

Agroecology for Sustainable Food Security

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INTRODUCTION

Agriculture is the backbone of our country and economy, which accounts for almost 30 per cent of GDP (Global Domestic Product) and employs 70 per cent of the population. Agricultural technology available in the 1940s could not have been able to meet the demand of food for today's population, in spite of the green revolution. The green revolution has not only increased productivity, but also caused several negative ecological consequences such as depletion of lands, decline in soil fertility, soil salinization, soil erosion, deterioration of environment, health hazards, poor sustainability of agricultural lands and degradation of biodiversity. In 1952, India had 0.33 ha of available land per capita, which is likely to be reduced to 0.15 ha by the end of year 2000. As the availability of land has decreased, application of fertilizers and pesticides became necessary to increase the production. The major effect is that our agriculture became chemicalized. In this situation, it is essential to develop eco-friendly technologies for maintaining crop productivity [1]. Agroecology is the only solution for sustainable food production and security. This system paves way for sustainable agriculture. According to CGIAR (Consultative Group on International Agriculture Research), 'Sustainable agriculture is the successful management of resources to satisfy the changing human needs, while maintaining or enhancing the quality of environmental and conserving natural resources'.

WHAT IS AGROECOLOGY?

The science of Agroecology, which is defined as the application of ecological concepts and principles to the design and management of sustainable agro ecosystems, provides a methodological framework to tackle this task. Drawing on the natural and social sciences, agroecology provides a framework for assessing four key systems properties of agriculture: productivity, resilience, sustainability and equity. The central idea of agroecology is to develop agroecosystems with minimal dependence on external inputs, emphasizing complex agricultural systems in which ecological interactions and synergisms between biological components provide the mechanisms for the systems to sponsor their own soil fertility, productivity and crop protection [2]. Agroecology serves as bridge to promote a dialogue of wisdoms between modern scientific agricultural knowledge and indigenous knowledge systems. It is a place-based, pragmatic science, uniquely suited to delivering on the promise of pro poor development. Agroecology goes beyond a one-dimensional view of agroecosystems - their genetics, agronomy, edaphology, and so on, to embrace an understanding of ecological and social levels of co-evolution structure and function. Instead of focusing on one particular component of the agroecosystem, it emphasizes the interrelatedness of all agroecosystem components and the complex dynamics of ecological processes. Agroecology is the holistic study of agroecosystems,

including environmental and human elements. It has emerged as the discipline that provides the basic ecological principles for how to study, design and manage agroecosystems that are both productive and natural resource conserving, and that are also culturally sensitive, socially just and economically viable [3].

AIM OF THIS ARTICLE

The aim of this paper to identify the ideas practices and policies that constitute our concept of agroecology for sustainable agriculture .We do so for two reasons to clarify the research agenda and priorities of our food programme and suggest others to take practical steps that may be appropriate for them in moving towards sustainable agriculture.

GOAL OF AGROECOLOGY

The ultimate goal of agroecology is to integrate components so that overall biological efficiency is improved, biodiversity is preserved, and the agroecosystem productivity and its self-regulating capacity are maintained. The goal is to design an agroecosystem that mimics the structure and function of local natural ecosystems; that is, a system with high species diversity and a biologically active soil, one that promotes natural pest control, nutrient recycling and high soil cover to prevent resource losses [4].

AGROECOLOGY Vs DIVERSIFIED AGROECOSYSTEMS

The problem of hunger and rural poverty in the developing countries has been perceived fundamentally as a problem of production. Attempts to solve the problem of hunger have focused on developing a system by which "low productivity subsistence oriented agriculture could be transformed in to high productivity commercial, cash crop oriented agriculture [5]. For economic and nutritionally vulnerable populations, agroecology supports production of both a greater quantity and diversity of high quality food, fiber and medicinal products, both for family consumption and the market. Agroecology provides guidelines to develop diversified agroecosystems that take advantage of the effects of the integration of plant and animal biodiversity such integration enhances complex interactions and synergisms and optimizes ecosystem functions and processes, such as biotic regulation of harmful organisms, nutrient recycling, and biomass production and accumulation, thus allowing agroecosystems to sponsor their own functioning. More over agroecology improves the adaptive capacity of agroecosystems and reduces vulnerability to natural disasters, climate change impacts, and new and emerging environmental and economic system stresses and shocks [6].

AGROECOLOGICAL MANAGEMENT

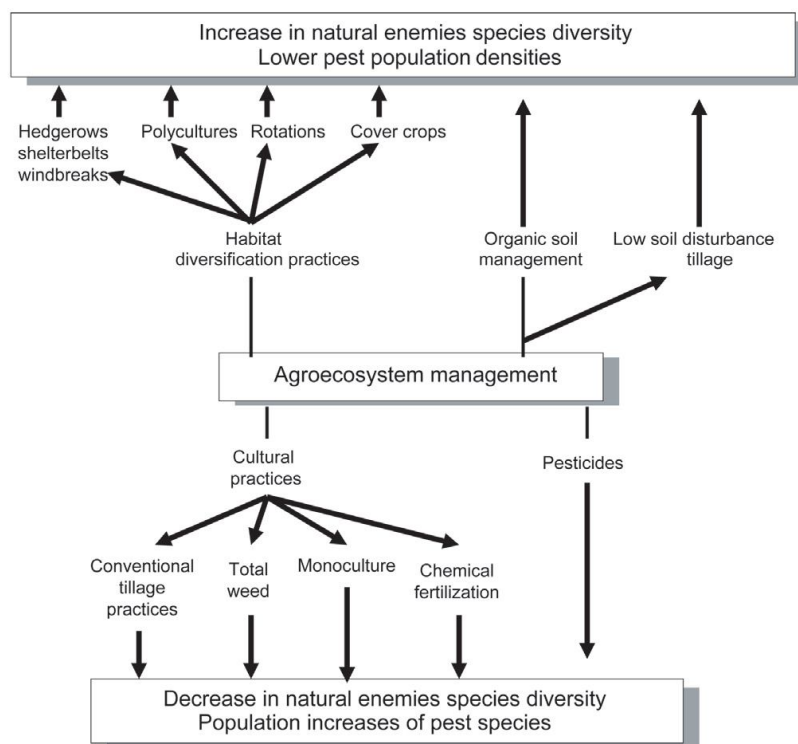
Agroecological management must lead management to optimal recycling of nutrients and organic matter turnover, closed energy flows, water and soil conservation and balance pest-natural enemy populations. Various strategies to restore agricultural diversity in time and space include crop rotations, cover crops, intercropping, crop/livestock mixtures, and so on, which exhibit the important ecological features (i.e.) Crop Rotations, Polycultures, Agroforestry Systems, Cover Crops and Animal integration [7].

All of the above diversified forms of agroecosystems share in common the features (i.e.)

1. Maintain vegetative cover as an effective soil and water conserving measure, met through the use of no-till practices, mulch farming, and use of cover crops and other appropriate methods.

2. Provide a regular supply of organic matter through the addition of organic matter (manure, compost, and promotion of soil biotic activity).
3. Enhance nutrient recycling mechanisms through the use of livestock systems based on legumes, etc.
4. Promote pest regulation through enhanced activity of biological control agents achieved by introducing and/or conserving natural enemies and antagonists [8].

Biodiversity is indeed an important regulator of agroecosystem function, not only in the strictly biological sense of impact on production, but also in the satisfying a variety of needs of the farmers and society at large. Understanding the lifecycles, ecological responses and interaction within and between the organisms that provide ecological services enables agroecosystems managers to build on and enhance the essential services provided by biodiversity. Agroecosystem managers can reduce external input requirement, increase productivity and improve the sustainability of the ecosystem [9].



Ecologically-based management of agroecosystems supports resource conservation and sustainable pest management.

Source: IAASTD Global Report Figure 3-6, from Altieri, M.A., and C.I. Nicholls, 1999. Biodiversity, ecosystem function, and insect pest management in agricultural systems. p. 69-84. In W.W. Collins and C.O. Qualset(ed) Biodiversity in Agroecosystems. CRC Press, NY.

INTENSIVE AND INCLUSIVE KNOWLEDGE

An agroecological approach is particularly well suited for rural communities and developing economies. It recognizes the value of high quality scientific research and of advanced technological exploration and innovation. It also emphasizes the societal and knowledge gains from dialogue between researchers, farmers and indigenous communities. Indigenous knowledge systems and traditional farming practices often yield site-specific insights that would otherwise be outside the purview of formal science. Successful agroecological research, education and extension programs have been building for decades on local and traditional knowledge systems, often through participatory and experiential learning processes and multi-organizational partnerships that integrate formal and informal Agricultural Knowledge, Science and Technology (AKST). Examples include Farmer Field Schools in Integrated Pest Management, Plant Health Clinics, farmer to-farmer extension programs, and agroecological studies in school and urban gardens. Agroecology combines scientific inquiry with indigenous and community-based experimentation, emphasizing technology and innovations that are knowledge-intensive, low cost and readily adaptable by small and medium-scale producers. These methods are considered likely to advance social equity, sustainability and agricultural productivity over the long term [10].

PROFITABLE PRODUCTION

Farmers adopting agroecological methods have produced equal and sometimes substantially increased yields per unit area compared to those using conventional methods in many parts of the world, although research challenges in specific crops and some agroecosystems remain. Similarly, a comprehensive examination of nearly 300 studies worldwide by the University of Michigan concluded that organic agriculture could produce enough food, on a per capita basis, to provide 2,640 to 4,380 kilocalories per person per day (more than the suggested intake for healthy adults). Organic farms in developing countries were found to outperform conventional practices by 57%. These promising findings may underestimate the full potential of agroecological farming to contribute to increased farm-level productivity, household income and food security, as only a very small fraction of public and private sector agricultural investment has thus far gone towards agroecological research [11].

FUTURE PROSPECTS

In future, agriculture will face formidable challenges to provide adequate nutrition for people. Therefore, it is the right time to take decisions, how to increase agricultural productivity, as the developing countries have the lowest productivity for most of the food crops. It is obvious that unless the latest tools of science and technology are applied for sustainable and distribution of natural resources of our country, poverty and hunger will persist. The new technology with agro ecology may be able to harness several newer possibilities in managing the farm sector precisely [12].

CONCLUSION

Agroecology based sustainable agriculture emphasizes farmer's participation, utilization of traditional knowledge, adaptation small farm enterprises, increased plant genetic diversity, soil fertility and sustainable productivity. There is need of basic research activity in agroecology. It should be included in academic curriculum. Integrated co operation of NGOs, Government agencies, community based organizations, policy makers, research institutions and

funding agencies creates frame work for implementing the sustainable based agriculture (Agroecology) among the farmers there by creating the hunger free India by safe guarding the food security. Thus agroecology provides the knowledge and methodology necessary for developing agriculture that is on the one hand environmentally sound and on the other hand highly productive, socially equitable and economically viable.

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Phytochemical Investigation and Anti Microbial Studies on the Leaf Extracts of *Cardiospermum halicacabum* L.

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ABSTRACT

The phytochemical screening and *in-vitro* anti-microbial activity of the leaves of the ethanol and aqueous extracts of *Cardiospermum halicacabum* L. (Sapindaceae) was investigated. The preliminary phytochemical analysis revealed the presence of alkaloids, carbohydrates, proteins and saponins. The extracts exhibited marked anti-microbial activity against Gram +ve and Gram -ve bacteria. Both aqueous and alcoholic extracts have significant antimicrobial activity. When the concentration is increased the zone of inhibition also increased.

Keywords: *Cardiospermum halicacabum* L., phytochemical studies, anti- microbial activity,

INTRODUCTION

Plant-based drugs have been used worldwide in traditional medicines for the treatment of various diseases. Approximately 60% of world's population still relies on medicinal plants for their primary healthcare. According to a survey by National cancer institute (NCI), USA, 61% of the 877 small-molecule new chemical entities introduced as drugs worldwide during 1981–2002 were inspired by natural products [1]. Plant species still serves as a rich source of many novel biologically active compounds, as very few plant species have been thoroughly investigated for their medicinal properties [2]. Thus, there is renewing interest in phytomedicine during last decade and now-a-days many medicinal plant species are being screened for pharmacological activities [3]. When we reviewed the number of articles published on the antimicrobial activity of medicinal plants in PubMed during the period between 1966 and 1994, we found 115; however, in the following decade between 1995 and 2004, this number was increased to 307. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine [4], essential oils [5] or

isolated compounds such as alkaloids [6], flavonoids [7], sesquiterpene lactones [8], diterpenes [9], triterpenes [10] or naphthoquinones [11] among others. Some of these compounds were isolated or obtained by bio-guided isolation after previously detecting antimicrobial activity on the part of the plant [12]. *Cardiospermum halicacabum* L., family Sapindaceae, is a deciduous, branching, herbaceous climber, which is distributed through out the plains of India. The whole plant has been used for several centuries in the treatment of rheumatism, stiffness of limbs, snake bite; its roots for nervous diseases, as a diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic and sudorific; its leaves and stalks are used in the treatment of diarrhoea, dysentery and headache and as a poultice for swellings. Phytochemical constituents such as flavones, aglycones, triterpenoids, glycosides and a range of fatty acids and volatile ester have been reported from the various extracts of this plant [13]. Most likely confused with *Physalis* spp (ground cherry), *Clematis occidentalis*, *Clematis virginiana*, *Campsis radicans*, and *Adlumia fungosa* [14]

MATERIALS AND METHODS

Plant collection and identification

Leaves of the plant *Cardiospermum halicacabum* L. was collected from Kunnam, 20 km away from Perambalur at Tamil Nadu, India. This collection of the plant was authenticated by Dr. Kosiba B.S., M.S, (Govt.Regd. no: 1209) Assistant Siddha medical officer, Government district head quarters, Perambalur-District, Tamil Nadu- state, India.

Chemicals

Dried leaves powder of *Cardiospermum halicacabum* L., ethanol (SD Fine Chemicals, Mumbai), chloroform water (SD fine, Mumbai, India.), beef extract (Merck Ltd, India), acid hydrolysate casein (Biomark laboratories, Maharashtra, India), starch (Vrajlal & sons Indore, India), agar-agar (Marine Chemicals, Kerala, India), sodium hydroxide (SD Fine Chemicals, Mumbai), hydrochloric acid (Merck Ltd, India), ciprofloxacin (Chatan & Chatan), distilled water.

Extraction of plant material

The choice of the plant material for extraction depends on its nature and the components required being isolated. The dried powder plant material is commonly used for extraction. The fresh plant parts when used are homogenized or moistures with a solvent such as alcohol by hot continuous percolation method using Soxhlet apparatus. The leaves of *Cardiospermum halicacabum* L. were dried in the shade. Then the dried leaves were powdered to get a coarse powder. About 25g of dry powder was extracted with 200ml of ethanol (95%) by hot continuous percolation method using Soxhlet apparatus. The extractions were continued for 72 hours at 50°C. The alcoholic extract was filtered and concentrated to a dry mass by using vacuum distillation method. 25g of dry powder extracted with 200ml of distilled water and 10 ml of chloroform duration for 72 hours at 50°C. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extract was then stored in a desiccators [15].

Qualitative phytochemical evaluation

The shade dried powder and various extracts of the leaves of *Cardiospermum halicacabum* L. were subjected to chemical test for its active constituents. Identification of the chemical constituents was carried out on the same extracts used in pharmacological tests according to the methodology proposed by Eyasu Makonnen and Jigna Parekh [16] [17].

Anti-microbial studies

Media – Muller Hinton Agar: Take beef extract (4gm), acid hydrolysate casein (7gm) and starch (0.6mg) are mixed and dissolve in distilled water (400ml). Check the pH 7.2 to 7.4. After that agar-agar (8gm) is included into this

mixture. The product is sterilized in autoclave at 15 lbs/sq. inch for 15 minutes. Then use the UV chamber to prepare Muller Hinton agar plate [18].

Cup plate or Cylinder plate method

Cup plate method using Mueller-Hinton agar medium was employed to study the antibacterial activity of the extracts against *Staphylococcus aureus*, *Bacillus subtilis*, and *E coli* [19]. The Mueller-Hinton agar is melted, after that cooled in suitable condition and then spreaded into petridishes. Take 0.2ml of known concentration of inoculums on the surface of the solidified agar. Cups are cavities are made by using sterile borer (4mm). Now 0.2ml drug is poured into the cups of agar plate. Then placed the sample both aqueous and alcoholic extracts, in different concentration in different Petri dish. Then incubated the plates at 37°C for 24 hours [20] [21].

RESULTS AND DISCUSSION

Leaf extraction

Leaf extract of *Cardiospermum halicacabum* L in ethanol (95%) was obtained by hot continuous percolation method using Soxhlet apparatus. Extract obtained by ethanol extraction for 12 hours at room temperature. After drying dark green residue was obtained. Other hand the same process continued with the aqueous extract. A greenish brown colour residue was obtained. Both aqueous and alcoholic extracts residue were subjected to the chemical test for identification of its active constituents and anti microbial study to know the potency of the leaves extracts.

Phytochemical screening

Preliminary phytochemical analysis revealed the presence of alkaloids (+ve test result for Wagneros), carbohydrates (+ve test result for Fehling's test, Benedict's test), proteins (+ve test result for Biuret test) and saponins. The other secondary metabolites like steroids (+ve test result for Liberman-Burchard, and Salkowski tests), cardiac glycosides (+ve result for Keller-Killani test), etc. present in trace amounts in the extracts (**Table 1**)

Anti-microbial study

The anti microbial study of the both alcoholic and aqueous extracts of the *Cardiospermum halicacabum* L was analyzed by using the Muller Hinton Agar medium (pH 7.2-7.4.). Ciprofloxacin (50mg/ml) was used as a standard drug to compare different concentration (50mg/ml, 75mg/ml, 100mg/ml) of the sample. There are three types of bacterial species are used for the anti bacterial activity. The activities of the extracts are measured by the zone of inhibition (mm) of the bacterial growth. The zone of inhibition of the standard drug is compare to the sample. The average zone of inhibition of aqueous extract 18mm (50 mg/ ml) is nearly close to the standard drug (50mg/ml) average 26mm. But it is too low in alcoholic extract (14mm). According to the result aqueous extract is effective than the alcoholic extract. The concentration of the sample is directly proposed to the zone of inhibition. The results are shown in **Figure 1** and **2**.

Conclusion

Plants continue to be used world-wide for the treatment of disease and novel drug entities continue to be developed through research into their constituents. In the present study, preliminary qualitative phytochemical tests revealed the presence of alkaloids, sterols, carbohydrates, protein, lignins and saponins in the extracts of *Cardiospermum halicacabum* L. Both aqueous and alcoholic extracts have significant antibacterial activity. But the aqueous extract has shown better anti-bacterial activity than that of alcoholic extract. When the concentration is increased the zone of inhibition also increased.

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Table 1: Phytochemical screening of the leaves extract of *Cardiospermum halicacabum* L

S. No	Chemical test	Aqueous extract	Alcoholic extract
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Fixed oils and fats	-	-
4	Saponins	+	+
5	Tannis and phenolic compounds	-	-
6	Proteins and free amino acids	+	+
7	Gums and mucilage	-	-
8	Flavonoids	-	-
9	Lignin	+	+
10	Phytosterol	+	+
11	Glycosides	-	-

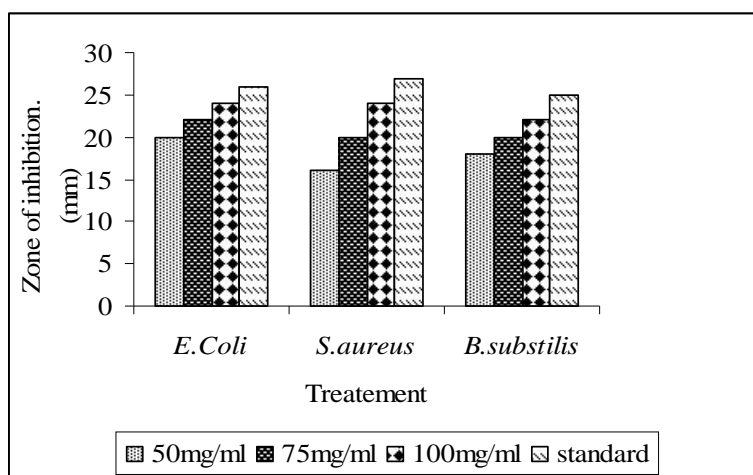


Figure 1: Zone of inhibition of various concentration of aqueous extract

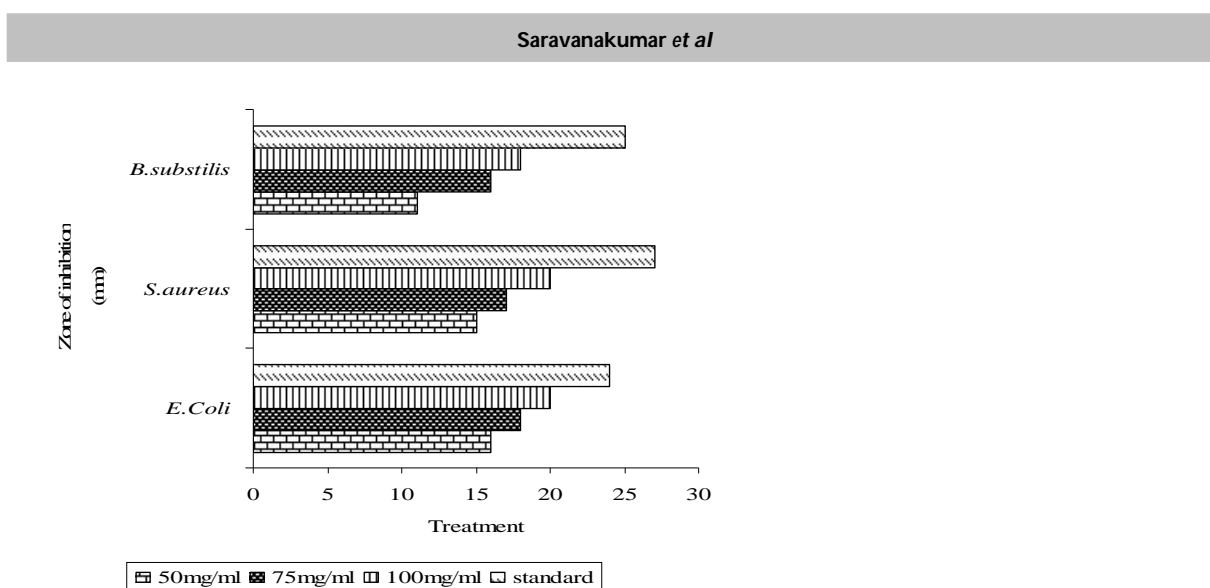


Figure 2: Zone of inhibition of various concentration of alcoholic extract

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RESEARCH ARTICLE

In Silico Modeling and Structural Analysis of NADPH Azoreductase of *Bacillus* sp. Involved in Azodye Degradation

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ABSTRACT

In present study, the 3D structure of NADPH azoreductase from *Bacillus* sp. (strain OY1-2) is generated based on the Putative NADPH-dependent Azobenzene FMN-Reductase (2GSW) of *Bacillus* sp. The protein like flavodoxin has used to bind with nucleotides and especially cofactor NAD. We have identified that the target protein has 8 helices, 6 sheets, 15 turns and secondary structure has the pattern of $\beta_1-\alpha_1-\beta_2-\alpha_2-\alpha_3-\beta_3-\alpha_4-\alpha_5-\beta_4-\alpha_6-\beta_5-\beta_6-\alpha_7-\alpha_8$. The generated target protein has high good quality scores during validation by using number of bioinformatics' tools.

Keywords: *Bacillus*, Homology modeling, Computational biology, Deep view.

INTRODUCTION

Azobenzene reductase (EC 1.7.1.6) belongs to the family of oxidoreductases, specifically acting on the azo dyes. The azoreductase was also found to reduce vat dyes like Indigo Carmine [1]. Amit *et al.*, have reported that *Bacillus velezensis* culture capable of degrading azo dye Direct Red 28 (DR28) [2]. The azoreductase from *Bacillus* sp. was not only able to decolorize MR but also able to decolorize sulfonated azo dyes such as Orange I and Acid Red 88 [3]. Suzuki *et al.* were isolated three bacterial strains which reduce the azo dyes from soil and sewage samples [4]. Among three bacterial strains, the *Bacillus* sp. OY1-2 was actively able to reduce azo dyes which catalyzes the reductive cleavage of azo bond in aromatic azo compounds to the corresponding amines and requires NADPH as an electron donor for its activity. But, the NADPH azoreductase from *Bacillus* sp. strain OY1-2 3D structure was not modeled. We have taken a novel and effective step to model a 3D structure of azoreductase of *Bacillus* sp. OY1-2 by using comparative homology modelling with helps of non-commercialized bioinformatics tools and also find the active site information of the same target protein through *in silico* analysis.

MATERIALS AND METHODS

The computational methods of 3D structure building involved in template selection, alignment with the target, building the model, validation and refinement the structure. The protein sequence of NADPH azoreductase from *Bacillus* sp. strain OY1-2 (Q9FAW5) was retrieved from Swiss-Prot. BLAST [5] and Protein Data Bank (PDB) database used to find an experimentally (X-ray diffraction and NMR) solved template structure by using Blosom 80 as scoring algorithm [6] and blastp 2.2.19 [7]. CLUSTAL X [8] was used to find a homologous 3D structure with more than 35% identical sequence with the target to do the multiple sequence alignment. Then, NJplot tree [9] was used to construct a phylogenetic tree to view the closely similar sequences among the hits which were obtained through blastp program. Bioedit [10] has been used to find hydrophobic and hydrophilic regions among retrieved sequences. The raw sequence of target was fit with 3D structure of template by using DEEVIEW V4.0 [11]. The structurally aligned backbone molecules submitted to SWISS-MODEL [12], an automated Comparative Modelling Server, to perform the molecular dynamics and energy minimization to the aligned model. This protein model was evaluated by using PROCHECK [13], ERRAT [14], VADAR [15], VERIFY3D [16]. To find the active sites of modeled target protein, CASTp [17] server was used. Then, Jmol, a free and open source molecule viewer, is used to view the pocket information from CASTp.

RESULTS AND DISCUSSION

In this study, NADPH azoreductase from *Bacillus* sp. strain OY1-2 (Q9FAW5) retrieved from the protein database Swiss-Prot. The template structure was taken from PDB by searching through the BLAST against the target sequence. Based on positive identities and similarity on residues, hydrophobic and hydrophilic were analyzed by using tools like CLUSTAL X, NJplot and Bioedit. Among the hits, Putative NADPH-dependent Azobenzene FMN-Reductase (2GSW) from *Bacillus subtilis* has 55% identical residues. The 3D structure of Putative NADPH-dependent Azobenzene FMN-Reductase from *Bacillus subtilis* was studied by X-ray diffraction at 2.92 Å resolution. It has four chains (A, B, C and D) and each chain has 182 residues. Among four chains, Chain A has 41% helical (9 helices, 76 residues) and 16% beta sheet (8 strands, 30 residues) but rest of the chains have 42% helical (9 helices; 78 residues) and 16% beta sheet (8 strands; 30 residues). But, all chains are $\alpha\beta\alpha$ type (sandwich) architecture and topologically Rossmann fold type. The 3D structure of NADPH azobenzene reductase generated at SWISS-ExPasy server based on the template (2GSW) from *Bacillus subtilis* as template. This model discloses the primary polypeptide chain with major secondary structure elements, α -helices and β -sheets. This model has 8 α helices and 6 β sheets, and 15 turns. The secondary structure of target found to be $\beta_1-\alpha_1-\beta_2-\alpha_2-\alpha_3-\beta_3-\alpha_4-\alpha_5-\beta_4-\alpha_6-\beta_5-\beta_6-\alpha_7-\alpha_8$ (Table 1). This model also has Rossmann fold type which is a structural motif found in proteins and used to bind nucleotides, especially the cofactor NAD. Rossmann fold can bind one nucleotide; binding domains for dinucleotides such as NAD consist of two paired Rossmann folds that each binds one nucleotide moiety of the cofactor molecule. Single Rossmann folds can bind mononucleotides such as the cofactor FMN [18]. This exactly reveals why these models of proteins need NAD or FMN as cofactors. In modeled target, the amino terminal end has $\beta\alpha\beta$ motif and carboxy terminal end has $\beta\alpha\alpha$ motif. Center core occupied the rest of the helices and sheets. Ribbon diagram of 3D model was displayed by Rasmol [19] visualization tool for protein and nucleic acid molecules.

Validation of structure

Procheck checks the stereochemical quality of a protein structure which gives ϕ and ψ distribution values of Ramachandran plot of non-glycine and non-proline residues and stereochemical quality of overall structure geometry. Mostly, 100% of the residues in favored and allowed regions (Figure 1) and VERIFY 3D result showed that 95.78% of residues had good compatibility of an atomic model (3D) with its own amino acid sequence which showed

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that good compatible and acceptable environment of this 3D model. The VERIFY-3D graph corresponded to acceptable environment of the model. The high score of 0.64 (**Figure 2**) indicated that the environment profile of the model is good. From ERRAT, 93.631 % of overall quality factor was obtained which showed that good high resolution of the 3D model of the target protein. Generally high resolution structures produce values around 95% or higher. From VADAR results, hydrogen bonds, dihedral angles, accessible surface area, accessible surface area for extended chain, volume had very good scores than expected value. The 3D profile quality index and 3D stereo/packing quality index scores were best. The results from the VADAR showed the coordination, compatibility environment of the protein was good. Overall, 3D structure of NADPH azoreductase from *bacillus* sp. strains OY1-2 was good scores (**Figure 3**). The active site of the target 3D structure was identified by using CASTp server which gives the information of active sites residues and its area and volume of the cavity (**Figure 4**). This effective method was carried out by using an advanced commercial and noncommercial bioinformatics tools. The overall quality factor showed very high quality about the target 3D structure of NADPH azoreductase from *bacillus* sp. strains OY1-2. From this study, we have observed that 3D structured Putative Azoreductase of *B. subtilis* played as a template in this study and also which should be used in further azoreductase homology modelling in laboratories studies because it may give a high quality structural protein.

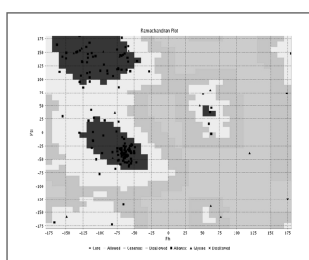


Figure 1: Ramachandran Plot of 3D structured of NADPH azoreductase of *Bacillus* sp., (strain OY1-2)

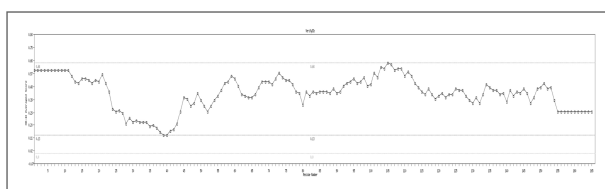


Figure 2: Verify 3D plot of 3D structure of NADPH azoreductase of *Bacillus* sp., (strain OY1-2)

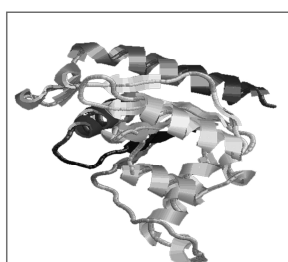


Figure 3: 3D structured of NADPH azoreductase of *Bacillus* sp., (strain OY1-2)

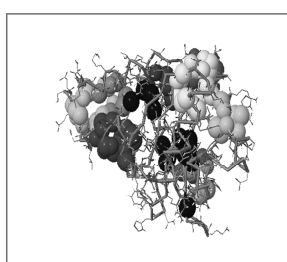


Figure 4: Active sites in the 3D structured of NADPH azoreductase of *Bacillus* sp., (strain OY1-2)

Table 1 : Residues of α helices and β strands of NADPH azoreductase of *Bacillus sp.*, (strain OY1-2)

α -helix	Amino acids	β -strands	Amino acids	Turns	Amino acids
α_1	Arg ₁₅ Thr ₁₆ Arg ₁₇ Val ₁₈ Val ₁₉	β_1	Val ₄ Val ₅ Ile ₆ Asn ₇	1	Lys ₁₂ Phe ₁₃
	Ala ₂₀ Lys ₂₁ Tyr ₂₂ Ile ₂₃ Ala ₂₄ Asp ₂₅ Gln ₂₆		β_2	Glu ₃₀ Leu ₃₁ Tyr ₃₂ Asp ₃₃	2
α_2	Gln ₄₈ Arg ₄₉ Asp ₅₀	β_3		Gly ₆₆ Val ₆₇ Val ₆₈ Leu ₆₉	4
	Ala ₅₃ Val ₅₄ Lys ₅₅ Lys ₅₆ Leu ₅₇ Lys ₅₈ Thr ₅₉ Leu ₆₀ Val ₆₁ Lys ₆₂ Ala ₆₃		β_4	Cys ₇₀ Thr ₇₁ Pro ₇₂	5
α_3	Gly ₈₀ Ala ₈₁ Leu ₈₂ Lys ₈₃ Asn ₈₄ Ser ₈₅ Leu ₈₆	β_5		Pro ₉₈ Val ₉₉ Ala ₁₀₀ Leu ₁₀₁	7
	α_4		Ser ₉₁ Ser ₉₂ Glu ₉₃ Phe ₉₄	Leu ₁₀₂ Ala ₁₀₃ Val ₁₀₄	8
α_5		Ile ₁₁₂ Asn ₁₁₃ Ala ₁₁₄ Leu ₁₁₅ Asn ₁₁₆ Ser ₁₁₇ Met ₁₁₈ His ₁₁₉ Ala ₁₂₀ Ser ₁₂₁	β_6	Asn ₁₂₈ Ala ₁₂₉ Ile ₁₃₀	9
	α_6	Leu ₁₂₂ Ala ₁₂₃ Gly ₁₂₄		Val ₁₃₄ Val ₁₃₅ Leu ₁₃₆	10
α_7		Val ₁₄₁ Gln ₁₄₂ Asp ₁₄₃	α_8		11
	Lys ₁₅₁ Pro ₁₅₂ Leu ₁₅₃ Ile ₁₅₄ His ₁₅₅ Asp ₁₅₆ Val ₁₅₇ Val ₁₅₈ Lys ₁₅₉ Glu ₁₆₀ Leu ₁₆₁ Lys ₁₆₂ Ala ₁₆₃ Tyr ₁₆₄ Met ₁₆₅ Ser ₁₆₆				12

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Phytoconstituents Evaluation and Nephroprotective Activity of *Sida spinosa* L.

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ABSTRACT

The revival of interest in herbal therapy has recently been witnessed in many countries and use of herbal drugs is on the increase of its low toxicity and potency. The objective of this study is to quantify the phytoconstituents and nephro protective activity of alcoholic leaf extract of *Sida spinosa* L. The results reveals that the presence of alkaloids, phytosterols, tannins, saponins, proteins and flavonoids. The alcoholic extract shows significant hepato protective activity in albino rats by Gentamicin induced nephro toxicity model.

Key Words: *Sida spinosa* L., Nephro protective activity, Gentamicin, alcoholic extract

INTRODUCTION

Today herbal medicines are coming in to prominence because of the conventional medicines such as antibiotics which have developed resistance to the many of the infectious organisms which have no longer responsive to the conventional medicines. The herbal drugs have been used throughout the world have received greater attention in recent times, because of it diversity of curing diseases, safety and well tolerated remedies compared to the conventional medicines. *Sida spinosa* L. (Malvaceae) occurs throughout the hotter parts of India from north-west to Ceylon. It is distributed over the tropical and subtropical regions of both hemispheres. The leaves demulcent and refrigerant and are useful for gonorrhoea, gleet and scalding urine administered in the form of a draught and the root is employed in mild cases of debility and fever and for some caoth diseases. A revival of interest in herbal therapy has recently been witnessed in many countries and use of herbal drugs is on the increase of its low toxicity and potency. In past few decades the modern and advanced stage scientific methods of evaluation using phytochemical investigation to isolate the components present and pharmacological, microbiological screening for their therapeutic efficacy have to tend to rational usage of the medicinal plants. The objective of the present study is to perform screening to elucidate the therapeutic potential of the selected plant for nephrotoxicity and quantitative phytochemical evaluations.

MATERIALS AND METHODS

Plant

A large number of *Sida spinosa* L. leaves were collected in Tiruchirappalli, botanically identified and confirmed. The collected leaves were washed with water and shade dried for two weeks, after milling as powder the leaves used for extraction.

Preparation of extraction

The dried plants materials were pulverized by mechanical grinder, sieved through 40 meshes. The powdered materials were extracted with ethanol using Soxhlet extraction apparatus for 48 hours at temperature of 50-60°C. This ethanol extract was then concentrated and dried under reduced pressure. The ethanol free semisolid mass thus obtained was preserved in desiccator and used for experiments.

Qualitative phytochemical evaluation

Ethanol extract was tested to determine the various phytochemical constituents using standard methods [1] [2]. The anti-microbial properties of the plants may be attributed to the secondary metabolites present in it, phytoconstituents like Phenolics [3] [4], tannins [5] and alkaloids [6] etc., is found to be effective anti-microbial substance against a wide range of micro organism [7].

Nephroprotective activity

Male Wister rats weighing 200-250gm were used and the animals were housed under conditions of controlled temperature and 12 hour day night cycle and were feed standard pellet diet (Hindustan liver Ltd, Bangalore). As per the protocol the animals were divided in to four groups of six animals each. Group -1 served as normal (only normal saline) throughout the course of the experiment, where no treatment was given, Group-2 served as control received daily intraperitoneal injection[8] of gentamicin (80 mg/kg) and 2 ml/kg of Carboxy Methyl Cellulose (CMC) orally for 8 days. This dose has already been shown to produce nephrotoxicity[8] Group-3 and 4 animals were administered gentamicin (80mg/kg) intraperitoneally for eight days in addition to this they also received 100mg and 200mg of *Sida spinosa* L in CMC orally respectively. This was started three days prior to the gentamicin injection and continued with the 8 days of gentamicin treatment.

Biochemical assays

At the end of the study, the animals were kept in individual metabolic cages for 24 -hour's urine collection. Before sacrificing the animals, blood was collected by orbital sinus under ether anaesthesia. Estimation of urinary sodium and potassium was done by using flame photometer (Systronic, M121). Blood urea concentration was determined by glutamate dehydrogenase (GLDH) Kinetic method, using Beckman Spectrophotometer. Creatinine clearance was calculated after estimating the serum and urinary creatinine [9] all the biochemical estimations were done by using semi-auto analyzer.

RESULTS AND DISCUSSION

The extract of *Sida spinosa* L. reveals that the presence of various phytoconstituents shown in **Table 1**. In gentamicin treated rat, body weight was significantly lower than control, and *Sida spinosa* L. plus gentamicin group. *Sida spinosa* L. alone had no effect on any renal parameters shown in (**Table 2**). A marked increase in blood urea (**Figure 1**), and serum creatinine (**Figure 2**) was noted in gentamicin treated group compared to control. Co administration of *Sida spinosa* L. decreased the rise in blood urea and serum creatinine level. Creatinine clearance in gentamicin group fell significantly compared to control (**Table 2**) and *Sida spinosa* L. significantly prevented the fall in creatinine clearance.

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Urinary glucose was significantly high, while there was less change in the urinary sodium and potassium excretion with gentamicin. Excretion of glucose in urine with gentamicin was significantly reduced with *Sida spinosa* L. treatment (Table 1).

Table 1: Phytochemical screening of the leaves extract of *Sida spinosa* L.

S. No	Chemical test	Alcoholic extract
1	Alkaloids	+
2	Carbohydrates	-
3	Fixed oils	+
4	Saponins	-
5	Tannis and phenolic compounds	+
6	Proteins and free amino acids	+
7	Gums and mucilage	-
8	Flavinoids	+
9	Lignin	-
10	Phytosterol	+
11	Glycosides	-
12	Volatile oils	-

Table: 2 Effects of Gentamicin and *Sida spinosa* L. extract on renal parameters

S. No	Treatment	Creatinine clearance (ml/min)	Urinary Glucose (mg/ay)	Urinary Sodium (meq/day)	Urinary Potassium (meq/day)
1	Group-1	0.42±0.02	0.0	0.76±0.07	0.89±0.06
2	Group-2	0.063±0.005	73.2±6.4	0.53±0.04	0.69±0.05
3	Group-3	0.19±0.08	8.31±3.2**	0.73±0.03*	0.77±0.06*
4	Group-4	0.38±0.06	0.0	0.91±0.08**	0.87±0.04**

Data are expressed mean ±S.E. n=6, *P<0.01Vs control, **P<0.001Vs control by student't' test

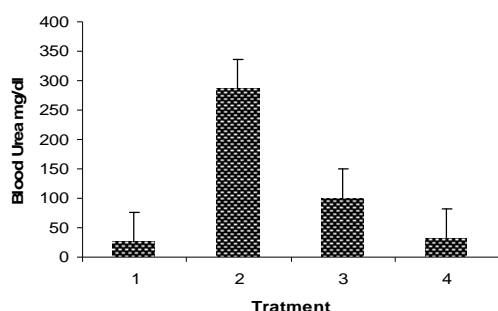


Figure 1: Blood urea levels of various concentrations of *Sida spinosa* L. compared with gentamycine.1=normal, 2= gentamicin, 3= *Sida spinosa* L. 100mg, 4= *Sida spinosa* L. 200mg

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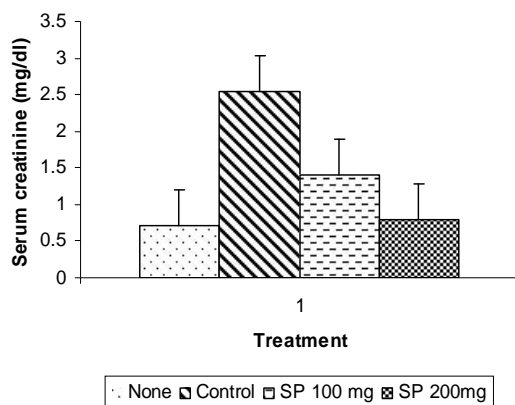


Figure 2: Serum creatinine levels of various concentrations of *Sida spinosa* L. compared with gentamicin.

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Analysis of Phytochemical Constituents and Antibacterial Activity of Neem Extract (*Azadirachta indica* A. Juss)

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ABSTRACT

Herbal plants are used for thousands of centuries by many cultures for their medicinal values. Herbal treatment is very popular because it is easily available, cheap and less toxic. Therapeutic value of many herbal plants has been described by practitioners of traditional medicine. *Azadirachta indica* A. Juss (neem) is an herbal plant widely distributed in India during all seasons. In the present study ethanolic extract of neem subjected to phytochemical analysis and antibacterial activity studies. The 75 mg/ml concentration of neem extract was applied against *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* and inhibition zones were measured which indicates the anti-bacterial activity of neem leaves extract.

Keywords: *Azadirachta indica* A. Juss, Phytochemicals, Antibacterial and Neem extract.

INTRODUCTION

Herbal medicines have been known to man for centuries. Therapeutic efficacy of many indigenous plants for several disorders has been described by practitioners of traditional medicine. *Azadirachta indica* A. Juss is a tree which has been used for a long time in herbal medicine. It is an indigenous plant widely distributed in India. *Azadirachta indica* A. Juss belongs to Meliaceae. The former is popularly known as Indian neem (margosa tree) or Indian lilac, and the latter as the Persian lilac. The medicinal properties of the plant *Azadirachta indica* A. Juss were studied by several workers. The antipyretic effect [1] [2], antimalarial effect [3] [4], antitumour effect [5], antiulcer effect [6], antidiabetic effect [7], antifertility effect [8] were some of the studies of the earlier workers. Antimicrobial properties of *Azadirachta indica* A. Juss were studied by several authors. Neem oil suppresses several species of pathogenic bacteria such as *Staphylococcus aureus* and *Staphylococcus typhosa*, all strains of *Mycobacterium tuberculosis* [9] [10]. The growth of *Salmonella paratyphi* and *Vibrio cholera* was inhibited [11]. Available antimicrobial agents can control the infection but they are expensive and rapid emergence of anti-microbial resistance. Neem may be used for its easy availability and

significant effect against bacteria. The neem tree is still regarded as 'village dispensary' [12]. The aim of the present study is to analyze phytochemical constituents and antibacterial activity of neem extract (*Azadirachta indica* A. Juss).

MATERIALS AND METHODS

Plant material

The plant materials used in this study were collected from Pudukkottai, TamilNadu. It was identified and authenticated by the Dr. S. Vijikumar, Head of Plant Science Research Division (PSRD), Tamil Nadu Scientific Research Organization, (TNSRO) Arimalam (Pudukkottai).

Chemicals

All chemicals used were the highest purity available and analytical grade purchased from Hi media laboratories, Mumbai, India.

Preparation of Neem extract

The plant extract was prepared by Osol [13] and Hymete [14] procedure with some modifications. Mature leaves of neem were collected and washed with distilled water. The leaves were shade dried and grind well. The powdered material was soaked in alcohol (70% ethanol). The material was filtered with filter paper after 25 days. The alcoholic extract was filtered and concentrated to a dry mass by using vacuum distillation method. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure.

Phytochemical Analysis

The qualitative and quantitative estimation of various phytoconstituents such as Alkaloids, Saponins, Tannins, Steroids, Flavonoids and Glycosides were carried out by the method of Brain and Turner [15].

Microorganism and medium used for antibacterial activity analysis

The pathogenic strains of *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* for antibacterial test were used. For bacterial sensitivity test Nutrient agar was used.

Analysis of antibacterial activity

The 75 mg/ml concentration of neem extract was prepared. The nutrient agar medium was prepared and sterilized. The sterilized media were poured on to sterile Petri dishes and the plates were inoculated with inoculums using a sterile swab is dipped in inoculums. Well was made at the center of the medium. Ciprofloxacin (50mg/ml) was used as a standard drug to compare antibacterial activity of the sample. Simultaneously the well was filled with 75 mg/ml concentration of neem extract. Then the plates were incubated at 32°C for 48 hours. The antibacterial activity was then measured as indicated by clear zones of inhibition with the help of zone reader.

RESULTS AND DISCUSSION

Phytochemical Analysis

The phytoconstituents of neem leaves extract showed 3.9% crude alkaloids, 4.92% Saponins, 3.1% steroid, 2.3% flavonoids, 4.5% glycosides and 0.6% tannic acid respectively as shown in (Table 1). Glycosides, flavonoids, Alkaloids, tannins, steroids were present in different parts of neem [16]. Neem leaves extract showed 4.1% crude alkaloids, 4.96% saponins 3% steroid, 2.5% flavonoids, 4.5% glycosides and 0.64% crude tannins [17].

Analysis of antibacterial activity

The ethanolic extract of neem showed different antibacterial activity towards test organisms and it was tabulated in **Table 2**. Neem extract showed high antibacterial activity towards *Staphylococcus aureus* represented in **Figure 1**. Ethanol extract of neem showed high antibacterial activity against *Staphylococcus aureus* where as low activity against *Pseudomonas aeruginosa* and moderate activity against *E. coli* and *Salmonella typhi*. The average zone of inhibition of standard drug Ciprofloxacin (50 mg/ml) is 25.5mm. Crude extract of neem plant was very effective against *Staphylococcus aureus* and *E. coli* [18]. The 10% chloroform extract of neem imported inhibitory effect against *Staphylococcus aureus* and *E. coli* [19].

CONCLUSION

The present study showed that ethanolic extract of neem having antibacterial activity and revealed that different phytoconstituents such as crude glycosides, flavonoids, tannins, steroids, alkaloids and saponins inhibited the growth of different bacteria. This finding supports the use of neem based products in the treatment of bacterial infections and diseases by alternative systems of medicine.

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Table-1: Quantity of Phytochemicals in neem extract

S.No	Phytochemicals	Result	Quantity in neem extract
1	Alkaloids	+	3.9%
2	Flavonoids	+	2.3%
3	Crude glycosides	+	4.5%
4	Steroids and Triterpinoids	+	3.1%
5	Tannic acid	+	0.6%
6	Saponins	+	4.92%

Table-2: Diameter of zone of inhibition by 75 mg concentration of neem extract against different bacteria

S.No	Bacteria	Diameter of inhibition zone (mm)
1	<i>E. coli</i>	18.5
2	<i>Salmonella typhi</i>	17.2
3	<i>Pseudomonas aeruginosa</i>	16.8
4	<i>Staphylococcus aureus</i>	19.2

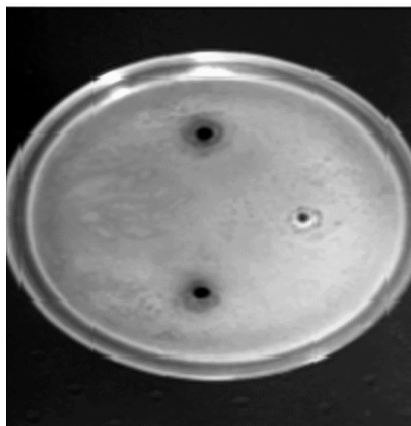


Figure 1: Neem extract showed high antibacterial activity towards *Staphylococcus aureus*

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Hepatoprotective Activity of *Coccinia grandis* L. against 1, 4 dichlorobenzene Induced Liver Injury

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ABSTRACT

The protective effect of the ethanolic leaf extract of *Coccinia grandis* L. on dichlorobenzene induced hepatic toxicity was studied. The hepatic marker enzymes such as Alanine Transaminase (ALT), Aspartate Transaminase (AST) and Alkaline phosphate (ALP), total bilirubin and histopathological changes were studied. A hepatic injury involved with possible necrosis which may have contributed to its possible pathogenesis was explored. Administration toxicant dichlorobenzene produced significant changes in physical (increased liver weight), biochemical parameters (ALT, AST, ALP and total protein) and histopathological changes (damage to hepatocytes of liver). The liver cells filled with uniformly distributed dense of small fat droplets, large nuclei, inflamed cells and evidence of necrosis and fibrosis while treating with the ethanolic extract of *Coccinia grandis* L. significantly decreased the level of all parameters. Pretreatment with 200 mg/kg of the extract showed micro vesicular fatty changes with no evidence of inflammation, necrosis or fibrosis. Therefore, the *Coccinia grandis* L. leaf extract has a strong moderately effect against the hepatic damage induced by 1, 4 dichlorobenzene.

Keywords: 1, 4 Dichlorobenzene induced hepatotoxicity, *Coccinia grandis* L., modulatory effects, hepatoprotective effect.

INTRODUCTION

The liver is the key organ regulating homeostasis in the body. It is involved with almost all the bio-chemical pathways related to growth; fight against to disease, nutrient supply, energy provision and reproduction. Liver is expected not only perform physiological function but also to protect against hazards of harmful drugs and chemicals. The liver is a major detoxifying organ in vertebrate body, which involves intense metabolic activities. Certain toxic chemicals and medicine can cause liver damage, which has been recognized as a toxicological problem. However, herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy

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[1]. Presently, only a few hepatoprotective drugs and that to from natural sources are available for the treatment of the liver disorder. Several plant materials (including roots, stem, barks, leaves, flowers etc.) were used to combat various ailments [2]. Human beings are exposed on a daily basis to certain toxic chemicals and pathogens, which are causing certain serious health problems, amongst them 1, 4 dichlorobenzene also one of the toxic substances widely used in printing ink, textiles, pesticides, and plastics. *Coccinia grandis* L. (Curcubitaceae) is a climber herb cultivated throughout India. The fruit is used to treat leprosy, fever, asthma, Jaundice and sore throats [3] [4]. The alcoholic extract of the plant is used as hypoglycemic and antioxidant agent [5], [6]. A compound polyphenol isolated from the ethanolic extract possesses anti-dyslipidemic activity [7]. Therefore, in the present investigation an attempt was made to evaluate the hepatoprotective activity of the leaf extract of *Coccinia grandis* L against 1, 4 dichlorobenzene induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant material

Fresh *Coccinia grandis* L. leaves were collected from Pudukkottai District in Tamil Nadu. The collected leaves were shade dried and then homogenized into fine particles. Then 250g powder form of leaves were extracted with ethanol using Soxhlet apparatus. The greenish black residues obtained were stored in sample bottles wrapped with aluminium foil, at 40° C for drying the excess solvent present in the extract. Finally got powder form and then extract was dissolved in saline before use.

Animals

Wistar albino rats (150 – 200g) of either sex were procured from Sri Venkateshwara Enterprises, Bangalore. They were maintained under standard environmental condition (Temperature 28°C) and allowed access to standard laboratory feed and water *ab libitum*. The animals were allowed to acclimatize to the laboratory condition for a week before they were used for the experiment.

Acute toxicity studies

Acute oral toxicity of *Coccinia grandis* L. were determined using Wistar albino rats. The animals were fasted overnight and provided only water, after which the extracts were administered orally at the dose (single) and then the animals were more observed for 14 days, when mortality was observed in 2 or 3 animals, the dose administered was recorded as a toxic dose. But when mortality was observed in one animal, then the same dose was repeated again for confirmation. However, if mortality was not observed, the procedure was repeated for further higher doses such as, 300 and 600, 1000 mg / kg body weight. Toxic symptoms for which the animals were observed for 72 hours include behavioral changes, locomotion, convulsions, and mortality.

Experimental Design

The experimental animals were divided into 4 groups of 6 animals each and treated as follows.

- | | | |
|-------------------|---|---|
| Group 1 (Control) | : | Animals that received only normal diet and Water <i>ab libitum</i> |
| Group 2 | : | Animals that received ethanolic leaf extract of <i>Coccinia grandis</i> L (200 mg / kg) per oral for 13 weeks |
| Group 3 | : | Animals received Dichlorobenzene (DCB) only 300mg / kg body weight for 13 weeks |
| Group 4 | : | Animals received ethanolic extract of <i>Coccinia grandis</i> L. (200 mg / kg) daily for 13 weeks along with Dichlorobenzene (300 mg / kg). |

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At the end of the treatment, the rats were sacrificed and blood was collected from the heart with the use of 5 ml sterile syringe individually for each animal and transformed into plain sample bottles immediately. The serum was separated by centrifugation at 2000 rpm for 15 minutes. The serum was then analyzed for hepatic marker enzymes, Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphatase (ALP) and total protein.

Histopathological Examination

Animals were sacrificed by cervical dislocation and the liver was removed, sliced and washed in saline and the pieces were preserved in 10% formaldehyde solution for histopathological evolution. Sections of pieces of the liver (about 4-6 mm in thickness) were processed and embedded in paraffin wax stained with haematoxylin and eosin. The slides were studied under a light microscope for any hepatocytes damage / protection.

Statistical Analysis

The results obtained were reported as mean (x) I standard deviation (SD) to 6 animals in each group. The data obtained from this study was analyzed statistically using Duncan's Multiple Range Test (DMRT). Differences were considered to be statistically significant at $p < 0.05$.

RESULTS

Preliminary phytochemical studied revealed the presence of alkaloids, steroids, carbohydrate and triterpenses. The animals treated with Dichlorobenzene showed significant increase in the levels of marker enzymes Alanine transaminase (ALT), Aspartate transaminase (AST), and Alkaline phosphatase (ALP) when compared with the control animals. For the animals given the *Coccinia grandis* L. leaf extract (200 mg / kg) the level of these enzymes were relatively normal when compared with dichlorobenzene treated group. Histopathological examination of liver section of the Group (1) rats showed normal hepatic architecture (**Figure A**). The section of liver taken from the animals (Group 2) treated ethanolic leaf extract showed hepatic architecture, which was similar to that of control (**Figure B**). Disarrangement of normal hepatic cells with intense Centrilobular necrosis and fatty vacuoles was observed in liver of DCB intoxicated rats (**Figure C**). Liver section of rat treated with ethanolic leaf extract and intoxicated with DCB showed an almost normal architecture with little deformation of hepatocytes and clearing of cytoplasm (**Figure D**).

Table 1: Effect of *Coccinia grandis* L. on serum enzymes in rats treated with 1, 4 -Dichlorobenzene.

Groups	Dose	ALT IU/L	AST IU/L	ALP IU/L	Total Protein mg/dl	Total Bilirubin (mg/dl)
Control	-	17.20±0.33	31.36±6.15	70.25±6.15	7.9±0.30	0.46±0.04
Control + Extract	200 mg / kg	16.13±1.52	30.24±1.20	69.72±3.18	8.0±0.28	0.42±0.03
Normal + DCB	0.3% DCB (300 mg/kg)	48.37±10.6	55.67±13.32	137.42±11.62	4.5±0.60	0.90±0.12
DCB + Extract	0.3% DCB + (CG) 200 mg / kg	20.39±4.5	41.33±5.57	71.54±6.27	7.3±0.30	0.44±0.06

DISCUSSION

Liver diseases are one of the serious health problems. Herbal drugs play a role in the management of various liver disorders in addition to other natural healing processes of the liver. The ALT, AST, are present in the hepatic and biliary cells [8] these enzyme are usually released from the hepatocyte and leak into circulation causing increase in their serum levels under hepatocellular injury or inflammation of the biliary track cells resulting pronominally an elevation of the ALP . Serum levels of these enzymes are particularly high in acute hepatocellular damage caused by drug toxicity and xenobiotics. The extinct of the enzyme, changes in related to the nature, closeness to toxic agent and duration of toxicity [9]. The DCB induce hepatic damage due to increased level of ALT, AST & ALP. Cell death is thought to takes place by at least 2 distinct processes, apoptosis and necrosis [10]. Some medicinal plants possess hepatoprotective effects. These effects present because, they contain some bioactive compounds [11]. The presence of saponins in variety of herbal preparation administered to human proved to be potent against cancer and hepatic cell proliferation. The increase in the levels of transaminase reflects a clear indication of cellular leakage and less of functional integrity of the cell membrane [12]. Assessment of liver function can be made by estimating the activities of SGOT and SGPT, which are originally present in higher concentrations in cytoplasm. Histopathological examination of liver section of normal rats showed normal hepatic cells with cytoplasm and nucleus where as DCB treated group showed that the liver cells are intoxicated with DCB the normal architecture of the liver was completely damaged. Treatment with ethanolic extract exhibited protection against liver damage by DCB, which is confirmed by the results of biochemical studies. Earlier report indicated that the flavonoids are phenolic compounds exert multiple biological effects, including antioxidant and free radical scavenging abilities. Therefore, the hepatoprotective activity of the extract may be due to the presence of chemical compound.

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Physico - Chemical and Statistical Analysis of Ground Water in Domestic Areas of Arsikere Taluk, Hassan District, South India.

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ABSTRACT

The quality of ground water samples collected from 12 representative dug wells in Arsikere's domestic zone was assessed in the pre-monsoon and monsoon season from February 2009 to September 2009. A total of 12 physico-chemical characteristics were analyzed. Four parameters: pH, Total alkalinity, Nitrate and Chloride were within and four parameters: Total Dissolved Solids (TDS), Electrical conductivity, Total hardness and fluoride were above the permissible limit for drinking purpose throughout the study period. Several water quality parameters showed significant correlation. Most significant linear relation were found among parameters (pH, Total alkalinity), (TDS, Electrical Conductivity (EC), Total hardness, Magnesium, Chloride), (Fluoride, Calcium, Nitrate), (Total hardness, Calcium, Magnesium), Dissolved oxygen (DO), Biochemical oxygen Demand (BOD) throughout the study period.

Keywords: Physico-chemical analysis, domestic areas, ground water, statistical analysis.

INTRODUCTION

Water is the most important natural resource without which life would be nonexistent. Availability of safe and reliable source of water is an essential prerequisite for sustainable development. Freshwater quality and availability remain one of the most critical environmental and sustainability issues of the twenty-first century [1]. Of all sources of freshwater on the earth, groundwater constitutes over 90% of the world's readily available freshwater resources [2] with remaining 10% in lakes, reservoirs, rivers and wetlands. Groundwater is also widely used as a source, of

drinking water supply and for irrigation [3]. However, groundwater is not only a valuable resource for water supply, but also a vital component of the global water cycle and the environment. As such, groundwater provides water to rivers, lakes, ponds and wetlands helping to maintain water levels and sustain the ecosystems. Groundwater pollution, often due to contaminant seepage from waste disposal sites, is a worldwide problem. Such contamination of groundwater resources potentially poses a substantial risk to local resource users and to the natural environment. Assessing risk involves identifying the hazard associated with a particular occurrence, action or circumstance and determining the probability of that hazard occurring due to rapid growth of population, industrialization and urbanization, there have been intense human activities and interference into nature leading to over-exploitation and severe pollution stress on natural water-bodies. Improper waste disposal and unscientific anthropogenic practices over the years adversely affected the surface and ground water quality. The major problem with the ground water is that once contaminated, it is difficult to restore its quality the solution is non-trivial because of complex dynamics involved in the ground water flow, which requires simultaneous solution of complicated geo-chemical and hydrological equation. Hence, there is a need for concern over the protection and management of ground water quality. The present study was urgently required to draw attention towards this region for taking necessary steps to minimize the adverse impacts which likely to occur due to drinking water contamination. This paper is an attempt to address the issue taking into account ground water quality parameters with respect of statistical analysis in domestic zone of Arsikere Taluk, Hassan District, Karnataka.

Study area

Arsikere is one of the important taluk of Hassan district, Karnataka state. It is a major railway junction on the South Western Railway and a central place to visit places of tourist interest, like Belur (40 km), Halebidu (25 km) and Shravanabelagola (80 km). The geographical location of Arsikere taluk lies between the latitude 13° 19' 48"N and longitude of 76° 15' 0" E. It has got an elevation of 807 m (2647 feet).

MATERIALS AND METHODS

Groundwater samples were collected at an interval of 30 days from February 2009 to September 2009 from all the 12 bore wells for physico-chemical analysis. Water samples were collected in plastic cans from bore wells of 2 liter capacity. Factors like pH and water temperature are recorded at the spot of collection. For Dissolved Oxygen (DO) the samples were fixed on the spot using Winkler's reagents. Later the samples were brought to the laboratory for the estimation of other chemical parameters. The final results were calculated by taking three consecutive readings. The standard prescribed methods were followed for the physico-chemical analysis of the water samples [4].

RESULTS AND DISCUSSION

The minimum, maximum, mean and standard deviation obtained for the pre-monsoon and monsoon period are reported in **Table 1**. The pH of a solution at any given temperature represents the hydrogen ion concentration at that temperature. In pH scale, the value of the water less than seven indicates acidic condition of that water and greater than seven indicates the alkaline condition of water. In the present study pH values ranged from 6.9 to 8.3 and 7.4 to 8.55 during the pre-monsoon and monsoon seasons respectively. During the present investigation the pH values were found well within the permissible limit of BIS (bureau of Indian standards) [5].

Low pH in pre-monsoon season may be due to acids, acid generated salts and dissolved carbon dioxide and high pH may be from carbonates, bicarbonates, hydroxides, phosphates and borates. Similar observations were made by Samir [6]. The result showed that pH has shown positive relationship with Total alkalinity (**Table 2 and 3**). The chief source of fluoride containing minerals viz., (fluorite, fluorapatite, cryolite, micas and hornblend) are rocks and

sediments. Fluoride bearing minerals occur in all geological factors such as sedimentary, metamorphic and igneous deposits [7] and [8]. The fluoride concentration ranged from 0.33 to 1.76 mg/L during pre-monsoon and 0.4 to 1.19 mg/L during monsoon season showed positive relationship with calcium and nitrate (**Table 1, 2 and 3**). The BIS [5] acceptable limit for fluoride is 1.5 mg/l. In the present study the concentration of fluoride in pre-monsoon exceeded permissible range as prescribed by BIS drinking water standards. The excessive concentration of fluoride (>1.5 mg/l) found which may be due to the geological formations of these regions associated with pegmatites, schist belt and igneous rocks such as granites in the form of [9].

The present study conformity with above findings. Total Dissolved Solids (TDS) denote the various types of minerals present in bore well water in the dissolved form. Total dissolved solids of water includes all soluble salts like carbonates, bicarbonates, chlorides, sulphates, phosphates and nitrates of calcium, magnesium, sodium, potassium, and iron. The high content of dissolved solids increases the density of water. TDS is an important parameter in drinking water and other water quality standards. In the current study, TDS ranged from 313 to 1038 mg/L during the pre-monsoon and from 450 to 1148 mg/L during the monsoon in both the seasons. The high values of TDS in both seasons may be due to the minerals pickup from the soil and rocks **Table1**. However, the amount of TDS is greater than permissible limits of BIS standards [5] **Table1**.

The statistical data revealed that TDS showed positive correlations with Electrical conductivity, Total hardness, Magnesium and Chloride. Electrical conductivity is a measure of the ability of water to conduct an electrical current. This ability depends on the concentration of dissolved ion in solution. Electrical conductivity values were found to be very high, ranging between 520 to 1700 $\mu\text{hos/cm}$ in the pre-monsoon and from 750 to 1950 $\mu\text{hos/cm}$ in the monsoon. In most of the well locations, EC values exceeded the permissible limits due to high concentration of dissolved solids in the water. The statistical data revealed that the electrical conductivity showed positive correlation with TDS, Total hardness, Magnesium and Chloride (**Table 2 and 3**).

Water hardness primarily represents the concentration of calcium and magnesium ions. Iron, aluminum and manganese may also contribute to hardness, but large amount of them are not usually present. In the present investigation, total hardness values ranged from 150 to 420 mg/L during the pre-monsoon and 258 to 638 mg/L during the monsoon in all wells, the total hardness values exceeded the permissible limit during the monsoon. The statistical data showed that the total hardness has showed positive correlation with Calcium, Magnesium and Chloride (**Table 2 and 3**). The amount of oxygen dissolved in water is referred as DO. It is an important parameter representing the quality of water. In the present investigation the DO was found in the range from 3.06 to 6.48 mg/L during the pre-monsoon and 3.62 to 7.3 mg/L during monsoon and showed negative correlation with BOD (**Table 2 and 3**). Our observation with the conclusions of [10]. The BOD values were found in the range from 2.42 to 9.29 mg/L during pre-monsoon and 0.59 to 2.03 mg/L during monsoon (**Table1**). Alkalinity is naturally added to water if it passes through soil and bedrock containing materials that are high in carbonates, bicarbonates and hydroxides. The total alkalinity values were found in the range between 148 to 280 mg/L during pre-monsoon and 249 to 464 mg/L during monsoon (**Table1**). The total alkalinity values found increasing in the monsoon period, compared to the pre-monsoon period in all wells. This may be due to the movement of pollutants into the ground water during rainfall and presence of bicarbonate or salt of weak acids (**Table1**). The present study agreement with Karthikeyan, K. et al [11]. The statistical data revealed that Total alkalinity showed positive correlation with pH **Table 2 and 3**. The presence of calcium in water results from passage through or over deposits of lime stones, dolomite, gypsum and such other calcium bearing rocks.

The calcium ion ranged between 27.25 to 126.7 mg/L during pre-monsoon season and 48.09 to 134 mg/L during monsoon season. The calcium ion concentration exceeded the limit in most of the places during the monsoon season

and showed positive correlation with some physico-chemical parameters such as Fluoride, Magnesium, Total hardness, Electrical conductivity and TDS (**Table 1, 2 and 3**).

Dissolved magnesium concentration is lower than calcium for a majority of the natural waters. Because of the high solubility of magnesium salts, the metal tends to remain in solution and is less readily precipitated than calcium. In the present investigation, the concentration of magnesium ranged between 13.53 to 49.3 mg/L during pre-monsoon and 27.45 to 88.57 mg/L during monsoon season. The magnesium values were found increasing in the monsoon season in all wells. The BIS [5] acceptable limit for total magnesium is 150 mg/l and in the present investigation, all the water samples in both seasons are within the permissible limits of drinking water standards and has showed positive correlation with Calcium, Total hardness, Electrical conductivity, TDS and Chloride (**Table 1, 2 and 3**). The chloride content normally increases as the mineral content increases. Chloride with high concentration may indicate pollution by weathering of some rocks, sewage and industrial effluents. The chloride concentration ranged from 56.73 to 278 mg/L during pre- monsoon and 120.55 to 382.93 mg/L in monsoon season and showed positive relationship with Electrical conductivity, TDS, Total Hardness, and magnesium (**Table 1, 2 and 3**). The observed values of chloride ions have shown an increasing trend during monsoon season as compared to pre monsoon season mainly due to dissolution of chloride contents in soil, agriculture run-off by infiltration. Similar observations have been made by Shivashankaran, [12] and Deepali. [13].

Nitrate is generally found in water due to bacterial action on ammonia and organic nitrogen. During decomposition, bacteria break down the protein molecules into ammonia. Ammonia is then oxidized by specialized bacteria to nitrite and then nitrate. Nitrates are then reduced to nitrogen gas. Fertilizers and sewage are the main source of ammonia and nitrate in ground water. The nitrate values were found between 0.04 to 3.2 during pre-monsoon and 0.02 to 0.16 mg/L during monsoon season. The high concentration of nitrate in pre-monsoon season compared with monsoon season may be due to increase in the decomposition of organic matters by bacteria. Similar observation has been made by Shivashnkaran [12]. The statistical data revealed that nitrate showed positive correlation with fluoride (**Table 2 and 3**).

Table 1: Ground Water Quality at Arsikere Taluk

Parameters	Pre-monsoon				Monsoon				BIS 1998	
	Minimum	Maximum	Mean	SD	Minimum	maximum	Mean	SD	Permissible limit	Excessive limit
PH	6.9	8.3	7.69	0.46	7.4	8.55	7.89	0.39	7.0-8.5	6.5-9.2
EC	520	1700	1195.83	437.19	750	1950	1315.83	449.66		
TDS	313	1038	726.10	261.01	450	1148	780.75	267.98	500	1000
TH	150	420	296.50	103.36	258	636	393.67	134.93	300	600
DO	3.06	6.48	4.98	0.98	3.62	7.3	5.36	1.17	4	6
BOD	2.42	9.29	6.70	1.92	0.59	2.03	1.24	0.42	2	3
Alka.	148	280	213.92	41.83	249	464	324.83	71.69	200	600
Ca	27.25	126.7	60.25	30.94	48.09	134	83.33	28.81	75	200
Mg	13.53	49.3	29.77	9.94	27.45	88.57	51.82	18.90	30	150
Cl	56.73	278	149.00	76.01	120.55	382.93	230.47	97.77	250	1000
No ₃	0.04	3.2	0.88	0.99	0.02	0.16	0.06	0.04	45	45
F	0.33	1.76	1.04	0.42	0.4	1.19	0.84	0.25	0.5	1.5

Table 2: Correlation coefficient calculated in pre-monsoon season at Arsikere Taluk

	pH	EC	TDS	TH	DO	BOD	T.Alk.	Ca	Mg	CL	No ₃	F
pH	1	-0.397	-0.389	-0.277	0.209	0.138	.678(*)	-0.458	-0.036	-0.552	0.124	-0.507
EC		1	.999(**)	.848(**)	0.108	-0.013	0.143	0.344	.787(**)	.831(**)	0.413	0.191
TDS			1	.851(**)	0.102	-0.028	0.162	0.332	.799(**)	.828(**)	0.399	0.18
TH				1	0.129	0.027	0.162	.617(*)	.670(*)	.594(*)	0.362	0.267
DO					1	-.917(**)	0.016	-0.132	-0.076	0.135	-0.508	-0.263
BOD						1	-0.149	0.287	-0.221	-0.232	.617(*)	0.431
T.Alk.							1	-0.365	0.324	-0.01	0.089	-0.536
Ca								1	-0.076	0.147	0.471	.809(**)
Mg									1	.588(*)	0.091	-0.196
CL										1	0.142	-0.036
No ₃											1	.636(*)
F												1

Table 3: Correlation coefficient calculated in monsoon season at Arsikere Taluk

	pH	EC	TDS	TH	DO	BOD	T.AIk.	Ca	Mg	CL	No3	F
pH	1	-0.085	-0.072	0.061	0.093	0.304	.711(**)	0.059	-0.094	-0.082	0.065	0.246
EC		1	.998(**)	.827(**)	-0.339	0.048	-0.12	.837(**)	.866(**)	.909(**)	-0.225	0.197
TDS			1	.804(**)	-0.364	0.053	-0.093	.814(**)	.843(**)	.901(**)	-0.204	0.201
TH				1	-0.136	0.19	-0.229	.995(**)	.916(**)	.883(**)	-0.503	0.306
DO					1	0.304	-0.098	-0.187	-0.122	-0.156	0.282	-0.471
BOD						1	0.251	0.157	0.031	0.017	0.056	0.251
T.AIk.							1	-0.244	-0.255	-0.346	0.556	0.324
Ca								1	.909(**)	.880(**)	-0.535	0.336
Mg									1	.878(**)	-0.397	0.022
CL										1	-0.375	0.033
No3											1	-0.088
F												1

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Cancer Cure Through Herbals – An Ethnobotanical Perspective

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ABSTRACT

Natural products are gaining increased applications in drug discovery and development. Being chemically diverse they are able to modulate several targets simultaneously in a complex system. Based on the natural resources available in India, many of the medicinal herbs being used effectively for their therapeutic potentials by the tribal and most of the rural and some of urban societies too in the management of cancer. Tumorigenesis or carcinogenesis is a multi-step process that is induced primarily by carcinogens leading to the development of cancer. Extensive research in the last few years has revealed that regular consumption of certain fruits and vegetables can reduce the risk of acquiring specific cancers. Phytochemicals derived from such fruits and vegetables, referred to as chemopreventive agents include genistein, resveratrol, diallyl sulfide, S-allyl cysteine, allicin, lycopene, capsaicin, curcumin, 6-gingerol, ellagic acid, ursolic acid, silymarin, anethol, catechins and eugenol. Worldwide effects are ongoing to identify new anticancer compounds from plants. The rapid increase in consumption of herbal remedies worldwide has been stimulated by several factors, including the notion that all herbal products are safe and effective.

Keywords: Natural products, Phytochemicals, carcinogenesis

INTRODUCTION

Cancer is a term for diseases in which there is uncontrolled multiplication of abnormal forms of the body's own cells and spread to the various parts of the body through the blood and lymph systems. The characteristic of cancer cells are uncontrolled growth, ability to invade local tissues, ability to spread (metastasis). The terms cancer, malignant neoplasm and malignant tumor are synonymous. Carcinogenesis is a multistage, multifactorial process that involves both genetic and environmental factors. The various stages are initiation, promotion and progression. Initiation involves exposure of normal cells to a carcinogen, producing genetic damage to cell. Promotion is the environment that becomes altered to allow preferential growth of mutated cells over normal cells.

THE GENESIS OF A CANCER CELL

A normal cells turns into a cancer cell because of one or more mutations in its DNA which can be inherited or acquired. The development of cancer is a complex multistage process, involving not only more than one genetic change but usually also other epigenetic factors such as hormonal action, co-carcinogen and tumor promoter effects. There are two main categories of genetic change that lead to cancer. (i) The activation of proto-oncogenes to (ii) The inactivation of tumor suppressor genes proto oncogenes is genes that normally control cell division, apoptosis and differentiation. Tumor suppressor genes are the one that have the ability to suppress malignant change. Tumors can be benign or malignant. Benign tumors are generally slow growing; resemble normal cells, localized, harmless. Malignant tumors often proliferate more rapidly, have a typical appearance, invade and destroy surrounding tissues, harmful if left untreated.

CATEGORIES OF CANCER

Solid tumors

Tumors of epithelial cells includes lung, colon, breast cancer etc.,

Tumors of connective tissue such as bone (osteosarcoma) or muscle (leiomyosarcoma)

Hematological malignancies

Lymphomas are tumors of lymphatic system (Hodgkin's and non-Hodgkin's lymphomas), Leukemia is tumors of blood forming elements, classified as acute or chronic, myeloid or lymphoid.

ETIOLOGY

- Viruses such as Epstein - Barr virus (EBV), hepatitis B Virus (HBV), human Papilloma Virus (HPV)
- Environmental and occupational exposure such as ionizing, UV radiation, exposure to chemicals including vinyl chloride, benzene, asbestos.
- Life style factors such as high-fat, low fiber diets, tobacco, ethanol.
- Medication such as alkylating agents, immunosuppressant
- Genetic factors such as inherited mutations, cancer causing genes, defective tumor suppressor genes.

ROLE OF MEDICINAL PLANTS IN CANCER TREATMENT

Plant materials have been used in the treatment of malignant diseases for centuries. Recent phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has induced often resulted in the isolation of principles with antitumour activity. An intensive survey of plants, micro organism and marine animals for antitumour activity began in the later 1950s mainly because the United States National Cancer Institute (NCI) instigated and fund a major screening programme. A random selection screening programme was adopted, since novel compounds may be found anywhere in the plant or animal kingdom. Soybean phytochemicals such as genistein (4',5,7-tribydroxy isoflavone) inhibit the growth of transplantable human prostate carcinoma [1] Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases such as cancer as the phytochemical extracts from it exhibit strong antioxidant activity [2]. Andrographolide is a potential cancer therapeutic agent isolated from *Andrographis paniculata* [3]. In the screening of Yemeni plants used in folk medicine for the anticancer potential, the methanolic extracts of *Dendrosicyos Socotrana*, *Withania aduensis*, *Withania riebeckii*, *Dracena Cinnabari* and *Buxus hildebrandlii* exhibited the highest toxicity on all tumor cell lines [4]. The four varieties of muscadine grape extract had the ability to inhibit activity of matrix metalloproteinases implying that these could be good inhibitors of carcinogenesis [5]. The limonoids isolated from the methanol extract of Khaya Senegalensis (Meliaceae) proved to

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have good anticancer activity [6]. The leaf extract of Ashwagandha selectively kills tumor cells and thus it is a natural source for safe anticancer medicine [7].

The fruit of deer berry (*Vaccinium stamineum*) exhibited the anticancer capability of human lung and leukemia cancer cells [8]. Polyphenolic extracts from *Vaccinium macrocarpon* inhibit the growth and proliferation of breast, colon, prostate, lung and other tumors as do flavonols, proanthocyanidin, oligomers and triterpenoids isolated from the fruit [9] *Morinda citrifolia* possess of cancer preventive effective in both clinical practice and laboratory animal models [10]. An alcoholic extract of *Biorhythms sensitivum* for antitumor activity could inhibit the solid tumor development in mice induced with DLA cells and increase the life span of mice bearing Ehrlich ascites carcinoma tumors [11]. Edible fruits and berries may serve as sources for novel anticancer agents, given that extracts of these foods have demonstrated cytotoxic activity against tumor cell lines [12]. Nimbolide, a triterpenoid extract from the flowers of the enema tree was found to have antiproliferative activity against some cancer cell lines [13]. *Semecarpus anacardium* Linn nut milk extract exerts its anticancer effect through quenching - reactive oxygen species [14]. The cytotoxic activities of two medicinal herbs *Linum persicum* and *Euphorbia cheradania* that are native to Iran showed cytotoxic activity on tumor cell lines [15].

The Pomegranate extracts inhibits the growth of breast cancer cells [16]. Brassinosteroids, steroid plant hormones are promising leads for potential anticancer drugs [17]. The *Careya arborea* bark significantly reduced the solid tumor volume induced by DLA cells [18]. The methanol extract of *Bauhinia racemosa* stem bark exhibited antitumor effect in EAC bearing mice [19]. The antitumor activity of the ethanol extract of *Indigofera aspalathoides* was established [20]. The extract of 12 Chinese medicinal herbs such as *Anemarrhena asphodeloides*(Root), *Artemisia argyi* (leaf), *Commiphora myrrha* (Resin), *Duchesnea indica* (Aerial Plants), *Gleditsia sinensis* (Fruit), *Ligustrum lucidum* (fruit) *Rheum palmatum* (Root, Rhizome), *Rubia cordifolia*(Root), *Salvia chinensis* (Aerial parts), *Scutellaria barbata* (Aerial Parts), *Uncaria rhychopylla* (Stem), *Vaccaria segetalis* (seed) have anticancer effects *in vitro* and these effects are markedly greater on cancer cells compared with normal cells [21] Phytoconstituents extracted from a large number of plants belonging to the genus *Hypericum* are known to possess potent anticancer nature [22] Cytotoxic activity of *Saururus cernuus* extract on human colon and breast carcinoma cultures was proved [23].

The natural antioxidant gallic acid (GA) isolated from the fruits of a Indonesian medicinal Plant, *Phaleria macrocarpa* was proved to be a potent anticancer compound [24]. The rhizome *Zingiber officinalis*, one of the most widely used species of the ginger family is a common condiment for various foods and beverages. The Pungent vallinoids (i.e.) 6-gingerol and 6-Paradol, Shogaols and Zingerone attributed to the anticancer properties of ginger [25]. The antineoplastic activity of methanolic extracts of six medicinal plants that are native to Iran including *Galium mite*, *Ferula angulata*, *Stachys obtuscrena*, *Grsium bracteosum* and *Echinophora cinerea* was investigated and proved to be active against tumor activity [26]. *Panax ginseng* and its extracts have long been used for medical purposes and there is increasing interest in developing ginseng products as cancer preventive agents [27]. Purified bioactive compounds derived from medicinal mushrooms are potentially important new source of anticancer agents [28]. The Saponins from the plant of china, *clematis manshrica* has obvious antitumor effects against various transplanted tumor in mice [29]. The Embelin derivatives such as 1,4 - benzoquinone derivative 5-0 ethyl embelin(1) and 5-0 methy embelin are promising antimitotic and anti cancer molecules [30]. Sesquiterpenes are a class of naturally occurring molecules that are 15-carbon isoprenoid compounds. These are typically found in plants and marine life. They have therapeutic potential in decreasing the progression of cancer [31]. The anticancer activity from *Platycodon grandiflorum* was proved and established [32]. The methanol extract of stem bark of *Dillenia pentagyna* appears to be more active against Dalton's lymphoma [33]. *Limonium vulgare*, *Artemisia maritima* and *Salicornia europaea* showed antineoplastic activities. The extracts of *Ononis spinosa*, *Trifolium fragiferum* and *T.repens* showed tumor growth inhibiting activities [34]. Methanol extract *Ledum groenandicum* Retzius (Labrador tea) leaf twig extract showed anticancer activity [35]. The anti-neoplastic activity of guduchi (*Tinospora cordifolia*) in Ehrlich ascities carcinoma was proved [36]. Some of the other plants reported to have anticancer activity are listed in **Table-1**.

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Table: 1 Some of the medicinal plants with anticancer activity

S.No.	Name of the Plant	Family	Parts used
1.	<i>Calotrophis gigantea</i>	Asclepiadaceae	Whole plant
2.	<i>Cajanus cajan</i>	Fabaceae	Leaves
3.	<i>Butea monosperma</i>	Fabaceae	Bark
4.	<i>Bauhinia variegata</i>	Caesalpinaceae	Root
5.	<i>Bacopa monnieri</i>	Scropulariaceae	Whole plant
6.	<i>Azadirachta indica</i>	Meliaceae	Bark
7.	<i>Asparagus racemosus</i>	Liliaceae	Root
8.	<i>Aphanamixis polystachya</i>	Meliaceae	Bark
9.	<i>Aloe barbadensis</i>	Liliaceae	Leaf juice
10.	<i>Allium cepa</i>	Liliaceae	Bulb
11.	<i>Acorus calamus</i>	Araceae	Rhizome
12.	<i>Cassia absus</i>	Caesalpinaceae	Leaves
13.	<i>Cassia auriculata</i>	Caesalpinaceae	Root
14.	<i>Cassia senna</i>	Caesalpinaceae	Leaves
15.	<i>Catunaregum spinosa</i>	Rubiaceae	Bark/Fruit
16.	<i>Citrullus colocynthis</i>	Cucurbitaceae	Root
17.	<i>Citrus medica</i>	Rutaceae	Root
18.	<i>Cissus quadrangularis</i>	Vitaceae	Whole plant
19.	<i>Clerodendrum serratum</i>	Verbanaceae	Root
20.	<i>Clerodendrum viscosum</i>	Verbanaceae	Leaves
21.	<i>Crinum asiaticum</i>	Amaryllidaceae	Bulb
22.	<i>Daucus carota</i>	Apiaceae	Root
23.	<i>Embelia ribes</i>	Myrsinaceae	Fruit
24.	<i>Flacourtia jangomos</i>	Flacourtiaceae	Bark/Leaf
25.	<i>Jatropha curcas</i>	Euphorbiaceae	Leaves,seed,oils
26.	<i>Kaempferia galanga</i>	Zingiberaceae	Rhizome
27.	<i>Kaempferia rotunda</i>	Zingiberaceae	Tubers
28.	<i>Lanata camara</i>	Verbanaceae	Whole plant
29.	<i>Lens culinaris medikus</i>	Fabaceae	Seed
30.	<i>Limonia acidissima</i>	Rutaceae	Fruit
31.	<i>Macrotyloma uniflorum</i>	Fabaceae	Seed
32.	<i>Mimosa pudica</i>	Mimosaceae	Whole plant
34.	<i>Operculina turpethum</i>	Convolvulaceae	Root
35.	<i>Rhinacanthus nasuta</i>	Acanthaceae	Whole plant
36.	<i>Salvadora persica</i>	Salvadoraceae	Bark,Leaf,Shoot,Fruit
39.	<i>Symplocos cochinchinensis</i>	Symplocaceae	Bark
40.	<i>Tylopora indica</i>	Asclepiadaceae	Root,Leaf
41.	<i>Vernonia cinerea</i>	Asteraceae	Whole plant
42.	<i>Vitex trifolia</i>	Verbanaceae	Leaf
43.	<i>Zanthoxylum armatum</i>	Rutaceae	Bark,Fruit
44.	<i>Xanthium strumarium</i>	Compositae	Root

CONCLUSION

This ethnobotanical survey reveals the role of various parts of the medicinal herbs in the treatment of various types of cancer. The available literature finds to be very impressive. This may be helpful for the researchers who are doing research in the area of cancer. However, there are several species unexplored. Searching the evidence for starting up the work is the key factor in every research. The literature surveys need to be done prior to proceed with. So this may be additional information to the society and research scholars.

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Plants and Air Pollution

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ABSTRACT

Air pollution has been known to have adverse effects on plants. At first it was only sulphur dioxide that was considered a dangerous pollutant. Now, with the advent of various pesticide and new industrial process, the range of harmful pollutants has multiplied tremendously. Plants can be injured when exposed to high concentrations of various air pollutants. Injury ranges from visible markings on the foliage, to reduced growth and yield, to premature death of the plant. The development and severity of the injury depends not only on the concentration of the particular pollutant, but also on a number of other factors. Such impacts on agricultural productivity can have serious implications where problems of food scarcity exist; studies in India have found that vulnerable sectors of society such as the poor and malnourished as well as those depending on sustainable agriculture for their livelihoods are more severely affected.

Key words: Air pollution, pollutant, sustainable agriculture.

EFFECTS OF AIR POLLUTION ON PLANTS

Air pollution has been known to have adverse effects on plants. At first it was only sulphur dioxide that was considered a dangerous pollutant. Now, with the advent of various pesticide and new industrial process, the range of harmful pollutants has multiplied tremendously. Sometimes, vegetation over 150km from the source of the pollutants has been found to be affected. Industrial pollution, particularly from smelters, has caused complete destruction of vegetation in some cases e.g. at Ducktown, Tennessee. The international dispute between Canada and the United States over the damage caused by the Trails, British Columbia Copper Smelter is well known. Los Angeles smog has caused wide spread damage to some agricultural crops and forests in Southern California. In fact many books, research articles and reports have been published in USA on air pollution injury to vegetation. In some cases, economics of the damage caused to agricultural crops and plants has been estimated .Compensation amounting to thousands of dollars has paid for damage done to the crops. In our own country, there are many reports of effect of pollutants like cement dust on plants [1].

KINDS OF INJURY TO PLANTS

Plants can be injured when exposed to high concentrations of various air pollutants. Injury ranges from visible markings on the foliage, to reduced growth and yield, to premature death of the plant. The development and severity of the injury depends not only on the concentration of the particular pollutant, but also on a number of other factors. These include the length of exposure to the pollutant, the plant species and its stage of development as well as the environmental factors conducive to a build-up of the pollutant and to the preconditioning of the plant, which make it either susceptible or resistant to injury.

a. Acute Injury

It results from short-time exposure to relative high concentration, such as might occur under fumigation condition. The effects are noted within a few hours to a few days and may result in visible markings on the leaves due to collapse and death of cells. This leads to necrotic patterns, i.e. area of dead tissue.

b. Chronic Injury

It results from long-time low level exposure and usually causes chlorosis or leaf abscission.

c. Growth or Yield Retardation

Here the injury is in the form of an effect on growth without visible markings (invisible injury). Usually a suppression of growth or yield occurs.

EFFECTS OF AIR POLLUTION ON PLANTS

Air pollution injury to plants can be evident in several ways. Injury to foliage may be visible in a short time and appear as necrotic lesions (dead tissue), or it can develop slowly as a yellowing or chlorosis of the leaf. There may be a reduction in growth of various portions of a plant. Plants may be killed outright, but they usually do not succumb until they have suffered recurrent injury. A list of air pollutants and their effects on plants are as follows

Air Pollutants Affecting Plants

Sulphur dioxide, Fluorides, Oxidants, Chlorine, Hydrogen Chloride, Nitrogen oxides, Ammonia, Hydrogen sulphide, Hydrogen cyanide, Mercury, Ethylene, PAN (Peroxy Acetyl Nitrate), Herbicides, Smog, Particulate Matter

Effects of Oxidants

Ozone is the main pollutant in the oxidant smog complex and is produced in the atmosphere during a complex reaction involving nitrogen oxides and reactive hydrocarbons, components of automobile exhausts and fossil fuel combustion. As this process proceeds only in sunlight, it is called a photo-chemical reaction. The vegetation injury, which can result from oxidant build-up in the air, can occur over large rural areas covering hundreds of square kilometers. Its effect on plants was first observed in the Los Angeles area in 1944. Since then, ozone injury to vegetation has been reported and documented in many areas throughout North America, including the southwestern and central regions of Ontario. Throughout the growing season, particularly July and August, ozone levels vary

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significantly. Periods of high ozone are associated with regional southerly air flows that are carried across the lower Great Lakes after passing over many urban and industrialized areas of the United States. Localized, domestic ozone levels also contribute to the already high background levels. Injury levels vary annually and white bean, which are particularly sensitive, are often used as an indicator of damage. Other sensitive species include cucumber, grape, green bean, lettuce, onion, potato, radish, rutabagas, spinach, sweet corn, tobacco and tomato. Resistant species include endive, pear and apricot.



Figure 1 Ozone injury to soybean foliage

Ozone symptoms (**Figure 1**) characteristically occur on the upper surface of affected leaves and appear as a flecking, bronzing or bleaching of the leaf tissues. Although yield reductions are usually with visible foliar injury, crop loss can also occur without any sign of pollutant stress. Conversely, some crops can sustain visible foliar injury without any adverse effect on yield. Susceptibility to ozone injury is influenced by many environmental and plant growth factors. High relative humidity, optimum soil-nitrogen levels and water availability increase susceptibility. Injury development on broad leaves also is influenced by the stage of maturity. The youngest leaves are resistant. With expansion, they become successively susceptible at middle and basal portions. The leaves become resistant again at complete maturation.

Effects of Sulfur Dioxide

Major sources of sulfur dioxide are coal-burning operations, especially those providing electric power and space heating. Sulfur dioxide emissions can also result from the burning of petroleum and the smelting of sulfur containing ores. Sulphur dioxide enters the leaves mainly through the stomata (microscopic openings) and the resultant injury is classified as either acute or chronic. Acute injury (**Figure 2**) is caused by absorption of high concentrations of sulfur dioxide in a relatively short time. The symptoms appear as 2-sided (bifacial) lesions that usually occur between the veins and occasionally along the margins of the leaves. The colour of the necrotic area can vary from a light tan or near white to an orange-red or brown depending on the time of year, the plant species affected and weather conditions. Recently expanded leaves usually are the most sensitive to acute sulfur dioxide injury, the very youngest and oldest being somewhat more resistant.



Figure 2 Acute sulfur dioxide injuries to raspberry

(Note that the injury occurs between the veins and that the tissue nearest the vein remains healthy)

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Chronic injury is caused by long-term absorption of sulfur dioxide at sub-lethal concentrations. The symptoms appear as a yellowing or chlorosis of the leaf, and occasionally as a bronzing on the under surface of the leaves. Different plant species and varieties and even individuals of the same species may vary considerably in their sensitivity to sulfur dioxide. These variations occur because of the differences in geographical location, climate, stage of growth and maturation. The agricultural crop plants which are generally considered susceptible to sulphur dioxide are alfalfa, barley, buckwheat, clover, oats, pumpkin, radish, rhubarb, spinach, squash, Swiss chard and tobacco. Resistant crop plants include asparagus, cabbage, celery, corn, onion and potato.

Effects of Fluoride

Fluorides are discharged into the atmosphere from the combustion of coal; the production of brick, tile, enamel frit, ceramics, and glass; the manufacture of aluminium and steel; and the production of hydrofluoric acid, phosphate chemicals and fertilizers. Fluorides absorbed by leaves are conducted towards the margins of broad leaves (grapes) and to the tips of monocotyledonous leaves (gladiolus). Little injury takes place at the site of absorption, whereas the margins or the tips of the leaves build up injurious concentrations [1]. The injury (**Figure 3**) starts as a gray or light-green water-soaked lesion, which turns tan to reddish-brown. With continued exposure the necrotic areas increase in size, spreading inward to the midrib on broad leaves and downward on monocotyledonous leaves. Studies of susceptibility of plant species to fluorides show that apricot, barley (young), blueberry, peach (fruit), gladiolus, grape, plum, prune, sweet corn and tulip are most sensitive. Resistant plants include alfalfa, asparagus, bean (snap), cabbage, carrot, cauliflower, celery, cucumber, eggplant, pea, pear, pepper, potato, squash, tobacco and wheat.



Figure 3 Fluoride injuries to plum foliage.

The fluoride enters the leaf through the stomata and is moved to the margins where it accumulates and causes tissue injury. Note, the characteristic dark band separating the healthy (green) and injured (brown) tissues of affected leaves.

Effects of Hydrogen Cyanide

Hydrogen Cyanide is used to fumigate green houses and trees in orchards for pest control. Sometimes this fumigation injures the vegetation [2].

Effects of Nitric Oxides

Injury to agricultural crop plants due to nitric acid vapours has observed near factories handing large amounts of this acid. The effects include brown margins and brownish-blank spots on the leaves. Concentration of about 25 ppm will cause these effects. Nitrogen oxides are important in photochemical reactions which cause smog.

Effects of Ammonia

Ammonia injury to vegetation has been observed frequently in Ontario in recent years following accidents involving the storage, transportation or application of anhydrous and aqua ammonia fertilizers. These episodes usually release large quantities of ammonia into the atmosphere for brief periods of time and cause severe injury to vegetation in the immediate vicinity. Complete system expression on affected vegetation usually takes several days to develop, and appears as irregular, bleached, bifacial, necrotic lesions. Grasses often show reddish, interveinal necrotic streaking or dark upper surface discolouration. Flowers, fruit and woody tissues usually are not affected, and in the case of severe injury to fruit trees, recovery through the production of new leaves can occur (**Figure 4**). Sensitive species include apple, barley, beans, clover, radish, raspberry and soybean. Resistant species include alfalfa, beet, carrot, corn, cucumber, eggplant, onion, peach, rhubarb and tomato.



Figure 4 Severe ammonia injuries to apple foliage and subsequent recovery through the production of new leaves following the fumigation

Effects of Ethylene

Ethylene causes injury to leaves of sensitive plants. The effects are epinasty, curling, chlorosis, leaf abscission, growth retardation [3].

Effects of Smog

London type smog is thought to be essentially a sulphur dioxide problem. But the gaseous constituents as well as the aerosols need further evaluation. The Los Angeles type smog is now fairly well understood, but the actual compounds that cause these effects are still unknown. Two types of smog injury to vegetation have been recognized in Los Angeles, one due to gases (smog gas) and the other due to deposition on the leaves of fog droplets (smog fog). The smog causes characteristic leaf lesions which are quite different from those produced by other pollutants, including ozone, which may be a constituent of the smog [3].

Effects of Particulate Matter

Particulate matter such as cement dust, magnesium-lime dust and carbon soot deposited on vegetation can inhibit the normal respiration and photosynthesis mechanisms within the leaf. Cement dust may cause chlorosis and death of leaf tissue by the combination of a thick crust and alkaline toxicity produced in wet weather. The dust coating (**Figure 5**) also may affect the normal action of pesticides and other agricultural chemicals applied as sprays to foliage. In addition, accumulation of alkaline dusts in the soil can increase soil pH to levels adverse to plant growth.



Figure 5 Cement-dust coating on apple leaves and fruit. The dust had no injurious effect on the foliage, but inhibited the action of a pre-harvest crop spray.

Effects of Hydrogen Fluoride

Hydrogen Fluoride behaves somewhat similar to sulphur dioxide except that with a few species of agricultural crop plants it is effective in causing lesions and interfering with photosynthesis in connections two or three order less than in the case of sulphur dioxide. With the most species it is up to 10 times as effective as sulphur dioxide. However, recovery of plants from the fluoride effects is much slower than from sulphur dioxide. This difference in rate of recovery is probably explained by the fact that sulphate, whereas, fluorides can be removed only by the slower process of volatilization or by some obscure chemical reaction. Forage may be rendered unsafe for animal feeding if more than 50 ppm of fluorine is absorbed.

Effects of Hydrogen Chloride

Hydrogen Chloride is considerably less toxic to vegetation than sulphur dioxide. It causes first a chlorotic margin in the leaf, which may become necrotic. At higher concentrations, lesions are produced. The threshold concentration is about 10 ppm a few hours exposure.

Effects of Chlorine

Chlorine is more toxic to vegetation than sulphur dioxide by a factor of two or three. Lesions are generally marginal and interveinal. Damage to vegetation caused by chlorine is rare, and most the reported cases are due to accidents or excessive use of gas for sterilizing [2].

SENSITIVITY OF PLANTS TO AIR POLLUTANTS

The sensitivity of agricultural crop plants to air pollutants is conditioned by many factors. Some of them are as follows

Genetic Factors

Plant responses to air pollutants vary between species of a given genus and between varieties within a given species. Such variation is simply a function of genetic variability as it affects the plants` morphological, physiological and biochemical characteristics. Plants do not necessarily show similar susceptibility to different pollutants. For example, some plants are sensitive to fluoride but resistant to sulphur dioxide.

Climatic Factors

The important climatic factors affecting the responses of plants to air pollutants are Duration of Light, Light quality (wave length), Temperature and Humidity

Miscellaneous Factor

Soil, water and fertility are some factors which affects the sensitivity of plants to air pollutants. However, these, factors have to be studied in detail before drawing any conclusion.

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Isolation and Characterization of Bioactive Metabolites in *Cuscuta reflexa* Roxb.

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ABSTRACT

The medical plant *Cuscuta reflexa* Roxb. was selected for analysis and Characterization of its medicinal value based on phytochemical studies. Phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, phytosterols, fixed oil and fats, proteins, phenolic compounds, tannis and saponins of ethanolic extract of *Cuscuta reflexa* Roxb. were analyzed qualitatively using UV-Vis and FT-IR. Our investigation suggests that *Cuscuta reflexa* Roxb. might be a source of large amount of metabolites such as phenolics. Therefore, this result may suggest that *Cuscuta reflexa* Roxb. extracts posses' compounds with antihelmithetic, antimicrobial and antioxidant properties which can be used as phytochemical agents in new drugs for therapy of microbial and other diseases in human.

Key words: *Cuscuta reflexa* Roxb., UV-VIS, FT-IR, phenolics

INTRODUCTION

The use of plant based drugs in the western world is increasing. India has a rich heritage of knowledge on plant based drugs both for use in curative and preventive medicine. Ancient Indian Scholars, Charak, Sushruta, Bagvatta and several others have given remarkable description of Indian medicinal plants in Achervaveda [1]. Medicinal plants posses secondary metabolism, hence forth it has secondary metabolic complex molecules because of these presence, medicinal plants have curative properties. These secondary metabolites grouped according to their chemical structures and properties as alkaloids, flavonoids, anthroquinones, phenolic compounds, cortico steroids, essential oils etc. [2]. There is a general feeling now a days that the bulk of the drugs in use are synthetic. This is however not true. Surveys carried out in USA have revealed that approximately 50% of all prescriptions dispensed

contained one or more products of natural origin. The importance of plant products in modern medicine even in highly advanced society as that of U.S.A can be seen from the data of National survey conducted in 1968. It was found that 25% of all prescriptions dispensed contained of crude plant material or a crude plant extract or a purified active principles [3]. Isolation, identification and quantification of secondary metabolic products, is known as Photochemistry [4]. Use of plant extracts in the treatment of various human infections was well known in ancient systems of medicine including the Indian and Chinese. The biologically active substances produced by plants are known as phytocides and complex molecules [5]. Many plants and their constituents have been screened for their antimicrobial activity. The plant constituents such as alkaloids, flavonoids, tannins, lactones are responsible for the antimicrobial activity. Extracts of various medicinal plants containing flavonoids have been reported to possess antimicrobial activity. The antimicrobial activity of Isoflavonoids and glycosides of luteolin and apigenin have been reported. Quercetin has been shown to inhibit viruses and bacteria [5]. *Cuscuta reflexa* Roxb. belongs to Cuscutaceae family found almost everywhere in the world, although *Cuscuta* is more often called dodder in English countries. Other names include hell weed, devil's gut, beggar weed, strangle tare, scald weed, dodder of thyme, greater dodder, and lesser dodder. In Chinese, *Cuscuta* seeds are called *tu si zi*. *Cuscuta* is a leafless plant with branching stems ranging in thickness from thread-like filaments to heavy cords. The mature plant lives its entire life without attachment to the ground. The stems of *Cuscuta* are used in Western herbalism and the seeds are used in Traditional Chinese Medicine (TCM). Contemporary Chinese herbalists use *Cuscuta* in formulas to treat a range of conditions, including: impotence, premature ejaculation, sperm leakage, frequent urination, ringing in the ears, lower back pain, sore knees, white discharge from the vagina (leucorrhea), dry eyes, blurred vision and tired eyes. *Cuscuta* is also used in the Indian system of Ayurvedic healing to treat jaundice, muscle pain, coughs, and problems with urination. The aim of the present study is to isolate, characterize and find out the bioactive metabolites through UV-VIS and FT-IR analysis method.

MATERIALS AND METHODS

Collection of plant sample

On the basis of its medicinal value which are available in the literature *Cuscuta reflexa* Roxb. was selected for phytochemical studies. The medicinal plant *Cuscuta reflexa* Roxb. was collected from Anaivari Village, Arimalam block, Pudukkottai district during the month of January – 2010. It was identified and authenticated by Dr. S. Vijikumar, Head of Plant Science Research Division (PSRD), Tamil Nadu Scientific Research Organization, (TNSRO) Arimalam (Pudukkottai).

Preparation of plant extract for phytochemical studies

About 100 g of dry stem powder of was *Cuscuta reflexa* Roxb. extracted with ethanol at 60°C to 70°C by continuous hot percolation using Soxhlet apparatus. The extraction was filtered and kept in oven at 50°C for 24 hours to evaporate the extracts from them. A greenish black waxy residue was obtained. These extracts were used for phytochemical analysis qualitatively through UV-VIS and FT-IR.

Phytochemical analysis

The plant extracts were screened for the presence of biologically active compounds like alkaloids, flavonoids, glycosides, carbohydrates, phytosteroids and fatty acids, proteins, phenolics, tannins and saponins [6-8].

Analysis of Phenolics Using UV-VIS and FT-IR Spectroscopy

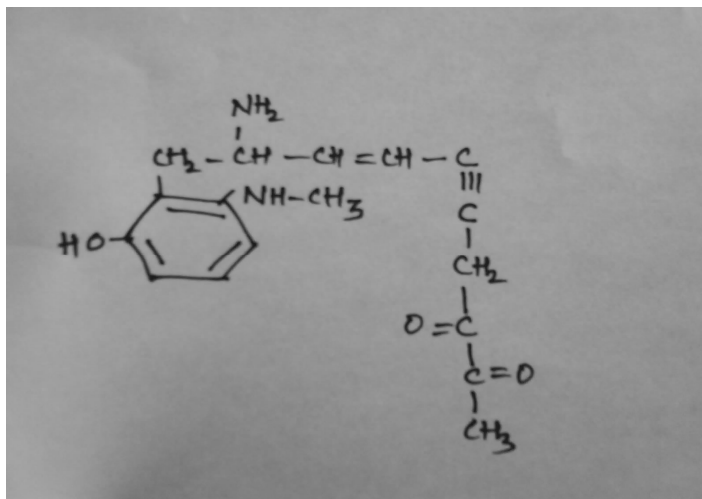
UV-VIS and FT-IR procedure was applied for the study plant of the *Cuscuta reflexa* Roxb. from the identified localities subjected to phenolics screening.

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RESULTS AND DISCUSSION

Phytochemical constituents like alkaloids, flavonoids, carbohydrates, glycosides, phytosterols, fixed oil and fats, proteins, phenolic compounds, and saponins of *Cuscuta reflexa* Roxb. were analyzed and reported in **(Table – 1)**. *Cuscuta reflexa* Roxb. contains a number of compounds like flavonoids (kaempferol, quercetin), coumarins, and flavonoid glycosides. Earlier studies have shown that both kaempferol and quercetin could significantly improve insulin-stimulated glucose uptake in mature 3T3-L1 adipocytes. It was further reported that these two compounds act at multiple targets to ameliorate hyperglycemia [9]. According to Fang [9] flavonoids are highly available in this plant; the separated compound is indeed phenolics, so the result is invariably correct right from isolation and characterization steps. According to Sakshy Sharma *et al.* [10] the plant has anti effects against spectrum of harmful activities; the separated compound is indeed having the proposed effects.

On the basis of UV-Vis and FT-IR spectral analysis on *Cuscuta reflexa* Roxb. we have found the following data. UV-Vis shown in **Figure 1** yielded 4 elevations (355.96nm, 1.2091 and 670.70nm, 0.17306) and the values were interpreted with table values **(Table 2)** and confirm the presence of phenolics in the given sample. FT-IR is shown in figure 2 yielded Maximum peak level 3930.36 cm^{-1} and Minimum peak level 646.80 cm^{-1} . FT-IR studies confirm the presence of functional groups in the compound listed in the table 2, So that the compound may be **phenolics**. The compound may be **9-amino, 10(1-hydroxy, 3-aminomethyl, phenyl) 2, 3-diketo dec-7-en-5yne** having molecular weight: 299 GMM, structure is given below



9-amino, 10(1-hydroxy, 3-aminomethyl, phenyl) 2, 3-diketo dec-7-en-5yne
Molecular weight: 299 GMM

Table 1: Phytochemical constituents of *Cuscuta reflexa* Roxb.

S. No	Metabolites	Results
1	Alkaloids	+++
2	Flavonoids	++
3	Terpenoids	++
4	Fixed oils	++
5	Phytosterols	++
6	Saponins	-
7	Phenolic compounds	++
8	Fats	++
9	Carbohydrates	++
10	Proteins	+++
11	Glycosides	+
12	Tannins	+

+++ present in high concentration

++ present in medium concentration

+ Present in low concentration

- not present in the sample

Table: 2 FT-IR results of *Cuscuta reflexa* Roxb.

Wavelength in cm ⁻¹	Functional group	Name of the functional group
3930.36	-NH	Imine
3817.77	-NH ₂	Amine
3420.22	-OH, Ph-OH	Alcohol, Phenol
2976.98	-CH ₂ -	Alkyl
2912.50	-CHO	Aldehyde
2139.56	-C C-	Alkyne
1571.39	-C=C-	Alkene
1411.90	-C=C-	Alkene
1243.58	-CH ₂ -CH ₂ -	Alkane
1058.67	-C=O	Ketone
881.05	-C=O	Ketone
646.80	-C-Cl	Chloro

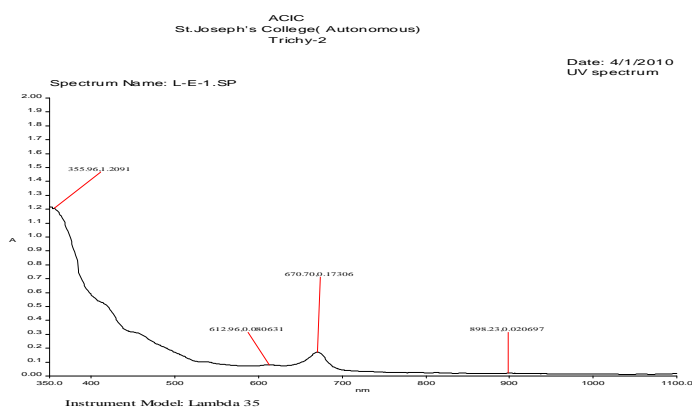


Figure 1: Depicts UV spectrum of phenolics of *Cuscuta reflexa* Roxb.

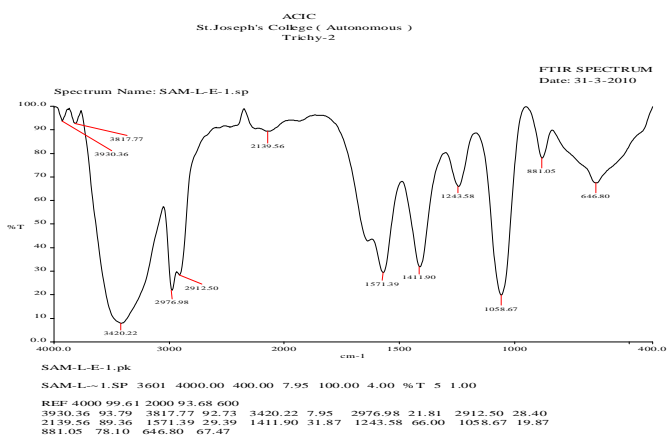


Figure2: Depicts that the FT-IR Spectrum of phenolics

CONCLUSION

These results suggest that *Cuscuta reflexa* Roxb. might be a source of large amount of metabolites such as phenolics. This may suggest that *Cuscuta reflexa* Roxb. extracts possess compounds with antihelmithetic, antimicrobial and antioxidant properties which can be used as phytochemical agents in new drugs for therapy of microbial and other diseases in human.

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Diabetes mellitus and Antidiabetic Activity of Medicinal Herbs

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder of endocrine system in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria, polydipsia and polyphagia. Several drugs such as biguanides and sulfonylurea are presently available to reduce hyperglycemia in diabetes mellitus. These drugs have side effects and thus searching for a new class of compounds is essential to overcome these problems. Management of diabetes without any side effects is still challenge to the medical community. The compounds found in Herbs help to control many of the pathological mechanisms that underlie diabetes and metabolic syndrome without any side effects. Herbs for diabetes include the spices *Cinnamomum verum* (cinnamon), *Brassica juncea* (mustard), *Allium sativum* (garlic), *Coriandrum sativum* (coriander), *Zingiber officinale* (ginger), etc. These are the spices most effective in lowering blood glucose and abnormal blood lipids in these diseases. The compounds contained in herbs are capable of directly counteracting the underlying disease mechanisms; by stimulating insulin production, raising insulin sensitivity and modulating the absorption of glucose in the intestines.

Key words: Anti-diabetes, coriander, hypoglycemic activity, *Allium sativum*, Ginger

INTRODUCTION

Diabetes mellitus has been known since ages and the sweetness of diabetic urine has been mentioned in Ayurveda by Sushruta. The word diabetes was coined by the Greek physician Aretaeus in the first century A.D. The presence of sugar in the urine of diabetics was demonstrated by Dobson in 1755. Diabetes mellitus, Diabetes—is a chronic metabolic disorder of endocrine system in which a person has high blood sugar, either because the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). This metabolic disorder is affecting approximately 4% of the population. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. Synthalin A, a biguanide was earliest Oral Hypoglycemic Agent (OHA),

to be used in therapy but was found to be too toxic. Currently available oral anti-diabetic drugs that are used clinically for glycemic control include Sulfonylureas, meglitinides, biguanides, α -glucosidase inhibitors, and thiazolidinediones (TZDs). Presently, there is growing interest in herbal remedies due to the side effects associated with the oral synthetic hypoglycemic agents for the treatment of diabetes mellitus. Herbal medicines have been long used for the treatment of diabetic patients and continue to be accepted as an alternative therapy. There are more than 1000 anti-diabetic plants have been described in the scientific literature. The plant kingdom is a wide field to search for a natural effective oral hypoglycemic or hypo lipidemic agent that has slight or no side effects. Natural products with both hypoglycemic and hypo lipidemic properties are useful anti-diabetic agents.

TYPES OF DIABETICS

Most cases of diabetes mellitus fall into three broad categories: type 1, type 2, and gestational diabetes.

Type 1 Diabetes

[Alternative names: Insulin-dependent diabetes mellitus; Juvenile onset diabetes; Diabetes - Type 1] Type 1 diabetes is an autoimmune disease. It results when the body's system for fighting infection (the immune system) turns against a part of the body. It is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas leading to insulin deficiency. It is a chronic (lifelong) disease. A person who has type 1 diabetes must take insulin daily to live. There is no known preventive measure against type 1 diabetes. Type 1 diabetes can affect children or adults but was traditionally termed "juvenile diabetes" because it represents a majority of the diabetes cases in children. Type 1 diabetes can occur at any age, but it usually starts in people younger than 30. Symptoms are usually severe and occur rapidly.

Signs and Symptoms

Symptoms include increased thirst (polydipsia) and frequent urination (polyuria), increased hunger (polyphagia), weight loss, blurred vision, nausea, vomiting, abdominal pain and extreme fatigue. If not diagnosed and treated with insulin, a person with type 1 diabetes can lapse into a life-threatening diabetic coma, also known as diabetic ketoacidosis.

Causes

Without adequate insulin, glucose builds up in the bloodstream instead of going into the cells. The body is unable to use this glucose for energy despite high levels in the bloodstream, leading to increased hunger. In addition, the high level of glucose in the blood causes the patient to urinate more, which in turn causes excessive thirst. Within 5 to 10 years after diagnosis, the insulin-producing beta cells of the pancreas are completely destroyed, and no more insulin is produced. Type 1 diabetes is also partly inherited and then triggered by certain infections.

Diagnosis

The following tests can be used to diagnose diabetes:

- urinalysis shows glucose and ketone bodies in the urine, but a blood test is required for diagnosis
- fasting blood glucose is 126 mg/dl or higher

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- random (non fasting) blood glucose exceeds 200 mg/dl (this must be confirmed with a fasting test)
- insulin test (low or undetectable level of insulin)

Treatment

The treatment is accomplished through education, insulin use, meal planning and weight control, exercise, foot care, and careful self-testing of blood glucose levels.

Type 2 diabetes

[Alternative names: Non insulin-dependent diabetes mellitus; Diabetes - Type 2]. Type 2 diabetes is a chronic, life-long disease that results when the body's insulin does not work effectively. Type 2 diabetes mellitus is characterized by insulin resistance which may be combined with relatively reduced insulin secretion. The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. Type 2 diabetes is the most common type. About 90 to 95 percent of people with diabetes have type 2. This form of diabetes is associated with older age, obesity, family history of diabetes, previous history of gestational diabetes, physical inactivity, and ethnicity. About 80 percent of people with type 2 diabetes are overweight. When type 2 diabetes is diagnosed, the pancreas is usually producing enough insulin, but for unknown reasons, the body cannot use the insulin effectively, a condition called insulin resistance. After several years, insulin production decreases.

Signs and Symptoms

The symptoms of type 2 diabetes develop gradually. Their onset is not as sudden as in type 1 diabetes. Symptoms may include fatigue or nausea, frequent urination, increased thirst, weight loss, increased appetite, blurred vision, frequent infections, and slow healing of wounds or sores. Some people have no symptoms.

Causes

A main component of type 2 diabetes is "insulin resistance". This means that the insulin produced by our pancreas cannot connect with fat and muscle cells to let glucose inside and produce energy. This causes hyperglycemia (high blood glucose). To compensate, the pancreas produces more insulin. The cells sense this flood of insulin and become even more resistant, resulting in a vicious cycle of high glucose levels and often high insulin levels. Genetics play a large role in type 2 diabetes and family history is a risk factor. Other risk factors include:

- Age greater than 45 years
- High blood pressure
- HDL - cholesterol of less than 35 and/or triglyceride level of greater than 250
- History of gestational diabetes

Diagnosis

Type 2 diabetes is diagnosed with the following blood tests:

- Fasting blood glucose level - diabetes is diagnosed if higher than 126 mg/dl on two occasions.

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- Random (non-fasting) blood glucose level - diabetes is suspected if higher than 200 mg/dl and accompanied by the classic symptoms of increased thirst, urination, and fatigue. (This test must be confirmed with a fasting blood glucose test.)
- Oral glucose tolerance test - diabetes is diagnosed if glucose level is higher than 200 mg/dl after 2 hours.

Treatment

The primary treatment for type 2 diabetes is exercise and diet.

Gestational diabetes

Gestational diabetes mellitus (GDM) resembles type 2 diabetes in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. Gestational diabetes develops only during pregnancy. It occurs in about 2%–5% of all pregnancies and may improve or disappear after delivery. About 20%–50% of affected women develop type 2 diabetes later in life.

Untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformations. Hyperbilirubinemia may result from red blood cell destruction.

Signs and Symptoms

One of the major problems a woman with gestational diabetes faces is a condition the baby may develop called macrosomia. Macrosomia means large body and refers to a baby that is considerably larger than normal. The combination of high blood glucose levels from the mother and high insulin levels in the fetus results in large deposits of fat which causes the fetus to grow excessively large, a condition known as macrosomia. In addition to macrosomia, gestational diabetes increases the risk of hypoglycemia (low blood sugar) in the baby immediately after delivery. This problem occurs if the mother's blood sugar levels have been consistently high causing the fetus to have a high level of insulin in its circulation.

Causes

The placenta performs the task of supplying the growing fetus with nutrients and water from the mother's circulation. It also produces a variety of hormones vital to the preservation of the pregnancy. Hormones such as estrogen, cortisol, and Human Placental Lactogen (HPL) have a blocking effect on insulin, a contra insulin effect. This contra insulin effect usually begins about midway (20 to 24 weeks) through pregnancy. The larger the placenta grows, the more these hormones are produced, and the greater the insulin resistance becomes. In most women the pancreas is able to make additional insulin to overcome the insulin resistance. When the pancreas makes all the insulin it can and there still isn't enough to overcome the effect of the placenta's hormones, gestational diabetes results.

Diagnosis

Several methods of diagnosis exist. The most common is the 50 gram glucose screening test. No special preparation is necessary for this test, and there is no need to fast before the test. The test is performed by giving 50 grams of a

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glucose drink and then measuring the blood sugar level 1 hour later. A woman with a blood sugar level of less than 140 milligrams per deciliter (mg/dl) at 1 hour is presumed not to have gestational diabetes and requires no further testing. If the blood sugar level is greater than 140 mg/dl the test is considered abnormal or positive. Not all women with a positive screening test have diabetes. Consequently, a 3 hour glucose tolerance test must be performed to establish the diagnosis of gestational diabetes.

Table: 1 Glucose Tolerance Test for Gestational Diabetes

	Diagnostic Criteria	Normal Mean Values
	Blood Glucose Level	Blood Glucose Level
Fasting	105 mg/dl	80 mg/dl
1 hour	190 mg/dl	120 mg/dl
2 hour	165 mg/dl	105 mg/dl
3 hour	145 mg/dl	90mg/dl

If two or more of tested blood sugar levels are higher than the diagnostic criteria, then the person have gestational diabetes. This testing is usually performed at the end of the second or the beginning of the third trimester (between the 24th and 28th weeks of pregnancy) when insulin resistance usually begins. The key to prevention is careful control of blood sugar levels just as soon as the diagnosis of gestational diabetes is made.

DIET

A typical healthy diet recommended to sufferers of diabetes mellitus is the one which have high fiber, with a variety of fruit and vegetables, and low in both sugar and fat, especially saturated fat. Care should be taken to avoid excess energy intake. Sucrose does not increase glycemia more than the same number of calories taken as starch. Although it is not recommended to use fructose as a sweetener, fruit should not be avoided because of its fructose content. Benefits may be obtained by consumption of dietary fibre in conjunction with carbohydrate. A low-carbohydrate diet or low GI (Glycemic Index) diet may be effective in dietary management of type 2 diabetes. For people with diabetes, healthy eating is not simply a matter of what one eats, but also when one eats. High fiber diet – It has been shown that a high fiber diet works better and control blood sugar levels with the same efficacy as oral diabetes drugs.

ANTIDIABETIC DRUGS IN ALLOPATHIC FIELD [1]

Anti-diabetic drugs treat diabetes mellitus by lowering glucose levels in the blood. With the exceptions of insulin, exenatide, and pramlintide, all are administered orally and are thus also called oral hypoglycemic agents or oral antihyperglycemic agents. There are different classes of anti-diabetic drugs, and their selection depends on the nature of the diabetes, age and situation of the person, as well as other factors. An ideal anti-diabetic drug should

- a) be effective orally
- b) be non toxic
- c) correct the other metabolic defects in diabetic.

Types

Insulin

Insulin is usually given subcutaneously, either by injections or by an insulin pump. In acute care settings, insulin may also be given intravenously. There are several types of insulin, characterized by the rate which they are metabolized by the body.

Sulfonylurea

Sulfonylurea was the first widely used oral anti-hyperglycemic medications. They are *insulin secretagogues*, triggering insulin release by direct action on the K_{ATP} channel of the pancreatic beta cells. The "second-generation" drugs are now more commonly used. They are more effective than first-generation drugs and have fewer side effects. All may cause weight gain. Sulfonylureas are only useful in Type II diabetes. They cannot be used with type I diabetes, or diabetes of pregnancy. The primary side effect is hypoglycemia.

Mechanism of action

Pancreas: Sulfonylureas stimulate release of insulin by pancreas β cells by increasing their sensitivity to glucose. These are effective only in presence of functioning pancreas. They act on 'Sulphonylurea receptors' linked to the ATP dependent potassium channels in cell membrane of β cells. Activation of receptors causes potassium channels to close and calcium influx into the cell, with exocytosis of insulin granules. Sulfonylureas release insulin slowly and for prolonged periods into portal circulation.

Liver: Sulfonylureas inhibit neoglucogenesis and glycogenolysis. The liver releases less glucose in response to Sulfonylurea induced hypoglycemia than that in response to insulin. The basic action of all Sulfonylureas is identical. They differ in their pharmacokinetic properties, potency and hypoglycemic activity of their metabolites.

Meglitinides

Meglitinides help the pancreas produce insulin and are often called "short-acting secretagogues." They act on the same potassium channels as sulfonylurea, but at a different binding site. By closing the potassium channels of the pancreatic beta cells, they open the calcium channels, hence enhancing insulin secretion. Adverse reactions include weight gain and hypoglycemia.

Sensitizers

Insulin sensitizers address the core problem in Type II diabetes—insulin resistance. Among oral hypoglycemic agents, insulin sensitizers are the largest category.

Biguanides

Biguanides reduce hepatic glucose output and increase uptake of glucose by the periphery, including skeletal muscle. Although it must be used with caution in patients with impaired liver or kidney function, metformin, a biguanide,

has become the most commonly used agent for type 2 diabetes in children and teenagers. Amongst common diabetic drugs, metformin is the only widely used oral drug that does not cause weight gain.

Thiazolidinediones

Thiazolidinediones (TZDs), also known as "glitazones," bind to a type of nuclear regulatory protein involved in transcription of genes regulating glucose and fat metabolism. These act on Peroxisome Proliferator Responsive Elements (PPRE). The PPREs influence insulin sensitive genes, which enhance production of mRNAs of insulin dependent enzymes. The final result is better use of glucose by the cells.

Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors are "diabetes pills" but not technically hypoglycemic agents because they do not have a direct effect on insulin secretion or sensitivity. These agents slow the digestion of starch in the small intestine, so that glucose from the starch of a meal enters the bloodstream more slowly, and can be matched more effectively by an impaired insulin response or sensitivity.

Peptide analogs

Incretin mimetics

Incretins are insulin secretagogues. The two main candidate molecules that fulfill criteria for being an incretin are Glucagon-like peptide-1 (GLP-1) and Gastric inhibitory peptide (aka glucose-dependent Insulinotropic peptide or GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4). GLP agonists bind to a membrane GLP receptor. As a consequence of this, insulin release from the pancreatic beta cells is increased.

DPP-4 (Dipeptidyl peptidase-4) inhibitors

Dipeptidyl peptidase-4 (DPP-4) inhibitors increase blood concentration of the incretin GLP-1 (glucagon-like peptide-1) by inhibiting its degradation by dipeptidyl peptidase-4 (DPP-4).

Amylin analogues

Amylin agonist analogues slow gastric emptying and suppress glucagon. They have all the incretins actions except stimulation of insulin secretion. Pramlintide is the only clinically available amylin analogue. Like insulin, it is administered by subcutaneous injection. The most frequent and severe adverse effect of pramlintide is nausea, which occurs mostly at the beginning of treatment and gradually reduces.

ANTIDIABETIC MEDICINAL HERBS [2]

Coriander



Scientific Classification	
Kingdom	Plantae
Division	Angiospermae
Order	Apiales
Family	Umbelliferae
Genus	Coriandrum
Species	<i>C.sativum</i>

Seed of Coriander is widely used for the medicinal purposes. It works both by enhancing the secretion of insulin from the pancreas and exhibiting insulin-like activity at cellular level. It is Yellowish–brown to brown in colour, aromatic, spicy and 2 to 4 mm in diameter and 4 to 30 mm in length. Coriander is a sub-globular cremocarpous fruit.

Cinnamon



Scientific Classification	
Kingdom	Plantae
Division	Magnoliophyta
Order	Lurales
Family	Lauraceae
Genus	Cinnamomum
Species	<i>C.zeylanicum</i>

Bark of Cinnamon is widely used for the medicinal purposes. It enhances the activity of the enzymes that increase cell receptor insulin sensitivity, and inhibits those that have the opposite effect. The outer surface is dull yellowish–brown, while the inner surface is dark yellowish-brown, Fragrant, Aromatic and sweet followed by warm sensation. Size is about 1 m in length and 1 cm in diameter and the thickness of the bark is approximately 0.5 mm. Its Shape is in the form of compound quills.

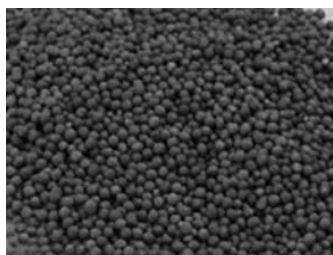
Garlic



Scientific Classification	
Kingdom	Plantae
Division	Angiospermae
Order	Asparagales
Family	Liliaceae
Genus	Allium
Species	<i>A.sativum</i>

Bulb of Garlic is widely used for the medicinal purposes. It has blood glucose-lowering properties and also has the ability to reduce the raised blood lipid levels. Bulbs are white to pink in colour, aromatic and pungent and 1.5 to 2.5 cm in Size

Mustard



Scientific Classification	
Kingdom	Plantae
Division	Angiospermae
Order	Brassicales
Family	Cruciferae
Genus	Brassica
Species	<i>B.juncea</i>

Seed of mustard is widely used for the medicinal purposes. It is a food adjuvant for diabetic patients. It is Black or dark brown or reddish-brown. Crushed seeds have pungent odour. Bitter to Taste. About 1mm in diameter in Size. Seeds are nearly spherical in shape.

Ginger



Scientific Classification	
Kingdom	Plantae
Division	Magnoliophyta
Order	Zingiberales
Family	Zingiberaceae
Genus	Zingiber
Species	<i>Z.officinale</i>

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Rhizome of Ginger is widely used for the medicinal purposes. It increases insulin level and decrease fasting glucose level. Externally, it is buff coloured and aromatic. Rhizomes of ginger are about 5 to 15 x 1.5 to 6.5 cm in size. The rhizomes are laterally compressed, bearing short flat, ovate and oblique branches on the upper side, with bud at the apex.

Table: 2 Anti-diabetic potential plants [3] [4] [5]

S No	Plant	Synonym	Family	Part Used
1	<i>Nymphaea pubescens</i>	Lotus	Nyctaginaceae	Whole Plant
2	<i>Helicteres isora</i>	East Indian Screw tree	Haloragaceae	Root bark, Leaf
3	<i>Linum usitatissimum</i>	Linseed	Linaceae	Flower, Seed oil
4	<i>Tinospora cordifolia</i>	Bulonga, Guduchi	Tiliaceae	Stem
5	<i>Cieba pentandra</i>	Kapok	Casuarinaceae	Stem bark
6	<i>Limonia acidissima</i>	Elephant apple	Liliaceae	Stem bark
7	<i>Alanthus excelsa</i>	Tree of heaven	Agavaceae	Stem bark
8	<i>Polialthia longifolia</i>	Indian fir	Poaceae	Stem bark
9	<i>Cissampelos pareira</i>	False paraira root	Chenopodiaceae	Root
10	<i>Sida cordifolia</i>	Country mallow	Scrophulariaceae	Root
11	<i>Bombax ceiba</i>	Silk cotton tree	Bombacaceae	Root
12	<i>Melia azedarach</i>	Persian lilac	Melastomaceae	Root
13	<i>Tribulus terrestris</i>	Calthrops	Tiliaceae	Root, Fruit
14	<i>Cocculus hirsutus</i>	Broom creeper	Clusiaceae	Leaf
15	<i>Aegle marmelos</i>	Bael fruit tree	Acanthaceae	Leaf
16	<i>Murraya koenigii</i>	Curry leaf plant	Moringaceae	Leaf
17	<i>Naringi crenulata</i>	Musk deer plant	Najadaceae	Leaf
18	<i>Nelumbo nucifera</i>	East Indian lotus	Najadaceae	Seed
19	<i>Portulaca oleracea</i>	Common purslane	Pontederiaceae	Seed
20	<i>Gossypium barbadense</i>	Cotton	Goodeniaceae	Seed
21	<i>Cocos nucifera</i>	Green gold tree	Cochlospermaceae	Flower
22	<i>Grewia tillaefolia</i>	Dhaman	Goodeniaceae	Wood
23	<i>Balanites aegyptiaca</i>	Zachun oil tree	Balanitaceae	Fruit

CONCLUSION

Diabetes is a metabolic disorder which is affecting approximately more than 4% of the population. The disease becomes a real problem of public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable. Moreover, uncontrolled diabetes leads to many chronic complications such as blindness, heart failure, and renal failure. In order to prevent this alarming health problem, the development of research into new hypoglycemic and potentially antidiabetic agents is of great interest. Currently available oral anti-diabetic drugs that are used clinically for glycemic control include Sulfonylureas, meglitinides, biguanides, α -glucosidase inhibitors, and thiazolidinediones (TZDs). Presently, there is growing interest in herbal remedies due to the side effects associated with the oral synthetic hypoglycemic agents for the treatment of diabetes mellitus. Herbal treatments for diabetes have been used in patients with insulin dependent and non-insulin dependent diabetes. Spices such as *Cinnamomum verum* (cinnamon), *Brassica juncea* (mustard), *Allium sativum* (garlic), *Coriandrum sativum* (coriander), *Zingiber officinale* (ginger), etc have been used widely as they contain antidiabetic

property. These are the spices most effective in lowering blood glucose and abnormal blood lipids in these diseases. Herbal drugs for the treatment of diabetes were found to be more effective and economical than allopathic drugs.

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