Amazon EC2 dataset. The exploratory worth through table and chart depicts the product fluctuations discovery parametric variables on cloud environment.

Table 2 and fig 5 illustrate the software anomaly detection ratio is measured based on the Run Id count. PB approach improves the detection ration by 3 – 8 % when compared with SPC Framework [1] because square root transformation is adequate, for proportions arcsine which detect the software anomaly. In PB approach, software track changes in the margins separately using simple process control techniques and run both adjusted and unadjusted versions and results in 7 – 17 % improved detection ratio when compared with TCB [2].

Table 4 and fig 6 illustrate the CPU utilization based on the File size. The size of file is measured in terms of Kilobytes (KB). Practical Bayes approach used in process control estimate Δ_{ABT} with δ_{ABT} and declare the ab^{th} cell of a software anomaly to reduce the CPU utilization. Deviations at time t are detected by comparing the observed values with the corresponding posterior analytical Practical Bayes distributions, so that it results in minimum CPU utilization of 15 – 32 % when compared to SPC Framework [1] and reduces from 8-18% when compared with TCB [2].

Table 5 and fig 7 illustrate the density rate which is measured based on the performance counter. The performance count ranges from 10, 20, 30...up to 70. The density rate is improved by imbalanced classified data stream from call logs that are added to the current database. When monitoring cells for deviations, the PB approach regulates the marginal statistics and increases density rate by 8 – 14 % when compared with SPC Framework [1]. Software variances which are direct consequences of changes in a small number of margins are detected with Gaussian mixture, and improve the density rate from 4 – 8 % when compared to the TCB [2].

CONCLUSION

In this paper, a product changes recognition model called the Practical Bayes approach is introduced which means to identify the product fluctuations in gigantic imbalanced characterized information streams. The upsides of these components from a guidance set of imperfection assemble a discriminative model utilizing AI. DLB removes the





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components in cloud that are conceivably related for successful positioning. The PB structure then, at that point, lessens excess by changing negligible changes and takes care of the various programming testing issues utilizing progressive Bayesian model inside a choice hypothetical system. PB approach demonstrates the predominance of H_mixture through reenactment utilizing the two part Gaussian blend for deviations in cloud climate. Moreover, the PB approach chips away at consolidating changed and unadjusted H_mixture to naturally produce programming fluctuations identification. The Fault distinguish instrument is then utilized as an arranged rundown of program components arranged dependent on their probability. The strategies regularly change program runtime states efficiently to limit flawed program components. It centers on issue limitation apparatuses that look at and right and flawed executions. Trial result accomplishes the 9.256% negligible runtime and CPU use. PB additionally further develops the thickness rate, versatility, and programming inconsistency recognition proportion on Amazon EC2 dataset.

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Table 1. Tabulation of Runtime

| No. of users | Runtime (sec) | | |
|--------------|---------------|-----|-------------|
| | SPC Framework | TCB | PB approach |
| 5 | 95 | 81 | 77 |
| 10 | 135 | 120 | 107 |
| 15 | 217 | 202 | 186 |
| 20 | 242 | 221 | 211 |
| 25 | 426 | 386 | 346 |
| 30 | 536 | 481 | 446 |
| 35 | 836 | 797 | 727 |

Table 4. Tabulation for CPU utilization

| File Size (KB) | CPU utilization (Kbps) | | |
|----------------|------------------------|------|-------------|
| | SPC Framework | TCB | PB approach |
| 35 | 6662 | 5651 | 4640 |
| 62 | 6735 | 6730 | 5720 |
| 90 | 7535 | 6526 | 5821 |
| 124 | 7852 | 6384 | 5920 |
| 189 | 8165 | 7155 | 6147 |
| 225 | 8232 | 7515 | 6596 |
| 387 | 9550 | 8528 | 7523 |

Table 5. Tabulation for Density Rate

| Performance Counter | Density Rate (%) | | |
|---------------------|------------------|-----|-------------|
| | SPC Framework | TCB | PB approach |
| 10 | 60 | 64 | 67 |
| 20 | 67 | 68 | 72 |
| 30 | 77 | 78 | 82 |
| 40 | 75 | 80 | 87 |
| 50 | 76 | 82 | 86 |
| 60 | 77 | 82 | 87 |
| 70 | 84 | 88 | 94 |





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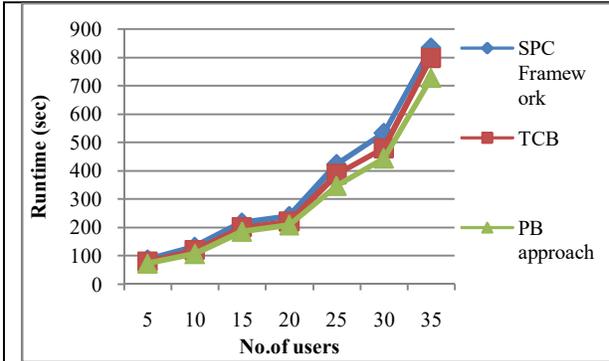


Fig 1. Measure of Runtime

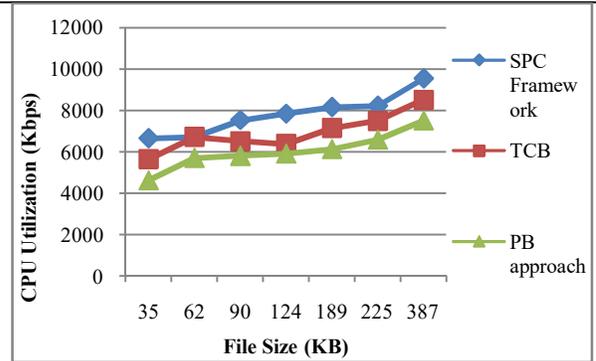


Fig 6. Performance of CPU utilization

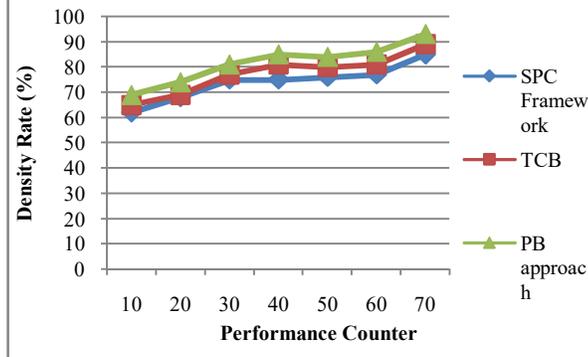


Fig 7. Density Rate Measure





A Survey on Awareness about Non Alcoholic Fatty Liver Disease among Indian Physiotherapists and Recommendations for Developing Structured Exercise Protocol

Sedhunivas R¹, Sridevi S^{2*}, Venkatesh N³ and Senthil Kumar T²

¹Former Post graduate student (SRIHER) and currently as Assistant Professor, Garden City University, Bangalore, Karnataka, India.

²Associate Professor, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

³Professor, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India

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*Address for Correspondence

Sridevi S

Associate Professor,

Sri Ramachandra Institute of Higher Education and Research,
Chennai, Tamil Nadu, India.

E.Mail: devibsmoorthy@sriramachandra.edu.in



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ABSTRACT

Non Alcoholic Fatty Liver Disease (NAFLD) is a common complication of obesity, associated with serum hypertriglyceridemia. There is a huge lacunae in exercise protocol for Indian Population and also awareness among Indian physiotherapists and structured Exercise protocol for NAFLD are yet to be established. This study aims to conduct a survey on awareness about NAFLD among Indian physiotherapists and provide recommendations for developing structured exercise protocol. A literature review was done looking forward for exercise protocols in available evidences between time period (Years 2013-2020). A cross sectional (survey) study was conducted online through WhatsApp and via emails among practicing Indian physiotherapists during period (April 2021 –June 2021). Awareness of NAFLD and exercise protocols prescribed were surveyed . Based on the responses of the survey and available evidences, recommendations for a structured exercise protocol was prepared . Data were collected from 112 physiotherapists and analyzed. 102 responses were finally analyzed and 10 were excluded. Overall, on awareness of NAFLD among physiotherapists, all of them had awareness on role of physiotherapy on obesity ,75 (73.5%) of them were aware of obesity related NAFLD,60 (58.8%) of them were aware of non obesity related NAFLD. Among them only 32 physiotherapists got opportunity to treat NAFLD patients and hence exercise protocols obtained from them were further analyzed. There is a need to create awareness and also develop structured exercise protocol for NAFLD Indian population.

Keywords: Awareness, Indian physiotherapists, Nonalcoholic fatty liver disease, Recommendations, Structured exercise protocol.



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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the commonest hepatic diseases, with macro vesicular fat deposition in more than 5% of hepatocytes at the lack of any secondary cause of hepatic steatosis, such as insignificant alcohol consumption (more than 10 g/day for women and 20 g/day for men), viral hepatitis, drugs induced steatosis, or any other cause (1). NAFLD is the most obesity linked complication, presented with serum hypertriglyceridemia and defects in liver lipoprotein metabolism(2). The severity of NAFLD was directly linked to anthropometric measurements inclusive of Body Mass Index, waist hip circumference, subcutaneous fat accumulation(3). The NAFLD is prevalent about 20-35% in the Western community and about 19-32% in Indian population, with the greater prevalence (70-90%) in obese and diabetic individuals.(4) Unhealthy dietary patterns and poor physical activity poses a major risk factor for Asian Indians to develop NAFLD. Evidences collected from various descriptive studies sources suggests that NAFLD may be linked with elevated risk of atherosclerotic deposits and cardiovascular disorder. Lifestyle interventions consisting in restriction of calorie intake, increasing physical activity weight reduction remain the cornerstone of treatment(5).

Mainstay protocol in NAFLD management consists of exercise training and physical activity. Therapeutic improvements have been with regular exercise by significant reduction in hepatic steatosis, improvements in energy expenditure, skeletal lipid oxidation, also decrease in total and abdominal adiposity and free fatty acid flux to liver. Regularly exercising obese NAFLD individuals have much reduced liver enzymes. Controlled diet intake along with exercise protocols collectively called as life style modification reduces intrahepatic triglycerides and proven to significantly improve the metabolism among NAFLD patients (6). Oh et al stated that hepatic functioning is improved by exercise training by reducing the amount of visceral adipose fat and body weight. Many western studies are available on exercise protocol for patients with NAFLD which would not be feasible for Indian population. Lack of Indian studies denotes there is lack of awareness among Indian physiotherapists also. Thus there is a huge lacunae in Indian Population and structured Exercise protocol for NAFLD yet to be established.

MATERIALS AND METHODS

Research design: Cross sectional study (online survey).

Ethical approval:

This study was approved by Research Advisory Committee of Faculty of Physiotherapy, Sri Ramachandra Institute of Higher Education And Research, Chennai.

Ethical approval no: CSP/21/AUG/98/434

A literature review was performed on available previous studies from the time period of years (2013-2020). The exercise protocols by various authors were considered and analyzed. An online survey was conducted from period of (27.4.2021-5.7.2021) among practicing Indian physiotherapists. Survey site was determined as online social platform through a Google form (<https://forms.gle/CztxpQGfxA2CD666>) and was executed through Online networking sites such as Facebook, What's app, email etc. Since it is an online survey we decided to accept maximum number of responses, but a minimum target of receiving 100 responses also was set. After obtaining prior informed consents, to participate in the study, the physiotherapists were allowed to fill the survey on awareness of NAFLD and its exercise protocols adapted. The questionnaire was developed with the aim of receiving appropriate responses that was in need for the study consisting mainly of basic details of participants including their experience in the physiotherapy field and also awareness and knowledge on NAFLD and exercise protocols for it. The questionnaire consisted of 33 questions enquiring the awareness of role of physiotherapy in obesity, obesity related NAFLD, non-obesity related NAFLD along with experience of treating NAFLD patients anytime in their career. The questionnaire also focused on questions related with framing exercise protocols for these patients according to FITT principle. Nutritional aspects, quality of life and barriers faced by exercising patients components were also added and enquired. The questionnaire was refined with a panel of experts before it was duly circulated in social platform.



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Then both the literature review and obtained survey data were taken in to account and proper recommendations for developing structured exercise protocol was provided.

RESULTS

The collected data were statistically analyzed through SPSS version 25.0 for descriptive statistics. Among the responses the physiotherapists who had both awareness of role of physiotherapy in obese related NAFLD and also treated NAFLD patients the exercise protocols described by them were further analyzed to maximum cumulative percentage. Among 112 responses received, 3 of them didn't give consent to the study, and 7 of them stated that they weren't practicing in India. Thus in total of 112 responses, 102 responses were finally taken for analysis. 44 physiotherapists stated that they are working in multidisciplinary hospitals and 37 of them are in private practice and 21 physiotherapists are in teaching hospitals. Among experience wise 25 of them were 1-5 years of experience, 3 of them were of 6-10 years of experience and 2 of them were 11-15 years of experience and 2 of them stated that they are experienced of 15 years and above.

DISCUSSION

Awareness on NAFLD is the need of the 21st century where in modern world technological advancement and excess intake of fatty junk food in combination with lack of physical activity increases the fat deposition in peripheral and abdominal areas and also among hepatocytes in liver. So in solution for increasing prevalence of NAFLD in India, an awareness survey and literature review was planned to devise exercise protocol suitable for Indian population. Thus extensive literature search was done for years (2013-2020) looking forward for exercise protocols, but only 24 international and 2 Indian Randomized controlled trials were found. A survey was conducted thorough social media Google forms were sent to about 1000 physiotherapists only 112 responses received owing to ignorance and lack of interest to participate in the study and this also denotes the lack of awareness on NAFLD among them. 102 responses was selected and on further analysis, Overall, all of them had awareness on role of physiotherapy on obesity, 75 (73.5) of them were aware of obesity related NAFLD, 60 (58.8) of them were aware of non-obesity related NAFLD. This concludes that average awareness is present among Indian physiotherapists, but there is also lack of experience among them on treating patients with only 32 physiotherapists were aware of obese related NAFLD and had also treated NAFLD patients and thus exercise protocols adapted by them were taken up for analysis.

Majority of the age of patient as observed by physiotherapists were between 31-50 years and Body mass index (51% patients) mostly with overweight and obese category (BMI 25-30) which denotes patients generally present to the treating physicians between fourth and sixth decade of life and also in advanced stage, which makes the resolution of condition difficult. 50% physiotherapists have recommended aerobic training is effective than resistance exercise in the most similar form of track walking(34.4%) which is in accordance with authors Mohammed Ibrahim et al., 2019 and A shamsoddini et al.,2019 who have concluded the same (7). Interval exercise training is being recommended by Indian physiotherapists and also evidence supports that high intensity interval training is more effective in reducing liver fat and improving quality of life(6,8). We found that higher evidences concluding that there was of reductions in liver enzymes and biomarkers when any exercise training performed for 12 weeks or more (8-11).

Though similar features have been observed in both survey results and literature review in terms of frequency of exercise sessions (3 sessions/week) and intensity of resistance exercises (1-RM), also several Variations have been noted which recommends moderate intensity (40-60% of THR) and moderate duration(20-30mins) of aerobic exercises, but the available evidences suggest higher intensity(60-80% of THR) for longer duration (30-40 mins) but literatures support more of equipment based training irrespective of aerobic or resistance training Equipment based training would be practically difficult for lower economy population of India, so the needs of equipment -free exercise training has to be worked out and this present study recommends exercise protocols for 3 days/week for



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Indian population in the form of aerobic exercises (60-80% of THR , duration of 30-40 mins) and resistance exercises(1-RM, 10 reps x 3sets) probably more of track walking and free weights.

LIMITATIONS AND RECOMMENDATIONS

The expected number of responses weren't received probably to due inexperience and lack of awareness on NAFLD among physiotherapists in treating NAFLD patients and also exercise protocol could only be face validated. We recommend the above exercise protocol can be taken up for content validation in future studies.

CONCLUSION

We recommend combined interventions inclusive of structured exercises in form of interval training, diet modification and physical activity.

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Table 1: Exercise Protocols Prescribed By Physiotherapists Who Had Treated Obesity Related NAFLD

| Exercise Protocols | Total Numbers (n) | Percentage (%) |
|--|-------------------|----------------|
| Frequency of Exercise Sessions | | |
| 2days/Week | 05 | 15.6 |
| 3days/Week | 20 | 62.5 |
| 5 Days/Week | 01 | 3.1 |
| 7 Days/Week | 06 | 18.8 |
| Treatment Interventions | | |
| Structured Exercises | 03 | 9.4 |
| Physical Activity | 04 | 12.5 |
| Diet Modification | 02 | 6.3 |
| All Of Above | 22 | 68.8 |
| Others | 01 | 3.1 |
| Type of Structured Exercises | | |
| Aerobic Exercise | 16 | 50.0 |
| Resistance Exercise | 01 | 3.1 |
| Flexibility Exercise | 0 | 0 |
| All Of The Above | 14 | 43.8 |
| Others | 01 | 3.1 |
| Type of Aerobic Exercise | | |
| Track Walking | 11 | 34.4 |
| Treadmill Walking | 07 | 21.9 |
| Cycle Ergometry | 04 | 12.5 |
| Jogging | 05 | 15.6 |
| Others | 05 | 15.6 |
| Intensity for Aerobic Exercises | | |
| 40%-60% Of THR | 25 | 78.1 |
| 60%-80% Of THR | 07 | 21.9 |
| Duration of Exercise per Session for Aerobic Exercises | | |
| Less Than 20 Mins | 05 | 15.6 |
| 20-30 Mins | 14 | 43.8 |
| 30-40mins | 10 | 31.3 |
| 40-60 Mins | 02 | 6.3 |
| Above 60 Mins | 0 | 0 |
| Others | 01 | 3.1 |
| Intensity for Resistance Exercises | | |
| 1-RM | 16 | 50.0 |
| 10-RM | 14 | 43.8 |
| Others | 02 | 6.2 |
| Type of Resistance Exercise | | |
| Free Weights | 16 | 50.0 |
| Body Weight | 09 | 28.1 |
| Plyometrics | 05 | 15.6 |
| Others | 02 | 6.3 |
| Repetition for Resistance Exercises | | |
| Less Than 10 Reps | 10 | 31.3 |
| 10-20 Reps | 17 | 53.1 |
| 20-30 Reps | 05 | 15.6 |
| More Than 30 Reps | 0 | 0 |





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| | | |
|------------------------------|----|-------------|
| Type of Flexibility Exercise | | |
| Static Stretching | 08 | 25.0 |
| Dynamic Stretching | 07 | 21.9 |
| Stretch, Hold And Relax | 13 | 40.6 |
| Ballistic Stretching | 02 | 6.3 |
| Others | 02 | 6.3 |
| The Type of Training | | |
| Circuit | 08 | 25 |
| Interval | 20 | 62.5 |
| Continuous | 02 | 6.3 |
| Fartlek | 01 | 3.1 |
| Others | 01 | 3.1 |

THR-“Target Heart Rate”; RM-“Repetition Maximum”; Reps-“Repetitions”

Table 2: Recommended FITT Exercise Protocol

| | |
|-----------------------|---------------------------------|
| Aerobic Exercises | Recommendations |
| Frequency | 3days/Week |
| Intensity | 60%-80% Of THR |
| Time | 30-40mins |
| Type | Track Walking, Jogging, Running |
| Resistance Exercises | Recommendations |
| Frequency | 3 Days/Week |
| Intensity | 1- RM |
| Time | 10 Reps X 3 Sets |
| Type | Free Weights |
| Flexibility Exercises | Static Stretching |

THR-“Target Heart Rate”; RM-“Repetition Maximum”; Reps-“Repetitions”

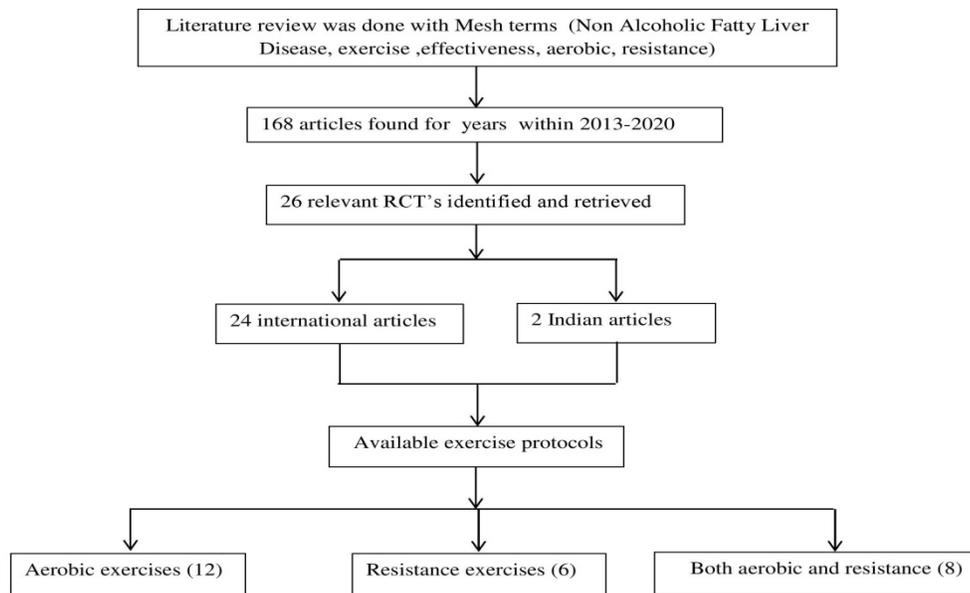


Figure 1: Flowchart-Literature Review





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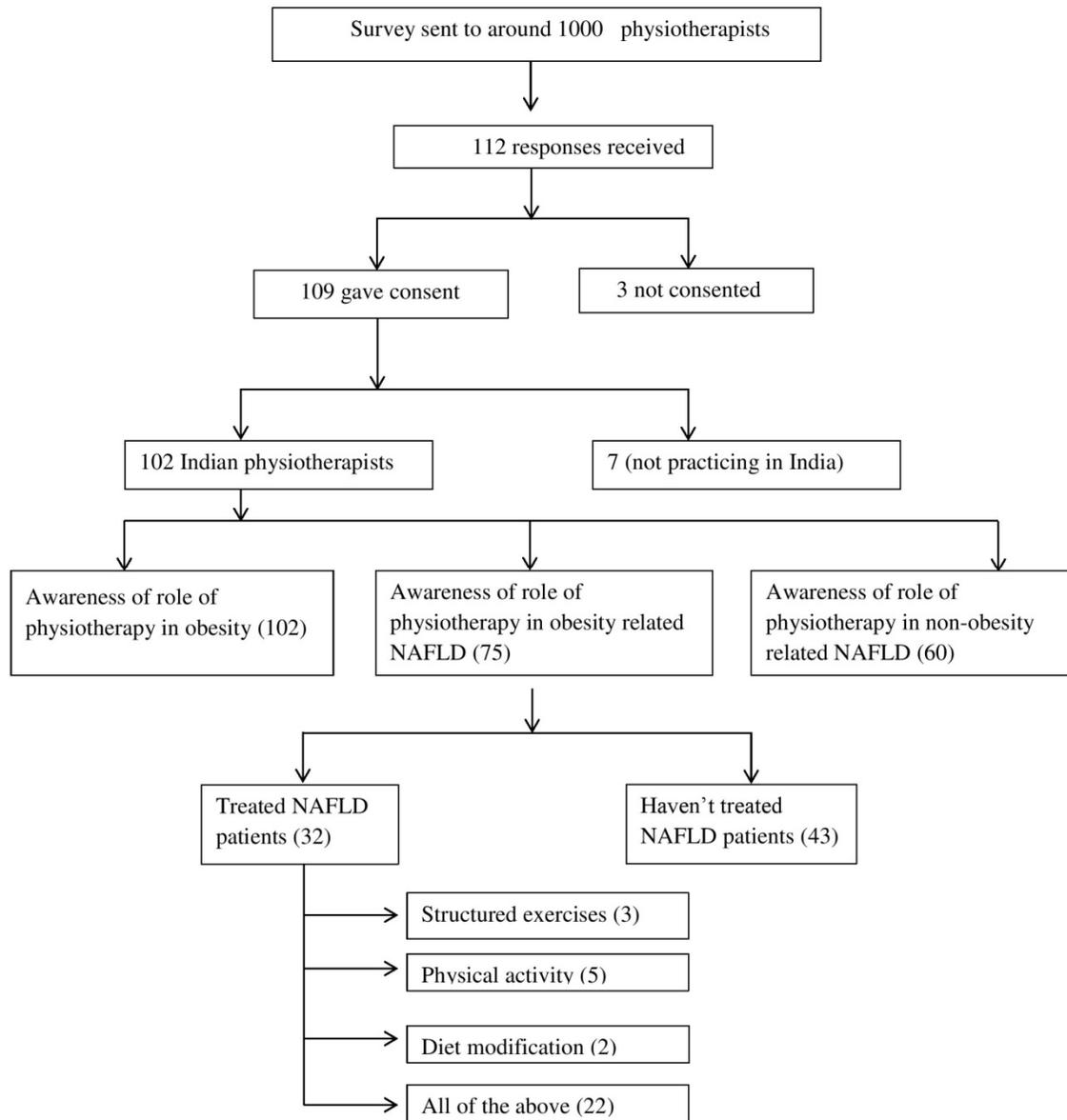


Figure 2: Flowchart-Survey





Determination of Biochemical Components of Fish Scales and Antibacterial Activity of Chitosan Extracted from Fish Scales

Pragathi. A.H¹ and C.M. Noorjahan^{2*}

¹Research Scholar, P.G and Research Department of Zoology, Justice Basheer Ahmed Sayeed College For Women (Autonomous), Teynampet, Chennai-600 018, Tamil Nadu, India.

²Assistant Professor, P.G and Research Department of Zoology, Justice Basheer Ahmed Sayeed College For Women (Autonomous), Teynampet, Chennai-600 018, Tamil Nadu, India.

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*Address for Correspondence

C.M. Noorjahan

Assistant Professor,

P.G and Research Department of Zoology,

Justice Basheer Ahmed Sayeed College For Women (Autonomous),

Teynampet, Chennai-600 018,

Tamil Nadu, India.

E.Mail: cmnoorjahan@gmail.com



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ABSTRACT

This research focused on the biochemical components of fish scales and antibacterial activity of chitosan extracted from scales of fish. Aims and objectives of the present investigation is to determine the amount of biochemical components such as protein, carbohydrates, lipid and ash present in fish scales, to extract the chitosan from the fish scales of *Tilapia mossambica* and to determine the antibacterial activity of chitosan extracted from fish scales against *Staphylococcus aureus* and *Pseudomonas* sp. The results of the estimation of biochemical components of the fish scales revealed that the amount of protein was higher than the carbohydrates, lipid and ash. The antimicrobial assay showed the potential of chitosan as an antibacterial agent.

Keywords: *Tilapia mossambica*, Fish scales, Biochemical components, Chitosan, Antibacterial activity.

INTRODUCTION

Marine waste management has been one of the problems having the greatest impact on the environment. Food industry waste is an important source of environmental contamination source. Around 33% , inevitability rises the amount of waste produced including head, spine, tail, viscera, skin and scale. The waste of fish processing industries have been discarded [1]. Chitosan can be utilized in various industrial applications. Its main features include



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biocompatibility, biodegradability, antibacterial, emulsifying and chelating properties and non-toxicity [2]. The benefits provided on consumption of chitosan are innumerable, eg., Regulation of the digestive system, fat absorption prevents its absorption by the body and reduction of sugar and cholesterol level in the body. Besides the series of its advantages, benefits and applications of chitosan, one underlines lack of information related to chitin extraction and chitosan deacetylation from fish scales [3],[4]. Hence the present study was undertaken to estimate the biochemical components -Protein, Carbohydrate, Lipid and Ash present in the fish scales quantitatively and to extract chitosan from the scales of *Tilapia mossambica* and to determine the antibacterial activity of chitosan against *Staphylococcus aureus* and *Pseudomonas* sp.

MATERIALS AND METHODS**MATERIALS****Collection of Samples**

Fishes *T. mossambica* were collected from the local market in Chennai, Tamilnadu, India. Fishes were washed with tap water to remove dust particles. Scales were scraped out from the body of the fishes, dried in an oven at 50°C for 12 hours and stored in sealed plastic bag for further study.

Collection of Microorganisms

The bacterial cultures *Staphylococcus aureus* and *Pseudomonas* sp were procured from Girishwari hospital, Chennai, Tamilnadu, India.

METHODS**Determination of the Bio-chemical components of fish scales**

The quantity of protein present in the fish scales were carried out by following the procedure [5] using Folin phenol reagent. The quantity of carbohydrates present in the fish scales were carried out as per the procedure [6]. The quantity of lipid present in the fish scales were carried out by following the method [7], [8]. The quantity of ash present in the fish scales were carried out [9].

Extraction of Chitosan from fish scales

The extraction of chitosan from fish scales were carried out by following the procedure [10].

Demineralization

Demineralization was carried out by adding 1 L of 0.5 M hydrochloric acid aqueous solution in 50 g of dried scales sample. The mixture was kept under stirring at 750 rpm for 2 hrs at 25°C. The scales were filtered, washed with water until neutrality and oven dried for 12 hrs at 30° C [11].

Deproteinization

The demineralized dried material was added to 100 mL of 1% sodium hydroxide aqueous solution under stirring at 250 rpm for 3 hrs at 50° C. Subsequently the material was filtered, washed with water until neutrality and oven dried for 12 hrs at 30°C [11].

Depigmentation and Deodorization

The deproteinized dried material was transferred to a 2 L beaker and treated with 1% sodium hypochlorite aqueous solution under stirring at 500 rpm for 2 hrs at 25° C. Subsequently, the material was filtered and washed with water until neutrality and oven dried for 12 hrs at 30° C. The dried material was named as crude chitin [11].



**Pragathi and Noorjahan****Deacetylation**

Deacetylated chitosan was obtained by suspending 10 gms of crude chitin in 400 mL of 40% NaOH aqueous solution, then kept under stirring for 6 hours at 117° C. The mixture was filtered, solid materials were washed with water until neutrality and chitosan was obtained. Then chitosan was washed with 30 mL of acetic acid (CH₃OH) and dried in an oven for 12 hours at 30° C. White coloured powdered form of chitosan was finally obtained [11].

Antibacterial Activity

Antibacterial activity of the extracted chitosan sample against two isolates of bacteria namely *Staphylococcus aureus* and *Pseudomonas* sp were carried out by following the agar well diffusion method [12].

Preparation of Culture Inoculum

The test organisms (*Staphylococcus aureus* and *Pseudomonas* sp) were inoculated in the nutrient agar medium and incubated for 24 hrs at 37° C and stock cultures were maintained. Active cultures of the isolates for experiment was prepared by transferring a loopful of culture from the stock culture to the tube containing nutrient broth and incubated at 37°C for 24 hrs.

Agar well diffusion method

The effect of chitosan on the test organisms were assayed by agar well diffusion method. Nutrient agar was poured into the petriplates aseptically and was allowed to solidify. The lawns of the test bacterial strain was done with the help of sterile cotton swab. Wells were made with the help of sterile micro pipette tips and the cut agar discs were removed aseptically with sterile needles. Four different concentrations (50 µg/µl, 100 µg/µl, 150 µg/µl and 200 µg/µl) of chitosan were added into the wells. The test plates were incubated aerobically at 37°C for 24 hrs. After incubation, the results were recorded as the presence or absence of inhibition zone formation. The antibacterial activity of chitosan was assayed by measuring the diameter of the inhibition zone formed around the wells.

STATISTICAL ANALYSIS

The data obtained from the above experiments were expressed as Mean and Standard deviation.

RESULTS AND DISCUSSION**Determination of the Biochemical components of the fish scales**

Tilapia mossambica is an cichlid fish native to southeastern Africa. Due to human introduction, it is now found in many tropical and subtropical habitats around the globe. It is a popular fish for aquaculture because it rapidly adapts to new situations. Hence *Tilapia mossambica* was selected for this study. The results of determination of the biochemical components of the fish scales were depicted in table 1. The amount of protein present in fish scales was 40.38±0.71mg. The amount of carbohydrates present in fish scales was 16.73±0.64mg. The amount of lipid present in fish

Fish processing industries generate different types of waste. Fish processing industry produces 30 to 40% of solid waste [1]. Which includes solid waste such as bones, shell, fins, skin, head and meat. Fishery waste is very useful and it contains high amount of protein, fats, minerals and chitin. Fishery wastes quickly undergo spoilage by enzymatic and bacteriological processes which accumulate flies and rodents. These waste materials also generate pollution in coastal areas and contaminate the environment.

Extraction of Chitosan

The result of the extraction of chitosan from fish scales was shown in plate 1. The result of the study showed that the chitosan extracted from the scales of fish was ash in colour.



**Pragathi and Noorjahan****Antibacterial Activity of Chitosan**

The result of antibacterial activity of chitosan extracted from fish scales were shown in Table 2. The results of the study showed that chitosan inhibits the growth of gram +ve and gram -ve bacteria tested and the antibacterial activity was increased with the increase in the concentration of chitosan. Zone of inhibition was observed well in bacterial culture - *Staphylococcus aureus* and *Pseudomonas* sp. The maximum diameter of zone of inhibition 10 ± 0.1 mm was recorded at $200\ \mu\text{g}/\mu\text{l}$ concentration and minimum diameter of zone of inhibition 7.4 ± 0.2 mm was recorded at $100\ \mu\text{g}/\mu\text{l}$ concentration against *Staphylococcus aureus*. Whereas the maximum diameter of zone of inhibition 10.4 ± 0.1 mm was recorded at $200\ \mu\text{g}/\mu\text{l}$ concentration and minimum diameter of zone of inhibition 7.6 ± 0.09 mm was recorded at $100\ \mu\text{g}/\mu\text{l}$ concentration against *Pseudomonas* sp thereby indicates that chitosan act as a potent antibacterial agent.

Chitin and Chitosan are polysaccharide polymer materials, chitin is the second most abundant renewable polysaccharide after cellulose. Shrimp, crab, squid, lobsters, fish, insect, cuticle, fungi and yeast [14] are the best naturally occurring sources of chitin and its derivatives were the biomolecules of great importance having versatile biological actions and exhibit biocompatibility and biodegradability as a result, derivatives are extensively used in pharmaceutical, cosmetics, food additives, agriculture and semi permeable membrane.

Chitosan is a versatile material with potential antimicrobial activity. Chitosan, a hydrophilic biopolymer industrially obtained by N-deacetylation of chitin and can be applied as an antibacterial agent. Bacteria act as an elicitor of plant defense mechanisms. A number of commercial applications of chitosan benefit from its antibacterial activity, including its use in food preservation, in density and ophthalmology, as well as in the manufacture of wound-dressings and antimicrobial-finished textiles. Chitosan seems to act on gram positive and gram negative bacteria satisfactorily. Water soluble derivatives of chitosan which can be attained by addition of methane, enhancing the chitosan applicability in a large pH range and also improve the antimicrobial activity, opening up a broad range of possibilities.

The data obtained for biochemical components of fish scales and antibacterial activity of chitosan were expressed in Mean and Standard deviation. Thus the results of the above study proves that the fish scales have chitosan and chitosan extracted from *Tilapia mossambica* scales acts as a potent antibacterial agent.

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Table 1: Estimation of Biochemical components present in fish scales.

| S.No | Constituents | Mean±Standard Deviation |
|------|-------------------|-------------------------|
| 1 | Protein (mg) | 40.38±0.71 |
| 2 | Carbohydrate (mg) | 16.73±0.64 |
| 3 | Lipid (mg) | 0.22±0.01 |
| 4 | Ash (g) | 0.64±0.01 |

Table 2: Antibacterial activity of Chitosan against Bacterial Cultures - *Staphylococcus aureus* and *Pseudomonas sp*

| S.No | Bacteria Culture | Concentrations (µg/µl) | Zone of Inhibition (mm) |
|------|------------------------------|------------------------|-------------------------|
| 1 | <i>Staphylococcus aureus</i> | CONTROL | |
| | | 50 | - |
| | | 100 | 7.4±0.2 |
| | | 150 | 9.01±0.07 |
| | | 200 | 10±0.1 |
| 2 | <i>Pseudomonas sp</i> | CONTROL | |
| | | 50 | - |
| | | 100 | 7.6±0.09 |
| | | 150 | 9.3±0.08 |
| | | 200 | 10.4±0.1 |

± = Standard deviation.

- = Absent



Plate 1: Chitosan extracted from the fish scales





A Scientometrics Analysis of Millets Research in India -Reflections from Web of Science Database during 2005-2023

Joseph M K^{1*}, Vijesh P.V² and Anupama A

¹The Project Director and Associate Professor, Rajagiri College of Social Sciences, Kalamassery, Kochi, Kerala, India

²Librarian, Rajagiri College of Social Sciences, Kalamassery, Kerala, India

³Document Specialist, Ernst and Young, Cochin, Kerala, India.

Received: 31 May 2023

Revised: 26 July 2023

Accepted: 05 Sep 2023

*Address for Correspondence

Joseph M K,

The Project Director and Associate Professor,

Rajagiri College of Social Sciences,

Kalamassery, Kochi, Kerala, India

E. Mail-emkay@rajagiri.edu



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ABSTRACT

Millets are a group of small-seeded grasses that have been an important food source for centuries in India. However, in recent years, there has been a decline in their cultivation and consumption due to the increasing popularity of rice and wheat. To understand the state of millets research in India, we conducted a scientometrics analysis using the Web of Science database for the period 2005-2023. The authors used biblioshiny software to analyse the millets research and publication output of Web of Science database during 2005-2023. A Total of 432 publications were taken from the web of science database for analysis. The study analysed the broad features of millet research output focusing on annual scientific publications, Average Citation Per Year, Most Relevant Source, Most Relevant Authors, Most Relevant Affiliations, Corresponding Author's Countries, Most Frequent Words, Trend Topics, Top cited institutions, etc. The study insights pave the way for further advancements in millets research, policy formulation, and the promotion of millet cultivation and utilization in India, ultimately contributing to the sustainable development of agriculture, nutrition, and food security.

Keywords: Millets, Millets research, Scientometric analysis, Crop research, Pearl Millets, SDG

INTRODUCTION

Millets, a group of small-seeded annual grasses, have been an integral part of our diets since the Vedic era, over 8000 years ago. However, the knowledge of their benefits has not been passed down for the last 400 years, leading to millets being stigmatized as the poor man's food. Fortunately, the United Nations' mission Sustainable Development



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Goals (SDGs) has given a new lease of life to traditional knowledge, prompting the Indian government to recognize millets as a sustainable crop in 2018. Grown primarily in dry areas of temperate, sub-tropical and tropical regions on marginal lands, millets are often referred to as "Nutri-Cereals" due to their higher nutritional content than commonly grown cereals like wheat, rice, or corn. They are an excellent source of nourishment for both humans and animals, especially mothers and their young (Joglekar,2022). Millets are a group of small-seeded grasses that offer a multitude of health benefits. Unlike some other grains, millets are non-allergenic and gluten-free, making them a safe option for individuals with dietary restrictions. In addition to being a rich source of nutrition, millets have been shown to have medicinal properties. Research suggests that millets can help decrease triglycerides and inflammation, which can prevent the development of cardiovascular disease. Millets are also a great source of dietary fiber, which can reduce the chances of developing inflammatory bowel disease. Additionally, the consumption of millets can help to detoxify the body, promoting overall health and wellness (Gupta,2023).

The role of science in agricultural development is paramount, and assessing science intensity in specific areas is crucial. Publication activity serves as a key indicator, revealing trends in scientific progress and international collaboration. By examining the number of articles published on a specific topic within a certain timeframe, we can gain insights into the development of agricultural science at both national and global levels. This article explores the importance of publication activity as a metric for evaluating science intensity in agriculture and its implications for scientific advancement (Boiarskii,2022).Crop science, especially cereal science is one of the major disciplines in the field of agriculture sciences in India (Garg,2014). Millets are crucial cereal crops that play a vital role in ensuring food and nutrition security, particularly in developing countries situated in the semi-arid tropics of Asia and Africa. Notably, countries like India, Nigeria, and Niger heavily rely on millets as a staple food source (Malathi,2016).Soybeans, with their high protein content, are a popular crop in Asia and globally consumed in forms like tofu and soymilk (Hasan,2023). Sustaining agricultural productivity is crucial for food security amid challenges like climate change, growing populations, and limited resources. Global research aims to develop innovative measures to safeguard and improve crop yields (Sisodia,2022).Regular consumption of whole grains like maize, oats, barley, and millets offers protection against chronic diseases like cancer, cardiovascular issues, obesity, and diabetes. These grains are rich in fiber, vitamins, and phenolic compounds, promoting health and well-being (Gunjan,2022).

Whole grains show promise in treating and preventing epidemic diseases. Analyzing research progress and trends using scientometric tools can provide a comprehensive understanding of the health effects of whole grains (Wei,2022). Pearl millet (*Pennisetum glaucum*) is an excellent crop choice to address water scarcity and ensure food security in regions with challenging climates. Its high drought tolerance and resource efficiency make it an ideal solution for harsh environmental conditions (Naorem,2023). The UN General Assembly declared 2023 as the International Year of Millets, recognizing their significance in enhancing food security. This resolution highlights the importance of millets and their role in promoting sustainable agricultural practices (Antony,2022).Scientific interventions utilizing molecular biomarkers, sequencing data, mapping populations, and mutants have facilitated the development and release of high-yielding millet varieties worldwide. These advancements have significantly contributed to the improvement of millet crops and agricultural productivity (Kumar,2018).Climate change poses a significant threat to the four pillars of food security: food availability, accessibility, utilization, and system stability. Its impact can have severe negative consequences on global food security, highlighting the urgent need for mitigation and adaptation strategies (Sweileh,2020).

Soil nutrient balance is influenced by the intricate interplay among soil fertilizer, soil water, climate change, and plant capabilities. Understanding these interaction mechanisms is crucial for maintaining optimal soil fertility and ensuring sustainable agricultural practices (Pan,2021).Indian millets are highly nutritious crops known for their drought tolerance, predominantly cultivated in arid and semi-arid regions of India. These millets play a vital role in providing sustenance and resilience in challenging agricultural environments (Punith Kumar,2023).A scientometric analysis of millet research in India, based on data from the Web of Science database spanning the period of 2005-2023, offers valuable insights into the growth, trends, and impact of research in this field. By identifying prolific authors, institutions, and countries, as well as the most frequently cited papers and popular research topics, this



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analysis provides a comprehensive and systematic overview of millet research in India. The comprehensive and systematic nature of this scientometric analysis contributes to the scientific literature by consolidating and organizing research findings, thereby providing a valuable resource for researchers and policy makers worldwide. This study aids in enhancing the global understanding of the state of millet research in India, which can, in turn, inspire and inform research initiatives in other countries facing similar challenges and aiming to harness the potential of millets as a sustainable and nutritious crop. Definitely this study offers a holistic view of research trends, growth, and impact. This analysis has implications for future research directions, funding decisions, and policy initiatives, while also contributing to the scientific literature and facilitating global collaboration and knowledge exchange in the field of millet research.

Objectives of the Study

The objectives of this scientometrics analysis of millets research in India are as follows:

- Assess the overall research output: Determine the number of publications related to millets research in India during the period 2005-2023 and analyze the trends in research output over time.
- Identify influential institutions: Identify the top institutions in India that have contributed significantly to millets research and assess their research productivity and impact.
- Explore subject areas: Determine the different subject areas within millets research in India and analyze the distribution of research across these areas. Identify the most prominent subject areas and assess their growth and evolution over time.
- Identify top authors: Identify the researchers/authors who have made significant contributions to millets research in India. Assess their publication output, citation impact, and collaborations.
- Analyze top journals: Identify the journals that have published a substantial number of articles on millets research in India. Assess the impact and influence of these journals in the field.
- Examine international collaboration: Analyze the extent of international collaboration in millets research conducted in India. Identify the countries with which Indian researchers have collaborated the most.
- Identify emerging trends and topics: Analyze the keywords and frequently occurring words in millets research publications to identify emerging trends and topics in the field. Determine the areas of current and future research focus in millets in India.

By addressing these objectives, the study aims to provide a comprehensive understanding of the state of millets research in India, highlighting the key contributors, research areas, and emerging trends in the field.

METHODOLOGY

This study employed a scientometric approach to analyze millets research in India using data collected from the Web of Science database during the period of 2005-2023. To gather the data, the biblioshiny software was utilized, enabling the extraction and analysis of relevant publications. The search was conducted using the keyword "Millets" to ensure the inclusion of publications specifically focused on this subject. Bibliometric indicators, such as the number of publications, citations, and co-citations, were employed to examine the research output. Additionally, various aspects were explored, including the most influential institutions, subject areas, authors, and journals related to millets research. Furthermore, the analysis encompassed the examination of contributing authors' countries, the identification of frequently occurring words, and the identification of trending topics. To visualize the bibliometric data and uncover patterns, clusters of related publications were identified using the biblioshiny software. The most influential publications in the field of millets research in India were also identified through this analysis. Overall, this methodology enabled a comprehensive assessment of millets research and its key contributors in India, offering valuable insights into the state of the field.

Data Analysis

From the Table 1 it is revealed that a total of 432 publications related to millets research were analyzed during the period, with an annual growth rate of 14.25%, indicating a steady increase in the number of publications on millets



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research in India over the years. This growth rate is a positive sign and shows that researchers are increasingly focusing on millets research, which could lead to better understanding of the nutritional and health benefits of millets and encourage farmers to cultivate them more. The average age of the documents analysed was 5.34 years, indicating that the majority of millets research publications in India are relatively recent. The average citations per document were found to be 8.134, indicating that millets research publications are relatively well-received and cited in the scientific community. This suggests that the research being conducted on millets in India is of high quality and significance. The analysis also revealed that a total of 1411 authors contributed to the publications analyzed, with only 23 single-authored documents. This high collaboration rate indicates that researchers are working together to produce better quality research on millets. Furthermore, the average number of co-authors per document was 4.04, indicating that the research being conducted on millets is often interdisciplinary and involves experts from various fields. International co-authorships accounted for 23.15% of all collaborations, highlighting the international interest in millets research in India. This is a positive sign as it shows that the research being conducted on millets in India is attracting the attention of researchers from around the world. Collaborations with international researchers can lead to the exchange of knowledge and ideas, and help to improve the quality of research. The majority of documents analyzed were articles (289), followed by article-book chapter (61), article-proceedings paper (14), and reviews (15). The high number of articles indicates that most millets research in India is published in peer-reviewed journals, which ensures that the research is of high quality and significance. The wide range of document types also indicates that the research on millets is multifaceted and involves various aspects such as agriculture, nutrition, health, and economics.

The table 2 presents the annual scientific production of millets research articles in India from 2005 to 2014. The number of articles published each year during this period provides insights into the research output and trends in the field. At the outset, the analysis reveals a relatively modest publication output on millets research in India during the initial years. In 2005 and 2006, only one article each was published, indicating a limited focus on millets as a subject of scientific inquiry. However, there seems to be a gradual upswing in interest and scholarly activity in subsequent years. From 2007 to 2009, there is a discernible rise in the number of articles published annually. Two articles were published in 2007, followed by a slight increase to three articles in 2009. This suggests a growing recognition of the significance of millets as a research topic within the scientific community. The subsequent years witnessed a more pronounced expansion in millets research. The annual publication count climbed steadily, with six articles published in 2010 and four in 2011. This upward trajectory continued in 2012, which saw a notable increase to nine articles. The years 2013 and 2014 marked a substantial surge in millets research output in India. In 2013, the publication count jumped to 19 articles, reflecting a significant intensification of scholarly interest in this area. The trend continued to accelerate in 2014, with 22 articles published, highlighting a robust growth in research activity. Overall, the analysis of the table indicates a progressive growth in the scientific production of millets research articles in India from 2005 to 2014. This upward trend suggests an increasing recognition of the importance of millets as a subject of study and highlights the growing efforts to understand and address the challenges related to millets cultivation, consumption, and their potential contributions to food security and nutrition in India.

Table 3 presents the average citation per year for millets research in India from 2005 to 2014. The table provides information on the mean number of citations per article, the number of articles (N), the mean number of citations per year, and the number of citable years. From the analysis, it can be observed that in 2005, there was only one publication with an average of 118 citations per article. The mean number of citations per year for that publication was 6.21, and it had been cited over a period of 19 years. In 2006, there was again only one publication, but the average number of citations per article dropped significantly to 8. The mean number of citations per year for that publication was 0.44, and it had been cited over a period of 18 years. The year 2007 showed an increase in the number of publications, with two articles. The average number of citations per article was 64.5, and the mean number of citations per year was 3.79. These publications had been cited over a period of 17 years. In 2008, there were two publications with an average of 3 citations per article. The mean number of citations per year for these publications was 0.19, and they had been cited over a period of 16 years. The trend continued with varying numbers



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of publications and corresponding citation averages in subsequent years. Notably, there were fluctuations in the average citation per year, indicating variations in the impact and recognition of millets research during different periods. Overall, the analysis of average citations per year provides an insight into the research impact and visibility of millets research in India. It indicates the extent to which the published articles are cited by other researchers, highlighting the relevance and influence of the research in the scientific community.

Table 4 Interprets the Most relevant sources, Among the top sources, the Journal of Pure and Applied Microbiology stands out with the highest number of articles, indicating a significant focus on the microbiological aspects of millets. This suggests that researchers in India are exploring the role of microorganisms in millet production, processing, and utilization. Another prominent source is Agricultural Research, which highlights the importance of agricultural practices and advancements in millet cultivation. This indicates a research emphasis on improving millet yields, pest management, and soil fertility to enhance overall agricultural productivity. The publication Millets and Sorghum: Biology and Genetic Improvement is another noteworthy source, suggesting a strong focus on genetic research and breeding strategies to enhance the quality, nutritional content, and resilience of millet crops. This reflects an effort to develop improved varieties of millets that can withstand various environmental challenges and meet the demands of changing consumer preferences. The presence of journals such as Bio-catalysis and Agricultural Biotechnology and Current Research in Nutrition and Food Science indicates an interest in exploring the potential of millets in biotechnological applications and their nutritional value. This suggests a growing recognition of millets as a valuable source of functional foods and their potential to address nutritional challenges and promote food security. Furthermore, the inclusion of the Indian Journal of Economics and Development suggests a multidisciplinary approach to millets research, acknowledging the economic and developmental aspects associated with millet cultivation, marketing, and policy implications. This indicates a holistic understanding of the role of millets in sustainable agriculture and rural development. Overall, the analysis of the relevant sources provides insights into the diverse aspects of millets research in India. It highlights the interdisciplinary nature of the field, with contributions from microbiology, agriculture, genetics, biotechnology, nutrition, and economics. These findings reflect the concerted efforts of researchers to explore the untapped potential of millets and promote their cultivation, consumption, and commercialization in India.

The table 5 presents the most relevant authors in the field of millets research in India, based on the analysis of publications from the Web of Science database during the period 2005-2023. The authors are listed in descending order based on the number of articles they have contributed to. At the top of the list is Author A Kumar, who has published 11 articles, representing approximately 2.82% of the total publications analyzed. Following closely behind is Author P Kumar with 10 articles (1.98%), and Author Ak Pokharia also with 10 articles (1.82%). Other notable authors include Author S Kumar with 8 articles (1.93%), Author R Singh with 7 articles (1.65%), and Author S Sharma with 6 articles (1.32%). Authors J Bates and Jd Haas both have contributed 5 articles each, accounting for 0.92% and 1.02% of the total publications respectively. Additionally, Authors R Kumar and V Kumar also have 5 articles each, representing 1.03% and 0.83% of the total publications respectively. These authors have made significant contributions to millets research in India, as evidenced by their high publication output. Their research work has likely contributed to the understanding of various aspects related to millets cultivation, nutrition, processing, and their potential impact on food security and sustainable agriculture. Their findings may have implications for policymakers, farmers, and other stakeholders interested in promoting millets as a viable and nutritious alternative to rice and wheat.

The table 6 showcases the most relevant affiliations in the field of millets research in India, derived from an analysis of publications extracted from the Web of Science database spanning the years 2005 to 2023. The affiliation that stands out with the highest number of articles is the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), with a total of 52 publications. ICRISAT is renowned for its significant contributions to agricultural research, particularly in the context of dry land crops. Their emphasis on millets research highlights the organization's commitment to addressing the challenges faced by semi-arid regions and promoting sustainable agriculture. The Indian Institute of Millets Research (IIMR), which focuses specifically on millets, occupies the



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second position with 43 publications. This institution plays a vital role in advancing millets research and development in India, aiming to enhance the productivity, profitability, and sustainability of millet-based farming systems. Tamil Nadu Agricultural University, with 35 articles, demonstrates its active involvement in millets research. The university's efforts in this domain are essential for addressing the specific agricultural needs and challenges faced by the state of Tamil Nadu. The Indian Agricultural Research Institute (IARI) holds the fourth position with 31 publications. As a premier national institute for agricultural research, IARI's contributions to millets research signify its dedication to enhancing agricultural practices and addressing food security in India. Other notable affiliations in the list include the University of Cambridge (25 publications), Periyar University (23 publications), College of Agriculture (16 publications), G.B. Pant University of Agriculture and Technology (16 publications), University of Agricultural Sciences (16 publications), and the University of Mysore (16 publications). These affiliations, both national and international, demonstrate the collaborative nature of millets research and highlight the diverse expertise involved in advancing this field. Overall, the data presented in the table showcases the significant contributions of various institutions in India towards millets research, underscoring their collective efforts to promote sustainable agriculture, enhance food security, and revive the importance of millets as a valuable food source.

The analysis reveals that India has emerged as the dominant contributor in millets research publications during the period 2005-2023. A total of 373 articles were authored by researchers affiliated with institutions in India, indicating a significant focus on millets research within the country. Among these publications, 328 were led by a single corresponding author (SCP), while 45 involved multiple corresponding authors (MCP). This suggests a substantial level of collaborative research in the field of millets within India. In comparison, the United States (USA) accounted for a relatively smaller number of publications with 17 articles. However, it is noteworthy that all 17 articles from the USA were MCPs, indicating a high level of collaborative international research involving millets. Other countries that made contributions to millets research include the United Kingdom, Italy, Australia, Brazil, Canada, Germany, and the Netherlands, each with a smaller number of publications ranging from 8 to 2. In all these cases, the publications had a 100% MCP ratio, implying collaborative efforts in millets research involving researchers from multiple institutions and countries. This analysis highlights the strong research focus on millets within India, with a significant number of publications being led by Indian institutions. The collaborative nature of millets research, as evident from the MCP publications, suggests active engagement and knowledge-sharing among researchers both within India and internationally, particularly with the United States. Such scientometric analysis provides valuable insights into the global landscape of millets research and helps identify key contributors and collaborative trends in this field.

Table 8 analysis of the most frequent words extracted from the scientometrics study on millets research in India reveals important insights into the focus and trends within the field. The data, gathered from the Web of Science database spanning the years 2005 to 2023, sheds light on the key areas of interest and research emphasis surrounding millets. At the forefront, pearl millet emerges as the most frequently mentioned term, appearing 28 times in the analyzed publications. This finding suggests that pearl millet has been a primary focus of research in India during the studied period, indicating its significance in the context of millet studies. Researchers may have explored various aspects of pearl millet, including its cultivation, nutritional value, processing, and potential applications. Quality and yield are two important factors that garnered substantial attention in the analyzed publications, with each term occurring 18 and 16 times, respectively. This indicates that researchers have been keen on investigating methods to improve the quality of millets, both in terms of nutritional composition and other desirable characteristics. The emphasis on yield suggests efforts to enhance the productivity and efficiency of millet cultivation, potentially to make it a more viable and sustainable food source. Other prominent terms in the analysis include sorghum, eleusine-coraana (finger millet), growth, wheat, millet, protein, and food. Sorghum, being another important cereal crop, demonstrates the interdisciplinary nature of millet research and the exploration of different crops that share common themes or interactions. The occurrence of eleusine-coraana highlights the attention given to this specific type of millet in the studies analyzed. The presence of growth and wheat suggests that researchers have been examining the growth patterns, development, and potential interactions between millets and wheat, a widely consumed grain in



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India. This comparison may shed light on the advantages, disadvantages, and potential complementarity of millets as alternatives or additions to the prevailing wheat-centric food systems. The terms millet, protein, and food indicate the overarching themes that permeate the analyzed publications. Researchers have been investigating millets as a valuable source of protein and their potential contribution to addressing food security and nutrition challenges. By exploring the diverse aspects of millets' composition, production, and utilization, researchers aim to position millets as an essential component of a sustainable and nutritious food system. In conclusion, the scientometrics analysis provides valuable insights into the predominant themes and areas of focus within millets research in India. The findings underscore the significance of pearl millet, quality, yield, and other related aspects, shedding light on the efforts to enhance millets' potential as a nutritious and sustainable food source. This analysis can guide researchers, policymakers, and stakeholders in further advancing millets research and promoting their cultivation, consumption, and utilization in India.

Table 9 reveals the different subject areas within millet research. Some prominent topics that emerged include grain yield, induction, growth, quantitative trait loci, accumulation, pearl millet, eleusine coracana, finger millet, sorghum, millet, fermentation, yield, antioxidant activity, climate change, quality, wheat, and protein. These topics reflect the diverse areas of focus in millet research, ranging from crop productivity and genetic traits to nutritional aspects and climate change adaptation. Furthermore, the study identified the top authors and journals in the field of millet research. These authors and journals have made substantial contributions to the scientific literature on millets, indicating their expertise and influence in the field. Overall, the scientometrics analysis provides insights into the state of millet research in India. It highlights the research areas that have received significant attention and the institutions, authors, and journals that have played key roles in advancing the knowledge and understanding of millets. This analysis can serve as a valuable reference for researchers, policymakers, and stakeholders interested in promoting millet cultivation, consumption, and further scientific advancements in India.

Findings

Millets are a group of small-seeded cereal crops that have gained recognition for their nutritional value and resilience in challenging agricultural environments. This manuscript aims to provide a comprehensive analysis of millets research in India from 2005 to 2014, focusing on scientific publications, citation impact, relevant sources, authors, affiliations, and key findings. Understanding the research landscape and trends surrounding millets is crucial for promoting sustainable agriculture, addressing food security challenges, and harnessing the potential of these crops. Some of the key findings are as follows:

- There was a progressive growth in the scientific production of millets research articles in India from 2005 to 2014.
- The average citations per year varied, reflecting fluctuations in the impact and recognition of millets research.
- The Journal of Pure and Applied Microbiology, Agricultural Research, and Millets and Sorghum: Biology and Genetic Improvement emerged as key sources for millets research.
- The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Institute of Millets Research (IIMR) were the most relevant authors and affiliations.
- India played a dominant role in millets research publications, followed by international collaborations involving the United States and other countries.
- The most frequently mentioned term was "pearl millet," indicating its primary focus in millet studies during the analysed period.

The findings demonstrate an increasing recognition of millets' importance and efforts to understand their cultivation, consumption, and potential contributions to food security and nutrition in India. Collaboration between Indian institutions and international partners indicates a global interest in millets research and knowledge-sharing. The identified sources, authors, and affiliations provide valuable insights for researchers, policymakers, and stakeholders involved in millets research and cultivation. This analysis lays the foundation for future research directions and strategies aimed at maximizing the potential of millets as a sustainable and nutritious food source.





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DISCUSSIONS

The scientometric analysis of millets research in India based on the Web of Science database from 2005 to 2023 reveals a robust growth trajectory and increasing research productivity in the field. The initial years showed limited focus on millets, with a gradual increase in scholarly activity from 2007 to 2009. However, the subsequent years witnessed a steady rise in annual publication counts, with a substantial surge in 2013 and 2014, indicating a growing interest and recognition of millets as an important research area. In terms of institutional contributions, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Institute of Millets Research (IIMR) emerged as prominent players in millets research, publishing 52 and 43 articles, respectively. These institutions have consistently demonstrated their leadership and commitment to advancing millets research, both in terms of quantity and quality of publications. Additionally, collaborations with other renowned institutions, such as Tamil Nadu Agricultural University and the Indian Agricultural Research Institute (IARI), further strengthen the research landscape and signify the collective efforts to drive millets research forward in India. The involvement of international collaborations in millets research is noteworthy.

Collaborations with institutions from the United States, United Kingdom, Italy, Australia, Brazil, Canada, Germany, and the Netherlands indicate a global interest and recognition of the importance of millets. These collaborations not only bring diverse perspectives and expertise but also foster knowledge exchange and enhance the overall impact and reach of millets research. Furthermore, the identification of the most relevant sources and authors provides insights into the research focus and expertise within the millets research domain. Journals such as the Journal of Pure and Applied Microbiology, Agricultural Research, and the book "Millets and Sorghum: Biology and Genetic Improvement" emerged as influential sources in their respective areas of emphasis, covering microbiological aspects, agricultural practices, and genetic research in millets. The analysis of corresponding author's countries highlights India's dominance in millets research publications, reaffirming its position as a key contributor in the field. Although the United States had a smaller number of publications, its active involvement in collaborative international research demonstrates the global significance and collaborative nature of millets research. The identification of frequently occurring words and trend topics provides a glimpse into the research themes and areas of focus within millets research. Terms such as "pearl millet," "quality," "yield," "sorghum," and "nutrition" reflect the core aspects of millets research, while emerging topics such as climate change adaptation, fermentation, and antioxidant activity indicate the evolving research directions and areas of exploration. In conclusion, the scientometric analysis of millets research in India sheds light on the substantial growth, institutional contributions, international collaborations, and emerging trends within the field. The findings underscore the importance of millets as a subject of research in India and highlight the key stakeholders, institutions, and areas of focus driving the advancements in millets research. This comprehensive understanding can guide future research endeavours, policy formulation, and efforts to promote millet cultivation and utilization in India.

Limitations & Future research

The study also identified some limitations as well

- **Data Source Limitations:** The study relied on data obtained exclusively from the Web of Science database, which may not include all relevant publications in the field of millets research in India. Exclusion of publications from other databases or sources could introduce a potential bias and limit the comprehensiveness of the analysis. Additionally, the availability and accessibility of publications in the database might vary, potentially leading to underrepresentation or overrepresentation of certain research outputs.
- **Language Bias:** The analysis focused on English-language publications, which could introduce a language bias and exclude non-English publications. This limitation may result in the omission of valuable research conducted in other languages, leading to an incomplete representation of millets research in India. Consequently, important contributions from researchers publishing in regional languages may not have been captured in the analysis.



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- **Timeframe Limitations:** The study covered the period from 2005 to 2023, which may not encompass the most recent developments and trends in millets research. By excluding recent publications, the analysis may fail to capture emerging topics, advancements, and shifts in research focus that have occurred beyond the study's timeframe. Researchers and institutions engaged in cutting-edge millets research during the post-2023 period would not be adequately represented in the analysis.
- **Quantitative Emphasis:** The scientometric analysis primarily focused on quantitative indicators such as publication counts, citations, and co-citations. While these indicators provide valuable insights into research productivity and impact, they do not capture the full breadth and depth of research quality and significance. Qualitative aspects, such as the policy impact of research, societal outcomes, and the practical application of findings, were not comprehensively explored. Therefore, the analysis may not fully reflect the real-world implications and practical implications of millets research.
- **Institutional and Geographic Focus:** Although the study examined influential institutions and collaborations, the analysis primarily focused on Indian institutions and their collaborations with a limited set of countries. This restricted focus may overlook contributions from researchers and institutions outside the identified countries. The collaborative landscape may be broader and more diverse, involving researchers from countries not covered in the analysis. Thus, the analysis may not fully capture the global nature and diversity of millets research collaborations.
- **Acknowledging these limitations is important for interpreting the findings accurately and understanding the potential gaps in the scientometric analysis of millets research in India. Future studies could address these limitations by incorporating additional databases, including non-English publications, expanding the timeframe, incorporating qualitative analysis, and conducting more extensive and inclusive analyses of collaborations across a wider range of countries.**

CONCLUSION

The scientometric analysis of millets research in India, based on data from the Web of Science database during the period 2005-2023, offers a comprehensive and vivid understanding of the growth, impact, collaboration, and emerging trends within the field. The findings provide valuable insights into various aspects of millets research in India, including research output, influential institutions, subject areas, top authors, top journals, international collaboration, and emerging trends. The analysis revealed a progressive growth in the scientific production of millets research articles in India over the years. While the focus on millets was initially limited, there was a gradual increase in scholarly activity from 2007 to 2009. Subsequently, there was a steady rise in annual publication counts, with a substantial surge in 2013 and 2014, indicating a growing interest and recognition of millets as an important research area in India. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Institute of Millets Research (IIMR) emerged as influential institutions, making significant contributions to millets research in India. These institutions demonstrated consistent leadership and commitment through their quantity and quality of publications. Collaborative efforts with other renowned institutions, such as Tamil Nadu Agricultural University and the Indian Agricultural Research Institute (IARI), further enhanced the research landscape and underscored the collective drive to advance millets research in India. International collaboration played a significant role in millets research conducted in India, with collaborations observed with institutions from the United States, United Kingdom, Italy, Australia, Brazil, Canada, Germany, and the Netherlands. This global collaboration not only brought diverse perspectives and expertise but also expanded the impact and reach of millets research, emphasizing the international recognition of the importance of millets. The identification of the most relevant sources, including the Journal of Pure and Applied Microbiology, Agricultural Research, and the book "Millets and Sorghum: Biology and Genetic Improvement," highlighted the various emphases within the field, ranging from microbiological aspects to agricultural practices and genetic research. These sources served as important platforms for disseminating millets research findings. Key authors and researchers were also identified, with the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and the Indian Institute of Millets Research (IIMR) leading the publications. Their contributions, along with other notable institutions such as Tamil Nadu Agricultural University and the Indian





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Agricultural Research Institute (IARI), showcased the expertise and dedication of researchers in driving millets research forward in India. The analysis of corresponding authors' countries revealed India's dominance in millets research publications, reaffirming its position as a key contributor to the field. The involvement of the United States in collaborative international research further exemplified the global significance and collaborative nature of millets research. The identification of frequently occurring words and emerging trends shed light on the core research areas and emerging topics within millets research. Terms such as "pearl millet," "quality," "yield," "sorghum," and "nutrition" reflected the primary focus areas, while emerging topics such as climate change adaptation, fermentation, and antioxidant activity indicated the evolving research directions and areas of exploration. While the study has provided valuable insights into millets research in India, it is essential to acknowledge the limitations. The reliance on the Web of Science database may have excluded relevant publications from other sources, potentially introducing biases and limiting the comprehensiveness of the analysis.

The focus on English-language publications may have also omitted valuable research published in other languages, resulting in an incomplete representation of millets research in India. Additionally, the study's timeframe may not encompass the most recent developments and trends in millets research. Despite these limitations, the scientometric analysis provides a tangible and comprehensive understanding of millets research in India. It highlights the growth and increasing research productivity in the field, the influential institutions and authors driving the advancements, the collaborative efforts both within India and internationally, and the emerging trends and topics shaping the future of millets research. These findings have significant implications for various stakeholders involved in millets research and utilization. Policymakers can use this knowledge to formulate evidence-based policies that promote millet cultivation, research funding, and sustainable agricultural practices. Researchers and institutions can identify gaps and opportunities for further research and collaborations, focusing on emerging areas such as climate change adaptation, fermentation, and nutritional aspects. The findings also provide valuable insights for industries and entrepreneurs looking to capitalize on the growing interest in millets and develop innovative millet-based products and technologies. In conclusion, the scientometric analysis of millets research in India offers a vivid and tangible understanding of the state of the field. It highlights the growth, impact, collaboration, and emerging trends, showcasing the contributions of influential institutions, authors, and international collaborations. These insights pave the way for further advancements in millets research, policy formulation, and the promotion of millet cultivation and utilization in India, ultimately contributing to the sustainable development of agriculture, nutrition, and food security.

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Table 1 -Main Information About Data

| Description | Results |
|---------------------------------|-----------|
| Timespan | 2005:2023 |
| Sources (Journals, Books, etc) | 200 |
| Documents | 432 |
| Annual Growth Rate % | 14.25 |
| Document Average Age | 5.34 |
| Average citations per doc | 8.134 |
| References | 19259 |
| Document Contents | |
| Keywords Plus (ID) | 1292 |
| Author's Keywords (DE) | 1388 |
| AUTHORS | |
| Authors | 1411 |
| Authors of single-authored docs | 23 |
| Authors Collaboration | |
| Single-authored docs | 26 |
| Co-Authors per Doc | 4.04 |
| International co-authorships % | 23.15 |
| Document Types | |
| article | 289 |
| article; book chapter | 61 |
| article; data paper | 1 |
| article; early access | 7 |





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| | |
|----------------------------------|----|
| article; proceedings paper | 14 |
| correction | 1 |
| editorial material | 3 |
| editorial material; book chapter | 9 |
| meeting abstract | 12 |
| proceedings paper | 14 |
| review | 15 |
| review; book chapter | 5 |
| review; early access | 1 |

Table 2-Annual Scientific Publications

| Year | Articles |
|------|----------|
| 2005 | 1 |
| 2006 | 1 |
| 2007 | 2 |
| 2008 | 2 |
| 2009 | 3 |
| 2010 | 6 |
| 2011 | 4 |
| 2012 | 9 |
| 2013 | 19 |
| 2014 | 22 |

Table 3 -Average Citation Per Year

| Year | Mean T Cper Art | N | MeanTCperYear | CitableYears |
|------|-----------------|-------|---------------|--------------|
| 2005 | 118 | 1.00 | 6.21 | 19 |
| 2006 | 8 | 1.00 | 0.44 | 18 |
| 2007 | 64.5 | 2.00 | 3.79 | 17 |
| 2008 | 3 | 2.00 | 0.19 | 16 |
| 2009 | 26.67 | 3.00 | 1.78 | 15 |
| 2010 | 7.67 | 6.00 | 0.55 | 14 |
| 2011 | 12.25 | 4.00 | 0.94 | 13 |
| 2012 | 15.67 | 9.00 | 1.31 | 12 |
| 2013 | 16.84 | 19.00 | 1.53 | 11 |
| 2014 | 39.36 | 22.00 | 3.94 | 10 |

Table 4 -Most Relevant Source

| Sources | Articles |
|--|----------|
| Journal of Pure and Applied Microbiology | 16 |
| Agricultural Research | 15 |
| Millets and Sorghum: Biology and Genetic Improvement | 13 |
| Biocatalysis And Agricultural Biotechnology | 11 |
| Sustainability | 11 |
| Current Research in Nutrition and Food Science | 10 |
| Indian Journal of Economics and Development | 10 |
| Nucleus-India | 9 |
| Faseb Journal | 8 |
| Journal of Environmental Biology | 8 |



Joseph *et al.*,**Table 5 -Most Relevant Authors**

| Authors | Articles | Articles Fractionalized |
|-------------|----------|-------------------------|
| Kumar A | 11 | 2.82 |
| Kumar P | 10 | 1.98 |
| Pokharia Ak | 10 | 1.82 |
| Kumar S | 8 | 1.93 |
| Singh R | 7 | 1.65 |
| Sharma S | 6 | 1.32 |
| Bates J | 5 | 0.92 |
| Haas Jd | 5 | 1.02 |
| Kumar R | 5 | 1.03 |
| Kumar V | 5 | 0.83 |

Table 6 -Most Relevant Affiliations

| Affiliation | Articles |
|-----------------------------------|----------|
| Int Crops Res Inst Semi Arid Trop | 52 |
| Icar Indian Inst Millets Res | 43 |
| Tamil Nadu Agr Univ | 35 |
| Icar Indian Agr Res Inst | 31 |
| Univ Cambridge | 25 |
| Periyar Univ | 23 |
| Coll Agr | 16 |
| Gb Pant Univ Agr And Technol | 16 |
| Univ Agr Sci | 16 |
| Univ Mysore | 16 |

Table 7 -Corresponding Author's Countries

| Country | Articles | SCP | MCP | Freq | MCP_Ratio |
|----------------|----------|-----|-----|-------|-----------|
| India | 373 | 328 | 45 | 0.863 | 0.121 |
| Usa | 17 | 0 | 17 | 0.039 | 1.000 |
| United Kingdom | 8 | 0 | 8 | 0.019 | 1.000 |
| | 8 | 4 | 4 | 0.019 | 0.500 |
| Italy | 4 | 0 | 4 | 0.009 | 1.000 |
| Australia | 2 | 0 | 2 | 0.005 | 1.000 |
| Brazil | 2 | 0 | 2 | 0.005 | 1.000 |
| Canada | 2 | 0 | 2 | 0.005 | 1.000 |
| Germany | 2 | 0 | 2 | 0.005 | 1.000 |
| Netherlands | 2 | 0 | 2 | 0.005 | 1.000 |

Table 8 -Most Frequent Words

| Words | Occurrences |
|-------------------|-------------|
| pearl-millet | 28 |
| quality | 18 |
| sorghum | 16 |
| yield | 16 |
| eleusine-coracana | 15 |
| growth | 15 |
| wheat | 15 |





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| | |
|---------|----|
| millet | 13 |
| protein | 13 |
| food | 12 |

Table 9 –Trend Topics

| item | freq | year_q1 | year_med | year_q3 |
|-------------------------|------|---------|----------|---------|
| grain-yield | 5 | 2009 | 2013 | 2017 |
| induction | 5 | 2014 | 2015 | 2019 |
| growth | 15 | 2014 | 2016 | 2021 |
| quantitative trait loci | 8 | 2012 | 2016 | 2017 |
| accumulation | 6 | 2015 | 2016 | 2017 |
| pearl-millet | 28 | 2012 | 2017 | 2020 |
| eleusine-coracana | 15 | 2016 | 2017 | 2020 |
| finger millet | 10 | 2016 | 2017 | 2019 |
| sorghum | 16 | 2016 | 2018 | 2020 |
| millet | 13 | 2017 | 2018 | 2019 |
| fermentation | 10 | 2017 | 2018 | 2021 |
| yield | 16 | 2016 | 2019 | 2020 |
| antioxidant activity | 11 | 2016 | 2019 | 2021 |
| climate-change | 10 | 2018 | 2019 | 2021 |
| quality | 18 | 2018 | 2020 | 2021 |
| wheat | 15 | 2018 | 2020 | 2021 |
| protein | 13 | 2018 | 2020 | 2021 |





Vermicomposting of Paper Mill and Sugar Mill Press Mud Wastes using *Perionyx excavatus* Earthworm

Nithya Srinivasan¹ and O.S. Sethu Raman^{2*}

¹Research scholar, Department of Zoology, Kandaswami Kandar's College, Namakkal, Tamil Nadu, India.

²Assistant Professor, Department of Zoology, Kandaswami Kandar's College, Namakkal, Tamil Nadu, India.

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*Address for Correspondence

O.S. Sethu Raman

Assistant Professor,

Department of Zoology,

Kandaswami Kandar's College,

Namakkal, Tamil Nadu, India.

E.Mail: sri.nithya11@gmail.com.



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ABSTRACT

In the present study, an effort has been made to utilize Paper mill waste and sugar mill press mud with cow dung through vermicomposting by using *Perionyx excavatus*. The experiments for vermicomposting were conducted in circular plastic pots. In each pot different combinations of paper mill waste and sugar mill press mud waste with cow dung were taken in three proportions viz. (T1) (Paper mill waste with cow dung in 1:2 ratio), (T2) - (Sugar mill press mud with cow dung in 1:2 ratio), (T3) - (Paper mill waste, Sugar mill press mud and cow dung in 1:1:1 ratio) for composting and vermicomposting. In the experimental period (90 days), different chemical parameters were analyzed within a time interval of 30 days. The Total Kjeldahl Nitrogen (%), Available Phosphorus (%) and Total Potassium (%) increased while the Organic carbon (%) decreased as the composting and vermicomposting processes progressed. 25 adult *Perionyx excavatus* were introduced in each mixture. Among the three treatment units E1 showed the best results.

Keywords: Paper mill waste, sugar mill press mud, vermicomposting, *Perionyx excavatus*, C:N ratio

INTRODUCTION

Paper industry is maximum polluting industries in India and is relatively water intensive. A variety of liquid and solid wastes are produced during different processes of paper manufacturing. Although many paper plants have



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begun cremation of their losses for energy recuperation, a significant sum (about 70%) of these strong squanders (side-effects) are as yet unloaded or spread straightforwardly ashore (Yadav et al.,2013) preserving in view of excessive percent of organic matter present in paper mill wastes, numbers of researchers have concentrated their efforts towards developing capacity packages for this solid paper mill wastes (Elvira et al.,1998). The strong wastes from pulp and paper industries are mainly remedy sludges, lime mud, lime slaker grits, boiler and furnace ash, scrubber sludges, and wooden processing residuals. The paper creation produces around 45% wastewater slop (0.2-1.2 kg dry count number (DM)/kg of biological oxygen interest (BOD) eliminated), 25% debris, (Zambrano et al.,2003), 15% wooden cuttings and squander, and 15% diverse solid waste. The sludge from wastewater treatment contraptions (20-60% stable divisions, pH 7) accommodates of fibers, biosludge, calcium carbonate, clay and different inorganic substances (Nurmesniemi et al., 2007). Dry sludge quantities to about 4.3% of the final product, growing to 20-40% for recycled paper turbines (international financial institution, 2007). The sludge generated in huge amount creates the problem of secure disposal. Sludge carries natural molecules and important plant vitamins like nitrogen, phosphorus, potassium and numerous hint factors. whilst stabilized via a composting process, it could become an amazing supply of organic fertilize and soil additive, free of chemicals and pathogens.

The utilization of this paper mill sludge can be done by vermistabilization. Earthworms feed simply upon the sludge additives, swiftly changing them into vermicompost, reduce the pathogens to safe stages and ingest the heavy metals. another most vital industry is Sugarcane processing industry is one of the most important agro processing industries in India. In India four hundred Sugar factories rank as the second one foremost agro industry in the country. The Sugar enterprise has several co-merchandise of colossal potential fee. The co merchandise encompass pressmud, molasses and spent wash. Wastewater treatment plants for sugar mills produce huge quantities of this solid sludge. After solidification, it is commonly known as press mud (PM), which is heavily polluting and also has harmful effects on the ambient environment. Pressmud is a smooth , spongy ,amorphous and dark brown fabric containingsugar , fiber and coagulated colloids consisting of canewax , albuminoids ,inorganic salt,sand, soil debris. It is composed 80p.cof water & 0.nine%-1.fivepercentsugar organic remember, nitrogen, phosphorus , potassium , calcium , coagulated colloids &different materials in various amounts.

The authors have found that, due to the prohibitive value of Press mud disposal, most of the mills in India keep the sludge in open grounds or along the roadside or railway tracks, where it can pollute surface or ground water causing public health hazards. Economically viable and environmentally sustainable technologies are therefore needed for its proper management.it's miles nicely established that a big quantity of organic wastes may be ingested by earthworms and egested as peat-like cloth termed vermicompost. Press mud contains a widespread percent of organic be counted and may be used as feed by using earthworms if mixed with some different natural waste in appropriate quantities. Vermicomposting is stabilization of natural cloth, related to the joint movement of earthworms and micro organisms.

Even though microbes are accountable for biochemical degradation of natural recall, earthworms are critical drivers of the approach, conditioning the substrate and altering the organic pastime (Aira et al., 2002). Earthworms normally devour half of decomposed natural wastes and preliminary aerobic composting plays an critical function to provide palatable food for earthworms. Earthworms play a vast eco useful function in soil surroundings through affecting physical, chemical and organic residences of the soil. Microorganisms hence making it plenty extra favorable for microbial pastime and similarly decomposition. Accordingly vermicomposting has grow to be the suitable opportunity for the secure hygienic and value powerful disposal of these wastes.

Vermicomposting, via earthworms, is an eco-biotechnological process that transforms power wealthy and complex organic substances in to stabilized vermicompost. The promising approach that may be implemented to treat the commercial sludge is vermicomposting by using a selected composting worm. the utilization of earthworms in sludge control has been named as vermistabilization. completed utilization of earthworms inside the breakdown of a broad scope of common buildups, for example, sewage sludge, creature squanders, crop deposits, and business won't deliver vermicompost, has been embraced (Hartenstein and Bisesi,1988; Van Gestel et al., 1992; Dominguez



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and Edwards, 1997). The significance of the worms in waste control, ecological protection, regular cultivating and feasible horticulture field.

The epigeic earthworm had been utilized for organic waste control (Suthar, 2006; Garg and Kaushik, 2005; Benitez et al., 2005). Epigeic types of earthworms can hurry the treating the soil system to a monster degree with assembling of better accessibility of vermicomposts (Ndegwa and Thomson, 2001). The assorted modern squanders which have been vermicomposted and changed into supplement rich excrement include paper waste (Elvira et al., 1998; Kaur et al. 2010), textilemill sludge (Garg and Kaushik, 2005), guar gum commercial wastes (Suthar, 2006), sugar enterprise wastes (Sen and Chandra, 2007). The investigation of the existing examine became to stabilize primary from a treatment unit of a sugar mill press mud and paper mill waste using the epigeic earthworm species *Perionyx excavatus*. The dried sugar mill press mud and paper mill waste was blended with cow dung at different ratios in order to optimize the waste mixture for better decomposition. The changes in physico-chemical parameters in waste materials were measured.

MATERIALS AND METHODS

Collection of material

The cattle dung (10 days old) was procured from nearby dairy farm. The moisture content of the medium was maintained at about 60%-70%. Paper mill waste (PMW) was collected from the Paper Mill Industry at TNPL, Pugalur, Tamil Nadu. Sugar mill Press mud (SPM) was collected from the Mohanur Sugar mill Factory, Mohanur, Namakkal, Tamilnadu.

Earthworm's cultures:

Perionyx excavatus Species were collected from Pugalur area around 10km From the TNPL, pugalur, Tamilnadu, India. For the prevailing examine, separate vermi-bed changed into made the usage of ten days (10 days) antique farm animals dung for mass tradition of *Perionyx excavatus*. The inventory tradition of the earthworm turned into maintained in plastic packing containers the usage of partly decomposed bio-waste and cow dung as increase medium in laboratory situation. This was further used in the vermicomposting experiment.

Pre-decomposition experiment

A plastic container plastic container of 55x40x25 cm measurement turned into full of a aggregate (5 kg) of cow dung and paper mill waste, also aggregate (5kg) of cow dung and sugar mill press mud. It turned into each day sprinkled with water so that it gets decomposed. Also this waste turned into grew to become up and down for correct aeration and decomposition. During the Pre- decomposition period temperature was recorded twice an afternoon. This experiment endured for 30 days.

Experimental layout

The vermibeds have been prepared using sugar mill press mud and paper mill waste in plastic containers and watering was accomplished frequently to moist the medium. The plastic boxes were filled with 1.5 cm thick sterilized soil layer at the bottom as soil is taken into consideration as an essential supporting material for vermicomposting, Control and remedies had been triplicate. To every plastic box, 60 grownup forty days antique earthworms *Perionyx excavatus* were brought from the inventory lifestyle after pre-composting of the raw materials. The experiment became carried out in dark room in ambient temperature. The moisture tiers within the experimental boxes had been maintained at 70±10% (Yadav and Garg, 2009). The containers have been blanketed by mesh garden fabric and had been located day by day in order to check the various parameters vital for the survival and reproduction of earthworms. This complete setup become maintained for 90 days until the finely granular vermicompost become organized.





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Experiment desine

| Experiment No | Specifications | Weight of the organic wastes taken | Number of earthworms Introduced |
|----------------|--|--|---------------------------------|
| Control - 1 | Paper mill Waste+ Cow dung (1:2) | 1 kg of Paper mill waste+ 2 Kg of Cow dung | |
| Experiment -1 | Paper mill Waste+ Cow dung (1:2)+ Earthworms | 1 kg of Paper mill waste+ 2 Kg of Cow dung | 25 |
| Control - 2 | Sugar mill Press mud+ Cow dung (1:2) | 1 kg of Sugar mill press mud+ 2 Kg of Cow dung | |
| Experiment - 2 | Sugar mill Press mud+ Cow dung (1:2)+ Earthworms | 1 kg of Sugar mill press mud+ 2 Kg of Cow dung | 25 |
| Control - 3 | Paper mill waste+ Sugar mill press mud+ Cow dung (1:1:1) | 1 kg of Paper mill waste+ 1 kg of Sugar mill press mud +1 Kg of Cow dung | |
| Experiment - 3 | Paper mill waste+ Sugar mill press mud+ Cow dung+ Earthworms (1:1:1) | 1 kg of Paper mill waste+ 1 kg of Sugar mill press mud +1 Kg of Cow dung | 25 |

Collection of Vermicompost and Compost

samples of all feed substrates were drawn at 0, 30, 60, 90 days. The vermicompost produced become air dried at room temperature and packed in hermetic plastic vials for further physicochemical evaluation.

Physico-chemical Analysis of Vermicompost

The vermicomposting test turned into continued for ninety days and the samples have been used to analyses on the cease of test for unique Physico-chemical houses like, moisture content, pH, organic carbon, C/N ratio and some macronutrients at the laboratory. The pH was determined using a double distilled water suspension of vermicompost in the ratio of 1:10 (W/V) that was agitated mechanically for 30 min and filtered through whatman no.1 filter paper (McLean EO 1982). Total organic carbon (TOC) of the vermicompost was estimated by the method of (Nelson et al., 1982); Total Kjeldahl nitrogen (TN) by (Bremner and Mulvaney 1982); Available phosphorus by (Anderson and Ingram, 1993), potassium by (Simard, 1993). C: N ratio was calculated by dividing the percent of carbon with percent of nitrogen.

Growth and reproductive performance of earthworms

The growth and reproductive performance of earthworms in different experimental containers were measured at the same interval. The earthworm population was counted by the hand-sorting method as suggested by (Gunadi et al., 2003) at 30, 60 and 90 days after the start of the experiment and the material was returned to the same containers.

Growth rate determination, $R = (N_2 - N_1) / T$

Where

R = Growth rate; N1= Initial earthworm biomass (mg); N2= Final earthworm biomass (mg); T= Time period of the experimental day.



**Nithya Srinivasan and Sethu Raman****Reproductive performance**

This was determined as the Net Reproductive Rate (Nrr) and was computed following (Dynes, 2003) as stated below:

$$Nrr = \frac{\text{Total number of earthworms harvested}}{\text{Total number of earthworm stocked} \times \text{experimental period (weeks)}}$$

Total number of earthworm stocked × experimental period (weeks)

Statistical Analysis

All the Data were analyzed using SPSS statistical package. Differences among different experimental parameters were calculated by using one way ANOVA ($p < 0.05$) and Duncan's multiple range test (DMRT).

RESULTS

Vermicomposting had a different treatment on the quality of the waste feedstock after 90 days. The end material became more stabilized, smell unfastened, and dark brown with a excessive stage to be had vitamins for plant increase. The percentage of pH decrease in the vermicompost was not significant, whereas the reduction in OC and C:N ratio was significant when compared to that of compost. A highly significant ($p < 0.001$) enhancement of N, P and K contents was found in the vermicompost when compared to compost except 90 day of analyses (Table 1).

pH is an important parameter in the vermicompost for promoting plant growth. There was a decrease in pH significantly ($P < 0.05$) in pH in all the Experiments (E1 -E3) relative to their initial values during vermicomposting (Table 1). Initially pH values in different Experiments were in range of 8.16, 7.69, and 8.10 and in final vermicompost, ranged from 7.05, 7.13 and 7.22. Maximum reduction was recorded in E1, while minimum was recorded in E3.

Total Nitrogen (TN)

The general nitrogen content material after 1st day and 90th days of *Perionyx excavatus* motion inside the specific vermibed changed into predicted. The results were compared with total nitrogen content E1, E2, E3 Experiments. In the Experiments nitrogen content was 1.27, 1.70, 1.50 on 1st day and 4.21, 3.68, 3.97 on the 90th day in vermicompost. The total nitrogen has shown significant increase in the vermicompost over the compost after supplementing the Paper mill waste with cow dung the total nitrogen content increased in all the experiments compared with control. But on the 90th day in all the Experiments (E1, E2, E3) the total nitrogen level increased significantly.

Total Phosphorus (TP) in vermicompost contributes the presence of phosphate, An crucial macronutrient. Phosphate content in the vermicompost have to be in an top of the line level to sell the vermicompost as a plant nutrient. In the Experiments phosphorus content was 0.52, 0.61, 0.50 on 1st day and 2.98, 2.58, 2.73 on the 90th day in vermicompost. In the 90th day vermibeds the amount of TP increased in E1, E2 and E3.

Total Potassium (TK) is one of the main nutrients essential for plant boom. The most excellent presence of TK in a vermicompost elevates its dietary value for utility to plants. The overall potassium content in all of the vermireactors tested in the variety 0.73, zero.95, 0.86 at 1st day. But on the 90th day of vermicomposting E1- 1.02, E2- 0.85, E3- 0.96 the entire potassium content level accelerated, The excessive attention of TK within the above mentioned vermibeds (E3, E1 and E2) need to be due to better mineralization fee because of more suitable microbial and enzymes within the guts of the earthworms as suggested in advance by (Parthasarathi and Ranganathan, 2000).

Total Organic Carbon (TOC) in 1st day and 90th days vermicompost was estimated in filter mud as well as filter mud and organic nutrient supplemented vermibeds. Total organic content in 90th day vermicompost was reduced when compared to 1st day old vermicompost. The TOC content in 90th day old vermicompost was in the range E1- 40.20, E2-37.24, E3- 36.61 where as it was in the range E1- 43.36, E2- 47.59, E3- 42.51 in 1st days of vermibeds. In all the treatment TOC decreased.



**Nithya Srinivasan and Sethu Raman****Carbon and Nitrogen Ratio (C/N)**

The C/N ratio is traditionally used to establish the maturity degree of compost (Bernal et al., 1998). The C/N ratio in all the experimental set up was in the range E1- 29.52, E2- 27.99, E3- 28.34 at 1st day of vermicomposting. But prolongation of *Perionyx excavatus* activity on vermibeds had reduced the C/N ratio significantly (Table 1). The C/N ratio was in the range E1- 9.54, E2- 10.11, E3- 9.22 after 90th of day vermicomposting. The decrease in C/N ratio was high in filter mud E3, whereas in other treatments, (E1 and E2), the C/N ratio on 90th day was reduced when compared to compost. The C/N ratio is a element related to the decomposition of the waste cloth and, even though it is diagnosed as a issue related negatively with the boom of earthworms and duplicate activities.

The parameter traditionally taken into consideration determining the degree of maturity of compost and to outline its agronomic satisfactory is the C: N ratio. Plants cannot assimilate mineral N until the C:N ratio is= 20:1, and this ratio is also an indicative of perfect maturity of compost (Morais and Queda, 2003). The C: N ratio of 20 or decrease being most desirable for agronomic use of composts.

Growth and reproductive performance of earthworms

The earthworm biomass of the current study is shown in (Table 2 and figure 1) with the highest numbers in E1 (154) and E3 (128) and the lowest numbers in E2 (117). Maximum average body weight lowest in E2 and recorded in E1 (20.01g) (17.84g). In E1, the maximum body length was specified (15.3cm) and the smallest in E2 (12.1cm). Earthworms' development rate primarily depends on based on the microbial flora and nutrient availability in vermibeds (Suthar, 2009). Fungi present during the vermicomposting process served as an extra food source for earthworms, increasing their population and weight. Fungi have chitin-based cell walls with high levels of endogenous protein and amino polysaccharide (Kumar, 1995). Particularly, it is believed that fungus and protozoa make up a significant portion of their food (Bonkowski and Schaefer, 1997). Crop residues typically differ in their C:N ratios, particle size, protein, crude fibre content, and even some concentrations of unique plant metabolites, such as poly-phenols and related compounds, which may affect the earthworm generation rate in the vermibeds (Ganesan et al., 2009). The earthworms cultivated in from paper mill and sugar mills press mud wastes (E1) exhibit higher reproductive performance than E2 in days 30, 60, and 90. (Table 3 and figure 2) In different diets, Domnguez et al. (Domnguez, 2000) discovered that *E. andrei* had varying rates of growth and reproduction.

DISCUSSION

The changes in extraordinary physicochemical properties over the duration of the test had been described in Tables. The pH, OC and C:N ratio decreased in the vermicompost when compared to the compost. pH is an important parameter in the vermicompost for promoting plant growth. (Gupta et al., 2007) also reported reduction in pH during vermicomposting of water hyacinth. The lower pH recorded inside the very last products could have been due to the manufacturing of CO₂ and natural acids by using microbial metabolism in the course of decomposition of the substrate within the feed aggregate (Chan and Griffiths 1988; Kaviraj and Sharma 2003). The decline in pH also is probably due to the better mineralization of nitrogen and phosphorous into nitrate/nitrates and orthophosphate (Kaviraj and Sharma, 2003). The vermicompost have more level N, P, K level. It is very important for soil health and plant growth. Earthworms can boost the nitrogen tiers of the substrate throughout digestion of their gut adding their nitrogenous excretory merchandise, mucus, frame fluid, enzymes, or even via the decaying dead tissues of worms in vermicomposting subsystem (Suthar, 2007a). The N enrichment system at some point of vermicomposting depends upon the microbial populations and proportion of commercial wastes which contains microbial increase retarding (Vasanthi et al., 2013a). (Hait and Tare, 2011) had postulated that the increase in total phosphorus content throughout vermicomposting changed into via mineralization, launch and mobilization of to be had phosphorus content from natural waste achieved partially by means of earthworm intestine phosphates and further launch of phosphorus might be because of phosphate solubilizing microorganisms found in worm cast (Esaivani et al., 2015). Total Potassium is one of the main nutrients essential for plant boom. The experiments better mineralization fee because of



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more suitable microbial and enzymes within the guts of the earthworms as suggested in advance by (Parthasarathi and Ranganathan, 2000).

Organic matter (which includes organic carbon) is very essential for soil health. The deficiency in OC reduces storage capacity of soil N, P, S and reduction in soil fertility (Kale, 1993c). Satchell and Martin (1984) reported a reduction in organic matter and a corresponding increase in mineral matter content in culture containing earthworms. The analysis of the results from the present study indicated that during the process of vermicomposting, the level of TOC was reduced to a lesser extent in the vermicompost obtained from various experiments (E1-E3) when compared to control. So, it can be concluded that OC in the vermicompost forms the main source of energy for both soil organisms and plant. Hervas *et al.* (1989) reported that the organic fraction of vermicompost constitutes upto over 50% of the total weight and contains several chemically defined compounds along with humic acids. Recently Suthar (2006) and Gupta and Garg (2009) reported that earthworms promoted such microclimatic conditions in the vermireactors that increased to loss of TOC from substrates through microbial respiration.

The availability of adequate organic carbon and nitrogen in assimilable form is essential for the survival and growth of organisms (Lee, 1985). The C:N ratio is one of the most widely used indices for compost maturation. The observed significant reduction in the level of C:N ratio in vermicompost of all treatments (T1-T5) in our present study, is in accordance with the observation of Mba (1983) who found that in *E. eugeniae* worked cassava peel compost the C:N ratio had decreased. The increased earthworm biomass in the presence of phosphobacteria points to the dual function of bacteria in providing food and enhancing the substrate. phosphorus through the solubilization of phosphorus. Additionally, this occurrence has been noted by (1972; Edwardset al.). According to numerous research, earthworms use microorganisms in their substrates. They can selectively digest them as a food source (Curry, 2006). (Domnguez, 2000) discovered that *E. andrei* had varying rates of growth and reproduction. They also discovered that earthworms allocated their energy preferentially toward either growth or reproduction depending on the quality of the food, and that the availability of carbon also constrains their ability to grow (Tiunov and Scheu, 2004). Cattle and pig dung with low C to N ratios were associated with enhanced growth and reproduction rates of *E. fetida*, according to (Gunadi *et al.*, 2003), while paper mulch with high C to N ratios was associated with lower growth rates. A good supply of readily metabolizable organic matter, unassimilated carbohydrates, and even a low quantity of growth-inhibiting chemicals in vermibeds contributed to a better worm growth pattern in *P. excavatus*. Earthworm survival in the waste-decomposing subsystem is primarily influenced by the physical and initial chemical composition of the feedstock (Suthar, 2009). The greatest indicators for measuring the effectiveness of the vermicomposting process are earthworm survival, biomass output, and reproduction (Suthar, 2006).

CONCLUSION

The physico-chemical and biological parameters of the vermicompost acquired from Experiment I (Paper mill waste with cow dung in 1:2 ratio) was regarded to be appropriate observed through Experiment III (Paper mill waste, Sugar mill press mud and cow dung in 1:1:1 ratio) and Experiment II (Sugar mill press mud with cow dung in 1:2 ratio). Number of worms turned into discovered to be relatively little better in the experiment I (Paper mill waste + cow dung in 1:2 ratio) than different experiments. The quantity of macro nutrients and biological composition of vermicompost obtained from E I experiment was appeared to be better for plant growth. Hence cow dung may be used for the production of vermicompost. Therefore it could be concluded that the Paper mill waste with cow dung in 1:2 ratio and it have to be transformed into vermicompost by way of making use of *Perionyx excavatus* under tradition conditions. In the present study highest earthworm biomass were observed in E1, E3 and least in E2. Based on the present study we conclude E1 waste vermicomposting shows better results of earthworm biomass and reproductive performance influence by the cow dung added in the substrate material. Thus the present experimental information affords a legitimate basis that vermicomposting is a appropriate for the conversion of Paper mill waste and Sugar mill press mud in to natural fertilizer and really demonstrates that the conversion of paper mill waste and press mud with bedding substances into vermicompost may not best reduce the burden of synthetic fertilizers but





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also acts as a good soil conditioners and a source of plant nutrients in agriculture. Further work is required to establish improve the production of earthworms for sustainable vermicomposting / vermiculture operations.

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Table 1. Vermicomposting of different chemical parameters in control and experimentals

| | Control | E1 | E2 | E3 |
|----------------|------------|------------|------------|------------|
| pH | 7.82±0.51 | 7.05±0.05 | 7.13±0.14 | 7.22±0.10 |
| Nitrogen | 2.21±0.08 | 4.21±0.43 | 3.68±0.16 | 3.97±0.21 |
| Phosphorus | 1.98±0.05 | 2.98±0.15 | 2.58±0.12 | 2.73±0.15 |
| Potassium | 0.57±0.02 | 1.02±0.5 | 0.85±0.02 | 0.96±0.04 |
| Organic carbon | 41.12±1.05 | 40.20±1.02 | 37.24±0.98 | 36.61±0.92 |
| C:N ratio | 18.60±1.21 | 9.54±0.51 | 10.11±1.02 | 9.22±0.97 |





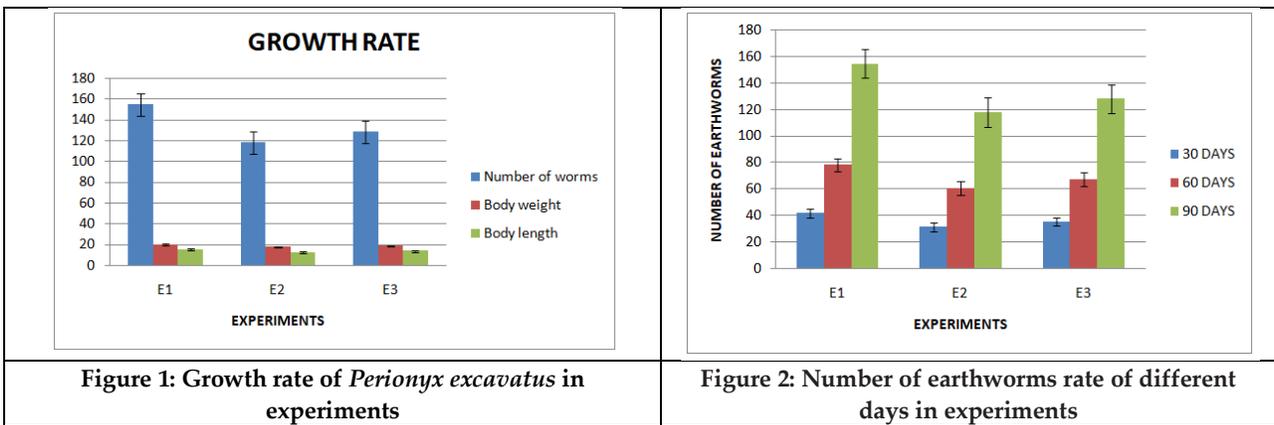
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Table 2. Relative growth rate of *Perionyx excavatus* in Paper mill waste and Sugar mill press mud vermicompost

| Experiments | Number of worms | | Average Body weight (g) | | Average Body length (cm) | |
|-------------|-----------------|------------|-------------------------|------------|--------------------------|-----------|
| | Initial | Final | Initial | Final | Initial | Final |
| E1 | 25±0.00 | 154.7±1.84 | 15.7±2.05 | 20.01±3.41 | 9.5±0.59 | 15.3±0.25 |
| E2 | 25±0.00 | 117.9±2.41 | 14.5±3.61 | 17.84±2.54 | 8.9±1.43 | 12.1±0.18 |
| E3 | 25±0.00 | 128.2±1.96 | 14.2±2.89 | 18.53±2.73 | 9.8±0.71 | 13.7±0.40 |

Table 3. Reproductive performance of *Perionyx excavatus* during vermicomposting (90 days)

| Experiments | Initial stocking | 30 th Days | | 60 th Days | | 90 th Days | |
|-------------|------------------|-----------------------|-----------|-----------------------|-----------|-----------------------|-----------|
| | | EW | RP | EW | RP | EW | RP |
| E1 | 25±0.00 | 41.53±1.47 | 0.41±0.04 | 78.05±1.49 | 0.39±0.13 | 154.7±1.84 | 0.51±0.24 |
| E2 | 25±0.00 | 31.69±1.86 | 0.31±0.05 | 60.28±1.93 | 0.30±0.09 | 117.9±2.41 | 0.39±0.18 |
| E3 | 25±0.00 | 35.05±2.04 | 0.35±0.10 | 67.34±2.68 | 0.33±0.07 | 128.2±1.96 | 0.42±0.12 |





Anti-Microbial Activity of Leaf and Bark Extract of *Alstonia scholaris* and Protein Profiling of its Associated Species

Jyoti Kumari* and Tanuja

Department of Botany, Patliputra University, Patna, Bihar -800020, India

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*Address for Correspondence

Jyoti Kumari

Department of Botany,
Patliputra University, Patna,
Bihar -800020, India

E.Mail: jyotikumari5379@gmail.com



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ABSTRACT

A vast spectrum of bioactive compounds are found in plants. *Alstonia scholaris*, a type of tree in the Apocynaceae family, is said to be a well-known herbal treatment for a number of ailments. Traditional medicine attributes pharmacological properties to this plant's leaves and bark. The aim of the study is to investigate the antimicrobial property of leaf and bark of *Alstonia scholaris* (ASLE and ASBE) against bacteria *E.coli* (MTCC 452), *S.aureus* (MTCC 96) and fungus *A.niger* (MTCC 282) and protein profiling of seeds of *Alstonia scholaris* (AS), *Alstonia venenata* (AV) and *Alstonia macrophylla* (AM). Anti-microbial study shows that the leaf extract was found to be more potent towards *E.coli* (MTCC 452) and bark extract was more effective against *S.aureus* (MTCC 96) whereas both the extract showed low zone of inhibition against the fungus *A.niger* (MTCC 282). The protein estimation of the seeds showed 509.17mg/g in AM, 389.09 mg/g in AS and 386.53 mg/g in AV protein concentration inferring maximum protein content in *Alstonia macrophylla* (AM) and minimum in *Alstonia venenata* (AV). On comparative analysis of seed protein profile of three species of *Alstonia* i.e., AS, AV and AM using 1-D electrophoresis gel analysis, inference was deduced that AS and AV share almost identical protein profiles while a substantial difference exist with AM protein profile.

Keywords: *Alstonia scholaris*, anti-microbial activity, leaf, bark, SDS

INTRODUCTION

A scientific field called phytochemistry examines compounds derived from plants with beneficial biological properties. These phytochemical rich species have been shown to be effective antimicrobial agents against a variety of pathogenic microorganisms. In order to treat a variety of illnesses and disorders, medicinal plants play vital roles



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in our daily lives. Despite the fact that many traditional chemical-based medicines are available. In developing nations like India, herbal and plant-based medicines—also referred to as herbal medicine, traditional medicine, or supplementary medicine—are very common. Since the beginning of time, plants have been a significant source of therapeutic agents, and traditional herbal medical systems like ayurveda have led to the Re-emergence of ancient medical practices (Misra *et al.*, 2011).

An enormous evergreen tree known as *Alstonia scholaris* (Apocynaceae) is typically found in the subtropical regions of South Asia and Africa. It is widely distributed in many parts of India and usually flowers in the months of October-december. Nearly all plant components are used in medicine. The bark is used to cure chronic diarrhoea, dysentery, diarrhoea, and bowel motions. The bark is also consumed as anti-helminthic, astringent, anti-periodic. Beri-beri, liver congestion, dropsy, and ulcers are all conditions that can be treated with leaves. The latex derived from the exudates of tree has been used to treat tumours, wounds, ulcers, and rheumatoid arthritis symptoms (Antony *et al.*, 2012). *Alstonia macrophylla*, also known as Hard alstonia, Hard milkwood, or big-leaved macrophyllum, is a species of tree native to South East Asia. Local names for it include "Pule batu," "Pulai penipu bukit," "Batino," and "Tung fa" in Thailand, Malaysia, Indonesia, and the Philippines. *Alstonia macrophylla*, is known in India as Chuharoi by Negrito group of the tiny Andaman Islands. *Alstonia macrophylla* is frequently mixed with *Alstonia scholaris*. Despite its restricted distribution, its bark is utilised in herbal pharmaceutical preparations in India in place of *Alstonia scholaris* bark (Khyadeet *et al.*, 2014).

Various plant parts of *Alstonia scholaris* such as leaf, bark, roots show excellent anti-bacterial activity against various gram positive (*Staphylococcus aureus*, *Bacillus cereus* & *Lactococcus lactis*) and gram-negative bacteria (*Aeromonas sp.*, *Enterobacter aerogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* & *Proteus mirabilis*) (Misra *et al.*, 2011). For determining the molecular weights of proteins, the sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) method is an effective resource. The speed with which a large number of test samples can be evaluated makes electrophoresis much superior than morphological examination. It concurrently takes use of variations in molecular size to resolve proteins with changes in their electrophoretic mobility through the gel matrix of as little as 1% (Saini and Sarin, 2012).

MATERIALS AND METHODS

Collection of plant

The leaf and bark of *Alstonia scholaris* were collected from local garden. The plant components were properly cleaned under running water to get rid of any dirt, and they were then allowed to dry in the shade for about a week. The dried plant material was grounded into fine powder separately by using an electric blender. The seeds of *Alstonia scholaris*, *Alstonia venenata* and *Alstonia macrophylla* were collected from local seed bank.

Extraction of plant material

Each powdered plant material went through a series of solvent extractions in distilled water using a Soxhlet Apparatus. 20g of leaf and bark powder was extracted with 100 ml of distilled water for 12 hours, coded as ASLE (*Alstonia scholaris* leaf extract) and ASBE (*Alstonia scholaris* bark extract) and stored at 4°C for evaluation of antibacterial activity against pathogens.

Screening for Anti-microbial activity: Anti-microbial activity of the extracts was determined by Agar well diffusion assay against gram negative *E.coli* (MTCC 452) and gram positive *S.aureus* (MTCC 96) bacteria and fungus *A.niger* (MTCC 282). Mueller Hinton Agar (MHA) Media for bacterial isolates and Sabouraud Dextrose Agar (SDA) media for fungus was prepared as per the standard composition given by HI-media. Then, 100µl of the culture broth of each isolate was added over the media and uniformly spread using sterile glass rod and wells were made by using sterile 6mm cork borer. The extract samples were prepared for concentration i.e., 1mg/L, 0.5mg/L, 0.25mg/L with DMSO. Then each well was loaded with 20µl of the respective sample and negative control well was loaded with



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DMSO. The positive control well was loaded with antibiotic ciprofloxacin of 100 ppm concentration and Luliconazole antifungal of 600ppm concentration. Zone diameter was measured after 48 hrs incubation at 37°C for bacterial tested plates while 4 days at 27°C for fungal tested plates (Mukherjee *et al.*, 2012).

Protein extraction from Seeds

Seed protein extraction from *Alstonia scholaris* (AS), *Alstonia venenata*(AV)and *Alstonia macrophylla*(AM). were done by homogenizing 100mg of seed sample with extraction buffer composed of 0.2% of SDS, 5M Urea, 0.5 M Tris HCL and 1% Mercaptoethanol. Extraction was followed by centrifugation at 8000 rpm-5min at 4°C. The supernatant was collected and used for the estimation of total protein and SDS-PAGE analysis.

Protein estimation of Alstonia seeds

The total protein content in the seeds of *Alstonia scholaris* , *Alstonia venenata* and *Alstonia macrophylla* was estimated by Lowry assay (Lowry *et al.*,1951) for which Bovine serum albumin was used as standard in concentration range of 0.1-0.5mg/L. Series of test tubes with concentrations at 0.1, 0.2, 0.3, 0.4 and 0.5mg/L of working standard were taken in the series of labeled test tubes and 100µl of the protein sample was taken in another test tube and volume was made up to 1mL with water, while the blank tube was filled with 1ml of distilled water. Then Folin-Ciocalteu reagent was added and colour change development was assessed absorbance. Optical density of the final complex was measured against the reagent blank at 630nm using spectrophotometer.

SDS-PAGE analysis of extracted protein

The protein samples were analysed by SDS-PAGE to determine the type of proteins and their respective molecular weight. The protein samples were loaded on the SDS-PAGE gel composed of 15% separating gel and 5% stacking gel. The gel solutions were prepared according the given composition and poured in the gel casting tray and allowed to solidify. The supernatant collected from the extraction step was mixed with sample buffer (gel loading dye) in 1:1 (v/v) and incubated at 95°C for 5minutes. After incubation, the sample (20µl) was loaded into separate wells on the stacking gel. The samples were allowed to run alongside protein marker till the end of the separating gel. Then the gel was stained with 0.1% CBB R-250 dye followed by de-staining to visualize the protein bands.

Gel Image Analysis

Gels stained with CBB R-250 dye and further detailed images were analyzed through GelAnalyzer 19.1 and PyElph 1.4. Features of the softwares Gel Analyzer 19.1 were used for automated lane detection, band detection, normalization, background removal and molecular weight determination. In PyElph 1.4, parameters were set for "width deviation", as automatic lane detection failed therefore lanes were defined manually with width at 10% for good accuracy, filter pass was set at 10 and filter width at 3. Band matching was done with distance set at 2% to consider 2 bands in single cluster. Protein profile was compared between seed samples of AS, AV and AM based on band matching.

RESULT

Results for anti-microbial activity as obtained from leaf and bark extracts of *A.scholaris* are presented in Table 1. For gram negative bacteria *E.coli* (MTCC 452), leaf extract showed zone of inhibition of 12mm at 1mg/L,9mm at 0.5 mg/L and no zone at 0.25 mg/L and bark extract showed no zone of inhibition at all concentrations against *E.coli* (MTCC 452)as shown in Fig 1. Thus, leaf extract of *A.scholaris* is effective anti-bacterial agent against *E.coli* (MTCC 452) (shown in fig 1[A-B]).For gram positive bacteria *S.aureus* (MTCC 96), bark extract show zone of inhibition of 12mm and leaf extract show zone of inhibition of 10mm at 1mg/L concentration only as shown in Fig2[C-D]. Thus, barks extract is more potent anti-bacterial agent against *S.aureus* (MTCC 96).Both leaf and bark extract showed very low zone of inhibition at 1.0 mg/L tested concentrations against the fungal species as shown in Fig 3[A-B]. Thus, low potential anti-fungal activity was observed in both the samples against *A.niger* (MTCC 282).



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Seed protein profiling of AS, AV and AM were done by 1D-electrophoresis. Protein extraction was done using the extraction buffer mentioned in above section (section 2.4) followed by its quantification, content showed 509.17mg/g in AM, 389.09 mg/g in AS and 386.53 mg/g in AV. Quantified samples were then separated on SDS-PAGE gel, stained and bands were further analyzed using GelAnalyzer 19.1 and PyElph1.4. Fig 4 shows the bands of protein observed in AS, AV and AM in comparison to the protein ladder. SDS denatured protein gels could resolve a total of 10 significant bands for AS with prominent bands of molecular weight 81,72, 53, 45, 36, 22, 20, 18, 15 and 13 KDa (shown in fig 5), AV bands showing at 81, 73, 54, 46, 36, 22, 20, 19, 16 and 13 KDa. AS and AV showed very similar pattern of bands indicating almost identical composition of protein. Whereas, a wide variation was seen in comparison to AM that showed distinguishable 4 bands at 73 37 27 23 KDa (shown in fig 4). Relative mobility ranged from 0.189 to 0.866 for AS, AV having Rm from 0.184 to 0.869 and in case of AM range of relative mobility was observed to be 0.217 to 0.66. Low, medium and high mobility bands were observed in all the cases (shown in fig 5).

DISCUSSION

This study reveals that the plant parts of *Alstonia scholaris* show good anti-bacterial property against both gram positive and gram-negative bacteria. In present study, leaf extract was more effective anti-bacterial agent against *E. coli* (MTCC 452) as compared to bark extract that is in line with the work of Misra *et al.* (2011). Both leaf and bark extract showed effective result against *S.aureus* (MTCC 96) unlike the work of Misra *et al.*, (2011) that shows only bark extract as an effective anti-bacterial agent against *S.aureus*. Bark and leaf extract of *Alstonia scholaris* in water showed excellent anti-bacterial activity against *Bacillus cereus* and *Pseudomonas aeruginosa*. Various plant parts of *A.scholaris* works as an effective anti-bacterial when extracted in other solvents such as acetone and petroleum ether against *S.aureus*. The petroleum ether leaf of *A.scholaris* showed no effect against *S.aureus* (MTCC 96) whereas acetone extract showed positive results where as both the extract where showed no result against *E.coli* (MTCC 452) (MTCC 452) in the findings of Badarudheen *et al.* (2014).

Findings of Kour *et al.* (2016) revealed that the alcoholic extract was effective anti-bacterial agent than aqueous extract when the alcoholic and aqueous leaf extracts of *Alstonia scholaris* were tested against Salmonella for their anti-bacterial properties. Present study revealed that both the leaf and bark extract were less effective anti-fungal agent against *A.niger* (MTCC 282) unlike the work of Shafique *et al.* (2014) that showed the anti-fungal properties of leaves extracted in Aqueous, methanol, ethanol, n-hexane and chloroform solvent against *F. oxysporum*, *A.alternate*, *A. solani*. The present investigation on SDS denatured proteins showed similarity in number of bands, band width and intensity between *Alstonia scholaris* and *Alstonia venenata* where as dissimilarity with banding pattern of *Alstonia macrophylla*. The present findings confirm the presence of polypeptide bands of heterogenous molecular weight and varying intensity in *A. scholaris* similar to the work of Saini and Sarin (2012) on leaf Galls of *Alstonia scholaris*.

CONCLUSION

Aqueous extracts of *Alstonia scholaris* plant parts leaf, bark were used to test the antimicrobial activities against Gram positive *S.aureus* (MTCC 96) and Gram negative *E.coli* (MTCC 452) bacteria. Only leaf extract was effective and bark extract was ineffective against gram negative *E. coli* (MTCC 452). However, growth of *S.aureus* (MTCC 96) was inhibited by both the extract of at the concentration of 1mg/L. With respect to the zone of inhibition, it can be concluded that the maximum range of zone of inhibition was exhibited by bark extract against *S.aureus*. Further research is needed to investigate the effect of various plant parts of *Alstonia scholaris* as an anti-fungal agent. From the present study it can be concluded that *Alstonia scholaris* contains some chemicals of medicinal importance due to which they show the antimicrobial against several pathogenic microorganisms and the seeds of the selected species of *Alstonia* can be used as an excellent source of protein.





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Conflict of Interest

There is no conflict with other interest in the manuscript content

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Table 1 Showing Zone of Inhibition of *E. coli* (MTCC 452), *S.aureus* (MTCC 96) and *A.niger* (MTCC 282) against leaf and bark extract of *Alstonia scholaris*

| S.No. | Sample | Organism | Well code | Concentration (mg/L) | Zone of Inhibition (mm) |
|-------|--------|--------------------------|-----------|----------------------|-------------------------|
| 1. | ASLE | <i>E.coli</i> (MTCC 452) | W1 | 1 | 12 |
| | | | W2 | 0.5 | 9 |
| | | | W3 | 0.25 | 0 |
| | | | W4 | PC | 29 |
| | | | W5 | NC | 0 |
| 2. | ASBE | <i>E.coli</i> (MTCC 452) | W1 | 1 | 0 |
| | | | W2 | 0.5 | 0 |
| | | | W3 | 0.25 | 0 |





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| | | | | | |
|----|------|---------------------------|----|------|----|
| | | | W4 | PC | 23 |
| | | | W5 | NC | 0 |
| 3. | ASLE | <i>S.aureus</i> (MTCC 96) | W1 | 1 | 10 |
| | | | W2 | 0.5 | 0 |
| | | | W3 | 0.25 | 0 |
| | | | W4 | PC | 24 |
| | | | W5 | NC | 0 |
| 4. | ASBE | <i>S.aureus</i> (MTCC 96) | W1 | 1 | 12 |
| | | | W2 | 0.5 | 0 |
| | | | W3 | 0.25 | 0 |
| | | | W4 | PC | 27 |
| | | | W5 | NC | 0 |
| 5. | ASLE | <i>A.niger</i> (MTCC 282) | W1 | 1 | 6 |
| | | | W2 | 0.5 | 0 |
| | | | W3 | 0.25 | 0 |
| | | | W4 | PC | 27 |
| | | | W5 | NC | 0 |
| 6. | ASBE | <i>A.niger</i> (MTCC 282) | W1 | 1 | 6 |
| | | | W2 | 0.5 | 0 |
| | | | W3 | 0.25 | 0 |
| | | | W4 | PC | 36 |
| | | | W5 | NC | 0 |

*ASLE (*Alstonia scholaris* leaf extract) and ASBE (*Alstonia scholaris* bark extract)

*PC: Positive control ; NC: Negative control

PC:100 ppm (Ciprofloxacin) against *E.coli* and *S.aureus* ; 800ppm (Luliconazole) against *A.niger*

NC:DMSO

W1- W5: Wells label in the plate

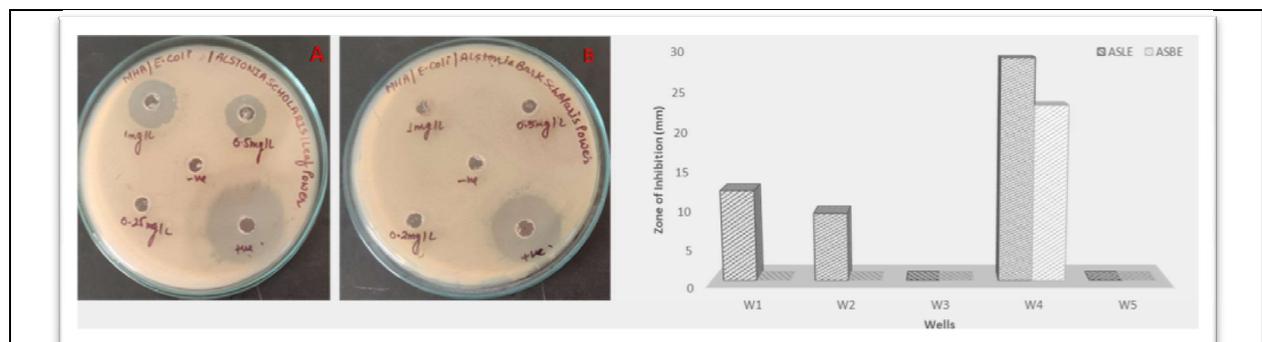


Fig. 1. Anti-bacterial activity of *A.scholaris* [A] leaf extract [B] bark extract against *E.coli* (MTCC 452); +ve indicates positive control, -ve indicates negative control, 1, 0.5 and 0.25 mg/L indicates different concentrations of sample extract. Well size \approx 5mm included in zone of inhibition





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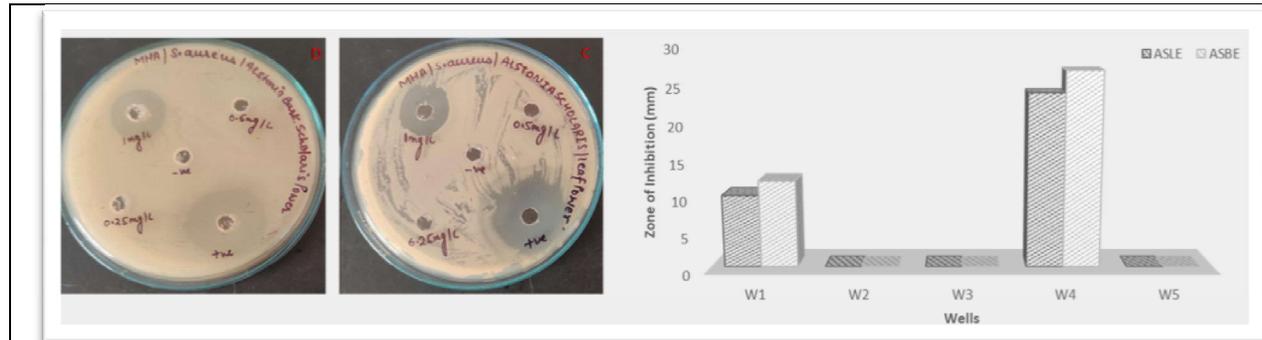


Fig. 2. Anti-bacterial activity of *A.scholaris* [A] leaf extract [B] bark extract against *S.aureus* (MTCC 96); +ve indicates positive control, -ve indicates negative control, 1, 0.5 and 0.25 mg/L indicates different concentrations of sample extract. Well size ≈5mm included in zone of inhibition

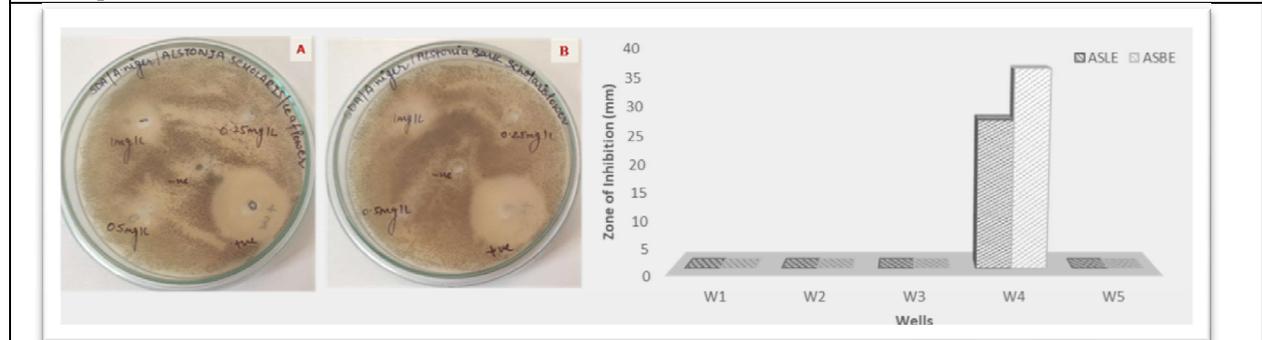


Fig 3 Anti-fungal activity of *A.scholaris* [A] leaf extract [B] bark extract against *A.niger* (MTCC 282); +ve indicates positive control, -ve indicates negative control, 1, 0.5 and 0.25 mg/L indicates different concentrations of sample extract. Well size ≈5mm included in zone of inhibition

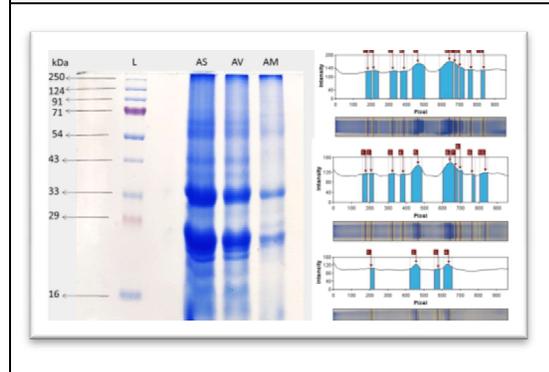


Fig 4 SDS-PAGE Gel images of the protein extracted from seed samples from *Alstonia scholaris* (AS), *Alstonia venenata* (AV) and *Alstonia macrophylla* (AM) while Protein ladder with size in kDa is the lane labeled as (L)

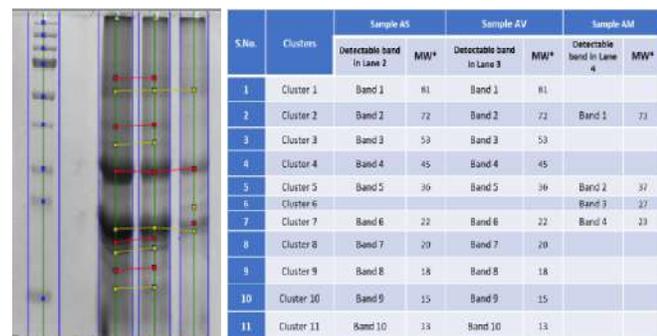


Fig 5: Band scoring and matching on PyElph 1.4, ladder 1 representing ladder while lane 2,3 and 4 represents sample AS, AV and AM respectively. In table shows clusters for closely appearing bands between 3 samples (AS, AV and AM). Bands having 2% or lesser closeness were kept in one cluster *MW: Molecular weight

