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Drug discovery, Threatened medicinal plants and strategies for sustainable use

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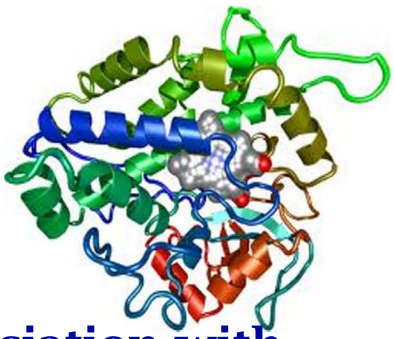


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"DRUG DISCOVERY, THREATENED MEDICINAL PLANTS AND STRATEGIES FOR SUSTAINABLE USE"

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DRUG DISCOVERY, THREATENED MEDICINAL PLANTS AND STRATEGIES FOR SUSTAINABLE USE

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PREFACE

We are pleased to introduce this book on "**DRUG DISCOVERY, THREATENED MEDICINAL PLANTS AND STRATEGIES FOR SUSTAINABLE USE".** This book provides an overview of a major ongoing scientific programme being conducted by various institutes and it also assesses the importance of conservation along with development. Medicinal plants have become the most important source of life-saving drugs for the majority of the world's population. Medicinal plants harvested from the wild remain of immense importance for the well-being of millions of people around the world. Over 70,000 plant species are thought to be medicinal**.**

For the people of the developing countries medicinal and aromatic plants continues to be a significant source of food, drug, herb and dietary supplement. About 50% of drugs used in modern medicine are of plant origin. The medicines for internal use prepared in the traditional manner involve simple methods such as hot- or cold-water extraction, extraction of juice after crushing, powdering of dried material, formulation of powder into pastes via such a vehicle as water, oil or honey, and even fermentation after adding sugar source.

Emerging new infectious, chronic and drug-resistant diseases have prompted scientists to look towards medicinal plants as agents for treatment and prevention. Medicinal plants' species are threatened by habitat loss, climate change, and species-specific, multipurpose over-harvesting and logging leading to potential extinction of useful medicinal plants in the continent. Securing supplies of quality products before the over-harvesting of wild stocks depletes the resource constitutes a concern. Declining wild stocks of medicinal plants are accompanied by adulteration and species substitutions, which in turn reduce efficacy, quality and safety.

The present publication reinforces the importance of medicinal plants diversity, particularly in the context of sustainable development. It attempts to give an overview of the issue, by analysing the main thematic areas and cross-cutting and strategic issues and exploring its direct and indirect links with the broader goal of sustainable development. The writers of the articles have been explicitly asked to focus on the threats faced by medicinal plants and their sustainable use. Each article highlights a priority area in an attempt to address a wide-ranging and pressing issue. Keeping these in mind the Department of Botany, St. Andrew's College (PG), Gorakhpur had conducted a national conference on "**Drug discovery, threatened medicinal plants and strategies for sustainable use"**. Many researchers participated and made their observations and contributed. The deliberations were thought provoking and we decided to publish a select list of papers presented in the conference. All their presentations are summed up in this book. In conclusion, we wish to express my deep gratitude to all those who have contributed articles to enrich this publication and trust that it would be both useful and informative to all readers.

We would like to gratefully acknowledge the fund received from central agencies such as,

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S. DOMINIC RAJKUMAR

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CONTENT

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CONCENTRATIONS AND CARCINOGENIC POTENCIES OF ORGANIC POLLUTANTS (PAHS) IN PM_{10} AT A SEMI ARID REGION OF INDIA

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ABSTRACT

Atmospheric aerosols are ubiquitous in the troposphere and play an important role in climate and atmospheric chemistry. They include inorganic and organic chemical species, which could have harmful effects on human health. Aerosols resulting from the emission of harmful gases and particles are serious environmental air pollution problems in many of the world's urban areas. Atmospheric particulate matter from four different areas within Agra city(a semi-arid region) were collected using respirable dust samplers during the 2006 summer season and were then extracted with dichloromethane using an automated Soxhlet Extraction System (Soxtherm®). The extracts were analyzed for 17 target Polycyclic Aromatic Hydrocarbons (PAHs). The total PAH (TPAH) concentrations were 76.6 ± 3.6, 27.9 ± 2.9, 23.7 ± 2.3 and 6.5 ± 1.7 nanograms per cubic meter (ng m^{-3}), respectively, at the industrial, residential, roadside and agricultural sites. The combined mean concentration of TPAH was 33.9 ng m^{-3} for all sites. The industrial site had the highest TPAH concentration followed in order by the residential, roadside and agricultural sites. Indeno(1,2,3-cd)pyrene, benzo(g,h,i)perylene and benzo(b)fluoranthene were the predominant compounds found in the samples collected from all of the sites. The carcinogenic potencies related to PAHs have been calculated by using Toxic Equivalent Factors (TEFs) compiled from the literature which corresponds to a B (a)P equivalent exposure of 8.9 ng m^{-3} and 8.6 ng m^{-3} with respect to carcinogenicity.

Keywords: *PAHs, Semi Arid Region, Particulate Pollution, Toxic Equivalent Factor*

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are chemicals containing two or more fused benzene rings in a linear, angular or cluster arrangement. PAH contain only carbon and hydrogen. They belong to the group - Persistent Organic Pollutants (POPs) known for their chemical carcinogenicity. They are formed during incomplete combustion of organic materials and geochemical formation of fossil fuels and are the products of thermal decomposition. Sources of PAHs include automobiles, re-suspended soils, refineries and power plants. It is well established that some PAHs have carcinogenic, mutagenic and immunotoxic effects on animals and can occur in low concentration in many parts of the environment (Grimmer, 1983). Due to their mutagenic and carcinogenic potential the atmospheric concentrations of PAHs in many geographical locations of the world have been measured and reported, e.g. Massachusetts, USA (Allen et al, 1996) Ontario, Canada (Katz et al, 1978), Athens, Greece (Viras et al, 1987), Antarctica (Caricchia et al, 1999), Lahore, Pakistan (Smith et al, 1996) and even in Mumbai, India (Kulkarni and Venkataraman,2000). In many circumstances the environmental occurrence of PAHs has been associated with adverse effects on public health (Grimmer, et al, 1983). Atmospheric PAHs are partitioned between the particulate matter and gas phases, depending on the PAH molecular weight. Low molecular weight PAHs have higher concentrations in the vapor phase while high molecular weight PAHs are often associated with particles. It is believed that there is no *"Threshold"* or "*Safe*" level for the mutagenic compounds, hence exposure to these PAHs at any level provide the risk of toxic effects.

Nowadays, there is a lot of concern about air quality in large urban areas, and it has been the subject of many studies. The harmful effects of Polycyclic Aromatic Hydrocarbons (PAHs) on human health have been especially studied, since some of these are carcinogenic and mutagenic, and some are associated with acute and chronic health problems (Dallarosa et al., 2005, Fang et al., 2004). PAHs are organic compounds of carbon and hydrogen arranged in combinations of aromatic rings. The contribution from natural sources of PAHs is limited, being restricted to spontaneous forest fires and volcanic emissions (Bourotte et al, 2005). In cities, the sources of PAHs are

exclusively anthropogenic, i.e. they are formed during incomplete combustion and pyrolysis of organic matter, such as coal, oil, wood and fuels like diesel and petrol. Automobile exhaust has been recognized as the major PAHs contributor in urban areas (Velasco et al., 2004). Their elevated concentrations in urban environments pose a risk of exposure to inhabitants (Sienra et al., 2005).

In Agra the most important source of PAHs is expected to be vehicular emission since motor vehicles contributed as much as 60% of pollution. As of August 2006 there were 3, 36,635 registered vehicles in Agra (News item, Amar Ujala, 2006). There are also other important potential sources of PAHs such as smoke coming from diesel generators which are 32,030 in Agra and are in use because of erratic power supply. The concentration determined for individual PAH will help Planners, Scientists, and Administrators to draw strategies to reduce PAH exposure to the people living in this area. The aim of this study is to investigate the concentration and distribution of particulate PAHs in Agra.

METHODOLOGY

Air sampling stations were set up in the four locations, representing industrial, residential, roadside and agricultural areas, were selected for study. Each station was monitored for ambient air quality twice a month in a scheduled manner. Particulate Matter (PM_{10}) in air were collected on 20.3 x 25.4 cm^2 glass fiber filter paper (EPM-2000) using respirable dust samplers (RSPM Envirotech Sampler RDS, 460 DX, New Delhi, India) at the rate of 1.0 cubic meter per minute (m^3/min). The air suction rate was verified every week using calibrated rotameters with an accuracy of ±1%. Samples were stored in a cool, dark place until analysis. Samples and blanks were extracted with 140 milliliters (mL) methylene chloride by Soxtherm®. Blank spike/blank spike duplicate (BS/BSD) samples (spiked with PAH spiking solution) were extracted using clean fibreglass thimbles. No surrogates were added. After the samples were extracted for one programmed cycle, 100 mL of additional solvent was added. All samples were extracted for another programmed cycle and then concentrated to 1.0 mL. Internal standards were added to all extracts prior to sample injection. The gas chromatograph (GC) oven was temperature programmed to separate the method analytes on a fused silica column,

which were then detected with a mass spectrometer (MS).

RESULTS AND DISCUSSION

Summer season comprises of four months i.e. from March to June. The atmospheric pollution load is high and because of the down ward wind, pollutants may be transported to the different areas mainly from an oil refinery situated in Mathura (50 kms from the centre of Agra City). This period is often characterized by strong dust storms caused due to low pressure developed in this area. The temperature and relative humidity ranges from 15.4°C to 48.8°C and 18.4%-62.7% respectively. The down ward wind is west and north-north-west and its speed ranges from 0.2 m s^{-1} to 9.2 m s^{-1} in summers (Parivesh, 2006). **Table 1** illustrates the individual concentrations of PAHs at different locations. The TPAH concentrations were 76.63, 27.96, 23.79 and 6.54 ng m^{-3} at industrial, residential, roadside, and agricultural sites, respectively. The combined mean concentration of TPAH was 33.95 ng m^{-3} for all sites. The industrial site had the highest TPAH concentration followed by the residential, roadside and agricultural sites. These results also indicate that PAH concentrations are strongly linked to land use.

The concentration trends of the major PAH found in present study are illustrated in **Figure 1**, which were: benzo (g,h,i)perylene > indeno(123-cd) pyrene > benzo(b)fluoranthene > benzo(a)pyrene at the industrial site; benzo(b)fluoranthene > indeno(123-cd)pyrene > benzo(k) fluoranthene > benzo(g,h,i)perylene at the residential site; benzo(g,h,i)perylene > benzo(b)fluoranthene > benzo(a)pyrene > indeno(123-cd)pyrene at the roadside site: and benzo(g,h,i)perylene > indeno(123-cd)pyrene > benzo(k)fluoranthene > benzo(b)fluoranthene at the agricultural site. At all the sites, indeno (123-cd)pyrene, benzo(g,h,i)perylene, and benzo(b)fluoranthene were the predominant compounds.

Figure 2 shows the relative contribution of 2-, 3-, 4-, 5-, and 6-ring PAHs in the atmosphere at the locations investigated in this study. The average TPAH percentage based on the number of rings were 0.67% (2-ring), 3.41% (3-ring), 21.72% (4-ring), 41.18% (5- ring), and 33.05% (6-ring). The major contributors to TPAH in the Agra region

were 5-ring and 6-ring PAHs (41.18% and 33.5% of the TPAH respectively).

An occupational exposure limit for TPAHs has not been established because of the complex chemical composition of PAH mixtures. Several PAH species including benzo(a)pyrene (the most carcinogenic compound) have been classified as probable (2A) or possible (2B) human carcinogens by the International Agency for Research on Cancer (IARC, 1987). B(a)P is a five ring ($C_{20}H_{12}$) compound, is mutagenic for human cells in culture (Osborne et al., 1987) and carcinogenic in whole animal assays (Cerna et al., 2000). One approach to estimating the carcinogenic potency associated with exposure to a given PAH compound is to assign a Toxic Equivalent Factor (TEF), a measure of its potency relative to that of B(a)P. The TEF for B(a)P is set at 1.0 (Bostrom et al., 2002). The concentration of each individual PAH compound is multiplied by its respective TEF to calculate the "B (a)P equivalent", or $B(a)P_{eq}$ concentration. The TEF approach has the main advantage of being relatively easy to apply, but it may underestimate risk because only a limited number of PAH compounds have assigned TEFs (WHO/IPCS, 1998). Two lists of TEFs, compiled by Tsai et al., (2004) and EPA Region III (2006), were used in this study.

Table 2 indicates the mean concentration of TPAHs in Agra to be 33.6 ng m^{-3}, which corresponds to a B(a)P equivalent exposure of 8.9 ng m^{-3} and 8.6 ng m^{-3} with respect to carcinogenicity, using TEFs given by Tsai et al. (2004) and EPA Region III (2006), respectively. Regardless of the TEF source, similar B(a)P exposure estimates were generated by the two sets of TEF values.

CONCLUSION

The TPAH concentrations were 76.6 ± 3.6, 27.9 ± 2.9, 23.7 ± 2.3 and 6.5 ± 1.7 ng m^{-3} at industrial, residential, roadside, and agricultural sites, respectively. The combined mean concentration of TPAH was 33.95 ng m^{-3} for all sites. The industrial site had the highest TPAH concentration followed by the residential, roadside and agricultural sites. These results also indicate that PAH concentrations are strongly linked to land use. The average TPAH percentage based on the number of rings were 0.67% (2-ring), 3.41% (3-ring), 21.72% (4-ring), 41.18% (5- ring), and 33.05% (6-ring). The major contributors to TPAH in the

Agra region were 5-ring and 6-ring PAHs (41.18% and 33.5% of the TPAH respectively). The result shows that the variation in the concentration of PAHs depends on different locations. The mean concentration of TPAHs in Agra to be 33.6 ng m^{-3}, which corresponds to a B(a)P equivalent exposure of 8.9 ng m^{-3} and 8.6 ng m^{-3} with respect to carcinogenicity. It is also clear that much variation is found in industrial and agricultural sites whereas the residential as well as roadside sites have almost similar concentration of PAHs.

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Table 1: Individual concentrations of PAHs at different locations (ng m^{-3})

PAHs	IND	RES	RDS	AGR
NAP	0.2	0.12	0.22	0.07
ACY	0.71	0.13	0.2	0.08
ACE	0	0	0.19	0
FLU	0.21	0.11	0	0
PHE	1.53	0.24	0.33	0.1
ANT	0.31	0.18	0.16	0.08
FLT	2.09	0.94	1.14	0.43
PYR	3.26	1.35	1.06	0.51
B(a)A	2.71	1.14	1.82	0.47
CRY	2.07	2.16	1.62	0.55
B(b)F	9.43	4.35	3.03	0.63
B(k)F	5.74	3.62	2.41	0.67
B(e)P	6.65	2.72	1.65	0.56
B(a)P	8.52	2.76	2.94	0.42
IND	11.63	3.8	2.72	0.69
DIB	2.11	1.13	1.12	0.18
B(ghi)P	19.46	3.21	3.18	1.1
TOTAL	**76.63**	**27.96**	**23.79**	**6.54**

Table 2: BaP toxic equivalency factors (TEFs) and BaP_{eq} exposure profiles (ng m^{-3})

PAHs	MEAN	*TEFs	BaP exposure	+TEFs	BaP exposure
Naphthalene	0.1525	0.001	0.0001525	--	--
Acenaphthylene	0.28	0.001	0.00028	--	--
Fluorene	0.08	0.001	0.00008	--	--
Phenanthrene	0.55	0.001	0.00055	--	--
Anthracene	0.1825	0.01	0.001825	--	--
Fluoranthene	1.15	0.001	0.00115	--	--
Pyrene	1.545	0.001	0.001545	--	--
Benzo(a)anthracene	1.535	0.1	0. 1535	0.1	0. 1535
Chrysene	1.6	0.01	0.016	0.001	0.0016
Benzo(b)fluoranthene	4.36	0.1	0.436	0.1	0.436
Benzo(k)fluoranthene	3.11	0.1	0.311	0.01	0.0311
Benzo(e)pyrene	2.895	1	2.895	1	2.895
Benzo(a)pyrene	3.66	1	3.66	1	3.66
Indeno(1,2,3-cd)pyrene	4.71	0.1	0.471	0.1	0.471
Dibenz(a,h)anthracene	1.135	1	1.135	1	1.135
Benzo(g,h,i)perylene	6.7375	0.01	0.067375	--	--
TOTAL	**33.6**		**8.9**		**8.6**

**TEFs cited by Tsai et al. (2004), +TEFs cited by EPA Region III (2006)*

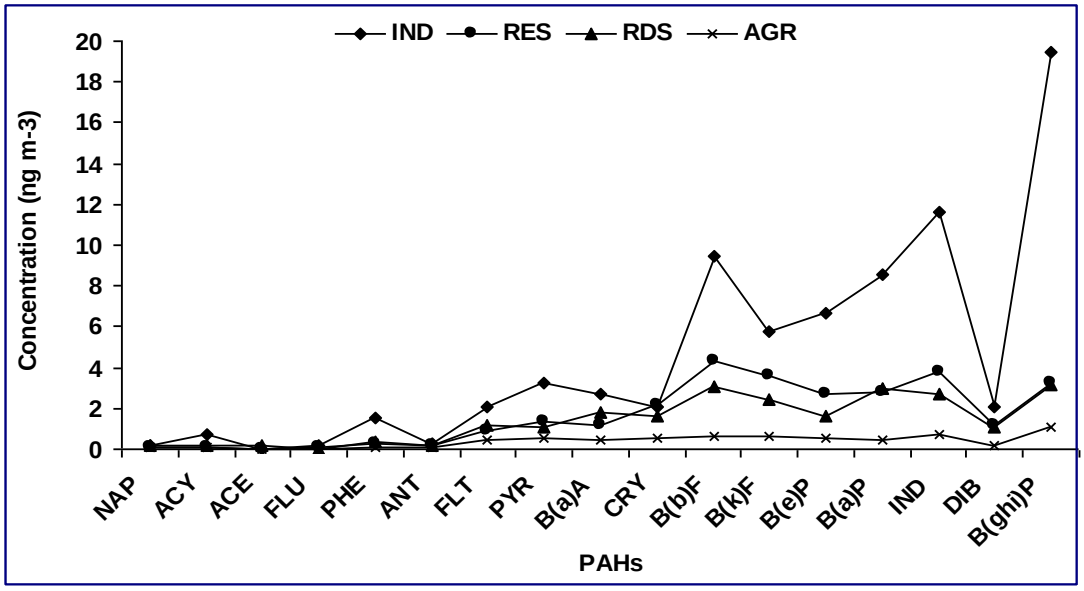

Figure 1: Trends of individual concentrations of PAHs in ambient air at different sites of Agra (ng m^{-3}) during summers

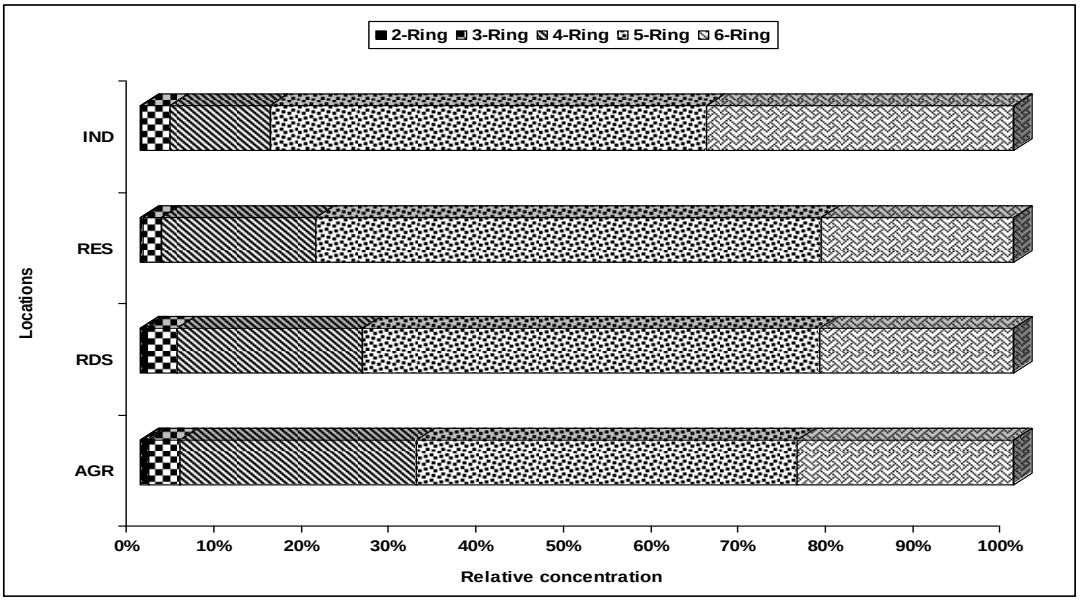

Figure 2: Distribution of PAHs based on benzene ring at different locations of Agra in summers

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POPULAR MUSHROOMS IN UTTAR PRADESH

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ABSTRACT

Mushroom is one of most nutritive food commodity. It preferred not only for its flavour, taste and aroma, but it also has a high nutrient value. Mushroom is rich in minerals and the essential aminoacids, equivalent to those obtained from animal protiens. As it lacks lipids and sugar, it is recommended to all type of patients. As a result of the present study, it has been concluded that three varietis of mushroom is cultivated in U.P. Availability and cultivation period of different mushroom has also been explored.

Keywords: Mushroom, minerals, *Agaricus bisporus*

The state of Uttar Pradesh is rich in bio-diversity and varied agro-climatic conditions. However, mushroom cultivation is unique as it is the most efficient and economically viable biotechnology process for the conversion of lignocelluloses waste materials into high quality protein rich food. Its cultivation started in 1974 by Uttar Pradesh Department of Agriculture (UPDA) on trial basis at Vivekanand Parvatiya Krishi Anusandhan (VPKA) Almora (Now in Uttaranchal).

Mushrooms are fruiting body of certain fungi belonging to the group of Basidiomycetes and Ascomycetes, some are edible and many are poisonous and non-edible. They are an accepted ideal food item, rich in protein, low in fat and carbohydrates and an efficient tool for recycling of organic wastes. There are more than 10,000 species of mushrooms and about 2000 of them are considered edible. Of these, less than twenty five species are widely accepted as food item and only about a dozen of them have been artificially cultivated (Arora, et al., 2013).

Button, oyster and paddy straw mushrooms are commonly used for human consumption, particularly in Uttar Pradesh. Due to their very low content of carbohydrate, mushrooms are suitable ingredients in the diet of diabetic individuals. They are also rich in vitamin and minerals. They have pleasant flavour and are delicious to eat. Mushrooms are referred to as "vegetarian meat" as it is rich in protein (35%). It can be called 'heart food' because they contain ergosterol, which converts into Vitamin D in due course of time, in the human body. The deadly cholesterol is absent in this food item. They are suitable diet for the obese persons as these are low in calories (32 KCal / 100g fresh mushroom) and low in fat (max. 0.3%). Most people eat mushrooms because of its flavour, meaty taste and medicinal value (Moore and Chiu, 2001). Mushrooms generally possess most of the attributes of nutritious food as they contain many essential nutrients in good quantity (Fukushima et al., 2000). It must however be emphasized that some mushrooms are poisonous and may kill within few hours after consumption (Phillips, 1985).

Species	**Production Period**
Agaricus spp.	November to March
Pleurotus spp.	February to April and September to November
Volvariella spp.	June to September

Considering mushroom's growth requirement, they grow well on a wide range of lignocellulosic wastes as substrates (Okhuoya and Okogbo, 1990; Kadiri, 1991). It has been established that they grow and fruit on various agricultural wastes (Moncaio et al., 2005).

Cultivation of Mushroom has been in vogue for almost 300 years. However, commercial cultivation in Uttar- Pradesh has started only recently. Its popularity is growing and it has become a business which is export- oriented. Mushroom is an excellent source of protein, vitamins, minerals, folic acid and is a good source of iron for anemic patient.

Some of the mushrooms cultivated in Uttar Pradesh are:

a) Button Mushroom (*Agaricus spp.*)

b) Oyster (*Pleurotus spp.*)

c) Paddy Straw Mushroom (*Volvariella spp.*)

Agaricus bisporus

White Button mushroom (*Agaricus bisporus*) is the most popular, economically best and is extensively cultivated. However, commercial production of this mushroom was initiated in the hilly regions of India (17-18°C) like Himachal Pradesh followed by Kashmir and Ooty, then slowly spread to North western plains of India. In controlled conditions it can be grown anywhere. These conditions include- controlled temperature, moisture, ventilation and good spawn. The temperature requirement for the spread of the mycelium or the vegetative growth is 22 to 25° C. For the reproductive stage the temperature requirement is 14 to 18° C. Within the limits prescribed, the temperature should be uniform throughout the growth of the crop. If it is too cold the development of

the spawn will be retarded. A high temperature will favour the development of moulds and bacteria, which will soon destroy the spawn or crop. On an average the growers can take 3-4 crops of white button mushrooms in a year depending upon the type and varieties cultivated. Factors affecting the yield of the crop both in terms of quality and quantity are incidence of pests or pathogens and non-availability of pure quality of spawn.

Pleurotus sajor caju

Common name oyster mushroom or Dhingri etc., this mushroom is not as popular as white button mushroom in the domestic market. It is widely cultivated due to their simple and low cost production technology and higher biological efficiency (Mane et al., 2007). *Pleurotus* species are efficient lignin degraders which can grow on wide variety of agricultural wastes with broad adaptability to varied agro-climatic conditions. It thrives well in a moderate range of temperature 20-30^{o}C and requires 80-85% humidity. Its growing season is longer that is February to April and September to November. *Pleurotus* species are rich source of proteins, minerals (Ca, P, Fe, K and Na) and vitamin C, B- complex (thiamine, riboflavin, folic acid and niacin) (Caglarırmak, 2007). They are consumed for their nutritive as well as medicinal values (Agrahar-Murugkar &Subbulakshmi, 2005). Mushroom protein is intermediate between that of animals and vegetables (Kurtzman, 1976) and is of superior quality because of the presence of all the essential amino acids (Purkayastha & Nayak, 1981). *Pleurotus* sp. contains high potassium to sodium ratio, which makes mushrooms an ideal (Jandaik & Goyal, 1995).

Volvariella volvacea

Common Name 'Chinese' or 'paddy straw' mushroom is commercially less attractive due to low yield per unit weight, of the substrate. As a kitchen garden crop it is preferred because of its taste and nutritional properties. In India, three species of *Volvariella* are commonly grown, namely *V. diplasia, V. volvacea and V. esculenta*. Out of these *Volvariella volvacea* is the common growing mushroom in Uttar Pradesh.

It is a high temperature mushroom grown largely in tropical and subtropical regions. This mushroom can be grown on a variety of agricultural wastes e.g. paddy straw, water hyacinth, banana, cotton or wood waste. (I.O. Fasidi (1995) Mushroom production is encouraged by heavy watering, temperature reduction and light. This mushroom can be grown up to 35°C and it should be harvested at egg stage.

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ANTIMICROBIAL ACTIVITY AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF *SESAMUM LACINATUM.* (Klein) ex willd

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ABSTRACT

In this study different solvents like ethanol, methanol, chloroform and aqueous fraction of selected angiosperms plant *Sesamum lacinatum.* (Kelin) ex willd, belongs to the family Pedalliaceae. The disc diffusion methods for different pathogenic bacterias like *Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae, Bacillus subtilis* and fungi species *Penicillium notatum, Aspergillus niger, Rhizopus nigricants* were studied with different solvent extracts. The ethanol and acetone stem extracts were studied for preliminary phytochemical analysis. *Bacillus subtilis* showed higher inhibition in stem extract and showed resistance in the leaf extract. Pathogenic fungi *Penicillium notatum* stem extract chloroform highly inhibition, aqueous extract did not show any activity. Chloroform and aqueous leaf extracts activity highly resistant. Preliminary phytochemical analysis was preformed which enabled identify the presence of different secondary metabolites and bioactive compounds.

Keywords: Antimicrobial activity, phytochemical analysis, *Sesamum laciniatum.*

INTRODUCTION

In the present scenario of every green of multiple drug resistance to human pathogenic organism, search for now, safe and effective therapeutically agent from another source including plants are urgently needed. Antimicrobial activity agents from plants are plentiful in many countries, especially in India, where thousands of tribal communities still use medicinal plants today to curse sickness. (Perumalsamy R and lgnacimuthu, S., 2000). In a recent year number of studies has been reported dealing with the antimicrobial screening of extracts of medicinal plants, previously with unknown. Pharmacological activities have been extensively investigated as a source of medicinal agents **(**Krishnaraju *et al.*, 2005).. Later, it was tested the bactericidal activity of anacardic acid and totarol on methicillin resistant strains of S. aureus (MRSA) and the synergistic effect of these compounds associated with methicillin (Muroi *et al.*, 1996). The synthetic antibiotics have the following limitation firstly; these are costly and are out of range from the patient belonging to developing countries. Secondly, with the passage of time microorganism develop resistance against antibiotics. Therefore, after some time these antibiotics are not effective against the microbes (Walsh and Alder. 2005) Phytochemical and antimicrobial activity estimated used in disc diffusion methods briefly investigated.

MATERIALS AND METHODS

Collection of Plants

The plant *Sesamum laciniatum* (Kelin) ex willd oil herbs. In Tamil it is called as ellu, kattu ellu and nallennai. The plants were collected from their natural habitats nearby T.kulathur, Thuraiyoor, Tiruchirappalli district, Tamilnadu. The sample was washed thoroughly in running tap water to remove soil particles and adhered debris and finally washed with sterile distilled water. The leaves and stem were shade dried, ground into fine powder and stored in air tight polythene bags until use.

Microorganism used

Four bacterial species were treated. The bacteria used in this study collected from P.G.P Arts and Science College Namakkal District. The bacteria species Gram positive bacteria: *Bacillus subtitles.* Gram negative bacterias: *Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae.* Fungi speciously were collected from Govt. Medical College, Tiruchirappalli, Tamil Nadu. The fungi

include *Penicillium notatum, Aspergillus Niger* and *Rhizopus nigricants.*

Preparation of Extracts

10g of the dried leaf and stem powdered materials were soaked with 150 ml of 80% methanol, ethanol, and chloroform and aqueous for 72 hrs, after that each extract was filtered by whatmann no. 1 paper and the filtrate was concentrated at room temperature in order to reduce the volume and kept in glass bottles in the refrigerator.

Evolution of antimicrobial activity

The stock culture was maintained at 4^0c on the slopes of nutrient agar, and potato dextrose agar. Active culture for experiments were prepared by transferring a loopful of cells from the stock culture to test tubes nutrient broth (NB) for bacteria and potato dextrose broth (POB) for fungi that were that were incubated without agitation for 24h at 37^0 c and 25^0 c respectively.

Antimicrobial screening

The Antimicrobial screening of the organic solvent extracts of leaves and stem of *Sesamum laciniatum* (Kelin) ex willd, was investigated through disc diffusion methods (Maruzella & Henry, 1958).

Phytochemical screening

Chemical test was carried out on the acetone and ethanol extracts for qualitative determination of phytochemical constituent as described by (Brindha., *et al.*, 1981).

RESULTS AND DISCUSSION

The methanol stem extract in *Bacillus Subtilis* (1.1cm) exhibited higher inhibition than the other bacterial species tested. In chloroform stem extract *Pseudomonas aeruginosa* and *Bacillus subtilis* showed higher inhibition but no activity against *Salmonella typhi* (Table: 1). *Indigofera linnaei*. L (Fabaceae) compare the methanolic leaf extract showed more activity against *P.aeruginosa* and minimum activity in *Klebsiella pneumoniae*, (Sahaya Sathish., *et al* 2012).In the methanolic leaf extract showed no antimicrobial activity against *Salmonella typhi* and *Bacillus subtilis* and *Vibrio cholerae*. The chloroform leaf extract showed antimicrobial activity against *Vibrio* sps. (1.0cm). It is evident from the (Table 1). Both gram positive and gram negative bacterial strains were found to be sensitive to the leaf extracts of all the solvents except chloroform at higher concentration (75% & 100 %). (Senthil kumar and Sahaya Satish., 2002). Ethanol and aqueous leaf extract did not any show

antibacterial activity against *Vibrio cholerae* (Table: 2). In determining the antifungal effect methanol stem extract against three fungal species *P. notatum* (1.8cm) exhibited higher inhibition then *A. niger* (1.1cm) and *R. nigricants* (0.3cm), ethanol extract against *P. notatum* (0.7cm), showed higher inhibition then *Aspergillus niger* (1.0cm), in chloroform stem extract *P. notatum* (1.8cm) exhibition then the remaining fungal species such *R. nigricants* (1.6cm) and *A. niger* (1.2cm), aqueous stem extracts did not show any activity against all three *fungal sps* (Table:3. The leaf extract *P. notatum* (2.2cm) exhibited higher inhibition towards the methanolic leaf extract then *R. nigricants* (1.0cm), there is no activity against, *A. niger*, ethanolic leaf extract three fungal species *A. niger* (1.8cm), exhibition then *P. notatum* (0.8cm) and *R. nigricants* (1.2cm), chlorophyll and aqueous leaf extract no showed antifungal activity against all the fungal sps, such as *P. notatum, A. niger, R. nigricants* (Table: 4).

Table 1. Effect *of Sesamum laciniatum*. (Kelin) ex willd. Stem extract on different pathogenic bacteria by disc diffusion method

Test Bacteria	Zone of Inhibition (cm) #				
	Ethanol	Methanol	Chlorophyll	Aqueous	Control*
Bacillus subtilis	0.9±05.	1.1±0.3	0.8±0.4	0.7±0.4	2.1
Pseudomonas aeruginosa	1.0±0.7	0.8±0.5	0.8±0.4	0.8±0.5	2.1
Salmonella typhii	0.9±0.5	0.9±0.5	_	_	1.5
Vibrio cholorae	0.8±0.4	0.8±0.2	1.0±0.6	0.7±0.1	1.9

Mean of triplicates, * Chloramphenicol, ± Standard deviation

Table 2. Effect of *Sesamum laciniatum*. (Kelin) ex willd. Leaf extract on different pathogenic bacteria Disc diffusion method

Test Bacteria	Zone of Inhibition (cm) #				
	Ethanol	Methanol	Chlorophyll	Aqueous	Control*
Bacillus subtilis	-	-	-	-	2.1
Pseudomonas eruginosa	-	1.2±0.1	-	-	2.1
Salmonella typhii	-	-	-	_	1.5
Vibrio cholorae	-	1.0±0.5	-	-	1.9

Mean of triplicates, * Chloramphenicol, ± Standard deviation

Table 3. Effect of *Sesamum laciniatum*. (Kelin) ex willd. Stem Extract on different pathogenic fungi Disc diffusion method

Test fungi	Zone of Inhibition in (cm) #				
	Ethanol	Methanol	Chlorophyll	Aqueous	Control*
Penicillium notatum	1.7±0.3	1.1±0.3	1.4±0.9	-	3.5
Aspergillus niger	1.0±0.5	1.1±0.6	1.2±0.6	-	3.0
Rhizopus nigricants	0.9±0.5	1.3±0.7	1.6±0.7	-	2.1

Mean of triplicates, * Chloramphenicol, ± Standard deviation

Table 4. Effect of *Sesamum laciniatum*. Kelin ex willd. Leaf extract on different pathogenic fungi Disc diffusion method

Test fungi	Zone of Inhibition in (cm) #				
	Ethanol	Methanol	Chlorophyll	Aqueous	Control*
Penicillium notatum	1.8±0.4	2.2±1.2	-	-	2.9
Aspergillus niger	1.8±0.5	-	-	-	2.4
Rhizopus nigricants	1.2±0.8	1.0±1.4	-	-	2.3

Mean of triplicates, * Chloramphenicol, ± Standard deviation.

Phytochemical Analysis

Analysis of ethanolic leaf extract of *Sesamum laciniatum* determined the presence of phytochemicals like steroids, triterpenoids, alkaloids, flavonoids and saponins. Biography of these chemicals that indicate they might be the agents of antimicrobial activity as reported in several of such systems (Parekh J. *et., al* 2005; Vinod *et., al* 2010). Preliminary phytochemical analyses were carried out on the ethanol extract for the qualitative determination, ethanol extracts in *S .laciniatum* is the experimental plant. The Ethanol extract large amount of carbohydrates, secondary metabolites compound by chemical substance like flavonoids, tannins, steroids, phenol. Acetone extract of this plant larger amount of phenol, secondary metabolite compounds present followed by the other chemical substance like glycosides, alkaloids, terpenoids and saponins (Table: 5).

Table 5. Qualitative Test in Plant extract

Chemicals	**Solvents**	
	Acetone	**Ethanol**
Tannins	-	+
Terpenoids	+	-
Steroids	-	+
Phenols	+	+
Glycosides	+	-
Carbohydrates	-	+
Alkaloids	+	-
Flavonoids	-	+
Saponins	+	-

CONCLUSION

The medicinal plant *Sesamum laciniatum*. (Kelin) ex wild, it may be concluded that. The study of antimicrobial activity and preliminary phytochemical studies. And antifungal activity were detected by dice diffusion methods assay by using organic solvents such has ethanol, ethanol, chloroform and aqueous for 4 different bacterial and 3fungal species. While leaf extracts of the plant did not produce any measurable antimicrobial activity. This study has the potency of the crude extract on the tested microorganism which indicates the medicinal value of the plant extract. This plant has certain bioactive principles and medicinal uses. The chemical compounds present this plant form the characteristic nature of its medicinal uses. The plant studies here can be seen as a potential source of useful drugs. The further studies will help to isolate, identify, characteristics and elucidate the structure of the bioactive compounds antimicrobial activities of the disease as claimed by traditional heater are also being investigated.

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MEDICINAL DIVERSITY OF THE FAMILY *ADIANTACEAE* (PRESL) CHING (PTERIDOPHYTA) OF EASTERN UTTAR PRADESH

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Abstract

The paper deals with medicinal value of family Adiantaceae. The Family Adiantaceae (Presl) Ching is with 4 species in the present study area. The species are ***Adiantum capillus- veneris***, ***A. caudatum, A. lunulatum*** and ***A. incism***. All four species are having a good medicinal value. On the basis of field surveys the traditional methods of curing of diseases and ailments like stomach disorders, Ulcers, cough and cold, fever, asthma etc. are presented.

Keywords: Medicinal ferns, Adiantaceae, Eastern Uttar Pradesh, India

INTRODUCTION

The Homosporous fern family Adiantaceae is well known group of ferns, commonly known as maidenhairs, which is widely distributed mainly in the tropical and subtropical regions. Twenty five species of *Adiantum* occur in India (Dixit 1984, Khullar 1994, Borthakur *et al.* 2001), 10 species are reported to be in South India (Dixit 1984, Manickam & Irudayaraj 1992) and nine species in Assam (Borthakur *et al*. 2001). Chandra (2000) has reported about 26 species from India. In the present study four species are reported. Pteridophytes are known to man for more than 2000 years for their medicinal values. Caius may be the first man who has described the medicinal uses of some ferns of India. Chaudhary (1973), Vyas and Sharma (1998) and Padala (1988) contributed to the knowledge of medicinal uses of pteridophytes. Nayar (1957), Kumar & Kaushik (1999) and Kaushik & Dhiman (1995) also told about medicinal uses of some Pteridophytes of India. Pteridophytes are used in Homeopathic, Ayurvedic, and Unani medicines. Different parts like rhizome, stem, fronds, pinnae and spores are useful in the treatment of various diseases.

Study Area

The Study area Eastern Uttar Pradesh (Fig. 1), which is at the foot hills of the Himalayas is bounded by Nepal on the North, Uttarakhand on the North-East, Himachal Pradesh on the North- West, Haryana on the West, Rajasthan on the South-West, Madhya Pradesh on the South & South-West & Bihar on the East. They are situated between 23°52' N & 31°28'N Latitudes and 77°30'E and 84°39'E Longitude. Being at the foot hills it is very rich in diversity and it has been poorly reported.

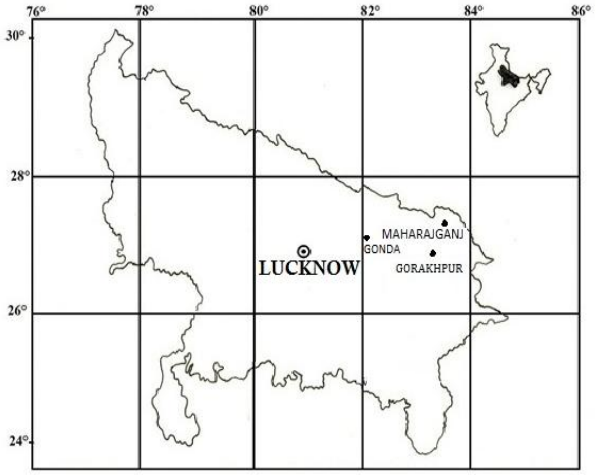


Fig. 1

MATERIALS AND METHODOLOGY

The present data is outcome of field work carried out in different parts of Eastern Uttar Pradesh. All the specimens were collected in duplicate and they are deposited in Centre for Plant Species Biology, Department of Botany, St.

Andrew's College (PG), Gorakhpur, Uttar Pradesh.

RESULTS

About four species of Pteridophytes with medicinal properties have been collected from Eastern Uttar Pradesh. Their Botanical Names of the plants, family, mode of use and parts used are given below.

1- Adiantum caudatum L.

N.V. Rajahamsa

Leaf paste is applied for burns, cuts and wounds. It is used as an expectorant and skin diseases, Diabetes, cough and fever.

2- Adiantum capillus-veneris L.

N.V. Hansraj

The decoction of leaves is taken for acute bronchitis and fever. The fronds are used against cough and cold and also chewed for the treatment of mouth blisters. Fronds extract mixed with honey is used as an eye ointment. It is used as a stimulant, expectorant, purgative, demulcent and hair tonic. It has anticancerous, hypoglycaemic, aphrodisiac, antibacterial, antifungal and antiviral properties.

3- Adiantum lunulatum Burm.

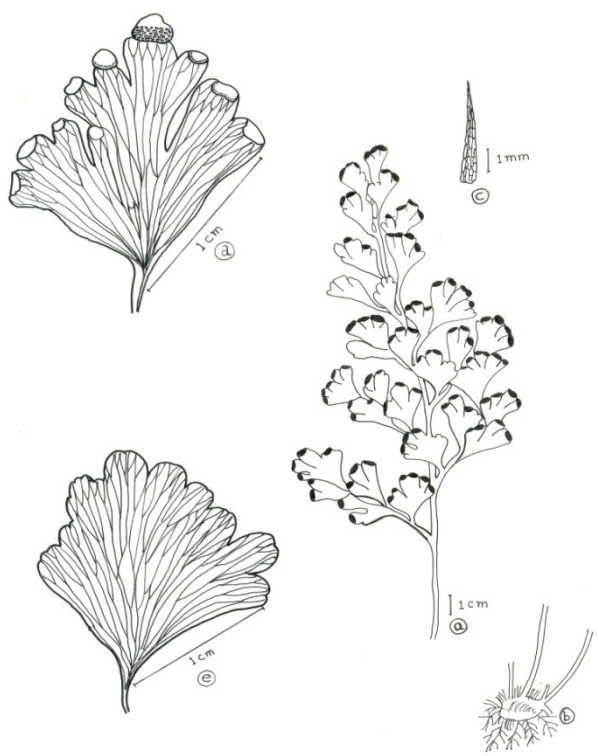
N.V. Chitrapada

The plant is useful in Dysentery, leprosy and fever. The paste of fronds and rhizomes is applied for centipede-bite. It is used in blood related diseases, in epileptic fits and in rabies, rhizomes prescribed for strangery and in fever due to elephantiasis.

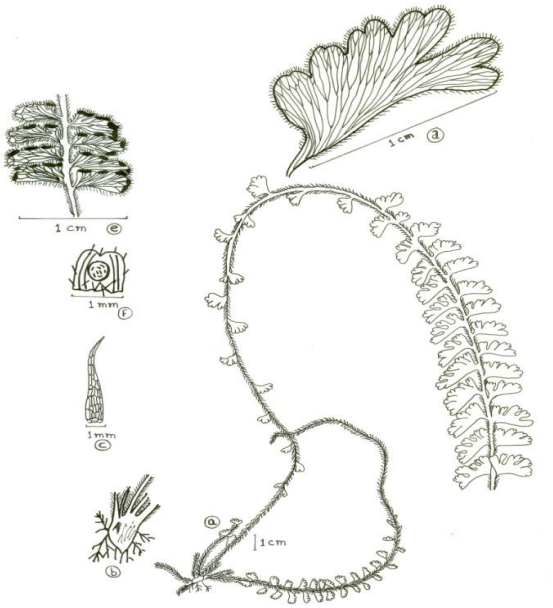
4- Adiantum incism Forsk.

N.V. Hanspadi

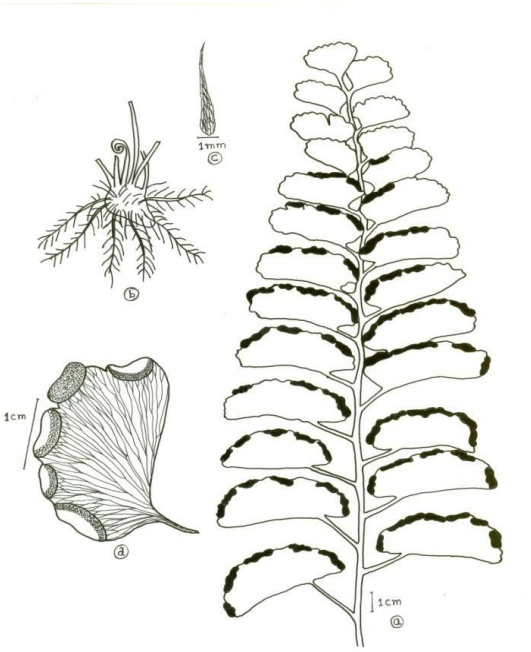
The leaf powder is mixed with butter and used for controlling the internal burning of the body. Also used in cough, diabetes, fever and skin diseases.



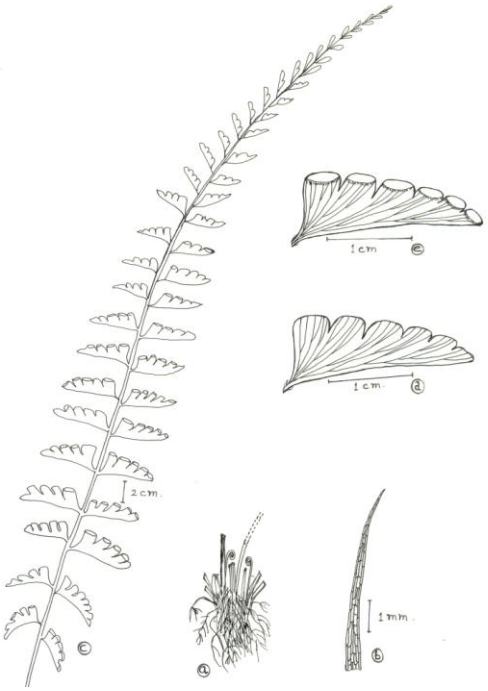
Adiantum capillus-veneris



Adiantum caudatum



Adiantum lunulatum



Andiatum incism

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LEGENDS

1. ***Adiantum capillus-veneris***
   a. Habit
   b. Rhizome
   c. Rhizome Scale
   d. Pinnule enlarged showing sori
   e. Pinnule enlarged showing venation
2. ***Adiantum caudatum***
   a. Habit
   b. Rhizome
   c. Rhizome Scale
   d. Pinna enlarged showing venation
   e. Two pairs of pinnae
   f. Sorus enlarged
3. ***Adiantum incism***
   a. Habit
   b. Rhizome scale
   c. Habit
   d. Pinnule enlarged showing venation
   e. Pinnule enlarge showing sori
4. ***Adiantum lunulatum***
   a. Habit
   b. Rhizome
   c. Rhizome Scale
   d. Pinna enlarged showing venation and sori

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STUDIES ON ELECTROSYNTHESIS AND PHOTORESPONSE OF THALLIUM DOPED CdSe FILMS

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ABSTRACT

The doping of thallium in cadmium selenide thin films have been carried out using a simple electrochemical codeposition method with the objective to study its effect on the photoelectrochemical properties of cadmium selenide thin films and identification of optimal deposition potential at which thin films of highest photoresponse and greatest stability is obtained. Electro synthesized these thallium doped CdSe films deposited at different deposition potential have been characterized by current voltage behavior, capacitance and photo action spectral studies. The doping of thallium lowers the band gap of the elctrodeposited material of thin films. Corrosion measurements revealed that the thin films were endowed with lowest corrosion rate when synthesis was carried out at optimal deposition potential.

Keywords: Thallium doped cadmium selenide, electro synthesis, deposition potential, band gap, capacitance, corrosion

INTRODUCTION

Industrialization and urbanization has taken a toll on the environment. The air is polluted, the water resources are getting depleted and the climate is being unpredictable [1-3]. Environmental degradation is now one of the greatest challenges before us.

We are dependent on the environment for all our needs. The uncontrolled exploitation of natural resources is continued from long period. Many natural resources, such as fossil fuels cannot be replenished. If this exploitation continues, there will soon be a time when there will be no natural resource left. Thus the protection of earth and environment and to save them for our future generation should be our prime duty [4].

As the non renewable resources of energy are in limited quantity on earth, it is important to conserve their current supply and to use renewable resources of energy so that natural resources of energy will be available for future use [5-7]. The most suitable renewable resource of energy is solar energy. It is nonpolluting and safe source of energy [8-10]. For conversion of solar energy into electricity solar cells are used. Currently these are the best sources of electric power in satellites.

In the solar cells various types of semiconducting materials are being used. Among them mixed metal chalcogenides are currently attracting attention because of their importance from the view of a variety of optoelectronic and photovoltaic applications [11-13]. Some such materials having variable band gaps have been investigated [14-16].

We have carried out electro synthesis of thallium doped cadmium selenide in the form of titanium supported thin films with the objective of determination of their photo electrochemical characteristics. In electro synthetic work the deposition potential as well as the composition of electroplating solution play a vital role in controlling the quality of the electrodeposited thin films. Corrosion behavior, capacitance measurement and current-voltage studies in the dark and under illumination along with photo action spectral studies have been used for the characterization of the electrodeposited thin films.

EXPERIMENTAL

For electrochemical codeposition three electrode cells was used. A flag shaped

titanium plate was cleaned with emery paper (john oakey), polished with diamond lapping paste (METSES diamond lapping 1.0 µm and 0.5 µm size) and Hifin Fluid-'OS' (Madras Metallurgical Services Pvt. Ltd.). It is then washed successively with acetone and deionized water. Its surface except the portion where material deposition was intended was covered with insulating tape. The electrode was then allowed to soak in an electroplating solution for an hour. Titanium foil was also used as counter electrode. The potential of working electrode was varied with respect to a saturated calomel electrode and the current between working and counter electrodes was measured using a digital multimeter (MASTECH, India Model MS 8220R). Cadmium sulphate, potassium iodide, cadmium acetate (all CDH, India) and selenium dioxide and thallium nitrate (Aldrich) were used for the preparation of solutions. Current voltage studies in appropriate electroplating solution were carried out using indigenously made power supply. For the measurement of photopotential a simple experimental arrangement was used in which when the dark potential between the working and counter electrodes became steady, the working electrode where CdSe thin film was deposited was illuminated with a beam of light from a 1000 watt tungsten lamp. Change in the potential was then recorded with a digital multimeter (Scientific Mes-Technik, India). Intensity of illumination was varied using a Dimmerstat (Automatic Electric Private Limited, Mumbai) to study the dependence of photopotential on relative light intensity.

RESULTS AND DISCUSSION

In order to identify the potential domain within which the deposition may take place, current voltage behavior is examined. The results show that the relevant electrochemical activity is expected within -0.5 V to -0.85 V versus saturated calomel electrode.

The thallium doped cadmium selenide films were prepared using electroplating solution containing 0.05 M $CdSO_4$, 0.01 M SeO_2 and 10^{-3} M Tl NO_3 by applying different deposition potential within -0.50 V to – 0.85 V range. The deposition condition of these thin films are presented in Table I. The film thickness values, h are calculated from current time plots using relationship [17]

$$h = Q (EW) / F A d$$

Where Q = charge in coulomb

(EW) = equivalent weight of deposited material

A = area of electrode and d = density

In the case of electroplating solution containing $TlNO_3$, the deposition current was fairly high. It was observed that in the case of deposition of (CdTl)Se films, the deposition current first decreases sharply, after few minutes it increases to certain value and again decreases. But steady current was always sufficiently higher than that in the case when $TlNO_3$, was not added in the electroplating solution deposited. A representative current time plot is shown in Fig. 1.

These films were tested for their optoelectronic behavior in 1.0 M cadmium acetate solution containing 0.01 M KI and 50 mM I_2 solution. The result is summarized in Table 2. These results show that a deposition potential of -0.70V is most suitable for obtaining the electrodeposited thin films of thallium doped cadmium selenide of better photo response.. The electrodeposited films at this potential shows maximum photo activity. Build up of photo potential and its decay was much faster in almost all cases. Further photo activity data shows that the electrodeposited films become anodic upon illumination indicating their p-type semiconducting nature. When deposition was carried out at -0.82V, the current measured between working and counter electrodes was very high.

The initial current was – 1.80 mA and steady current was – 0.79 mA. Due to very high current the deposited films were amorphous in nature and a little part of it was dropped into the solution during electro synthesis.

The value of dark potential E_D gives valuable information about the quality of the electrodeposited films. The dark potential E_D of electrodeposited film first decreases rapidly and then attains constant value. This happens in almost all cases. A representative plot is given in Fig. 2. It is observed in almost all cases that if E_D has low positive value then the electrodeposited film exhibit better photo response

Current voltage behavior of the thallium doped cadmium selenide thin film semiconductor electrodes in dark and under illumination was also studied to obtain the information concerning the nature of the semiconducting material. This clearly shows that the thallium doped cadmium selenide

films are endowed with p-type semiconductivity.

Thallium doped cadmium selenide deposited films are likely to be susceptible to corrosion. With a view to ascertain ability of these films to withstand photocorrosion, the thin films were subjected to uninterrupted illumination in I_3^-/ I_2 redox solution. The results show that the electrodeposited films are resistant towards photocorrosion in substantial measure.

With a view to study the influence of thallium inclusion on band gap, photo action spectra in 300 nm to 1000 nm range were employed. A representative plot is shown in Fig.3. Band gap values were obtained from Ep^2 versus ƛ curves in accordance to the equation

$$Eg = \frac{hc}{\lambda \tau e}$$

Where Eg = band gap

c = velocity of light

e = electronic charge.

h = Plank constant

Cadmium selenide has a band gap of 1.7 eV. Whereas in the case of thallium selenide it is 0.75 eV. A lowering of band gap is expected with inclusion of thallium in the cadmium selenide lattice. It was indeed found experimently the band gap values of thallium doped CdSe thin films were found to be close to 1.39 eV.

Light intensity dependence of photopotential has been examined in Fig.4. The photopotential initially increases with light intensity but at higher light intensity, a stage of saturation is observed. On the other hand the photocurrent increases with increase in light intensity (Fig. 5.). However a linear relation exist between photopotential and ln(Light Intensity). The result is presented in Fig. 6. This behaviour indicates semiconducting nature of these thin films. The data are also consistent with equation.

$$Ep = \frac{2.303AKT}{} \log I_L + Be$$

In this equation B is a constant and A is ideality factor. I_L denotes light intensity.

Examination of these results reveals that thallium doped cadmium selenide film under consideration is endowed with ideality factor 1.22. This shows that the above preparation was not ideal. For ideal systems this factor should be unity.

In order to investigate electrochemical corrosion behavior of the thin films variation of current with potential

was studied to obtain Tafel plots. The anodic and cathodic Tafel plots are described by

$$\eta = \beta \log \frac{i}{icorr}$$

Where $\eta =$ overvoltage of the thim film electrode with respect to its value at equilibrium, the so called corrosion polential E_{corr}

$i = current\ at\ applied\ polential$

i_{corr} = corrosion current

E_{corr} and i_{corr} were obtained using paralac data analysis technique [18]. Cathodic and anodic Tafels β_c and β_A are obtained from the slopes of Tafel plots .The corrosion rate is generally expressed is g/s and is measured using equation.

$$\mathcal{R}corr = \frac{icorr\ \chi\ (E.W)}{\mathcal{F}}$$

Where EW = equivalent weight of the deposited film . F = Faraday constant .

The values of corrosion current, corrosion rate, β_c and β_A for (CdTl)Se thin films deposited at different deposition potential were calculated from Tafel plots. The results are presented in Table.3.These results show that the thin films deposited at depositon potential -0.70 V versus saturated calomel electrode exhibit lowest corrosion rate.

A semiconductor when kept in a redox system behaves as a capacitor because of the formation of depletion layer. This capacitance varies with the potential to which the electrode is subjected and this variation is given by the Mott-Schottky relationship

$$1/C^2 = 2\ (E - E_{fb}\ ) / e\ \varepsilon\ \varepsilon_0\ N_A$$

Where C is the capacitance of space charge region, e is the electronic charge, ε is the semiconductor dielectric constant, ε_0 is the 8.85 x 10^{-14} C/V cm, E is the applied voltage and E_{fb} is the potential at which the semiconductor bands are flat.

The charge carrier density, N_A and the flat band potential E_{fb} may be obtained by plotting $1/C^2$ against E vs SCE in the form of Mott-Schottky plots [19]. A representative Mott-Schottky plot is presented in the Fig.7. The slope of the plot is given by

$$Slope = 2 / [e\varepsilon\varepsilon_0 N_A]$$

So that

$$N_A = 2 / [\ e\varepsilon\varepsilon_0\ slope]$$

The capacitance behavior of (CdTl)Se thin films prepared in the electroplating solution containing 0.05 M $CdSO_4$, 0.01 M $Se0_2$ and 10^{-3} M $TlNO_3$

using different deposition potentials was studied. The results are presented in Table 4. These results show that highest flat band potential and charge carrier density is obtained for the thin films which were deposited at -0.70 V. The positive value of E_{fb} in all cases show that the deposited (CdTl)Se thin films are endowed with p-type of semiconductivity, the result already derived on the basis of photoactivity and current voltage behavior in dark and under illumination.

With the knowledge of flat band potential E_{fb}, the band bending and hence the depletion layer width, W_D, can be calculated [20-21]. We know that

$$E_b = E_{F,\,redox} - E_f \quad \text{and}$$

$$W_D = [2\,\epsilon_{SC}\,\epsilon_o\,E_b / e\,N_A]^{1/2}$$

Value of $E_{F,redox,}$ the redox potential of I_3^-/I_2 redox couple is 0.295 V vs SCE. Results are also included in Table 4.

CONCLUSION

The above studies illustrate the possibility of doping of thallium in cadmium selenide thin films. The preparation of (CdTl)Se thin films may be carried out by electrochemical codeposition technique. The doping of thallium improves the quality of thin films in terms of their photoresponse and corrosion characteristics. Further the doping of thallium lowers the band gap of the deposited semiconducting material.

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Table 1. Deposition conditions of (CdTl)Se thin films obtained at different deposition potentials

Electroplating solution: 0.05M $CdSO_4$, 0.01M SeO_2 and 10^{-3}M $TlNO_3$

Deposition Time = 2 hours

Deposition Potential (V)	Initial current (mA)	Steady current (mA)	Film thickness (10^{-5}cm)
-0.55	0.68	0.34	2.36
-0.60	0.83	0.42	2.98
-0.65	1.07	0.49	5.82
-0.70	1.01	0.50	6.53
-0.75	1.15	0.54	8.25
-0.80	2.24	0.58	9.97

Table 2. Variation of photoactivity of (CdTl)Se thin films with deposition potential

Electroplating solution: 0.05M $CdSO_4$, 0.01M SeO_2 and 10^{-3}M $TlNO_3$

Deposition Potential(V)	E_D (mV)	E_L (mV)	E_P (mV)
-0.55	-616 -599 -537	-406 -356 -267	210 243 250
-0.60	-565 -521 -484	-369 -314 -266	196 207 218
-0.65	-575 -522 -440	-402 -302 -216	173 220 224
-0.70	-498 -519 44	-206 -218 370	292 301 326
-0.75	-460 -423 -512	-239 -220 -218	221 196 204
-0.80	-635 -525 -570	-402 -405 -466	233 220 204

Table 3. Corrosion characteristics obtained from Tafel plots

Electroplating solution: 0.05M $CdSO_4$, 0.01M SeO_2 and 10^{-3} M $TlNO_3$

Testing solution: 1.0M $(CH_3COO)_2Cd$, 0.1M KI and 50mM I_2

Deposition potential (V)	E_{corr} (i = 0) (mV)	Cathodic Tafel β_C(mV)	Anodic Tafel β_A(mV)	i_{corr} (μ_A cm^{-2})	Corrosion rate (10^{-9} g/s)
-0.55	-202.96	277.77	312.50	2.65	2.92
-0.60	-83.54	166.66	385.05	2.75	2.72
-0.65	-55.47	300.01	454.24	2.45	2.42
-0.70	-50.88	449.39	375.12	1.97	1.95
-0.75	-136.47	100.65	212.78	2.29	2.26
-0.80	-253.63	249.61	167.31	2.88	2.85

Table 4. Capacitance characteristics of (CdTl)Se films of synthesized at different deposition Potentials.

Electroplating solution: 0.05M $CdSO_4$, 0.01M SeO_2 and 10^{-3} M $TlNO_3$

Testing solution: 1.0M $(CH_3COO)_2$ Cd, 0.1M KI and 50mM I_2

Deposition potential(V)	E_{fb} (V)	N_A ($10^{17}cm^{-3}$)	E_b (V)	W_D (10^{-12}cm)
-0.55	1.12	1.10	0.825	1.91
-0.60	1.21	1.36	0.915	1.81
-0.65	1.32	2.11	1.025	1.54
-0.70	1.55	5.36	1.255	1.07
-0.75	1.39	2.82	1.095	1.37

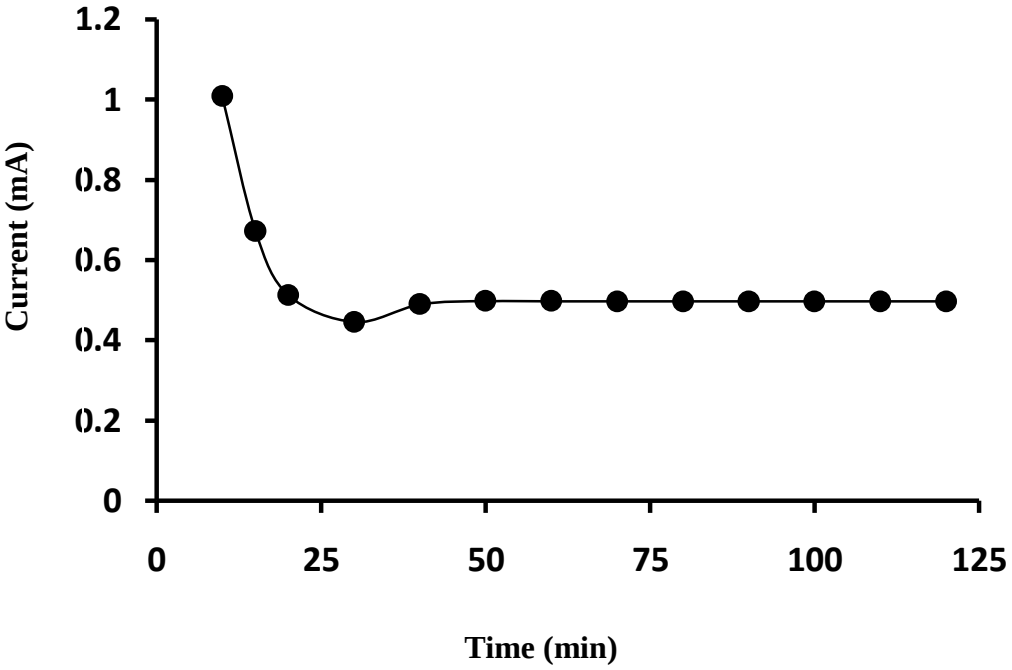

Fig.1. A representative current time curve

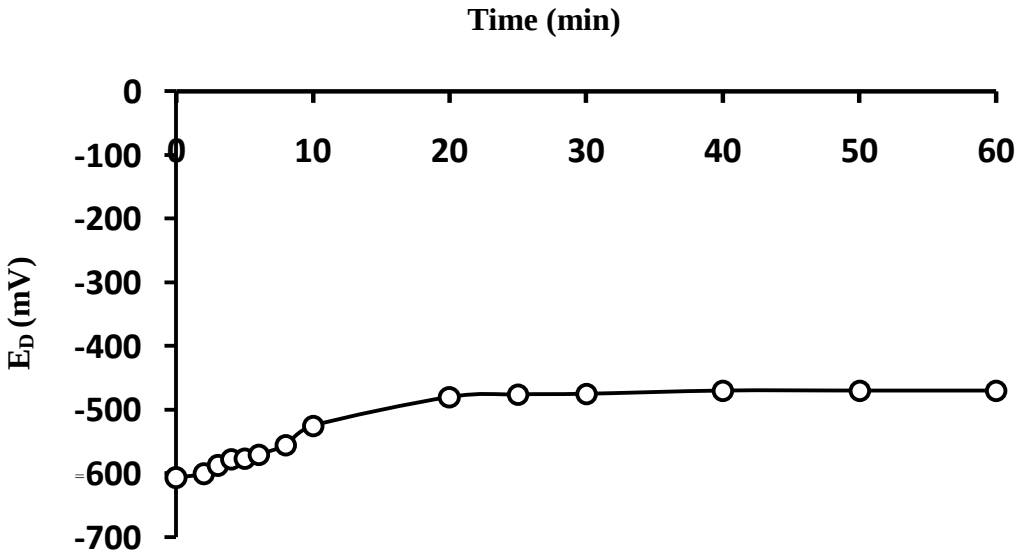

Fig. 2. Variation of dark potential with time

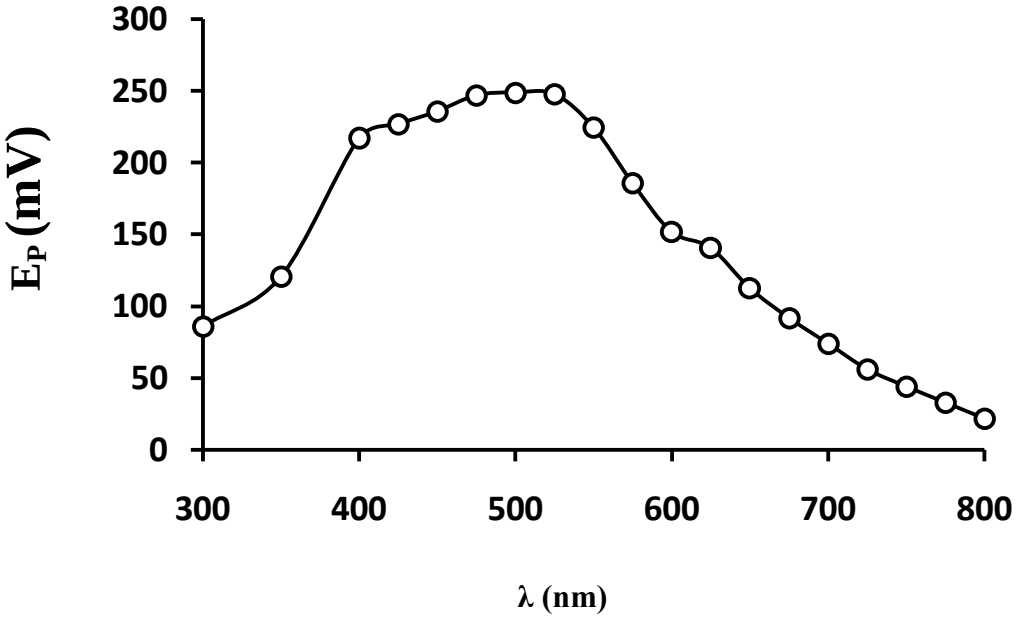

Fig. 3. Photoaction spectrum of a typical thallium doped cadmium selenide thin film

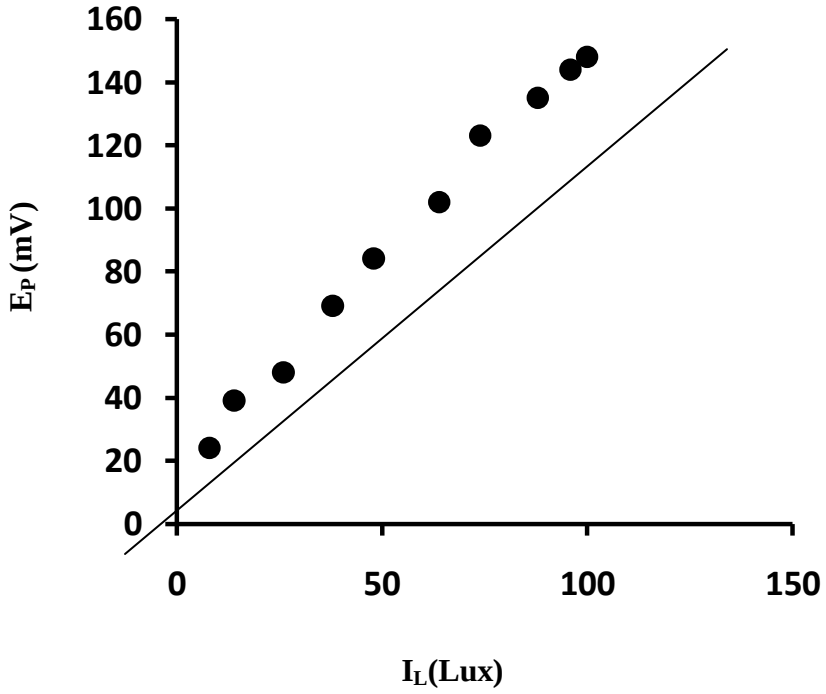

Fig. 4. Variation of photopotential E_P, with light intensity

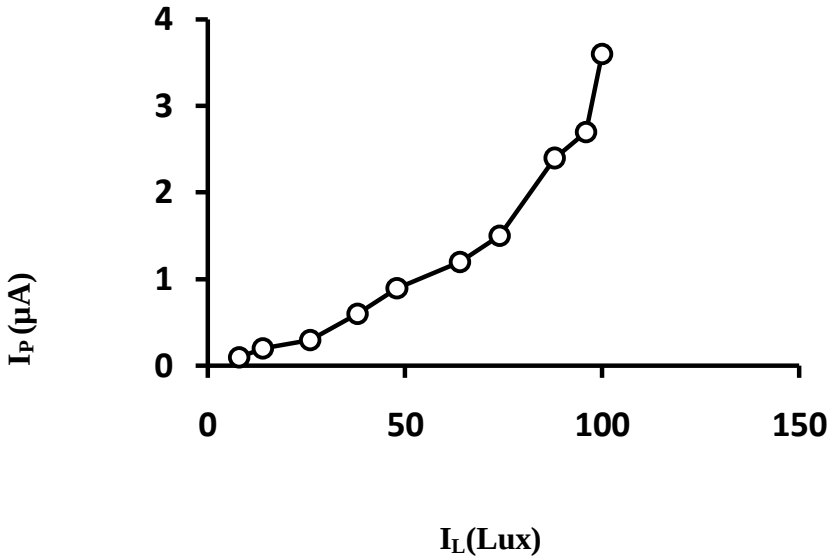

Fig. 5. Variation of photocurrent with light intensity

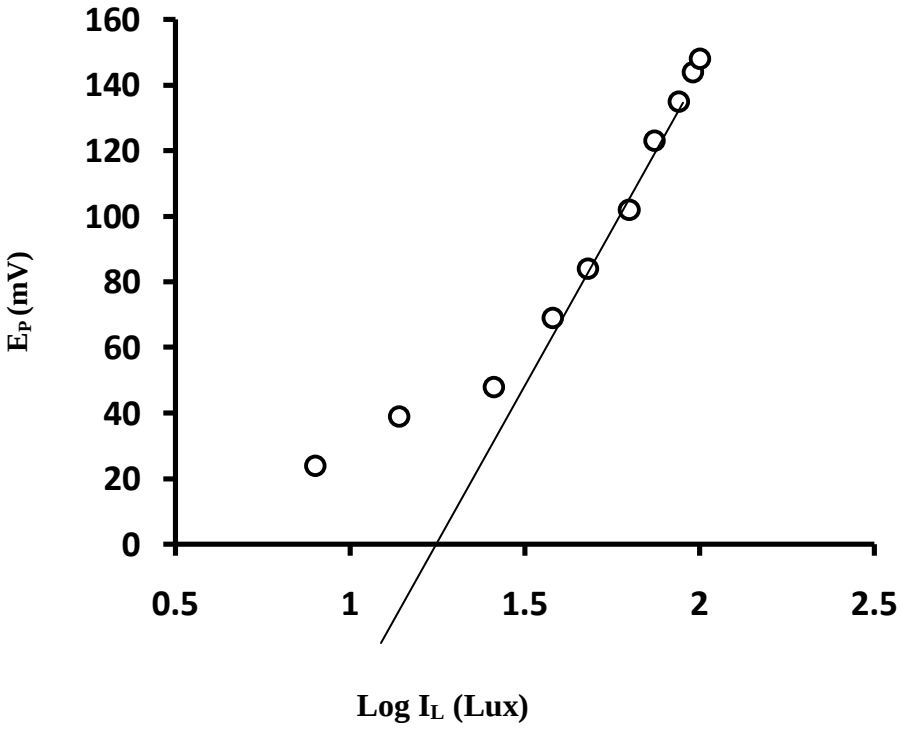

Fig.6. Variation of photopotential with log(light intensity)

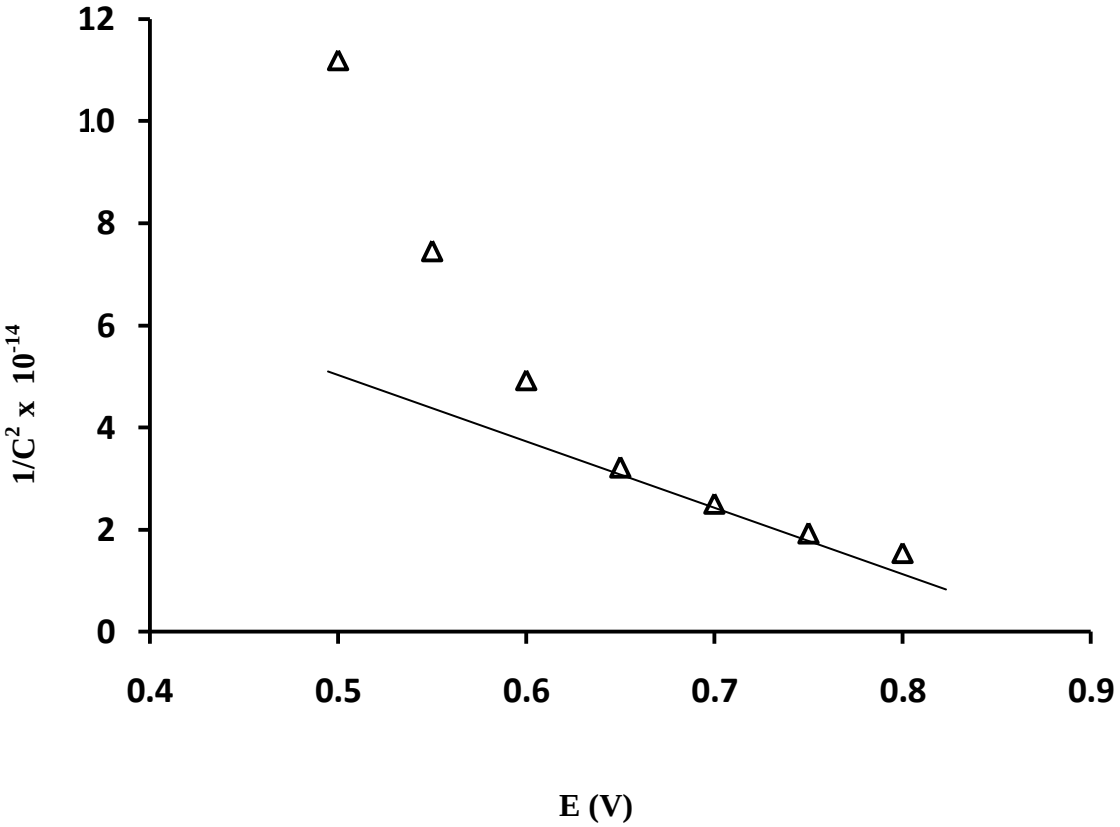

Fig. 7 A representative Mott-Schottky plot

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DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAHS) IN GOMTI RIVER, LUCKNOW, INDIA

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ABSTRACT

The Gomti river, one of the major tributaries of the river Ganga serves as one of the major source of drinking water for the Lucknow City, the State capital of Uttar Pradesh, India. The river receives the untreated wastewater and effluents from Lucknow city directly in its course through more than 25 drains. Polycyclic aromatic hydrocarbons have been identified as exhibiting toxic/hazardous properties.Our study shows that Gomti river is highly contaminated with PAHs and poses high risk to the aquatic life. The study area covers seven different locations. Grab samples of water and bed sediments were collected from each of the locations in the month of November and December. Total PAHs in water at all the seven locations studied ranged between0.467-5.342 µg/l. In the river water the most abundant hydrocarbon was acenaphthene followed by acenaphthene. The most abundant hydrocarbon among all the analysed hydrocarbon in the river sediments was acenaphthene.

Keywords: The River Gomti, Lucknow City, PAHs

INTRODUCTION

Polycyclic aromatic hydrocarbons are neutral, non-polar organic molecules that comprise two or more benzene rings arranged in various configurations. Members of this class compounds have been identified as exhibiting toxic/hazardous properties (Masih *et al*, 2010) moreover they are the major culprits in urban areas causing human lung cancer (Lighty *et al*., 2000). On the basis of their molecular masses and properties, three classes of PAHs can be distinguished. 2-3 ring PAHs are defined as low molecular weight PAHs (LMW-PAHs), 4 ring PAHs are known as middle molecular weight PAHs (MMW-PAHs), whereas 5-6 ring PAHs are defined as high molecular weight PAHs (HMW-PAHs). The LMW-PAHs and MMW-PAHs have a significant acute toxicity, while some of HMW-PAHs show high carcinogenic and mutagenic potentials. Most of these are formed during incomplete combustion of organic materials such as wood and fossil fuels, petroleum products, coal and the composition of PAHs mixture varies with the source(s) and also due to selective weathering effects in the environments (Neff 1979). Forest fires, which may or may not be the consequence of human activity, are a significant and usually unpredictable source of PAHs. In general, environmental contamination is by complex mixtures of PAHs, not by single compounds. The Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants frequently found in different environment such as freshwater and marine sediment (Fernandez *et al*, 2000).

PAHs have been detected in the atmosphere, water, soil, sediments and food (Masih & Taneja, 2006). During their atmospheric residence, PAHs are redistributed between the gas and particulate phase (Bourotte *et al*, 2005) and therefore be transported through atmosphere over long distances and enter water bodies by wet and dry deposition and/or gas-water interchange (Fang *et al*., 2004). On entering the aquatic systems, most of the PAHs are adhered to the suspended particles due to their hydrophobic properties, and therefore sink to the bottom and accumulate in the sediments (Masih, 2011) and from a long-term source of potential pollution. Atmospheric deposition has been regarded as a main pathway for the loading of PAHs to many water bodies (Masih *et al*, 2008).

Due to their wide distribution, the environmental pollution by PAHs has aroused

global concern. Combination of their physico-chemical properties such as low aqueous solubility, moderate vapour pressure, high octanol-water partition coefficient (Kow) and persistence in environment make them capable of long range transport. Once in the atmosphere, they can travel long distances and deposit in remote areas such as mountains, lakes and even the Arctic and Antarctic (Fernandez *et al*, 1999). Atmospheric transport is a major pathway for the loading of these contaminants to the systems in remote regions with no history of their use. Wet and dry depositions are the common removal modes for PAHs. The critical effect of many PAHs in mammals is their carcinogenic potential. Benzo(a)pyrene, benzo(a)anthracene, dibenzo (a,h)anthracene, benzo(b)fluoranthene and indeno(1,2,3-cd) pyrene have been classified as probable possible carcinogens to humans (IARC, 1987). Due to tendency of PAHs to accumulate in the food chain, their release during dredging operations, episodes of high scouring, or leaching from confined disposal facilities poses a threat to aquatic ecosystem and consequently a potential threat to human health (Tabak *et al*, 2003).Although several studies on behaviour of PAHs in various aquatic systems (Fernandez *et al*, 1999; Watanabe *et al*, 2003; Dodder *et al*, 2003) have been conducted but data on distribution and level of PAHs in the Indian water resources are rare.

This study was undertaken with a view to generate data on distribution of PAHs in river system in India and presents information on the concentration and distribution of PAHs in the water and bed sediments of the river Gomti, Uttar Pradesh.

MATERIALS AND METHOD

The Gomti river, one of the major tributaries of the river Ganga originates from a natural reservoir in the swampy and densely forested area (Miankot, elevation of about 200m; North latitude $28^0$34' and east longitude $80^0$07') in Pilibhit district of Uttar Pradesh, India. The soil sediments here are silty sands. The river flowing through the districts of Pilibhit, Shajahnpur, Sitapur, Lucknow , Barabanki , Sultanpur , Janpur and Ghazipur in Uttar Pradesh traverses a total distance of about 730 km before finally merging with the Ganga river in Ghazipur district about 30 km north of Varansi**.** Throughout its stretch , there are a few small tributaries (Kathna, Sarayan,Reth, Kalyani and Sai) originating

within short distances and carrying the wastewater and industrial effluents from different towns and industrial units in the basin. Lucknow, Sultanpur and Janpur are the three major urban settlements on the banks of the river and there are several industrial units in the catchments of the river in this region. Further, the river serves as one of the major source of drinking water for the Lucknow City, the State capital of Uttar Pradesh with a population of about 3.5 million. The river receives the untreated wastewater and effluents from Lucknow city directly in its course through more than 25 drains. The study area covers seven different locations namely Gaughat (I), Mohan Meaking (II), Martyr's Memorial (III), Hanuman Setu (IV), Nisatganj Bridge (V), Pipraghat (VI) and Malhaur (VII) **(fig. 1)** on the river spread over about 30 km stretch of the river in Lucknow city (State capital).

Grab samples of water and bed sediments were collected from each of the locations in the month of November and December. The sediments samples were collected from three points (1/4,1/2 and 3/4) across the river width at each of the seven locations using Ekman sediments sampler. The samples collected in the polyethylene bags were transported to the laboratory, pebbles, shells and vegetable matter were removed and the samples were air-dried. The air-dried samples then ground with pestle-mortar and sieved to 200 BSS mesh size. The organic carbon content of the sediment was determined using back titration method (Walkely and Black 1934, Pandey *et al*.2013a). The river water samples collected from midstream of the river (30 cm below the surface) in brown glass bottles were transported to the laboratory under ice conditions. The Total organic Carbon of the water was determined using TOC Analyzer (Schimadzu, Model No. - 5000A).

Extraction

For PAHs analysis 10.0 ± 0.05 g sediment sample from each location were soxhlet extracted with 100 ml of dichloromethane for 16 hr. the extracts were demoisturised using activated anhydrous granular sodium sulphate and concentrated in a rotary evaporator and go for Silica-gel column cleanup**.**

For PAHs analysis, 1 Lit. of the water sample from each location was

triply extracted by liquid- liquid extraction in a 2 Lit. Separatory funnel using 50, 30 and 30 ml of dichloromethane solvent. The combined solvent extracts were demoisturised using anhydrous granular sodium sulphate and concentrated in a rotary evaporator and go for Silica gel column cleanup.

Cleanup

Exchanged the Dichloromethane extract with Cyclohexane. After setting the column Eluted column by 15 ml. n-Pentane. Now, passed the sample through the column. Transfered by rinsing flask three times by 2 ml (Cyclohexane) then eluted the column by 25 ml n-Pentane. Threw away the above two elutes. Now, eluted the column by 4:6 mixture of Methylene chloride plus n-Pentane (25ml.). Collected the elute and reduced upto 2 ml. The working solvent was exchanged to acetonitrile making up final volume to 2 ml.

The samples were stored in dark at 4^0C till the analysis was performed. All the sediments and water samples were analysed for 9 PAHs viz. acenaphthylene , acenaphthene , phenanthrene, anthracene, fluoranthene ,pyrene, chrysene , benzo(a)pyrene and benzo(g,h,i) perylene using the high purity grade water-acetonitrile solvent system on HPLC (Waters. USA) equipped with UV-VIS detector (Model 2486). The PAHs standards (99.9 % purity) were supplied by Sigma-Aldrich,USA. All the analysis were carried out in duplicate and the recoveries of individual PAHs were determined through spiked sample method which were found between 90-93 percent. Recovery correction factors were applied to the final results. The results given in Table 1 and 2 is the mean value of three points (1/4, ½ and ¾) at each sampling sites.

RESULTS AND DISCUSSION

The level of different individual and total PAHs contents in water and sediments of Gomti river at all the selected locations in winter season are presented in table 1 and 2 respectively. Total PAHs in water at all the seven locations studied ranged between 0.467-5.342 µg/l. In the river water the most abundant hydrocarbon was acenaphthylene followed by acenaphthene. Highest concentrations of ΣPAHs in the river water at all the locations were found higher than the Bureau of Indian Standards (BIS) guideline value of 0.2 µg/l (BIS 1982). The

ΣPAHs contents in the river sediments at different locations ranged between 290.3-25457 ng/g. The most abundant hydrocarbon among the entire analysed hydrocarbon in the river sediments was acenaphthene in winter. The highest concentration of ΣPAHs in the river sediment was found at Site- V (25457 ng/g, in winter). Between Gaughat and Malhaur location, there are some 25 drains carrying about 400 million litres per day (mld) of untreated sewage and industrial wastewater from different parts of the city, discharging directly into the river. Moreover, a little upstream of the Pipraghat, there is barrage to restrict the river flow to maintain water level in the river for abstraction at Gaughat for urban water supply. The ΣPAHs content in the sediment at Pipraghat was found to be 12390 ng/g in winter at upstream of the Pipraghat (downstream of Lucknow). There is a crematoria on the river bank, where funeral activities are carried out throughout the year and may be a major source of the hydrocarbon to the river through surface run-off and atmospheric transport. A comparisons of the ΣPAHs burden of Gomti river with other aquatic resources (Table-3) suggests that Gomti river is heavily polluted.

There was found a correlation in winter (R2=0.2343) between the concentration of ΣPAHs in the water and sediment of the river Gomti (Fig. 2 –a). Further, at each of the locations, ΣPAHs contents of the sediments were found several times higher as compared to the ΣPAHs as contents in the water of the Gomti river. The reason for the lower concentrations of PAHs in river water as compared to the sediment may be due to the low aqueous solubility, volatilization, and affinity of these compounds to the organic matter. The spatial and temporal distribution of ΣPAHs generally followed the distribution pattern of organic carbon (%) content as reflected by observed correlation in winter (R2=0.1991) among the ΣPAHs and the organic carbon (%) contents of the sediments of the Gomti river (Fig. 2-b). It was observed that these possible carcinogenic hydrocarbons contributed about 8.67 % and 7.33 % of the total PAHs contents of the river water and sediments, respectively. It was observed that the 3-ringed hydrocarbons were the most abundant ones followed by the 4- and 5-ringed hydrocarbons, both in the sediments and in the water of the Gomti River. But in winter (water) 4-ringed hydrocarbons were

the most abundant ones followed by the 3- and 5-ringed hydrocarbon.

In the Todos Santos Bay, more than 75 % of the total PAHs concentrations were represented by three- and four- ringed hydrocarbons (Zamora *et al*. 2002). The abundance of three- and four- ringed hydrocarbons may be attributed to the partitioning of these hydrocarbons from the dissolved to organic carbon-rich settling particles, whereas higher molecular weight PAHs exist primarily in the particulate phase in both the atmosphere and water (Bidleman 1988).

Some molecular ratio of specific hydrocarbons has been developed to distinguish between the PAHs originating from various sources (pyrolytic, petroleum hydrocarbons and diagenetic). Combustion of organic matter at high temperature generates PAHs characterized by a low Phenanthrene/Anthracene ratio (<10), whereas the slow maturation of organic matter during catagenesis leads to much higher Phenanthrene/Anthracene ratio (>15) (Soclo 1986). Catagenesis is the process by which organic material in sedimentary rocks is thermally altered by increasing temperature resulting in the generation of oil and gas. The isomer ratio of fluoranthene and pyrene concentrations greater than 1 is characteristic of pyrolytic origin, whereas, values lower than 1 are related to the petroleum hydrocarbons (Sicre *et al*. 1987).

In the Gomti river sediments, ratio of fluoranthene to pyrene concentrations in winter season was given in Table-5. The ratio which was found <1 are related to petroleum hydrocarbon but > 1 shows its pryolytic origin at all the locations which also suggests that majority of the hydrocarbons have originated from combustion and open burning of common activities in the region and these have been identified as among the major contributors to the PAHs release in the region (UNEP 2003).

CONCLUSION

Our study shows that the Gomti river is highly contaminated with PAHs and poses high risk to the aquatic life. The findings support that the PAHs present in the river system at most of the locations have their origin from combustion processes in the catchments.

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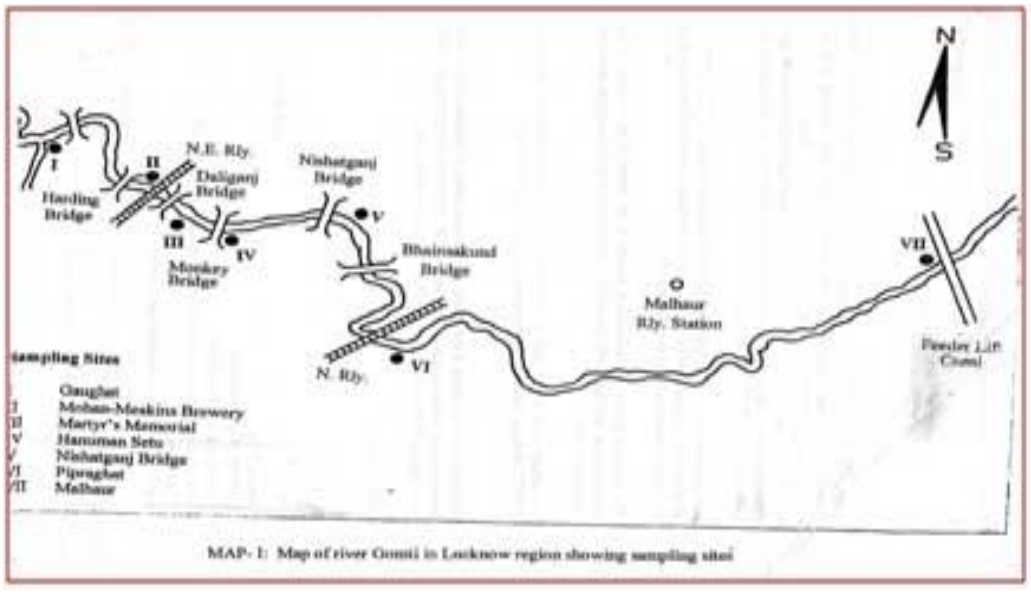
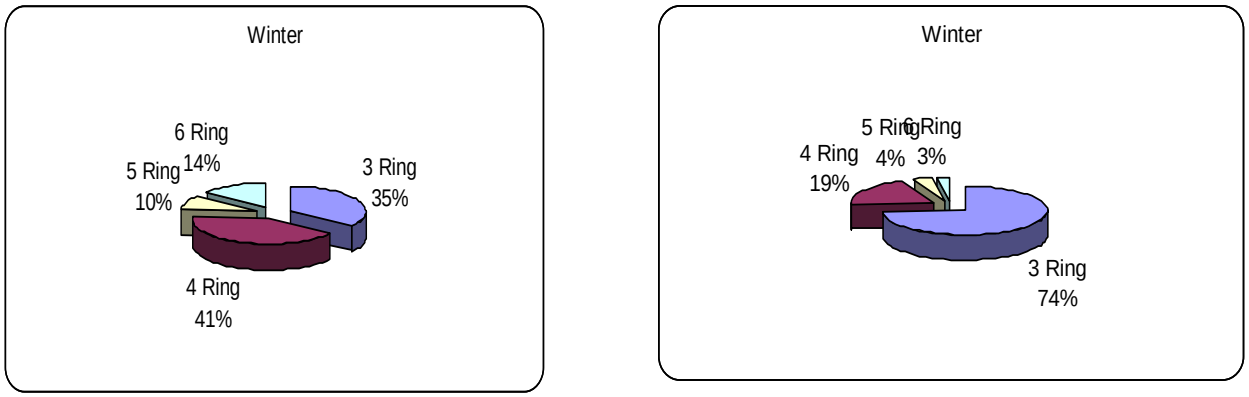


Fig.1:Map of river Gomti in Lucknow region showing sampling sites



(a) **Water** (b) **Sediments**

Fig 2: Contribution of the different groups of PAHs to the total PAHs burden in river water and sediments in winter.

Table-1: Concentration of different PAH in water (ng/ml) of river Gomti

Sampling Site	Acenaphthylene	Acenaphthene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Chrysene	Benzo (a) Pyrene	Benzo (g,h,i) Perylene
I	1.437 (0.00-4.310)	0.688 (0.000-1.710)	0.067 (0.010-0.163)	0.106 (0.003-0.203)	0.218 (0.00-0.547)	0.541 (0.00-1.164)	1.971 (0.00-4.125)	0.000 (0.00-0.00)	0.356 (0.00-0.196)
II	0.098 (0.062-0.149)	0.098 (0.029-0.213)	0.079 (0.069-0.095)	0.004 (0.00-0.007)	0.538 (0.190-1.130)	0.050 (0.013-0.106)	0.026 (0.023-0.031)	0.091 (0.023-0.202)	0.045 (0.00-0.133)
III	0.211 (0.098-0.639)	0.042 (0.022-0.072)	0.166 (0.146-0.200)	0.002 (0.00-0.004)	0.576 (0.187-1.140)	0.021 (0.007-0.033)	0.029 (0.015-0.047)	0.056 (0.023-0.082)	0.107 (0.003-0.278)
IV	0.000 (0.000-0.000)	0.568 (0.375-0.897)	0.001 (0.000-0.002)	0.041 (0.039-0.045)	0.070 (0.014-0.103)	0.044 (0.000-0.133)	0.073 (0.048-0.112)	0.019 (0.00-0.056)	0.000 (0.00-0.00)
V	0.067 (0.000-0.127)	0.140 (0.000-0.273)	0.005 (0.00-0.010)	0.030 (0.00-0.054)	0.019 (0.017-0.021)	0.154 (0.097-0.204)	0.048 (0.029-0.061)	0.001 (0.00-0.004)	0.003 (0.00-0.005)
VI	0.142 (0.00-0.307)	0.039 (0.000-0.117)	0.013 (0.00-0.032)	0.019 (0.002-0.053)	0.082 (0.019-0.190)	0.134 (0.005-0.215)	0.029 (0.000-0.058)	0.018 (0.00-0.046)	0.000 (0.000-0.000)
VII	0.407 (0.025-0.785)	0.544 (0.257-1.098)	0.010 (0.005-0.017)	0.000 (0.00-0.001)	0.058 (0.034-0.097)	0.063 (0.029-0.127)	0.025 (0.012-0.049)	0.184 (0.018-0.343)	0.032 (0.00-0.071)

Table-2: Concentration of different PAH in sediment (ng/g)of river Gomti

Sampling Site	Acenaphthylene	Acenaphthene	Phenanthrene	Anthracene	Fluoranthene	Pyrene	Chrysene	Benzo (a) Pyrene	Benzo (g,h,i) Perylene
I	112.097 (2.27-212.68)	116.763 (59.13-181.93)	0.330 (0.0-0.99)	3.739 (2.75-4.428)	16.630 (14.16-19.34)	39.060 (23.14-52.58)	0.987 (0.52-1.35)	0.233 (0.0-0.70)	0.356 (0.00-0.196)
II	275.217 (175.61-390.24)	724.633 (447.59-1246.52)	0.000 (0.0-0.0)	14.883 (8.94-21.30)	44.993 (29.99-55.81)	166.217 (94.36-217.10)	5.250 (4.19-6.41)	0.707 (0.0-1.93)	0.045 (0.00-0.133)
III	235.283 (151.87-362.64)	597.270 (194.22-1398.29)	1161.837 (0.0-3399.50)	284.247 (0.0-836.16)	1217.873 (27.84-1929.68)	159.310 (87.77-262.73)	86.670 (7.70-149.79)	95.977 (0.0-283.37)	0.107 (0.003-0.278)
IV	356.413 (0.0-927.90)	262.887 (0.0-583.83)	1750.463 (0.0-5191.02)	9.430 (0.0-18.57)	922.037 (0.0-2726.12)	1387.030 (158.93-3801.13)	52.087 (0.0-144.63)	109.347 (0.56-310.36)	0.000 (0.00-0.00)
V	167.983 (0.0-345.99)	23623.177 (44.63-59176.12)	28.557 (0.0-85.67)	5.997 (0.0-17.99)	1336.327 (850.19-1627.17)	22.213 (15.45-28.64)	179.517 (123.77-275.38)	90.993 (60.49-120.01)	0.003 (0.00-0.005)
VI	112.887 (0.0-338.66)	10185.053 (0.0-30376.44)	2.627 (0.0-7.88)	46.533 (15.76-123.84)	1530.617 (9.79-3297.84)	161.017 (0.0-357.11)	67.183 (0.0-196.37)	92.360 (0.0-277.08)	0.000 (0.000-0.000)
VII	131.093 (0.0-393.28)	443.383 (0.0-875.36)	1163.363 (0.0-3453.30)	34.207 (0.0-102.62)	701.187 (9.14-1110.02)	25.493 (0.0-38.59)	25.080 (0.0-61.74)	100.743 (0.0-200.99)	0.032 (0.00-0.071)

Table 3: Concentration (ng/g dry wt) of possible mutagenic/carcinogenic hydrocarbons in the sediments of Gomti river and other aquatic systems.

Aquatic System	n*	PAHs (ng/g dry wt.)	Reference
Gomti River. India	9	10.51-35953.16	Present Study
East River. USA	18	1069910	Tabak et al. 2003
NY/NJ Harbor. USA	4	1100	Tabak et al. 2003
Todos Santos Bay. Mexico	16	7.6-813.1	Zamora et al. 2002
Morava river. Czeck Republic	16	636- 13205	Vondracek et.al 2001
Remote Mt. Lakes	-	13000-18000	Fernandez et al.1999
Passic River.USA	19	0.22-8000	Huntley et al.1995

n*= Number of hydrocarbon taken in the analysis.

Table 4 : Phenanthrene/Anthracene & Fluoranthene/Pyrene Ratio in the sediments of the Gomti River in Winter Season.

Location	Phenanthrene/Anthracene	Fluoranthene/Pyrene
I	0.09	0.43
II	0.0	0.27
III	4.09	7.64
IV	185.63	0.66
V	4.76	60.17
VI	0.06	9.51
VII	34.01	27.51

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PHYTOCHEMICAL STUDIES ON SELECTIVE MEDICINAL PLANTS

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ABSTRACT

The present study was carried out to characterize the bioactive constituents presence in different plant leaves of *Nyctanthes arbour-tristis, Calotropis gigantea*, *Adhatoda vasica*, *Lantana camera* and *Nerium oleander.* Presence of compounds Alkaloids, Tannins, Phenolic compounds, Steroids, Flavonoids, Coumarins, Terpenoids and Volatile oil Protein, Amino acids and Lipid were identified by the qualitative analysis. Biochemical estimations were also carried.

Keywords: Medicinal plants, phytochemical screening, biochemical compounds.

INTRODUCTION

According to World Health Organisation medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries lies traditional medicine. Natural products from microbial sources have been the primary source antibiotics with the increasing recognition of herbal medicine as an attractive form of healthcare. The screening of medicinal plants for active compounds has become very significant. (Koduru *et al.*, 2006), some Phytochemicals product produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs (Nascimento GGF *et al.*, 2000). These substances serve as plant defense mechanism against production by microbes. Insects , herbivores some Terpenoids, give plants order some of the plant pigments, some plant flavour and some herbs, species used by humans season food yield and useful medicinal compounds(Adamu *et al.*, 2005).

Plants as a source of medicine have been inherited and are important compounds of the healthcare system in India. In the Indian system of medicine most practitioners formulate and disperse their own recipes, hence this requires proper documentation and research. In western world also, the use of herbal medicine is steadily growing with approximately 40% population response to use of hers to treat medical illnesses within the fast year. Public academic and government interest in traditional medicines is growing exponential due to the increased incidence of the advance drug reaction and economic burden of the modern system of medicines. Therefore such plants should be investigated to better understand the properties, safety and efficiency. The antimicrobial properties of plant have been investigated by member of researchers worldwide.

Today investigated for phytochemical screening studies selective medicinal plants. In different plants part extract collected for further analyses used for (Brindha *et al.*, 1981). Method the investigation carried out tat preliminary Phytochemical analyses was aqueous extract of five different plants extract, the *Calotropis gigantea* (L.) more Phytochemicals such as Alkaloids, Tannins, Phenolic compounds, Volatile oil, steroids, saponins. When compare to other plant extract. Some biochemical compound present in Calotropis sps protein, amino acids and lipids valuable

secondary metabolites. To compared the therapeutic purpose and commercial interest to both pharmaceutical companies and research institutes production of new drugs.

MATERIALS AND METHODS

Collection of plant materials

The healthy plant samples were collected from Anbil of Trichirappalli District. The plants were *Nyctanthes arbour-tristis* (L.), *Nerium oleander* (L.), *Calotropis gigantea* (L.), *Adhatoda vasica* (L.) and *Lantana camera* (L.).

Medicinal plant samples

1gm of fresh extract collected from the leaves, fresh sample were extracted with 10ml of 80% ethanol solvent with gentle stirring for 72 hr. The sample kept in dark for 72 h with intermittent shaking. After incubation, the solution was filtered through whatmann no.1 filter paper and the filtrate was collected (crude extract) it was then transferred to glass vials and kept at 4˚c before use.

Phytochemical analyses

Phytochemical analyses for major phytochemical constituents of the plant extract were undertaken using standard method as described by (Brindha *et al.*, 1981). The plants extracts were screened for the present of biological active compounds like sugars, amino acids, proteins, phenols, steroid, Terpenoids etc.

Estimation of proteins

The protein in solution can be measured quantitatively (Bradford method).

Estimation of Lipids

The lipids in solution can be measured quantitatively by using (TPA Devasagayam *et al*., 2003) method.

Estimation of total free amino acid:

Amino acids in solution can be measured quantitatively by using **(**Troll and canon, 1953) method**.**

RESULTS AND DISCUSSION

Medicinal plants which form the backbone of traditional medicine, in the last few decades have been the subject for very intense pharmacological studies. The plants as potential source of new compounds of therapeutic value and as sources of lead compounds in drug development in developing countries, it is estimated that above 80% of population rarely depends on traditional medicine for the primary healthcare. There arises a need to screen medicinal plants for bioactive compounds as a basic for further pharmacological studies.

The biological activities were based on phytochemical analyses; extract

of *Nyctanthes arbour-tristis* (L.) showed the presence of Alkaloids, Tannins, and Phenolic compounds. In Leaves extract of *Nerium oleander* (L.), Steroids, Tannins, and Phenolic compounds were identified. Leaf extract of *Lantana camera* (L.) have Alkaloids, steroids Tannins and Phenolic compounds. Leaves extract of *Calotropis gigantea* (L.) contain Alkaloids, Tannins and Phenolic compounds, Flavonoids and Volatile oil were present in the *Adhatoda vasica* (L.) leaf extract. By the estimation of biochemical parameters, the plant extracts have high content of protein, amino acids and lipids. The biochemical compounds highly present in *Calotropis gigantea* (L.). It used for various therapeutic purposes. The commercial interest to the pharmaceuticals companies and research institutes in the production of new drug from this plant. Medicinal plants are of great importance to the health of individuals and communities. The medicinal value of these plants is because of some chemical substances that produce a definite physiological action on the human body.

Table 1: Preliminary Phytochemical analysis of selected medicinal plants leaf extracts

Phytochemical constituents	***N. arbour tristis* (L)**	***N. oleander* (L)**	***L. camera* (L)**	***C. gigantea* (L)**	***A. vasica* (L)**
Alkaloids	+	-	+	+	+
Terpenoids	+	-	-	+	+
Steroids	-	+	+	-	-
Coumarins	-	-	-	-	+
Tannins	-	+	+	+	-
Flavonoids	-	-	-	+	-
Phenols	+	+	+	+	+
Volatile oil	-	-	-	-	+
Quinines	-	-	-	-	-
Sugar	-	-	-	-	-

(+)Present, (-) Absent

Table 2: Quantitative Biochemical parameters of selected plants leaf extracts

S.No	Selected medicinal plants	Biochemical parameters (µgm/gm.fwt.tissue)		
		Proteins	Amino acids	Lipids
1	*N. arbour- tristis*	0.75	0.52	0.22
2	*N. oleander*	0.50	0.48	0.51
3	*L. camera*	0.80	0.44	0.11
4	*C. gigantea*	0.55	0.22	0.11
5	*A. vasica*	0.95	0.90	0.22

CONCLUSION

Phytochemicals protect human from a host of diseases. The present investigation carried out Preliminary Phytochemical analysis from ethanol extract of five different plants. The *Calotropis gigantea* (L) and *Adhatoda vasica* (L.) contain more Phytochemicals such as Alkaloids, Terpenoids, Coumarins, Flavonoids, Tannins, Phenolic compounds and Volatile oil when compared to other plant extracts. Some biochemical compounds are conformed in biochemical parameter analysis. *Calotropis gigantea* (L.) have high amount of protein and amino acid. *Nerium oleander* (L.) have high amount of Lipids compare to other plants. It can be conclude that the *Calotropis gigantea* (L.) and *Adhatoda vasica* (L.) possess the valuable secondary metabolites and biochemical compounds when compared to other plants. Hence it is used for various therapeutic purposes. The findings could also commercial interest to both pharmaceutical companies and research institutes in the production of new drugs

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EVALUATION OF PHYTOCHEMICAL PROPERTIES AND NITRIC OXIDE SCAVENGING ACTIVITY OF LEAFY VEGETABLES OF SOME TREES

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ABSTRACT

The biochemical content and nitric oxide scavenging potential of leafy vegetables of some trees like *Moringa oleifera* L. (Moringaceae), *Sesbania grandiflora* (L.) Pers (Fabaceae) and *Pisonia grandis* R.Br (Nyctaginaceae) were studied. The nitric oxide scavenging activity of ethanolic extracts from shade dried leaves of *Moringa oleifera* showed IC_{50} values 226.24 ± 0.52µg/ml against the standard of BHT 280µg/ml. The rest of the plant extracts showed higher IC_{50} values than BHT indicates their scavenging activities were lesser than BHT in the following order *Sesbania grandiflora* > *Pisonia grandis.* The heirarchy of total poly phenols in different plant extracts were found to be *Moringa olifera* > *Pisonia grandis* > *Sesbania grandiflora* ranging between 34.54 ± 0.99 tannic acid equivalent mg/g in *Sesbania grandiflora* and a maximum amount of 129.85 ± 0.79 tannic acid equivalent mg/g in *Moringa olifera.* Similarly the flavonoids (19.91± 0.81mgQE/g), and flavonols (13.73 ± 0.52 mg QEequivalents /g) were also maximum in *Moringa olifera.* But flavonoids and flavonols were minimum in *Sesbania grandiflora* (13.48 ± 0.74mg QE/g) and *Pisonia grandis* (4.48 ± 0.57) respectively. The concentration of Total Proanthocyanidin in leafy vegetables was in the range of 3.78 ± 0.48 equivalents mg/g in *Sesbania grandiflora* and a maximum of 6.26 ± 0.54 equivalents mg/g in *Pisonia* grandis.

Keywords: Tree leafy vegetables, *Moringa olifera, Pisonia grandis, Sesbania grandiflora, BHT,* Nitric oxide radical scavenging, toatal phenols, flavonoids, flavonols, and Proanthocyanidin.

INTRODUCTION

Reactive oxygen species (ROS) including superoxide (O_2), hydrogen peroxide (H_2O_2), hydroxyl (OH), nitric oxide (NO) exert oxidative stress in the cells of human body rendering each cell to face about 10,000 oxidative hits per second (Halliwell, 1989; Lata and Ahuja, 2003). When generation of ROS overtakes the antioxidant defense of the cells, the free radicals start attacking the cell proteins, lipids and carbohydrates and this leads to development of degenerative diseases. Hence the rationale for the use of antioxidants is well established in prevention and treatment of diseases where oxidative stress plays a major role. Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by bringing interruption in ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules (Sen, 1995; Hegde, and Joshi, 2009). Therefore the uses of antioxidants, both natural and synthetic are gaining wide importance in prevention of diseases.

Flavonoids are present in most plant tissues and often in vacuoles (Croteau *et al*.,2000). Flavonoids are divided into classes according to their substitutes and oxidation level on the middle ring. The main subclasses and their respective food sources are anthocyanidins (red, purple and blue berries), flavanols (tea, red grapes and red wine), flavones (green leafy species) flavanones (citrus), and isoflavones (soybeans). Flavonoids in plants can function as color definitions and attractants to pollinators and seed dispersers, as antioxidants to protect plants against UV-radiation, as insect feeding attractants in host-species recognition, as signal molecules to facilitate nitrogen fixation, in inducible defense against bacterial and fungal attack; and as bitter or astringent taste attributes to repel birds and other animals (Croteau *et al*.,2000; Wildman, 2001; Winkel-Shirley, 2001, 2002). For humans, several health beneficial properties of dietary flavonoids are recognized for their antioxidant and anti proliferative effects which may protect the body from various diseases, such as cancers, cardiovascular disease and inflammatory (Middleton *et al*.,2000; Nijveldt *et al*.,2001). Flavonoids and flavonols are also stabilizing lipid oxidation with antioxidant activity (Yen and Duh, 1993).

Polyphenolic substances possess many biological effects which are mainly attributed to their antioxidant activities in scavenging free radicals, inhibition of peroxidation and chelating transition metals (Bahman *et al.*, 2007). For examples, flavonols, cinnamic acids, coumarins and caffeic acids are well known polyphenolic compounds with strong antioxidant properties. Hence they play an important role in protecting food, cells and organs from oxidative damage (Osawa, 1999). These compounds (phenolic substances) all share the same chemical patterns, with one or more phenolic groups for hydrogen proton donors and neutralize free radicals (Parejo *et al.*, 2002; Milliauskas *et al.*, 2004; Atoui *et al.*, 2005; Galvez *et al.*, 2005). Keeping the above back ground information in mind the present study was taken up to evaluate the Phytochemical contents and nutritive value of edible greens of tree species and their antioxidant/Nitric oxide radical scavenging potential.

MATERIALS AND MEHODS

Plants selected for this study are *Pisonia grandis* R.Br (synonym: *Pisonia alba*, *Pisonia morindifolia*) belongs to the family Nyctaginaceae; *Moringa oleifera* L., commonly Known as Drum stick, belongs to the family Moringaceae and *Sesbina grandiflora* (L.) Pers (Leguminosae) commonly called as Agati (Fig. 1).

Fig.1 *Pisonia grandis* *Moringa oleifera* *Sesbania grandiflora*

Preparation of extract

The dried powder (20 g) was extracted with ethanol (200 mL) in an orbital shaker for 48 hours. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper and evaporated

to dryness. The resulting extract was reconstituted with sterile distilled water to give desired concentrations used in this study.

Phytochemical screening of the plant extract

Total Phenolics Content

The total phenolics content of the extract were determined by Folin-Ciocalteu method described by Wolfe *et al.* (2003), with little modification. The amount of total phenolic content was expressed as mg/g tannic acid equivalent using the expression obtained from the calibration curve: Y = 0.1216x, R2 = 0.936512, where x is the absorbance and Y is the tannic acid equivalent in mg/g.

Total Flavonoids

The total flavonoids were determined using the method of Ordonez *et al.*, (2006). Total flavonoids content was calculated as quercetin equivalent (mg/g) using the equation obtained from the curve: Y = 0.255x, R2 = 0.9812, where x is the absorbance and Y is the quercetin equivalent.

Total Flavonols

The total flavonols content were determined using the method of Kumaran and Karunakaran (2007). Total flavonoids were calculated as quercetin (mg/g) using the following equation based on the calibration curve Y = 0.0255x, R2 = 0.9812, where x is the absorbance and Y is the quercetin equivalent.

Proanthocyanidins

The total proanthocyanidin were determined using the procedure reported by Sun *et al*. (2005). Total proanthocyanidin contents were expressed as catechin (mg/g) using the following equation of the curve: Y= 0.5825x, R2 = 0.9277, where x is the absorbance and Y is the catechin equivalent.

Nitric Oxide Scavenging Activity

The method of Garrat (1964) was used to determine the nitric oxide radical scavenging activity of extracts of sample.The amount of nitric oxide radical was calculated using the equation: % Inhibition of nitric oxide radical scavenging activity = $[Ao - A1]/Ao \times 100$

Estimation of pigments: Chlorophylls and carotenoids

The concentrations for Chl a (C_a) 664nm, Chl b (C_b) 648nm, and the sum of

leaf carotenoids ($C_{(x+c)}$) can be calculated (Lichtenthaler, 1987) with the following equations

Ethanol with 5% (v/v) water:

$$c_a\,(\mu g/ml) = 13.36\,A_{664.1} - 5.19\,A_{648.6}$$
$$c_b\,(\mu g/ml) = 27.43\,A_{648.6} - 8.12\,A_{664.1}$$
$$c_{(x+c)}\,(\mu g/ml) = \left(1000\,A_{470} - 2.13\,c_a - 97.64\,c_b\right)/209$$

Anthocyanins

Anthocyanins were estimated by adapting method of Mónica Giusti and Wrolstad, 2001.

Statistical analysis

Experiments were carried out in triplicate and the data were subjected to statistical analysis like mean and standard error using SPSS 10.0.

RESULTS AND DISCUSSION

Over the years, exploration of plant products has been on the increase leading to the identification and improvement of plant products beneficial to mankind. *Moringa oleifera* L. (Moringaceae), *Sesbania grandiflora* (Fabaceae) and *Pisonia grandis* (Nyctaginaceae) are recognized as a multifunctional versatile plants with enormous economic, nutritional and health potentials and hence was considered for the antioxidants study.

Total phenols (TP) and Flavonoid (TF)

Phenolic compounds may contribute directly to antioxidative action and free radical terminators (Duh *et al.*, 1999). Polyphenolic compounds may have an inhibitory effect on mutagenesis and carcinogenesis in humans when as much as 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka *et al.*, 1998). They also involved in retardation of oxidative degradation of lipids (Pourmorad *et al.*, 2006).

The trend of total poly phenol and flavonoid contents in the studied plant extracts were *Moringa olifera* > *Pisonia* > *Sesbania grandiflora* (Table 1). *Moringa olifera* leaf showed 129.85 ± 0.79 tannic acid equivalent mg/g, a maximum among the tested plants. Sharma *et al.*, (2009) reported that the total phenol content in *Moringa olifera* fruit 125 GAE mg/g and the oil has 40.17 ± 0.01mg GAE g^{-1} (Ogbunugafor *et al.*,2011).

The total phenol content in *Sesbania grandiflora* was 34.54 ± 0.99 tannic acid equivalent (TAE) mg/g which is almost

similar to the observations made by Padmaja *et al*., (2011) which was 31.34 ± 0.577 GAE/gm. The content may vary from species to species, as *Sesbania rostrata* - 18mg/g (Dc - Ouattara *et al*. 2011) and *Sesbania pachycarpa* - 47.50 ± 2.0mg/g (Ouattara *et al.*, 2011).

In the Present study Total flavonoid content of *Moringa olifera* was 19.91± 0.81QEmg/g, almost equal in seed oil, 18.24 ± 0.01mg RE g^1 (Ogbunugafor *et al*.,2011) but slightly lesser than fruit (Sharma *et al.*, 2009). Flavonoids in *Pisonia grandis* and *Sesbania grandiflora* were 14.76 ± 0.75 and 13.48 ± 0.74mg QE/g respectively.

Table1. Total phenol and Flavonoid content of leafy vegetables

Plants	**TP mg/g**	**TF mg/g**	**TFL mg/g**	**PAC mg/g**
Moringa olifera	129.85 ± 0.79	19.91± 0.81	13.73 ± 0.52	5.56 ± 0.56
Pisonia grandis	36.54 ± 0.89	14.76 ± 0.75	4.48 ± 0.57	6.26 ± 0.54
Sesbania grandiflora	34.54 ± 0.99	13.48 ± 0.74	11.45 ± 0.5	3.78 ± 0.48

Total Flavonol content (TFL)

The concentration of total flavonol in leafy vegetables was in the range of 4.48 ± 0.57 mg QE equivalents/g in *Pisonia grandis* and a maximum of 13.73 ± 0.52 mg QE equivalents /g in *Moringa olifera* (Table 1).

In terms of Polyphenols, Flavonoids and Flavonol contents *Moringa oleifera* shows maximum content among the tested plants and expected to have maximum antioxidant activity possibly due to synergistic effects of different compounds.

Total Proanthocyanidin (PAC)

In the tested plants, unlike the other compounds, the concentration of total PAC in leafy vegetables was maximum in *Pisonia grandis* and least in *Sesbania grandiflora* (Table 1) The unique polyhydroxy phenolic nature of proanthocyanidins and the resulting electronic configuration allows relatively easy release of protons and, as a result, they have substantial antioxidant activity, which is greater than vitamins C and E, the gold standards (Bagchi *et al.*, 1997; Ho *et al*., 1999; Santos-Buelga & Scalbert, 2000; Bors *et al*., 2001; Hatano *et al.*, 2002; Beninger & Hosfield, 2003).

Cataract formation and occurrence of retinopathy are two common complications of type 2 diabetes. Cocoa derived proanthocyanidins fed to diabetes-induced (streptozotocin treated) rats nearly completely inhibited cataract formation (Osakabe *et al*., 2002).

Nitric oxide scavenging assay

Nitric oxide is a very unstable species under the aerobic condition. It reacts with O_2 to produce the stable product nitrates and nitrite through intermediates likeNO_2, N_2O_4 and N_3O_4. In the presence of test compound, which is a scavenger, the amount of nitrous acid will decrease. The extent of decrease will reflect the extent of scavenging. Here the extent of inhibition of ethanolic extract of *Moringa oleifera* showed the IC_{50} values 226.24 ± 0.52µg/ml as against the standard of BHT 280µg/ml. while the rest of the plant extracts showed higher IC_{50} values than BHT indicating that their nitrous oxide scavenging activity lesser than BHT in the following decreasing order *Sesbania grandiflora* > *Pisonia grandis* (Table 2).

Table 2. Nitric oxide scavenging activity of leafy vegetables

Standard / Plants	**IC_{50} (**µg/ml)
BHT	280 ± 0.28
Moringa olifera	226.24 ± 0.52
Sesbania grandiflora	550.27 ± 0.48
Pisonia grandis	965.74 ± 0.92

Pigments in Leafy vegetables

Anthocyanins

Among the leafy vegetables *Pisonia grandis* had a maximum concentration of anthocyanin (0.759mg/g) followed by *Moringa olifera* and *Sesbania grandiflora* (fig.2). The phenolic structure of anthocyanins is responsible for their antioxidant activity; i.e., ability to scavenge reactive oxygen species (ROS) such as superoxide (O2 −), singlet oxygen (‘O2), peroxide (ROO−), hydrogen peroxide (H2O2), and hydroxyl radical (OH) (Wang and Jiao, 2000) and by chelating metals, direct binding to proteins (Kong *et al*., 2003). They play an important role in the prevention against mutagenesis and carcinogenesis mediating some physiological functions related to cancer

suppression (Omenn, 1995). Anthocyanins show inhibitory effects on the growth of some cancer cells (Kamei *et al*., 1995; Koide *et al*., 1997; Nagase *et al*., 1998; Meiers *et al*., 2001) and also inhibit cell transformation (Hou *et al*., 2004).

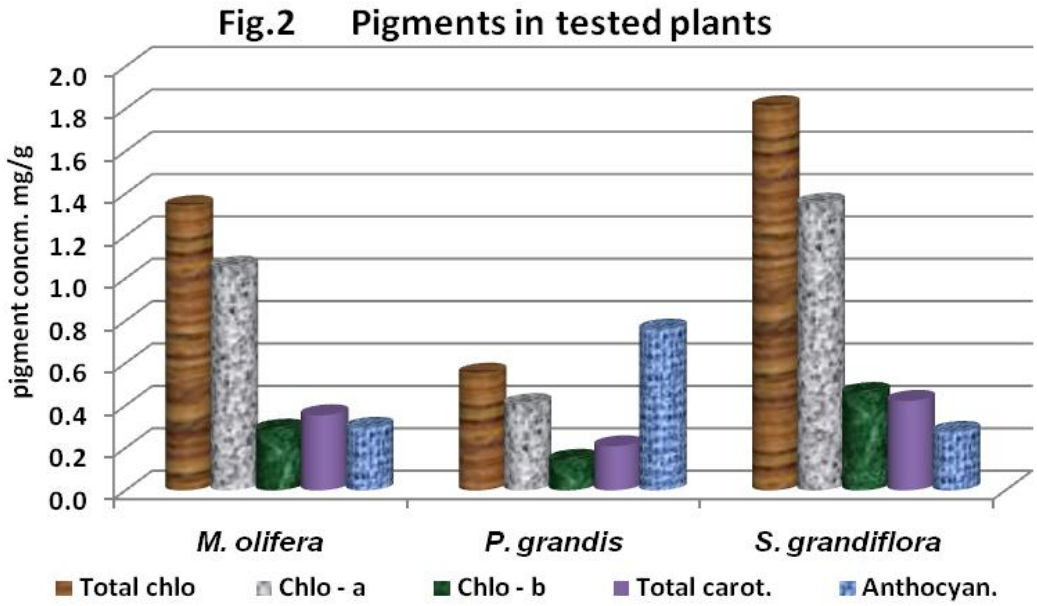
The antioxidant effects of anthocyanins in vitro have been demonstrated using several cell culture systems including colon (Parry *et al*., 2006; Renis *et al*., 2007), endothelial (Bagchi *et al*., 2004), liver (Meyers *et al*., 2003) and leukemic cells (Feng *et a*l.,2007), and keratinocytes (Afaq *et al*., 2007).

Carotenoids

Among the leafy vegetables *Sesbania grandiflora* Showed a maximum (0.424mg/g) total carotenoid pigments and least in *Pisonia grandis* (fig.2).

Chlorophyll pigments

Among the leafy vegetables *Sesbania grandiflora* Showed (fig.2) maximum (1.812mg/g) total chlorophyll pigments and *Pisonia grandis* observed to have least amount (0.550mg/g) of total chlorophyll pigments.



Thus, the presence of phenolic compounds and flavonoids in the tested greens are an added value to its nutritional and health potential and having a preventive

action against cancer and heart disease. The maximum content of anthocyanin and proanthocyanidin in *Pisonia grandis* possibly have a role in reducing joint pain and arthritis as it has strong anti-inflammatory, antioxidant, and radical scavenging activity. Among all tree leafy vegetables *Moringa olifera* is the cheap and best.

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IN VITRO DETERMINATION OF ANTIBACTERIAL ACTIVITY OF *LEPIDAGATHIS CRISTATA* WILLD

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ABSTRACT

This investigation is carried out to determine the antibacterial activity of different solvent extracts of *Lepedagathis cristata* Willd. Two different sets of solvents i.e. polar (methanol and ethanol) and non polar (toluene and acetone) were selected for the extraction of plant material using Soxhlet apparatus. The extracts were tested against human pathogenic bacteria namely, *Bacillus subtilis, Proteus vulgaris, Klebsiella pneumonia, Salmonella typhi* and *Pseudomonas aeruginosa*. Among the extracts tested, polar solvent extract showed significant activity than the non polar solvent extracts. It was further observed that the extracts of *L.cristata* seemed to be more effective against Gram positive bacteria than Gram negative bacteria tested.

Keywords: *Lepedagathis cristata,* antibacterial activity, solvent extract

INTRODUCTION

The use of natural products with therapeutic properties has a long history (Kao, 1980). Nearly all cultures from ancient times to the present day have used plants as one of the sources of medicines (Lino and Deogracious, 2006). A considerable percentage of the peoples in both developed and developing nations use medicinal plant remedies. This revival of interest in plant-derived drugs is mainly due to the current widespread belief that "green medicine" is safe and more dependable than the costly synthetic drugs many of which have adverse side effects (Shariff, 2001; Parekh and Sumitra, 2006). Many approaches have been made to discover new biologically active principles higher plants (Farnsworth and Loub, 1983). One such approach is systematic screening of plants which may result in the discovery of novel effective biologically active compounds (Farnsworth *et al.*, 2002; Janovska *et al.*, 2003).

In India herbalists traditionally use various herbal preparations to treat a variety of diseases. In this line of herbal usage people in remote places of Chhattisgarh have been traditionally exploited the herb, *Lepedagathis cristata* Willd. for various curative treatments particularly malarial fever. They also use the species for the treatment of itching. Cattle owners of this region use the decoction of this herb to wash their cattles in order to keep away the flies. Since the herb has been exploited tremendously in this region in many ways for various curative purposes, it is necessary to evaluate in a scientific base, the potential use of folk medicine for the treatment of infectious diseases.

Biological studies are very much essential to substantiate the therapeutic properties of medicinal herbs used in folk medicine on scientific line (Girish, 2008). Literature survey on *L.cristata* revealed that the therapeutic properties of this herb have not been established so far. Hence an attempt was made in the present study to investigate the feasibility of using *L.cristata* against various human pathogenic bacterial strains. *L.cristata* (Acanthaceae) is a perennial herb with almost no stem. Branches arise out of a globose head on the ground and spread out.

MATERIALS AND METHODS

Plant material Preparation

L.cristata was collected from Chhattisgarh, washed, shade dried, packed properly and transported to the Department of Botany, St.Joseph's College, Tiruchirappalli, Tamil Nadu, India. The name was authenticated with the help of expert from the Rapinat Herbarium of St.Joseph's College, Tiruchirappalli and a voucher specimen (Collection no: URT-001) was deposited.

Preparation of extracts

The dried plant materials were pulverized by a mechanical grinder. The powdered plant material (500 g) was successively extracted with acetone, toluene, ethanol and methanol up to 48 hrs at room temperature using a Soxhlet apparatus. The extracts were filtered and concentrated at 35^{o}C.

Test Microorganisms

Authentic pure cultures of human pathogenic bacteria like *Bacillus subtilis, Klebsiella pneumonia, Proteus vulgaris, Pseudomonas aeruginosa* and *Salmonella typhi* were obtained from National College, Tiruchirappalli, Tamil Nadu, India.

Antibacterial activity assay

Antibacterial activity of solvent extracts of the entire plant was determined by disc diffusion method. Standard discs (6 mm diameter) were autoclaved and soaked separately in different solvent extract over night. The organism to be tested was uniformly spread on the sterile nutrient agar medium. Three solvent extract soaked discs were carefully placed on the inoculated medium aseptically. The plates were incubated for 24 hrs at 37^{o}C and zone of inhibition if any around the disc was measured in millimeter (mm). The treatment also includes the antibacterial agent Gentamicin as the positive control and the respective solvents as the negative controls. For each treatment three replicates were maintained. The data was subjected to statistical analysis.

RESULTS AND DISCUSSION

The observations and the results of antibacterial activity of various solvent extracts of *L.cristata* against human pathogenic bacterial strains are shown in the Table. The results revealed that toluene, acetone, ethanol and methanol solvent extracts were active against all the tested five pathogenic bacterial strains. The literature indicates that the antimicrobial activity is due to different chemical agents in the extract. These are classified as active

antimicrobial compounds (Rojas, *et al.*, 1992). The extraction of the active antimicrobial compound from the plant material is largely dependent on the type of solvent used. The traditional healers make use of water primarily as a solvent to extract the active compound from the plant material. However in the present investigation four different solvents were used with different polarity. Because the solubility of the active components of the plant material may be vary from solvent to solvent (Boer, *et al.*, 2005). Therefore polar components of the plant material can be extracted using polar solvent and vice versa. When the results are compared between the two different solvent systems used in the present investigation the extracts of polar solvents seemed to be greater activity than the extracts of non polar solvents.

Review of literature reveals lack of information on the antibacterial potential of *L.cristata* extracts. In the present investigation the antibacterial activity of this whole plant extracts has been demonstrated for the first time. The results reveal that the extracts of *L.cristata* were significantly effective against tested Gram-positive organism than Gram-negative organisms. This is in agreement with previous reports that plant extracts are more active against Gram-positive bacteria than Gram-negative bacteria (Vlietinck, *et al.*, 1995; Rabe and Van Staden, 1997). Among the Gram-negative bacterial strains tested *P.aeruginosa* seemed to be lesser susceptible to the solvent extracts. This could be attributed to the fact that this bacterium is naturally resistant to many antibiotics due to the permeability barrier afforded by its outer membrane. Also its tendency to colonize in a biofilm form makes the cells impervious to therapeutic concentrations of antibiotics. Since its natural habitat is the soil, living in association with bacilli, actinomycetes and molds it develops resistance to a variety of their naturally occurring antibiotics.

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Table 1 Antibacterial activity of different solvent extracts of *Lepidagathis cristata* against human pathogenic bacteria

Sl. No.	Pathogens	Toluene extract	'-' control	Acetone extract	'-' control	Methanol extract	'-' control	Ethanol extract	'-' control	'+' control
1	*B.sublitis*	0.5±0.2	0±0.00	0.6±0.18	0±0.00	11.6±0.24	0±0.00	12.4±0.14	0±0.00	24±0.00
2	*K. pneumoniae*	0.6±0.2	0±0.00	0.6±0.17	0±0.00	7.54±0.32	0±0.00	8.5±0.36	0±0.00	20.27±0.27
3	*P.vulgaris*	0.72±0.12	0±0.00	0.75±0.26	0±0.00	6.33±0.14	0±0.00	7±0.00	0±0.00	19.33±0.14
4	*P.aeruginosa*	0.25±0.16	0±0.00	0.36±0.17	0±0.00	3.5.38±0.27	0±0.00	4.7.83±0.14	0±0.00	12.37±0.12
5	*S.typhi*	0.53±0.16	0±0.00	0.67±0.11	0±0.00	6.5.12±0.24	0±0.00	7.8.37±0.15	0±0.00	20.17±0.27

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ANTIMICROBIAL AND PHYTOCHEMICAL SCREENING OF *TRAGIA INVOLUCRATA* L. USING UV-VIS AND FTIR

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ABSTRACT

India is endowed with a rich wealth of medicinal plants. Some of these plants are the potential source of antibiotics for various infections. The antimicrobial activity of *Tragia involucrata* L. stem and leaf extracts was determined against 7 pathogenic bacteria and fungi by disc diffusion method. The Chloroform stem extract and Ethanol leaf extract has showed higher inhibition in bacterias like *Pseudomonas aeruginosa* and *Vibrio cholerae*. Ethanol stem extract and Methanol stem extract has more effect against fungi like *Aspergillus niger* and *Rhizopus arrhizus*. There was no activity against *Penicillium chrysogenum*. Preliminary phytochemical screening was carried out for qualitative determination of plant extracts. The secondary metabolites such as Alkaloids, Flavonoids, Steroids, Lignin's, Quinines and Triterpenoides. The different peaks were obtained in the UV-VIS profile and by the FTIR, it was confirmed that the presence of Alcohol, Amides, Phosphines, Anhydrides, Carboxylic acids, Alkenes, Sulfoxides and Alkyl halides.

Keywords: *Tragia involucrata* L, Antimicrobial activity, Phytochemical analyzes, UV-Vis, FTIR.

INTRODUCTION

Medicinal plants constitute an important natural wealth of a country. India is endowed with a rich wealth of medicinal plants. Plants have been an essential part of human society since the start of civilization. Medicinal plants are a boon of nature to cure a number of ailments of human beings. Ayurveda and Unani systems of medicine regularly employ a large number of Indian medicinal plants as antibiotic agents. Our country represents a store house of genetic diversity of plants (Perumal *et al*., 2004). In India around 20,000 medicinal plants have been recorded recently, but more than 500 traditional communities use about 800 plant species for curing different diseases (Kalaivani *et al*., 2012).

Uses of antimicrobial agents against harmful pathogen are known to have been common practice at least 2000 years. In order to promote the use of medicinal plants as potential sources of antimicrobial compounds, it is important to thoroughly investigate their composition and activity and thus validate their use (Nair *et al*., 2006). Some Phytochemicals produced by plants have antimicrobial activity and used for the development of new antimicrobial drugs (Sahaya Sathish *et al*., 2012). Higher plants are much more important in the production of economically important organic compounds and pharmaceuticals. The rising incidence of multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. The use of plant extracts and Phytochemicals with known antimicrobial properties can be of great significance in therapeutic treatments (Sahaya Sathish *et al*., 2011).

In recent years attempts are being made to screen out the efficiency of better known plants which have medicinal values, especially to find out their antimicrobial properties against different pathogenic microbes. Several countries have already done analytical studies on better known medicinal plants for controlling diseases with this motive. The screening of plants extracts and natural products for antimicrobial activity has revealed the potential of higher plants as a source of the new anti-infective agents as well as serving drug discovered from natural products for primary and secondary compounds. Antimicrobial agents include all classes of secondary metabolites. These principles play an important role in the biochemical resistance against pathogenic organisms. Some of these plants are the potential source of antibiotics for various

infections. Keeping all this in mind, in the present investigation an attempt was made in the medicinal plant to study the antimicrobial activity and phytochemical constituents of *T. involucrata* L. using UV-VIS, FTIR.

MATERIAL AND METHODS

Collection and Preparation of different Plant extracts

Healthy, disease free and mature plant collected from Madurai. Madurai district, Tamil Nadu, India. Successive solvent extraction-the *T. invocrata* L. plant were taken and subjected to successive solvent extraction. The plant leaves and stem were collected, washed with distilled water and dried under shed. The dried samples were grinded to fine powder. 30 gm of powdered sample was mixed with 50 ml of solvent (Ethanol, Methanol, Chloroform and Aqueous) kept undisturbed for 3 days. Finally the solvents were filtered to collect the plant extract.

Tested Microorganisms

Tested Microorganisms were *Bacillus subtilis,* (Gram positive bacteria), *Pseudomonas aeruginosa, Salmonella typhi, Vibrio cholerae* (Gram negative bacteria) and *Penicillium chrysogenum, Aspergillum niger, Rhizopus arrhizus* (Fungus). The Microorganisms were obtained collected from Govt. Medical College, Tiruchirappalli, Tamil nadu, India.

Antibacterial activity assay

The antibacterial activity of the plant extracts were tested by the modified disc diffusion method (Baur *et al.,* 1966). The bacterial inoculums (20 h broth) were uniformly spread over the agar plates using a glass L-rod. A total of 0.2ml of each extract was aseptically added to the discs (0.5mm diameter) and allowed to dry before being placed on the top of the agar plate. The plates were incubated at 37°C for 24h and the diameter of growth inhibition zone was recorded. A standard antibiotic for Bacteria, Chloramphenicol and Fungi, Gentamicine was used as positive controls.

Phytochemical analysis

The Ethanol leaf extract was selected for Preliminary Phytochemical screening because of their better antimicrobial activity. Compounds identification by chemical tests. Tests for Steroids, Triterpenoides, Glycosides, Carbohydrate, Alkaloids, Phenolic compound, Catachins, Flavanoids, Saponins, and Tannins were carried out according to the methods of (Brindha *et al.,* 1981).

UV-VIS and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis For UV-VIS and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 min and filtered through Whitman No. 1 filter paper by using high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 300-1100 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks and their functional groups. The peak values of the UV-VIS and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.

RESULTS AND DISCUSSION

In Ethanolic leaf extract, the antimicrobial activity in *P. aeruginosa, B. subtilis, V. cholerae, A. niger, R. arrhizus* showed higher inhibition zone (1.63cm, Table-1). The inhibitory action was observed in terms of inhibition zone formed around each disc caused by the diffusion of antimicrobial substances from the paper disc into the surrounding media. Chloroform stem extract showed the higher inhibition zone (1.26cm) in *P. aeruginosa, B. subtilis, V. cholerae, and R. arrhizus* (Table-2). Hence the antimicrobial activity of various solvent extracts of leaf and stem on selected microorganisms clearly revealed that the aqueous extract was found to possess significant inhibitory property. Both aqueous extracts showed the less inhibition on pathogenic strains (0.7cm). The antimicrobial activity was maximum of Ethanolic extract while minimum activity is in Aqueous extract (Table-1 & 2). However *P. chrysogenum* are resistance to all the solvent extracts.

The qualitative UV-VIS spectrum profile of *T. invocrata* L. Ethanolic stem extract was selected at wavelength from 350 to 1100 nm due to sharpness of the Ethanolic peaks and proper baseline. The profile showed the peaks at 350 and 975nm with the absorption of 353.68 and 974.14 respectively (Fig. 1; Table- 5) and UV-VIS profile of Ethanolic leaf extract of *T. invocrata* L. was chosen at a wavelength of 400 to 1100 nm and the profile showed the peaks at 423.26, 617.64 and 676.47nm with the absorption 425.52, 616.18 and 674.77 respectively (Fig. 2; Table-5). Performing the next advanced phytochemical analysis technique of FTIR,

the presence of various functional group of different compound was found. Every solvent had its respective functional group like Alcohol, Amides, Phosphines, Carboxylic acids, Alkenes, Sulfoxides, Alkyl halides. Hence, the crude extracts subjected to UV-VIS and FTIR analysis is used for the identification of chemical constituents present in *T. invocrata* L (Fig.3; Table-4). In addition, UV-VIS and FTIR spectroscopy is proved to be a reliable and sensitive method for detection of biomolecular composition. (Komal Kumar *et al.*, 2011).

Table: 1. Antimicrobial activity of leaf extract of *T. involucrata* L.

Selected Microbes (Bacteria & Fungi)	Zone of Inhibition (cm)*				
	Ethanol extract	Methanol extract	Chloroform extract	Aqueous extract	Standard antibiotics
P. aeruginosa	1.2±0.28	1.26±0.15	0.76±0.15	_	2
B. subtilis	1.0±0.95	0.86±0.75	0.56±0.51	0.53±.0.47	2.1
S. typhi	_	_	0.93±0.32	_	2
V. cholerae	1.63±0.20	1.26±0.05	1.16±0.25	_	2
P. chrysogenum	_	_	_	_	1.9
A. niger	0.86±0.30	0.86±0.30	_	0.83±0.15	2
R. arrhizus	1.5±0.20	1.3±0.20	0.96±0.56	0.73±0.20	2.1

Note: *values are the mean of triplicate, Standard antibiotics (Bacteria Chloramphenicol, Fungi Gentamicine), ± standard deviation

Table: 2. Antimicrobial activity of stem extract of *T. involucrata* L.

Selected Microbes (Bacteria & Fungi)	Zone of Inhibition (cm)*				
	Ethanol extract	Methanol extract	Chloroform extract	Aqueous extract	Standard antibiotics
P. aeruginosa	1.06±0.45	0.95±0.15	1.3±0.26	0.8±0.43	1.9
B. subtilis	_	_	1.03±0.55	0.7±.0.35	2.1
S. typhi	0.83±0.46	1.16±0.46	0.93±0.35	_	2
V. cholerae	_	_	0.93±0.55	0.83±0.11	1.9
P. chrysogenum	_	_	_	_	1.9
A. niger	0.8±0.3	0.8±0.3	_	0.63±0.15	2
R. arrhizus	1.13±0.20	1.26±0.20	0.96±0.56	0.66±0.20	1.9

Note: *values are the mean of triplicate, Standard antibiotics (Bacteria Chloramphenicol, Fungi Gentamicine), ± standard deviation

Table: 3. Phytochemicals screenings of *T. involucrata L.* Ethanol leaf extract.

S. No	Phytochemicals	Ethanol leaf extract
1	Steroids	+
2	Triterpenoides	+
3	Glycosides	+
4	Carbohydrate	+
5	Alkaloids	+
6	Phenolic compound	-
7	Catachins	-
8	Flavanoids	+
9	Saponins	-
10	Tannins	-

Note: + represent as present; - represents as absent

Table: 4. FTIR peak values of Ethanol leaf extracts of *T. involucrata L.*

Function group	Molecular motion	Wave number (cm^{-1})
Alcohol	O-H stretch	~3696.72
Amides	N-H stretch	~3434.77
Phosphines	P-H stretch	~2355.95
Anhydrides	C=O stretch	1813.91
Carboxylic acids	C=O stretch	1613.87
Alkenes	C=C stretch (conjugated)	1613.87
Alkenes	C-H in –plane bent	1404.51
Sulfoxides	S=O stretch	1030.40
Alkyl halides	C-CI stretch	776.33
Alkynes	Acetylenic C-H bent	685.24

Table 5: UV-VIS peak values of Ethanolic stem & leaf extracts of *T. involucrata* L.

S. No	Ethanolic stem extract		Ethanolic leaf extract	
	nm	Absorption	nm	Absorption
1.	350.00	353.69	425.52	423.26
2.	506.25	505.22	617.64	616.18
3.	525.00	535.89	676.47	674.77
4.	606.25	605.86	__	__
5.	656.25	661.94	__	__
6.	975.00	974.14	__	__

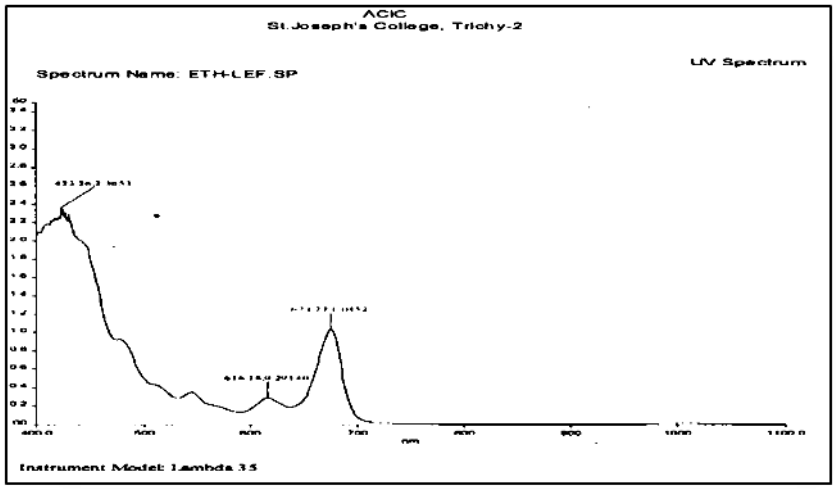

Fig. 1: UV-VIS Spectrum of Ethanolic stem extracts of *T. involucrata L*

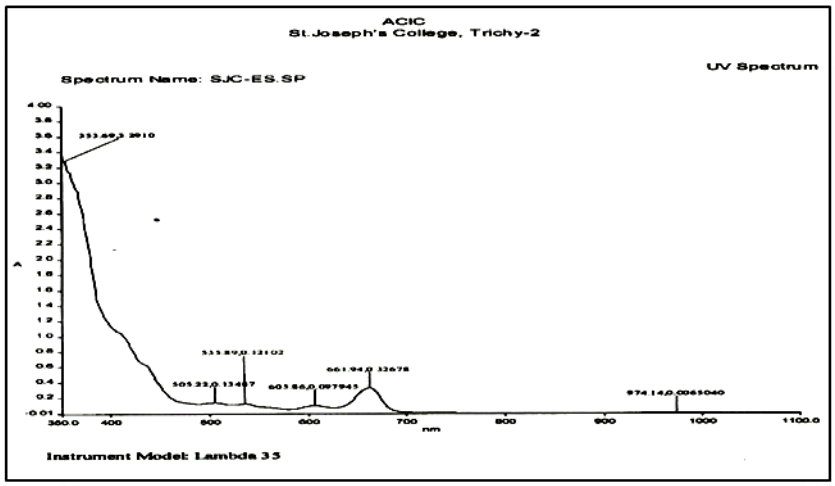

Fig. 2: UV-VIS Spectrum of Ethanolic leaf extracts of *T. involucrata L.*

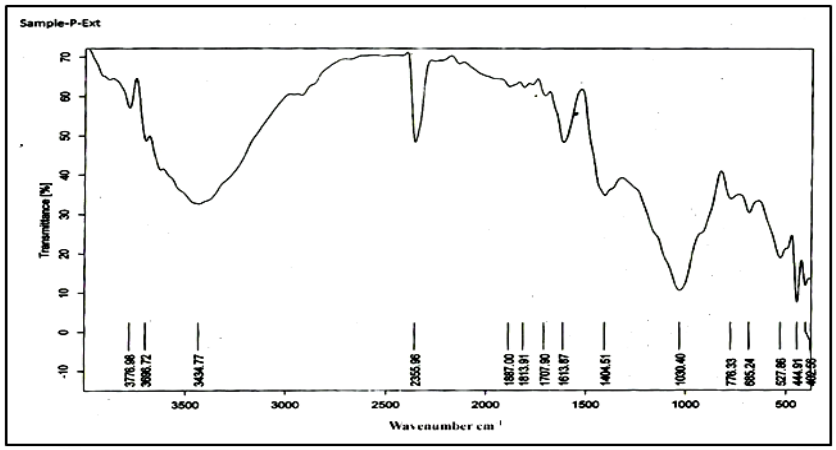

Fig. 3: FTIR Spectrum of Ethanolic leaf extracts of *T. involucrata* L.

SUMMARY AND CONCLUSION

The phytochemical analysis and antimicrobial activity was performed in the leaf and stem of *T. involucrata* L. the antimicrobial activity was studied by disc diffusion method in the 3 different solvent extract and Aqueous extract of the plant *T. involucrata* L. leaf and stem. There was antimicrobial activity observed in the all the 4 tested bacterial and 2 fungal species. The Chloroform stem extract and Ethanol leaf extract has showed higher inhibition in *P. aeruginosa* (1.3cm) and *V. cholera* (1.6cm). Ethanol stem extract and Methanol stem extract has more effect against *A. niger* (1.5cm) and *R. arrhizus* (1.26cm). There was no activity against *P. chrysogenum*. On taking leaf extract, preliminary phytochemical analysis (Brindha *et al.*, 1981) was performed which enabled to indentify the presence of different secondary metabolites and principle bioactive compounds. Further studies comprising of phytochemical investigation of the taken plant *T. involucrata* L. was done by UV-VIS and FTIR. The peaks for the UV-Vis and FTIR for Ethanolic leaf extract were obtained and further interpretation is done. In FTIR, the peaks were analyzed and presences of different group of chemical compounds were found. From the phytochemical analysis of *T. involucrata* L is to be concluded that this plant have bioactive compounds like Steroids, Triterpenoides, Glycosides, Carbohydrate, Alkaloids, Phenolic compound, Catachins, Flavanoids, Saponins, and Tannins form the characteristic nature for its medicinal uses. These compounds also possess antimicrobial activity against pathogenic microbes as well. This Indian herb serves to cure many diseases an also used for various other herbal treatment.

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ANALYSE OF THE EFFECT OF SUGAR MILL EFFLUENT IN THE GROWTH BLACK GRAM AND SORGHUM BY GERMINATION STUDIES

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ABSTRACT

The present study investigated about the efficiency of sugar mill effluent on the germination of monocot (*Sorghum vulgare*) and dicot seeds(*Vigna mungo*) The physico-chemical parameters of sugar mill effluent were analyzed revealed the presence of rich macro & micro nutrients which favored the growth of living organism. Trace amount of heavy metals were also in the soil. On constant irrigation deposition of heavy metal occur which favored the inhibition of plant growth during consecutive cultivating the seeds of monocot & dicot in polluted normal soils. The seeds cultivated in polluted soil showed low response than the seeds cultured in the normal soil. The shoot length, dry weight, chlorophyll estimation were high in the seeds cultured in the normal soil than the polluted soil. Germination studies were also studied with the different concentrations of sugar mill effluent. As the concentration increases, the germination percentage of seed decreases. Thus the present study can be concluded that the effluent cannot be utilized for irrigation. Since the heavy metals get accumulated in the soil on consecutive irrigations.

Keywords: Physico-chemical parameter, Sugar mill effluent, polluted and Non polluted soil, germination studies, chlorophyll estimation.

INTRODUCTION

Soil is an important edaphic factor it play a versatile role in growth of the living organisms. Now days due to industrialization many industries are emerged out and it is created pollutions Sugar industry is back bone of rural, agricultural and socioeconomic development in India. Many industries directly (or) indirectly depend on sugar industry which in turn is responsible for overall development of state in this context sugarcane production is vital importance for its products and by products. Disposal of industrial effluent is the major cause of soil pollution. The content organic and inorganic as well as non biodegradable material touch toxic chemicals affect the soil parameters and soil fertility farmers have been using these irrigation and found that the growth, yield and soil health were reduced. Contaminants such as Chloride, Sulphate, Phosphate, Magnesium and Nitrate are discharged with the effluent of various industries, which create a nuisance due to a physical appearance, odour and taste. Such harmful water is injurious to plant, animals and human beings. Diverse sugar industry effluents disposed in soil and water cause major pollution problem. Sugar factory effluent that has not been treated properly has an unpleasant odour when released in to the environment farmers using this effluent for irrigation to reduce water demand have found that plant growth and crop yield were reduced and soil health was compromised. Sugar industry effluent is commonly used for irrigation. It is essential to determine how crops respond when exposed to industrial effluent. The effluent not only affects the plant growth but also deteriorate the soil properties when used for irrigation. The polluted soil become unsuitable for further cultivation so there is needs to conduct some kind of source of environment in that way microorganism play a major role in bioremediation of contaminated soil.

Deterioration of soil fertility

Contamination of soil in cultivated fields by industrial effluent loaded with toxic heavy metals has emerged as a new threat to agriculture. Most of the effluent and wastes contain heavy metals in an amount sufficient enough to cause toxicity to crop plants. Excessive accumulation of heavy metals like nickel, copper, cobalt in soil originating from metal mining and processing and other technological activities of man have been reported on a number of occasions. The quality of irrigation water has great importance since

many soil properties may be affected. The important quality parameter of waste water from an agricultural point of view physical properties such as Tds, Ec, Temperature, Turbidity, Hardness and Sediments, chemical properties such as Alkalinity, Acidity. The discharge of sugar mills waste to surface bodies with a high Tds adversely affects aquatic life, damage crop yields if used for irrigation. There are however possibilities of accumulation of sludge in the irrigated soils. The present study was investigated about the physico-chemical parameter of the sugar effluent, germination studies, chlorophyll estimation of Monocot and Dicot seeds

MATERIALS AND METHOD

Collection of sugar mill effluent

The sugar mill effluent was collected from the sugar industry, The Salem co-operative sugar mill at Mohanur, Namakkal District, Tamil Nadu. The fresh samples were collected in a sterile plastic cane for the study. The sample was brought to the laboratory to keep a for the further analysis.

Collection of soil sample

The soil samples of both polluted and non polluted were collected from the cultivable land. The physico-chemical parameters of both the soil samples were analyzed.

Collection of seeds

Healthy certified seeds of black gram and sorghum were procured from seed seller in Namakkal.

Analysis physico-chemical parameter of sugar mill effluent and polluted and non polluted soil

The collected sugar mill effluent assessed for various physico-chemical parameters like Colour, Odour, Turbidity, pH, Electrical conductivity, Total suspended solids (TSS), Total Dissolved Solids (TDS), Total solids (TS),Biological Oxygen Demand (BOD), Chemical Oxygen demand(COD), Chloride, Sulphate, cadmium, Magnesium, Sodium, Potassium and heavy metal Zinc were measured using standard methods APHA, (1990). Standard methods for examination of water and wastewater. 20thedition American Health Association, Washington, DC.

The collected soil samples assessed for various physico-chemical parameters like pH, Electrical conductivity (EC), Organic carbon, Available Nitrogen, Available Phosphorous, Available Zinc, Available Potassium, Available Copper, Available Iron, Available Magnesium and

heavy metal Chromium, Lead, Cadmium. Jackson, M.L. (1973), Linsay, W.L. and Norwell, W.A. (1978), MAFF. (1986), Piper, C.S. (1994).

Germination studies

Petri dish Experiments (Thamizhiniyan *et al.*, 2009)

Culture experiment (Ezhilvannan *et al.*, 2011)

Germination percentage

Germination percentage determined by this formula,

Germination perccentage = Number of seeds geminted/ Total number of seeds shown x 100

Seedling length: The length of root and shoot was measured with wetted (for flexibility) and scale.

Fresh and dry weight: The removed seedling were wrapped with aluminium foil and kept in the oven at 80°C for 24 hours. A single pan electrical balance was used for weighing.

Estimation of pigment content of primary leaves: The pigments were extracted according to the procedure of Arnons method.

RESULTS AND DISCUSSION

The effect of sugar mill effluent was studied through germination studies with the seeds of monocot and dicot plants. Prior to this study the physico-chemical parameters were analyzed on both sugar mill effluent and soil samples (polluted &non polluted soil).The effluent was alkaline in nature. Required amount of macro & micro elements were present. The results obtained were tabulated in TABLE-1. The heavy metals were also present. Sallequzzman *et al.*, 2008 stated that the sugar mill effluent has high TDS value, high BOD value, high conductivity value like high Ca, Mg, K, So_4, Cl^-, etc. contents indicates high pollution of effluent.

Table: 1 Physico-chemical parameters of the sugar mill effluent

S.NO	Name of the parameter	Sample
1.	Colour	Deep brown
2.	Odour	Dis. Agre
3.	Turbidity	Highly turbid
4.	Total solids (mg/1)	2159

5.	Total suspended solids (mg/1)	489
6.	Total dissolved solids (mg/1)	1683
7.	PH	8.45
8.	Electrical conductivity (dsm^{-1})	2.63
9.	BOD (mg/1)	2250
10.	COD (mg/1)	3200
	Anions	
11.	Chloride (mg/1)	539
12.	Sulphate (mg/1)	126
13.	Calcium (mg/1)	589
14.	Magnesium (mg/1)	126
15.	Sodium (mg/1)	236
16.	Potassium (mg/1)	0.11
	Heavy metals	
17.	Zinc (mg/1)	0.96

Table: 2-Physico-chemical parameters of the polluted and non polluted soil

S.NO	Name of the parameter	Sample	
		NP	P
1.	pH	8.06	8.12
2.	Electrical conductivity(dsm^{-1})	0.18	1.52
3.	Organic carbon (%)	0.26	0.32
4.	Available Nitrogen (Kg/ac)	132.0	189.3
5.	Available Phosphorus (kg/ac)	3.89	6.38
6.	Available Potassium (Kg/ac)	125	206
7.	Available Zinc (ppm)	0.96	1.72
8.	Available Copper (ppm)	1.06	2.36
9.	Available Iron (ppm)	5.69	15.63

10.	Available Magnesium	11.3	12.5
	Heavy metals (ppm)		
11.	Chromium	0.02	0.29
12.	Lead	0.02	0.23
13.	Cadmium	NIL	0.06

Detection of physico-chemical parameters of soil samples

Both the soil samples were slightly alkaline in nature, which is suitable for the growth of the plant. The experimental results indicated that most of the physico-chemical parameters such as silt, clay, electrical conductivity, water holding capacity, organic matter and total nitrogen, phosphorous and potassium, microbial population high in the test sample than in the control (venkateshwar Reddy *et al.*, 2013) was compared with the obtained results. The obtained results were tabulated inTABLE-2.

Germination studies

Depending on the concentration of effluent the shoot length, dry matter& germination percentage showed variation. The results were inversely proportionate with the effluent concentration. The shoot length, dry matter & germination percentage showed variations. The results obtained were similar to the work done by (Siva Shanthi and Suja Pandian, 2012) which stated that as the germination percentage & germination value decreases with the increase in concentration of effluent.

Petri dish experiments were conducted to test the efficacy of seeds in different concentration of effluent. The results obtained were tabulated as (TABLE -3& 4). Both Monocot and Dicot seeds were used to test the germination percentage. Comparatively Dicot seeds (seeds of *Vigna mungo*) showed a higher percentage in germination than monocot seeds (seeds of *Sorghum spp*). In combined with this, shoot length, and dry matter were also studied. Dicot seeds showed good results than monocot seeds.

Chlorophyll estimation in Petri dish experiment

The pigment analyses, chlorophyll a, chlorophyll b & total chlorophyll were estimated after 15 days of germination, grown in the different concentrations of effluent (Baskaran *et al.*, 2009). Similar to this work, the work was carried out results were obtained, tabulated in the chlorophyll content were increased as decrease in the concentration of the effluent.

TABLE - 3 Estimation of germination percentage, shoot length and dry matter, chlorophyll content of dicot plant in Petridish culture at different concentration Black gram (*Vigna mungo* L) Vamban.

S.No	Treatment	Germination	Shoot length (cm)	Dry matter (g/plant)	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total chlorophyll (mg/g Fw)
1	Control	100	11.8	0.192	1.159	0.462	1.397
2	20%	70	9.8	0.183	1.091	0.458	1.341
3	40%	50	8.3	0.136	1.060	0.450	1.308
4	60%	40	6.5	0.121	0.984	0.452	1.251
5	80%	30	4.3	0.115	0.936	0.467	1.231
6	100%	10	3.3	0.096	0.861	0.466	1.172

TABLE:4-Estimation of germination percentage, shoot length and dry weight, chlorophyll content of monocot plant in petridish culture at different concentration-Jowar (*Sorghum vulgare* Pers.) Heera

S.No	Treatment	Germination (%)	Shoot length (cm)	Drymatter (g/plant)	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total chlorophyll (mg/g Fw)
1	Control	90	8.6	0.582	1.601	1.397	2.757
2	20%	70	6.3	0.511	1.518	1.381	2.676
3	40%	60	5.4	0.467	1.364	1.302	2.471
4	60%	50	4.8	0.416	1.271	1.299	2.396
5	80%	30	3.9	0.362	1.099	1.229	2.187
6	100%	20	3.1	0.325	1.043	0.903	1.789

Pot culture experiment

Pot culture experiment was also conducted. Germination percentage, shoot length and dry matter were high in normal soil than polluted soils (Vijayaragavan *et al.*, 2011) reported that as the concentrations of effluent increases, there was decrease in the chlorophyll content, similar to this, chlorophyll content was high than the polluted soil. The results obtained were recorded in the (TABLE -5 & 6).

TABLE: 5-Estimation of germination percentage, shoot length, dry matter, chlorophyll content of dicot plant in pot culture - Black gram (*Vigna mungo* L) Vamban-2

S.No	Treatment	Germination (%)	Shoot length (cm)	Dry matter (g/plant)	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total chlorophyll (mg/g Fw)
1	Normal soil	80	13.97	0.212	1.295	0.434	1.471
2	Polluted soil	40	9.84	0.186	0.979	0.379	1.168

TABLE: 6-Estimation of germination percentage, shoot length, dry matter, chlorophyll content of monocot plant in pot culture - Jowar (*sorghum vulgare* Pers.) Heera.

S.No	Treatment	Germination (%)	Shoot length (cm)	Dry matter (g/plant)	Chlorophyll a (mg/g Fw)	Chlorophyll b (mg/g Fw)	Total chlorophyll (mg/g Fw)
1	Normal soil	90	16.42	0.602	1.631	1.585	2.985
2	Polluted soil	50	11.58	0. 524	1.151	0.894	1.862

CONCLUSION

The study concluded that physico-chemical parameter such as the pH, EC, Turbidity, TS, TDS, TSS, BOD, COD, Chloride, Sulphate, calcium, Magnesium, Sodium, Potassium and heavy metal Zinc these are high level in sugar mill effluent. When effluent irrigated in the agricultural land was severely affected the soil properties. The germination studies were studied the seed showed the polluted soil shows the low response compared to the non polluted soil. The shoot length, dry weight, chlorophyll content high response compared to the polluted soil. As the concentration increase the germination percentage decrease. The growth response shows the deposition toxic substance from effluent to soil. So the continuous irrigation should be avoided. So the effluent cannot be utilized for irrigation.

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SCREENING OF MARINE GREEN ALGAE *ULVA SPECIES* FOR ANTICOAGULANAT ACTIVITY

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ABSTRACT

The marine green algae *Ulva* sp were collected from Muttam, Kanyakumari District, Tamil Nadu. The algal extract was prepared from the dried, depingmented samples using sterile distilled water and concentrated by precipitation with Acetone. The prepared algal extract was used for anticoagulant assay against human blood plasma and the results of clotting test were expressed as a clotting time ratio, the prepared algal extract was found to be 2.3 PT (Prothrombin time) time ratio. In chemical analysis, algal extract contains 24.10% total carbohydrate and 4.82% proteins. The FTIR analysis 23 functional ions/groups were found between 400-4000 nm and showed the presence of sulphated ions.

Keywords: Green algae, anticoagulant activity, *Ulva* sp., sulphated polysaccharides

INTRODUCTION

Heparin is the most widely known and therapeutically used glycosoaminoglycan and has powerful blood anticoagulant activity. It can be used from an inner anticoagulant surface on various experimental and medical devices such as test tubes and renal dialysis machines. Heparin acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the blood (Bjork and Lindahl 1982). Although heparin is used as a primary anticoagulant drug, it has disadvantages such as production difficulties, hemorrhagic-like side effects (Shanmugam and Mody 2000), an osteoporosis effect with long-term use, and lack of oral bioavailability (Albuquerque *et al*. 2004). Because of the above limitations of heparin, there is a need for antithrombosis research for discovering effective, safer, and easier-to-use anticoagulant agents for short-term treatment of arterial and venous thromboembolic disorders and for long-term prevention of recurrences (Srivastava and Kulshreshtha 1989).

The discovery of alternative anticoagulant molecules must be an important task for scientists. It has been done research in anticoagulant activity of polysaccharides and glycosaminoglycans of diverse sources such as: ascidians (Pavão *et al.*, 1998), sea cucumbers (Vieira *et al*., 1991), echinoderms (Mourão *et al*., 1996), tunicates (Cavalcante *et al*., 2000). These molecules have different degree of sulfate in their structure and therefore are capable of substitute heparin (Farias *et al*., 2000). In 1913, scientists investigated blood anticoagulant properties of marine brown algae (Killing, 1913).

In recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products, and their blood anticoagulant, antimutagenic, antiviral, hypoglycemic, and anti-inflammatory properties (Srivastava and Kulshreshtha 1989) have been reported. Marine algae are a rich source of sulfated polysaccharides with novel structures, and these compounds have anticoagulant properties. Various anticoagulant-active polysaccharides, especially from red and brown algae, have been isolated and characterized (McLellan&Jurd, 1992). They contain a variety of sulfated galactans and sulfatedfucans, which are among the

most abundant non mammalian sulfated polysaccharides found in nature (Harada *et al.*, 1998). In this present study, an algal extracts from the marine green algae *Ulva* species (it's consumed by local inhabitants as a marine vegetable) was screened for anticoagulant activity.

MATERIALS AND METHODS

Sample collection and processing

Marine green algae *Ulva sp*) was collected from sea shore of Muttom, Kanyakumari district, Tamil Nadu. A matured, healthy sample was collected in a sterile polythene bag and immediately it was transferred into laboratory for further processing.

Salt, sand and epiphytes present on the surface of sample were removed by cleaning the sample with tap water. After cleaning, it was dried at room temperature and made fine powder using electric mixer grinder. Then the powdered sample was depigmented by using methanol.

Preparation of algal extracts

One gram of the ground algal powder was mixed with 50 ml of water and placed in shaking incubator for 12 hours at 70°C. The mixtures were centrifuged at 3500rpm for 20 min at 4°C and filtered with Whatman filter paper. Finally, the supernatant was preliminarily subjected for anticoagulant assay.

The extract was concentrated under reduced pressure. The concentrated samples were precipitated with acetone (1:4 v/v) dehydrated with acetone and dried at 40^0C. The dried product was dissolved in minimum distilled water, and lyophilized to obtain crude sulphated polysaccharide product. The Yield of crude sulphated polysaccharide was calculated by dry weight basis (Shanmugam *et al*., 2001).

Blood coagulation assay

Preparation of plasma: Human blood was collected from healthy volunteers and normal human plasma was prepared as follows: blood was anticoagulant using 3.8% tri sodium citrate solution and it was centrifuged immediately at 3000rpm for 15 minutes. The Plasma was separated and pooled. The pooled plasma was stored at 4^0C and it was used for further process.

Preparation of algal sulphated polysaccharide: The Algal sulphated polysaccharide sample was prepared in normal saline solution. 0.85 g of sodium

chloride was mixed in 100 ml of distilled water. From this solution 1 ml was used for Algal sulphated polysaccharide preparation (Yasantha Athukorala *et al*. 2001).

Anticoagulant activity: Anticoagulant activity of algal sulphated polysaccharide was tested using prothrombin time test with 750ug/ ml concentration. All clotting and control test were performed in duplicate, and average of the two duplicate was recorded. Standard blood anticoagulant heparin140.3 units/mg was used for comparative study. The results of the clotting test were expressed as a clotting time ratio. The ratio was obtained by dividing the clotting time achieved with algal sulphated polysaccharide included in the system by the time achieved under similar conditions with normal saline solution (Shanmugam *et al*., 2001).

Chemical analysis

The carbohydrates concentration was measured by phenol-sulphuric acid method (Krishnaveni *et al*., 1984) and total protein content was estimated using the protocol of Lowry *et al*., (1951). Then the sample was subjected to Fourier Transform Infrared Spectrophotometer (FTIR) analysis. The spectrum (400-4000 nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement (Williams and Fleming, 1989).

RESULT AND DISCUSSION

In recent years, investigation of anticoagulant, thrombolytic, and antithrombic reagents from various sources has become a high priority in biomedical research because of the many disorders of blood clotting and fibrinolysis (Matsubara *et al*. 2000). In the present study, anticoagulant activity of the green algae *Ulva* species was screened by prothrombin test (Shanmugam *et al*., 2001).

Blood coagulation assay

All clotting and control test were performed in duplicate, and average of the two duplicate was recorded and heparin for comparative study also recorded. The results were mentioned in table 1.

Clotting time ratio = clotting time achieved with algal sulphated polysaccharide / clotting time achieved normal saline solution = 46/20 = **2.3**

Table: 1 Prothrombin time test

Test sample	Concentration of samples (ul)	Concentration of plasma (ul)	No. of test	Prothrombin time (min)	Average	PT time ratio
Control	-	200	1	8	8	-
			2	8		
Algal SPS	10	190	1	47	46	2.3
			2	45		
Heparin	10	190	1	>120	>120	>11
			2	>120		
Normal saline	10	190	1	20	20	-
			2	20		

The results of the clotting test were expressed as a clotting time ratio. The ratio was obtained by dividing the clotting time achieved with algal sulphated polysaccharide included in the system by the time achieved under similar conditions with normal saline solution

Chemical analyses

The sulfated polysaccharides extracted from *Ulva sp.* have high carbohydrate contents (24.10%) than total protein (4.82%). The sulfated polysaccharides mainly are composed of rhamnose (Wenjun Mao *et al.*, 2006). In FTIR analysis, there are 23 functional ions/groups were detected between 400-4000 nm spectra (Fig. 1).

The two peaks seem to be obvious basic knowledge in the sulphate group detection, the absorption bands can be observed at 3434.37 cm^{-1} (ascribed to O-H stretching), 2925.15 cm^{-1} (C-H stretching), 1632.80 and 1099.46 cm^{-1} (symmetrical and asymmetrical stretching of S-O in SO_3 heparin groups, respectively), 1100-1000 cm^{-1} (Si-O-Si stretching) and at 850 cm^{-1} (=C-H bending). However, observations of the type described can be invaluable in determining the presence of specific functional groups when used in combination with the infrared spectrum.

Similarly, the use of negative spectral curves, viz. the absence of a characteristic group frequency, is equally important. For example, if extracted polysaccharide compound have honey like viscosity and color. In spectra it have a strong peak in between 1200 and 1000 cm-1, if the compound is more sharp in its curve it maybe a sulphate compound or benzaldehyde. If an aldehydic carbonyl group is absent, then the compound probably contains a sulphate group. In context with heparin compound detection the values are the absorptions at 1023.27 and 1124.54 cm^{-1} are considered as a fingerprint of heparin. Therefore, the presence of the absorptions recognized to sulfur-containing groups in the spectrum denotes the preservation of the heparin structure.

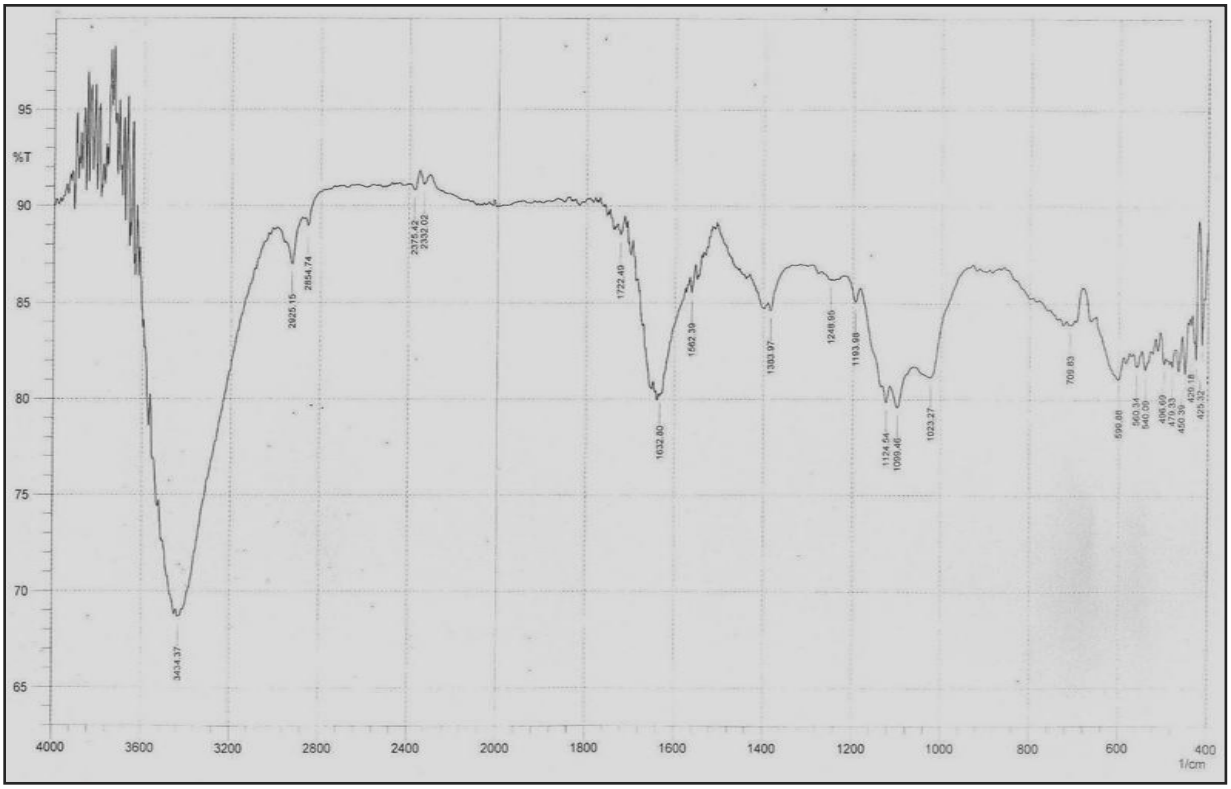

Fig: 1 FTIR chromatogram of Algal extract

Spectrum is of a film deposited from a heparin/acetone mixture. Owing to the presence of OH groups in the monomer and therefore probably also in the film, a band at around 3434 cm^{-1} is expected, but is in medium peak. Two peaks, at 2925.15, 2854.74, confirm the presence of CH groups. The band centered around

1632.80cm^{-1} is indicating the presence of C = O groups. These interpretations are reliable with the features of infrared spectra of films produced. Bands characteristic of heparin (at 1250 and 1020 cm^{-1}) are very little amount, possibly representing poor preservation of the heparin in the deposited material.

Most of green algae exert their anticoagulant activity through polysaccharide, or proteoglycan. In many cases, arabinose and its sulphate derivatives elicit anticoagulant activity of green algae. The anticoagulant activity of most green seaweeds are due to sulphated arabinan, however, sulphated arabinogalactan also have been reported as possible anticoagulant compound in green algae. Therefore, arabinan and sulphate groups of the isolated compounds play a crucial role in prolonging anticoagulant activity of these algal species. As a conclusion, this study prevailed use of Algal biomass for the preparation of medicinally valuable products.

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PHYTOCHEMICAL STUDIES IN THE LEAVES OF *ECBOLIUM LINNEANUM*- THE MEDICINAL PLANT

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ABSTRACT

The Medical plant, Ecbolium Linneanum, belongs to the botanical family of acanthaceae. All the parts of this plant have traditional medicinal values. In the present work, the extracts from the different solvents (ethanol, water, chlototorrm) can be subjected to the separation of compounds like, xanthoprotein, alkaloid, reducing sugar, steroids, saponins, tannins, phenolic compounds and carbohydrate. Quantitative determinations like moisture content, total ash, acid insoluable ash, water soluble ash and residue on ignition can also be determined. From the results, it is cleat that ethanol is the best solvent to show chemical constituents than the other solvents.

Keywords: *Ecbolium Linneanum*, extract, solvents, Acanthaceae)

INTRODUCTION

Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich health, wealth and a large number of diverse plants grown in different parts of the country. India is one of the richest countries in the world with regard to the diversity of medicinal plants.

The importance of medicinal plants has been emphasized from time to time due to their more safety and less side effects. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Medicinal plants are claimed to be useful for wound healing in the traditional system of medicine. The discipline of medicines in medicinal chemistry is devoted to the discovery and development of new agents for treating diseases.

Large numbers of medicinal plants are constantly being screened for their possible pharmacological value particularly of their anti- inflammatory, hypersensitive, hypoglycemic, anti- fertility, anti biotic and cytotoxic properties.

Chemical constituents

Chemical constituents of herb may be therapeutically active or inactive. The curative actions of the medicinal plants are also to be identified for the therapeutic value of the bioactive ingredients which are characterized. The most important of these bioactive compounds of plants are Alkaloids, Phenolic compounds, Flavannoids, Tannins and Saponins.

Alkaloids

Alkaloids are a structurally diverse group of over 12,000 cyclic nitrogen containing compounds that are found in over 20% of plant species. Alkaloids are very important in medicines and they constitute most of the valuable drugs. They have physiological effect on the human body, For instance, individual alkaloids act as agonists and antagonists to a variety of neurotransmitter systems. The specific biological functions of alkaloids are (i) They may act as reserved substances to supply nitrogen (ii) They may be the end products of detoxification mechanisms (iii) They may act as poisonous substances which afford plants safely from herbivores and insects, (iv) They may act as reservoirs for protein synthesis.

Phenolic Compounds

Phenolic compounds are widely distributed in the plant kingdom. Presence of phenols is considered to be potentially

toxic to the growth and development of pathogen. Most of these chemical groups are expectorants and emulsifying agents, Phenols are found across the plant kingdom, with 10,000 structures identified to date with a few notable exceptions.

Flavanoids

Flavanoids represent the largest and the most diverse groups, encompassing some 6000 compounds, all of which share a common underlying structure of two hexagonal rings, with a 3 - carbon bridge, which usually forms a third ring. Flavanoids can then be subdivided according to modification of this basic skeleton into chalcones, flavones, flavanones, isoflavones, flavan - 3- ols and anthocyanins. Flavanoid are 15 - carbon compounds generally distributed throughout the plant kingdom. Flavanoids constitute one of the most characteristic classes of compounds in higher plants. Many flavanoids are easily recognized as flower pigment in most angiosperm families. Flavanoids are the most important plant pigment for flower coloration producing yellow or red / blue pigmentation in petals designed to attract pollinator animals.

Tannins

Tannins are fairly potent bioactive compounds of vegetable origin found in medicinal plants frequently encountered in food products of plant parts such as tea leaves and fruits, that can be used for therapeutic purposes. Tannins have molecular weights ranging from 500 to over 3000 (gallic acid esters) and upto 20,000 (proanthocyanidins). They are commonly found in both gymnosperms as well as Angiosperms. Tannins are mostly located in the vacuoles or surface wax of plants these storage sites keep tannins active against plant predators, but also keep some tannin from affecting plant metabolism while the plant tissue is alive; it is only after cell breakdown and death that the tannins are active in metabolic effects.

Saponins

Saponins are a class of chemical compounds one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species. The aglycone (glycoside - free portion) of the saponins are termed as sapogenins. The number of saccharide chains attached to the sapogenin / aglycone core can vary, giving rise to another dimension of nomenclature as can the length of each chain. Aglycone derivatives can also incorporate nitrogen,

so that some saponins also present chemical and pharmacologic characteristics of alkaloidal products

A lot of medicinally important attributes have been assigned to this family of plants, to which Ecbolium Linneanum belongs. The Taxonomic classification of Ecbolium Linneanum is Vital in furthur understanding their accurate botanical description and to the magnitude of their diversity and usefulness

Taxonomical classification

Kingdom: Plantae

Class: Angiosperm

Phylum: Eudicots

Order: Lamiales

Family: Acanthaceae

Genum: *Ecbolium*

Species: *Linneanum*

Binomial Name: *Ecobolium Linneanum*

Common Name: Blue fox tail

Ecbolium Linneanum (Family: Acanthaceae)

It is an indigenous Indian plant that grows naturally along the eastern parts of India. It has been found in Africa and tropical Asia and also in Mumbai and konkan region. It is a shrubby plant with 4 sides flower spikes at the end of branches. Bracts are oval, entire and mucronate leaves are elliptic, oblong narrowed at both ends, velvety. Flowers are large, greenish blue in color. Upper lip of the flower is linear, reflexed. It is an erect shrub with smooth, hairless stems and leaves.

Physical Characteristics

The plant prefers light (sandy) and medium (loamy) soils and requires well - drained soil. The plant prefers neutral and basic (alkaline) and can grow in very alkaline soil. It cannot grow in the shade. It requires dry and moist soil.

Uses

The medicinal uses of this plant are that, it is used in the treatment of cancer, cardiovascular disease and weak immune system. The roots of this plant are used to cure jaundice, menorrhagia and rheumatism. It is also used as an anti inflammatory activity. Root juice is used as antihelmintic and also to treat premenstrual colic. It possess some pharma cological properties, they may be toxic or mutagenic leaves are used as wound healing and also used as an anticancer agent.

MATERIALS AND METHODS

Ecbolium Linneanum was collected from Marthandam in Kanyakumari District. The leaves of *Ecbolium Linneanum* were washed with water and air dried over a period of two weeks. The dries leaves were finally powdered and extracted with various solvents such as ethanol, water and chloroform for the determination of phytochemical characters.

Table -1

S.No	Experiment	Observation	Inference
1.	Test Solution + minimum amount of $CHCL_3$ +3 drops of Conc. H_2SO_4.	Purple colour changes to blue or green	Presence of steroids
2.	Test solution + equal volume of Fehling solution A and Fehling solution B and heated in a water bath.	Red CU_2O precipitate is formed	Presence of reducing sugars.
3.	Test solution + 10% NaOH solution and heated	Solution is burned	Presence of carbohydrate
4.	Test solution is shaken with 2N HCl. A queous layer formed is separated and to which one or two drops of mayer's reagent are added	White precipitate is formed	Presence of Alkaloids
5.	Test Solution + conc. HNO_3+ Excess of NH_3.	Reddish orange precipitate is formed	Presence of Xanthoproteins
6.	Water soluble portion of the extract is treated with lead acetate solution	White precipitate is formed.	Presence of Tannins.
7.	Test Solution + Mg powder and treated with con. HCl while cooling the test tube under running water.	Orange colour is formed	Presence of Flavanoids
8.	Test solution + Tollens reagent	Silver mirror	Presence of Reducing sugars
9.	Test solution + Molisch's reagent	Purple colour	Presence of Reducing Sugars
10.	Test solution + Water + Shaken well	Foamy lather	Presence of saponins
11.	Test solution + neutral $Fecl_3$+1ml ethanol	Any changes	Presence of Phenol

Quantitative determination

The percentage of moisture content on leaves, total ash, acid insoluble ash, water soluble ash and residue on ignition were obtained by employing standard method of analysis described in pharmacopoeia of India (1966).

Determination of moisture content

A known quantity of leaves were weighed and allowed to dry under shade until a constant weight was obtained. From the initial and final weights, the loss of weight on drying was calculated.

Determination of total ash

A known quantity of dried samples of the leaves of *Ecbolium Linneanum* was taken in previously weighed silica crucible and ignited carefully not exceeding dull red heat until the ash was free from carbon. The crucible was cooled and weighed. The percentage of ash with reference to the air - dried was calculated.

Determination of acid - insoluble ash

A known weight of ash (about 0.lg) was boiled with 25ml of dilute hydrochloric acid (2N). The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to constant weight and weighed. The percentage of acid insoluble ash with reference to the air - dried sample was calculated.

Determination of water soluble ash

A known weight of the ash (0.2g) was boiled with 25ml of distilled water. The insoluble matter was collected in a previously weighed sintered crucible, washed with hot water, dried to a constant weight and weighed. The percentage of water soluble ash with reference to the air - dried sample was calculated.

Determination of residue on ignition

A known weight of the air dried samples (2g) was taken in a previously weighed silica crucible and carefully incinerated till the ash was strongly ignited, cooled and weighed. The percentage of ignited ash with reference to the air dried sample was calculated.

Extraction method

Chemical method

Extractive value of the leaves of *Ecbolium Linneanum* in water, ethanol and chloroform were determined by employing the methods of analysis in pharmacopoeia of India (1996). About 1g of air dried samples were taken in a stopper flask, 20ml of the solvent was added, shaken well and allowed to stand for a hour with occasional shaking, then the content was filtered. 10ml of the filtrate was pipette out

into a clean previously weighed china dish and evaporated in a water bath. Finally it was dried at 105°C cooled and weighed. The percentage of solvent soluble extract with reference to the air dried sample was calculated.

RESULTS AND DISCUSSION

The systematic chemical analysis and phytochemical investigations were carried out on the leaves of *Ecbolium Linneanum*

All the tests shows that the leaves contain predominant amount of ash total (9.4%), acid - insoluble ash (30%), water soluble ash (38.5%) and residue on ignition contents (12.15%) and moisture content (44%).

Table -2

Sl. No	Particulars	Leaves (%)
1	Moisture content	44%
2	Total ash	9.4%
3	Acid insoluble ash	30%
4	Water soluble ash	38.5%
5	Residue on ignition	12.15%

Table -3

Sl. No	Solvents	Leaves (%)
1	Water	33.20
2	Ethanol	43.14
3	Chloroform	15.8%

The above results show that the amount of extract is larger in Ethanol as a solvent than the other solvents.

Table – 4

Preliminary Phytochemical Analysis of the leaves of *Ecobolium Linneanum*

S.No	Extracts	Steroids	Reducing Sugars	Alkaloids	Phenolic Compounds	Saponins	Xantho Proteins	Tannins	Flavanoids	Carbohydrates
1	Water	-	+	-	+	+	-	-	-	-
2	Ethanol	-	-	+	+	-	-	-	+	-
3	Chloroform	+	-	-	+	-	-	-	-	-

+ present; - absent

The water as the solvent shows the presence of saponins, reducing sugars and phenols. The test for steroids, tannins, flavanoids, alkaloids gave negative results i.e, the group of compounds are absent in water extract. The ethanol extract gave the positive results for Alkaloids, Flavanoids and phenolic compounds, while the other compounds are absent.

The chloroform extract indicates the presence of phenols and steroids. The test for tannins, Reducing sugars and alkaloids gave negative results i.e, the group of compounds are absent in chloroform extract. It is found that the water and chloroform is failed to extract the alkaloids, whereas ethanol extracted it. From the table, it is clear that from the leaves of *Ecbolium Linneanum* , Ethanol is

the better extracting solvent than the other two solvents.

CONCLUSION

The Medicinal plant, *Ecbolium Linneanum* belongs to the botanical family of Acanthaceae. All the parts of these plants have traditional medicinal values. The extracts from the different solvents can also be subjected to the separation of compounds like xanthoprotein, alkaloids, reducing sugar, Steroids, saponins, tannins, phenolic compounds and carbohydrate.

In the present work, the extraction, characterisations of the leaves of *Ecbolium Linneanum* were carried out. From the above discussion, it is clear that ethanol is the best extracting solvent than the other solvents. However, further work is needed to isolate the chemical constituents from the plant extract and to carry out pharmaceutical studies.

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FOLIICOLOUS FUNGI COLLECTED FROM NORTH-EASTERN UTTAR PRADESH, INDIA PARASITIZING *CALAMUS TENUIS* ROXB, A MEDICINAL PLANT

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ABSTRACT

Two species of foliicolous fungi have been collected and described from the leaves and leaf sheath of *Calamus tenuis* Roxb., a medicinal plant, named *Dwibahubeeja indica* and *Melanographium calami.*

Keywords: Foliicolous Fungi, Medicinal Plant, *Calamus tenuis.*

INTRODUCTION

Calamus tenuis Roxb is a plant of Family – Arecaceae. It is used as a medicinal plant by the folk medicinal healers of Bangladesh (Rahmatullah *et al.* 2010). Juice from shoot tips and roots are taken in the morning and evening for seven days and used as antidote to poisoning.

The North-Eastern Uttar Pradesh of India experiences a humid sub-tropical climate and is adorned with lush green vegetation represented by an array of angiosperms and ferns. On these plants, foliicolous fungi, particularly those belonging to the Deuteromycotina frequently occur. Consequently, our earlier surveys of this region have resulted in the description and illustration of several such fungi which were new to science (Chandra *et al.* 1991; Srivastava *et al.* 1994 & 1995; Srivastava and Morgan-Jones, 1996; Misra *et al.* 1997).

In one of our recent surveys of North-Eastern Uttar Pradesh, a number of foliicolous fungi were collected. Amongst these collections were two hyphomycetous forms causing leaf spot diseases to *Calamus tenuis*, which are described and illustrated in this communication.

MATERIALS AND METHODS

The infected specimens were viewed with unaided eyes so as to have an idea about the nature of symptoms produced, and shape and size of lesions formed on the leaf surface. Scrap mounts of infected portions of the leaves were prepared in Lactophenol-Cotton Blue for preliminary examination and detailed observations and drawing purposes as well. The preparations were examined with the help of compound microscope using different eye pieces (15x) and objective (10x, 40x, 45x and 100x) combinations. Figures showing all the morphological details of reproductive propagules were drawn carefully with the help of Camera Lucida. The measurements of different relevant structures were also taken side by side.

RESULTS AND DISCUSSION

Two different foliicolous hyphomycetes have been described and illustrated which are pathogenic to the medicinal plant *Calamus tenuis* Roxb., as follows:

Dwibahubeeja indica (Fig. 1-3)

Infection spots amphigenous, primarily small, then coalescing to form irregular, large patches. Colonies

epiphyllous, brownish-black with straw-yellow margin. Fungal mycelia are branched at right angles, sparingly euseptate, dark brown. Hyphopodia capitates, globose, thick-walled, 4-8 *u*m wide. Conidiogenous cells intercalary on external hyphae, cylindrical to doliform, 1-2 *u*m long. Conidia bifurcate, conidial base 6-21 *u*m wide, arms cylindrical, each arm 4-20 euseptate, 30-280 *u*m X 3-10 *u*m.

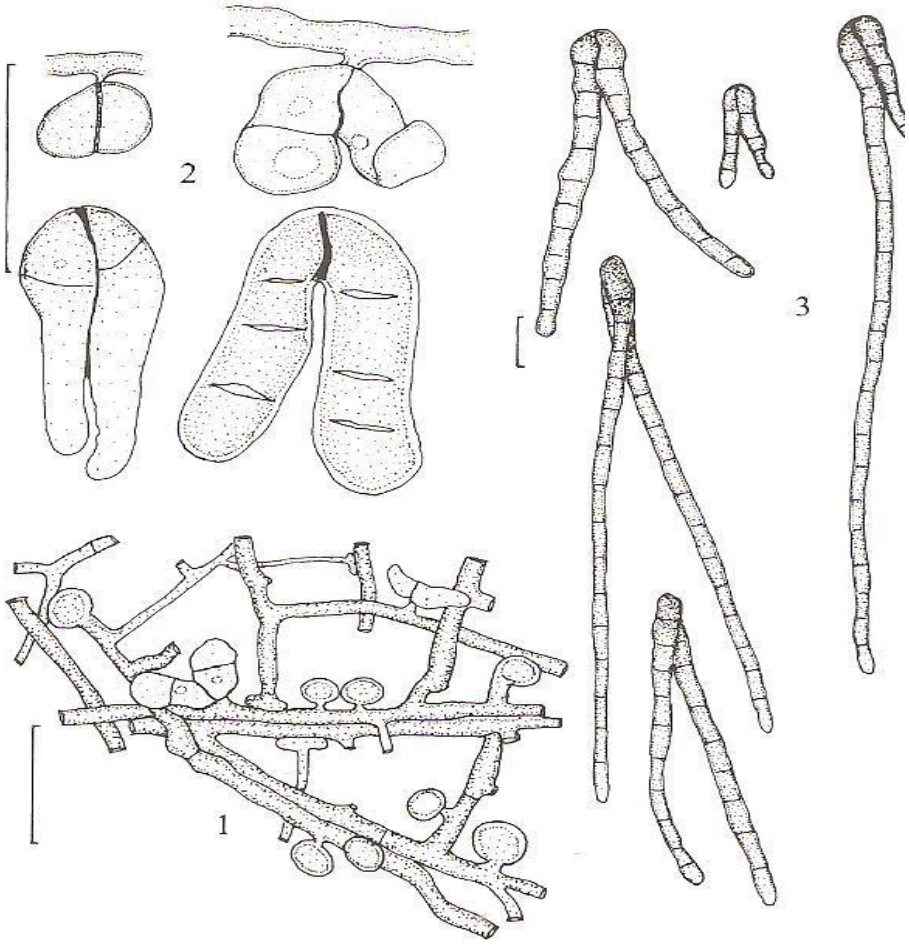

Fig. 1-3. *Dwibahubeeja indica.* Fig. 1. Mycelium; Fig. 2. Young Conidia;Fig.3. Mature Conidia. Scale Bars = 20 *u*m.

Melanographium calami (Fig. A, B and C) Colonies effuse velvety, dark blackish-brown. Mycelium immersed, composed of branched, septate, smooth thick-walled, pale brown to brown, 2-4.5 *u*m wide hyphae. Stromata distinct, partly immersed to erumpent and more or less superficial, pseudoparenchymatous, composed of mostly

isodiametric, brown to dark-brown cells, upto 80 *u*m wide X 60 *u*m high (30-80 X 35-60 *u*m). Conidiophores originating from the upper, outer cells of the stromata in loose or somewhat dense 80-300 *u*m wide fascicles, macronematous, mononematous, caespitose, spreading, usually gently flexuous or straight, simple, erect to procumbent, smooth, brown to dark brown, somewhat paler towards the apex up to 6 septate, 100-300 *u*m long, 5-7 *u*m wide at the base, 5-8 *u*m wide towards the apex. Conidiogenous cells terminal, integrated, polyblastic, indeterminate, sympodial, cylindrical, bearing a number of unthickened , inconspicuous conidial scars. Conidia acrogenous, formed holoblastically at the apex of each conidiophores and from successively produced conidiogenous loci, solitary, dry, unicellular, smooth, thick-walled, very strongly curved giving a more or less horse-shoe aspect, brown to dark brown, rarely with a hyaline longitudinal slit, 16-25 *u*m in diameter, 10-13 *u*m wide at the broadest central part.

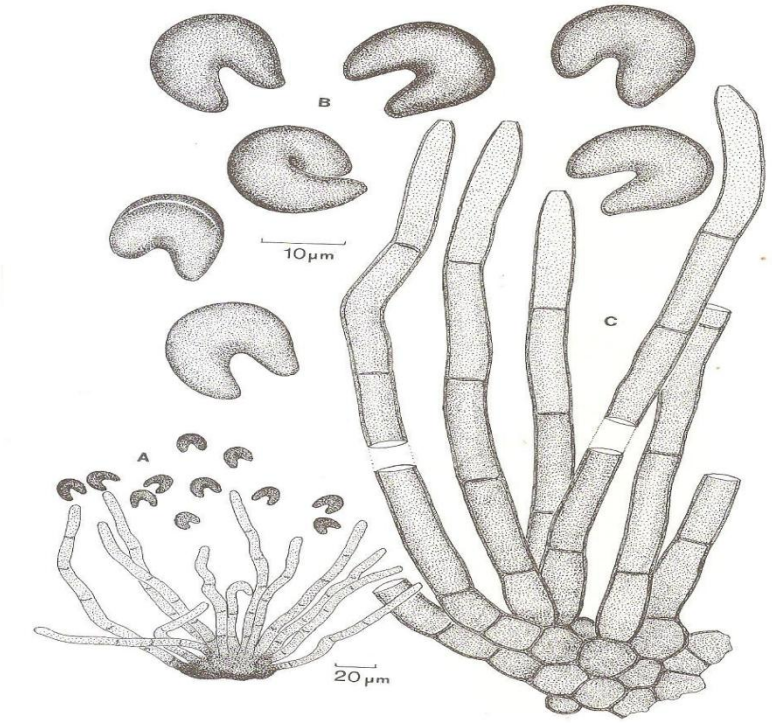

Fig. A-C. *Melanographium calami.* Fig. A. Stroma, Conidiophore fascicle and Conidia; Fig. B. Conidia; Fig. C. Portion of Stroma and Conidiophores. Scale Bars = 10 *u*m.

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BRYOFLORA OF MUKTESHWAR

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ABSTRACT

Present study is an attempt to explore the bryoflora of almost undisturbed and natural habitat of Mukteshwar (Kumaon hills). For this study several regular and periodic surveys were made to Mukteshwar in a span of more than one decade. Bryophytes species were collected identified in lab or inter laboratory and described. It was observed that some species were growing luxuriantly in one ecological niche and were totally absent at neighboring niche. It may be due to influence of edapho-climatic factors, micronutrients and man-made activities.

Keywords: Bryoflora, Natural habitat, Mukteshwar

INTRODUCTION

Bryophytes form an important and striking part of cool and humid Himalayan scenario. They impart lush greenery and a verdant cover in every possible shade of green tinged with hues of brown, red and yellow to almost all kinds of habitat like rocks, boulders, stones, hill slides, tree trunk, forest cover and various artificial substrates in and around Kumaon hills (Pant and Tiwari, 1989). Bryophytes are ecologically important, diversified plant communities; differ morphologically and physiologically from vascular plants (Saxena and Saxena, 2005). Although bryophytes form a minor component of the total biomass of biota, even though they play a major role in nutrient cycling (Raeymaekers, 1987). The percentage of occurrence of mosses in India is quite high as compared to any other plant group. About 27.5% of world mosses and 11.26% of liverworts are present in India (Banerjee, 1998).

Due to rapid urbanization and with the pressure inflicted by the inexorable growth of the human population, the natural vegetation of the Himalaya is disappearing at an alarming rate. Rapid deforestation has resulted into a loss of an appreciable resource, the gene pool inherent in these Himalayan plants. On the other hand we have to realize the harsh reality that the study of our Himalayan cryptogamic flora still is in a neglected state of infancy. Therefore, there is an urgent need to explore, collect, study and protect this unique cryptogamic mosaic of our fragile Himalayan ecosystem. In the present day, in the state of utter disturbance, these plants are fast vanishing from their habitats without being scientifically catalogued or studied. No attempt has been made in last few years to study the bryoflora and species richness on Kumaon hills. Therefore, present study was undertaken to explore the bryophyte wealth on Kumaon Himalaya.

MATERIALS AND METHODS

Bryophytes were collected from different pockets of Muketshwar in different seasons (Figure 1). The collected bryophytes were tentatively identified and send for different laboratories for inter-laboratory identification. The voucher specimens were prepared and placed in the bryology laboratory of Bareilly College, Bareilly.

RESULTS AND DISCUSSION

Poor and localized bryo-vegetation was observed at lower elevation, while increasing altitude with mesic condition promotes species richness and luxuriance. In present study a total of 44 bryophytes

species are recorded from the study area Table 1). The study further demonstrates that mosses are the predominate component of bryo-vegetation of study area. These findings are in broad agreement with earlier studies conducted by Pandey, 1984 and Tiwari, 1984.

Study revealed the presence of *Frullania sp., Conocephalum sp., Lajunia sp.* in pure and undisturbed habitats while *Plagiochasma sp., Polytrichum sp., Hydrogonium sp. and Hypnum sp.* were growing well along the roadside environment. Thus such biomapping studies can be used for pollution monitoring studies and for constructing the pollution map of study area.

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Figure 1: Map of the Kumaon Hills showing study area

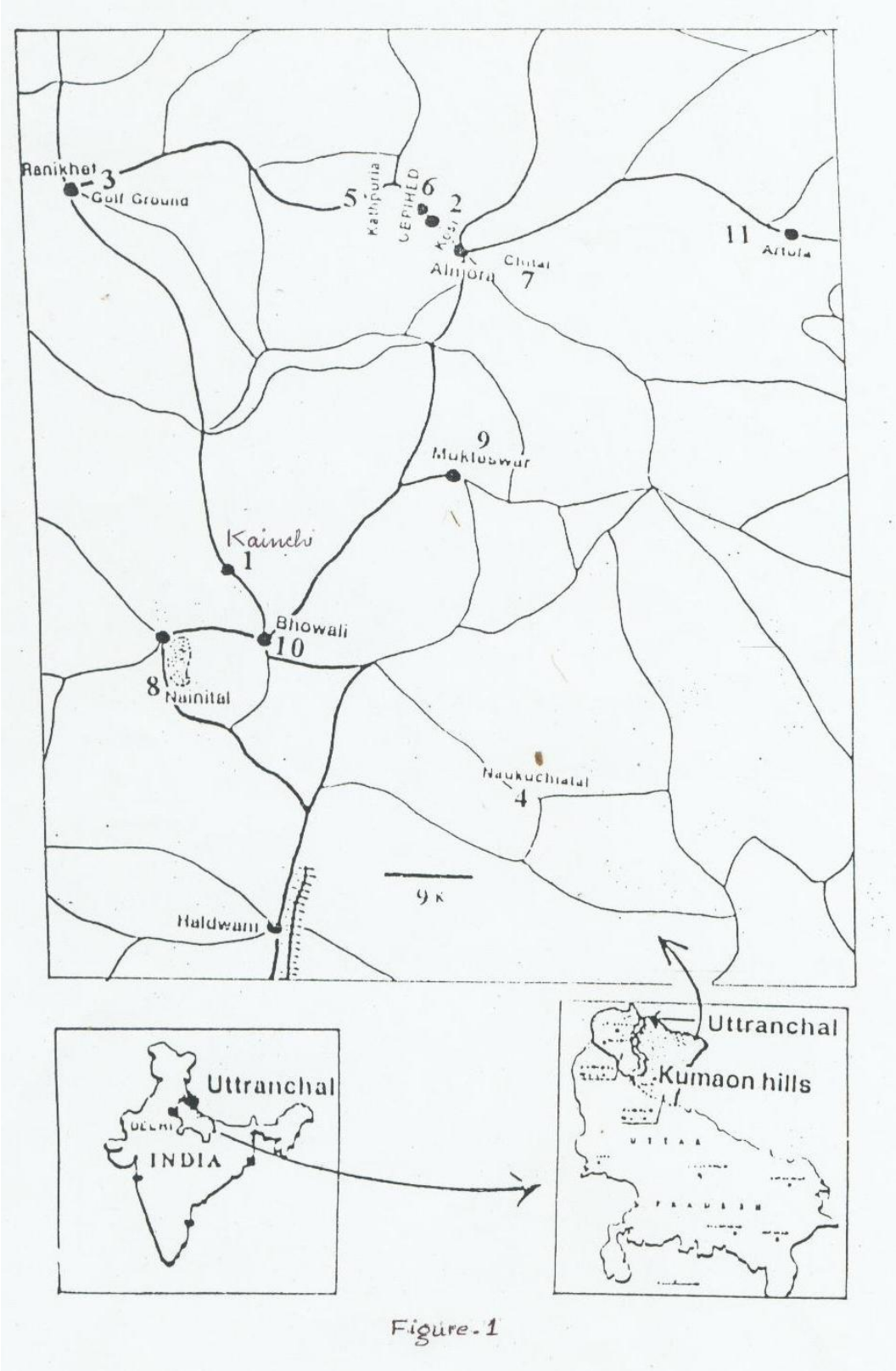

Figure-1

Table 1: Bryoflora of Kumaon hills

S.No.	*Bryophyte*	Location	Habitat	Altitude
1	*Asterella wallichiana*	On the way to Mukteshwar	Along the road side on stone wall	1000
2	*Atrichum pallidum*	Near IVRI, Mukteshwar	On soil in forest	1800
3	*Rachithecium perpusillum*	Natural forest of Mukteshwar	Epiphytic, on rock, on stone wall	2200-2240
4	*Barbula unguiculata*	On the way to Mukteshwar	On rocks	
5	*Brachythecium buchananii*	On the way Bhimtal and Mukteshwar	On soil in forest, rotting tree base	2210-2240
6	*Brachythecium sp.*	IVRI, Mukteshwar	On humus over soil, rocks	2250
7	*Brothera leana*	On the way Bhimtal and Mukteshwar	Epiphytic	
8	*Bryum sp.*	Along road side, on the way to Mukteshwar	On rocks	
9	*Campylium sp.*	Mukteshwar	On soil over humus	2215
10	*Campylopus subulatus*	Mukteshwar	between grass on a small hill	2215
11	*Campylopus umbellatus*	On the way from Bhimtal to Mukteshwar	On rock	
12	*Claopodium cf prinophyllum*	Mukteshwar, natural forest	on soil	2230
13	*Conocephalum conicum*	Mukteshwar	On rocks	
14	*Cryptoleptodon sp.*	Mukteshwar, natural forest	Epiphyte	2230
15	*Dicranum sp.*	Mukteshwar, natural Forest	On soil	
16	*Dumortiera sp.*	Mukteshwar, natural forest	On soil	
17	*Entodon sp.*	Mukteshwar	On rock	2215
18	*Fissidens sp.*	Mukteshwar, natural research forest	On soil	2240
19	*Floribundaria*	Mukteshwar, natural research forest	Epiphyte	2230
20	*Herprtineuron toccoae*	Bhimtal and Mukteshwar	Epiphytic	
21	*Homalia*	Bhimtal and Mukteshwar	Epiphytic	
22	*Hydrogonium sp.*	On the way to Mukteshwar	On rock	
23	*Hylocomium sp.*	On the way to Mukteshwar	On soil	
24	*Hymenostylium recurvirostrum*	Mukteshwar, natural forest,	On soil	2240
25	*Hypnum sp.*	between Bhimtal and Mukteshwar	On soil in forest	
26	*Hypophila javanica*	Mukteshwar, natural forest	On soil in forest	

27	*Lejunia sp.*	Mukteshwar, natural forest	On soil in forest	
28	*Lindbergia koelzii*	Mukteshwar	Epiphyte	2215
29	*Marchantia polymorpha*	On the way to Mukteshwar	On rock	
30	*Meteorium buchananii*	Mukteshwar, natural forest	Epiphyte	2230
31	*Mnium thomsonii*	Mukteshwar, natural forest	On soil	2230
32	*Notothylus sp.*	Mukteshwar, natural forest	Epiphyte	
33	*Orthotrichum pumilum*	Mukteshwar, hill behind hotel	Epiphyte	2215
34	*Palamocladium hilgheriense*	Mukteshwar, natural forest	Epiphyte	2230
35	*Plagiochasma appenticulatum*	IVRI, Mukteshwar	On soil	2280
36	*Plagiomnium rhynchophorum*	On the way from Bhimtal to Mukteshwar	On rock	
37	*Polytrichum commune*	Mukteshwar, natural forest	On soil	
38	*Rhynchostegium celebicum*	Mukteshwar, natural forest	on soil on a N-exp. steep slope	2240
39	*Rhynchostegium herbaceum*	Mukteshwar, natural forest	over a small stone on forest floor	2230
40	*Rhytidialphus squarrosus*	IVRI, Mukteshwar	On soil	
41	*Sciuro-hypnum plumosum*	Mukteshwar, natural forest	over flat rock on forest floor	2230
42	*Sematophyllum sp.*	between Bhimtal and Mukteshwar	epiphytic	
43	*Thamnobryum subseriatum*	Mukteshwar, natural forest	On rock	2230
44	*Trachypodopsis serrulata*	Mukteshwar, natural forest	On dead tree	2230

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PROTECTIVE EFFECTS OF *PHYLLANTHUS POLYPHYLLUS* EXTRACT ON CARBON TETRACHLORIDE - INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The study was aimed to investigate the hepatoprotective potential of *Phyllanthus polyphyllus* (whole plant) leaf methanol extract (PPLME) on CCl_4-induced hepatotoxicity in male Wistar rats. CCl_4 injection induced liver damage by a significant rise in serum marker enzymes. The hepatoprotection was assessed in terms reduction in histological damage, changes in serum enzymes (SGOT, SGPT, ALP) and metabolites bilirubin (BL). Pre-treatment of rats with different doses of plant extract (200, 300 and 400 mg/kg) significantly lowered SGOT, SGPT, ALP and BL levels against CCl_4-induced rats. Histopathological examinations showed extensive liver injuries, characterized by extensive hepatocellular degeneration/necrosis, inflammatory cell infiltration, congestion, and sinusoidal dilatation. Oral administration of the leaf extract at a dose of 200, 300 and 400 mg/kg body weight significantly reduced the toxic effects of CCl_4. The activity of leaf extract at the dose of 300 and 400 mg/kg was comparable to the standard drug, silymarin. Based on these results, it was observed that *P. polyphyllus* extract protects liver from hepatotoxicity induced by CCl_4 and thus helps in evaluation of traditional claim on this plant.

Keywords: ethnomedicine, *Phyllanthus polyphyllus,* hepatoprotective activity, carbon tetrachloride, silymarin

INTRODUCTION

Phyllanthus polyphyllus Willd. (Euphorbiaceae) is a small tree widely distributed in tropical and subtropical regions in India and Sri Lanka (Gamble, 1935). *P. polyphyllus* is one of the predominant elements found in abundance in the semiarid belts of Eastern and Western Ghats of South India. It used for the treatment of jaundice and locally called Keelkainelli (Viswanathan et al., 2005). A plant paste (15g) is consumed either with cow milk or Coconut milk once a day for 2 to 3 days for jaundice. The patient should take a bath daily. Consumption of salt, Pepper, fish and dry fish is prohibited. It is also used as an anti-inflammatory drug in folk medicine (Rao *et al*., 2006). The traditional healers (Nattu vaidhyas) of Kolli hills claim this plant to have certain important medicinal properties i.e., a potential antidiabetic and antidepressant drug.

Increase in scientific investigations into indigenous wealth of herbal medicines gives ample evidence of medicinal plants as sources of drugs (Bannerman, 1980). Liver damage in animal models can be induced by CCl_4, from which free radical derivatives are biotransformed and lead to increasing lipid peroxidation as well as cell death. Therefore, a large amount of transaminases leakage in the blood can be detected, which is often associated with hepatonecrosis. Herein, we report the hepatoprotective properties of the leaves of *P. polyphyllus* on CCl_4 - induced liver damage in Wistar rats.

MATERIALS AND METHODS

Plant Material

Phyllanthus polyphyllus Willd. was collected from the Kolli hills (Eastern Ghats) of Namakkal district of Tamil Nadu, India, during the month of March 2003, identified and authenticated by Dr. P. Jayaraman, Plant Anatomy Research Centre (PARC), Tambaram - 600 045, Tamil Nadu, India. The voucher specimen (XCH 24909) has been deposited in the herbarium of St. Xavier's College Herbarium (XCH) of the St. Xavier's College (Autonomous), Palayamkottai, Tamil Nadu, India for reference.

Extraction

The shade-dried and powdered whole plant (2 kg) was extracted with methanol using a Soxhlet apparatus. The extract was filtered through Whatman No. 1 filter paper, concentrated on a water bath and obtained syrupy mass weighing 35.47 g after removing the last traces of solvents *in vacuo*.

Preliminary Phytochemical Screening

The methanol extract was tested for preliminary phytochemical screening (Brindha *et al*., 1982).

Animals

Healthy Male Wistar rats (150-175 g) were procured from Tamil Nadu Veterinary College, Chennai, India. They were kept in the Departmental Animal House, Department of Pharmacy, Vel's College of Pharmacy, Pallavaram, Chennai- 600 117, at 26±2 °C and relative humidity 44 – 55%, 10-h light: 14-h dark cycles for one week before the experiment. Animals were provided with rodent diet (Lipton India Ltd., Bombay) and water *ad libitum.*

Drugs and Chemicals

All the drugs and chemicals were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents were of analytical grade and were obtained from S.D. Fine Chemicals, Mumbai, India.

Evaluation of Hepatoprotective Activity

Animals were divided into six groups of six animals in each group. Normal control group I animals were administered a single dose of liquid paraffin daily (1 ml/kg body weight, p.o.). Negative control group II received carbon tetrachloride (1.25 ml/kg body weight, i.p.). Dosage of carbon tetrachloride was administered as 30% solution in liquid paraffin for every 72 h. Positive control group III received silymarin at a dose of 100 mg/kg, p.o., along with carbon tetrachloride. Test groups IV, V and VI were administered orally 100, 200 and 300 mg/kg body weight of methanol extracts along with carbon tetrachloride respectively, in the form of aqueous suspension once a day. On 16th day, blood samples (48 h after the last injection) were collected, allowed to clot and serum were separated and analysed for various biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT) Reitman and Frankel (1957), alkaline phosphatase (ALP) Kind and King (1954) and total bilirubin Mallay and Evelyn (1937).

Histopathoglogical Examination

On 16th day, after withdrawal of the blood, the animals were sacrificed and liver were dissected out from the animals and washed separately with normal saline for examined grossly and weighed, processed for dehydration, infiltration and embedding. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. They were infiltered and embedded with

paraffin. The microtome sections were taken at 5 µ thickness, processed in alcohol–xylene series, stained with alum haematoxylene and eosin and examined under microscope for the evaluation of histopathological changes.

Statistical Analysis

The values were expressed as mean ± SEM. Data were analysed by one way analysis of variance (ANOVA) followed by Dunnett test. $P < 0.05$ value were considered to be significant.

RESULTS AND DISCUSSION

The level of various biochemical enzymes and liver weight in normal, CCl_4 control and treated groups were represented in Table 1 & 2. The activities of SGOT, SGPT, ALP and BL were significantly increased in CCl_4 control compared to normal control. The levels of the above enzymes were significantly reversed on treatment with PPLME in a dose-dependent manner. The activity of the leaf extract at the dose of 300 and 400 mg/kg was comparable to that of the reference drug silymarin. The liver protection (Table 1) in terms of % protection the methanol extract treated group with reference to SGOT was 62.14%, 73.79% and 80.58% for 200, 300 and 400mg dose of extract per kg body weight, respectively. The percent protection for SGPT was 75.37%, 85.96% and 89.14%, ALP was 67.71%, 84.68% and 87.43% and BL was 83.57%, 97.14% and 101.43%.

The histopathological studies of the liver showed (Fig. 1) fatty changes, swelling and necrosis with loss of hepatocytes in CCl_4 control rats in comparison with normal control. The treated groups of PPME showed regeneration of hepatocytes, normalization of fatty changes and necrosis of the liver. The silymarin treated group showed almost normalization of fatty accumulation and necrosis. The maximum protection against hepatic damage was achieved with the PPLME at dose of 400 mg/kg.

Carbon tetrachloride is well-known hepatotoxic agent in liver diseases. The biochemical mechanism of CCl_4 toxicity is based on mitochondrial damage that leads to an accumulation of fat within 60 min, damage of endoplasmic reticulum within 30 min (Christie and Judah, 1954) and damage of lysosomes leading to the death of hepatocytes (Judah, 1969). Liver microsomal oxidizing systems are connected with cytochrome P_{450} system in the endoplasmic reticulum producing trichloromethyl free radicals (CCl_3^{-})

which then bind covalently to neighboring proteins and lipids in the presence of oxygen to form a trichloromethyl peroxyl radicals, which initiate lipid peroxidation, and finally result in the death of cells (De Groot and Noll, 1986; Clawson, 1989; Reckengel et al., 1989). *Phyllanthus* species reported to have potent hepatoprotecive property inhibiting various hepatotoxins (Gulati *et al.* 1995; Prakash *et al.* 1995; Unander *et al.* 1995; Asha *et al.* 2004; Harish and Shivanandappa, 2006; and Pramyothin *et al.* 2006). In the present investigation, the rats treated with an overdose of CCl_4 developed significant hepatic damage, which was observed through a substantial increase of serum enzymes. Treatment of the rats with PPLME at 200, 300 and 400mg/kg for 15 days resulted in a significant protection of CCl_4-induced by the elevation of serum marker enzymes. The hepatoprotective effect of PPLME against CCl_4-hepatotoxicity is attributed to the presence of flavonoids, triterpenoids and steroids which are known to be hepatoprotective and antioxidants (Banskota et al., 2000; DeFeudis et al., 2003) that act as free radical scavengers for the lipoperoxidants. However, the preliminary phytochemical studies reveal the presence of these secondary metabolites in PPLME. Hence, the possible mechanism of hepatoprotective effect of PPLME may be due to its flavonoid content. On the basis of the study, the present results conclude that of PPLME exhibited significant hepatoprotective activity on dose dependent manner. Further studies are needed to isolate the active principle of *P. polyphyllus* and establish chemical nature, which are responsible for their hepatoprotective properties.

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Table 1: Effect of PPLME on biochemical parameters in CCl_4 - induced rats

Treatments	SGPT (U/L)	% protection	SGOT (U/L)	% protection	ALP (U/L)	% protection	BL (mg%)	% protection
Normal control	138.50		45.00		176.60		0.70	
CCl_4 (1.25 ml/kg, b.w.)	327.30		148.00		486.00		2.10	
PPME (200 mg)+CCl_4	185.00^c	75.37	84.00^b	62.14	276.50^c	67.71	0.93^c	83.57
PPME (300 mg)+CCl_4	165.00^c	85.96	72.00^c	73.79	224.00^c	84.68	0.74^c	97.14
PPME (400 mg)+CCl_4	159.00^c	89.14	65.00^c	80.58	215.50^c	87.43	0.68^c	101.43
Silymarin (50 mg)+CCl_4	178.00^c	79.08	59.00^c	86.41	198.00^c	93.08	0.80^c	92.86

The values represent the mean ± S. E. M. for six rats per group. $^a p<0.05$; $^b p<0.05$; $^c p<0.05$ with respect to disease control (One way ANOVA followed by Dunnet *t*-test)

Table 2: Effect of PPLME on liver weight variation in CCl_4-induced rats

Treatments (mg/kg)	Liver wt/100 g body weight
Normal control	3.4±0.10
CCl_4 (1.25 ml/kg, b.w.)	6.8±0.28
PPME (200 mg)+CCl_4	4.9±0.05^b
PPME (300 mg)+CCl_4	4.5±0.04^c
PPME (400 mg)+CCl_4	4.1±0.06^c
Silymarin (50 mg)+CCl_4	3.8±0.26^c

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BIOCHEMICAL PROFILE AND POTENTIAL MEDICINAL USE OF *IPOMEA BILOBA* PRESENT IN THE WESTERN COASTAL LINE OF ARABIAN SEA AT SOUTH INDIA

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ABSTRACT

Ipomea biloba is an aquatic perennial runner plant used as a medical herb for various diseases. The plant were collected and extracted for biochemical compound isolation. In this present study the medicinal uses and biochemical parameters such as enzymes, amino acids, antioxidants, chlorophyll content, calcium, iron and total phenol present in the plant by using different methods.

Keywords: *Ipomea biloba*, amino acids, sap, erosion, enzymes, minerals

INTRODUCTION

In India, large diversity of plant species is available; of these species some are either beneficial or harmful. Fruits and vegetables generally form indispensable constituents of human diets supplying the body with minerals, vitamins and certain hormone precursors, in addition to small amounts of protein and energy. The plant *Ipomea biloba* belongs to the plant family Convoluvulacea, a moving glory family. It is a semi-aquatic tropical plant. In Tamil it was known as "Kadamba Valli". *I.biloba* was mainly located on the west coast of India, bounded by Arabian Sea. It is established worldwide on many tropical beaches including those of Australia and the Caribbean [1][2]. The predominant soils in the district are laetrile soils. The plant size may be up to 100 feet long.

It grows well on nutrient-poor, moist, sandy, or calcareous soils [3]. The growth of *I.biloba* was extensive on the sand dunes near the shore "cyanodon dactylon" a gramiane member was also found growing luxuriously along *I.biloba*. This type of plant species are normally seen under the coastal areas of Arabian Sea. The leaves of the plants are greenish in color [4]. The flower of *I.biloba* is pink in nature. The plant produces adventitious roots at the nodes and run horizontally rather than vertically [5]. It does not tolerate prolonged frost conditions. It provides habitat for many diverse animal species including gopher tortoise, the endangered beach mice, scrub jay and the threatened kestrel.

MATERIALS AND METHODS

I.biloba was collected from west costal line of Manavalakurichi village,

Kanyakumari district, Tamilnadu, India. The leaves were separated and cleaned well. Cleaned leaves were then dried under shade. The drying process was continued until all the water molecules evaporated and leaves became well dried for grinding. The leaves were finely powdered and extracted with acetone solvent using Soxhlet apparatus.

In this study the presence of Carbohydrates and Pentose was analyzed by using Molisch test and Bial's test method. The presence of Tryptophan and Histidine were analyzed by using Hopkins cole and Pauly's method. The estimation of Protein was done by Biuret method. Total Phenol was estimated by using Bray and Thorpe method. The minerals such as iron and calcium were estimated using Clark & Coltip method and Dipyridyl method. The enzymes amylase and catalase were estimated by using Caraway and Sodium Perborate method. The Estimation of Lipid was done by using Bilgh's & Dyes method.

RESULTS

I.biloba is a plant with an immense medicinal property in it. The plant plays a vital role for both human as well as environment. The sap from the succulent leaves has been used as a first-aid to treat jelly fish stings. Both leaves and stems exude a watery white sap that may be a chemical protection against insect pests and grazing animals. The plant also serves nature by preventing soil erosion. The plant also provides Food & Shelter for some sea insects and animals. The qualitative and quantitative screeing of the I.biloba were carried out and the results were tabulated in table 1 and table 2 respectively.

Table: 1 Qualitative biochemical screening of *I.biloba*

S.No	Compounds	Inference
1	Carbohydrates	+
2	Pentose	+
3	Tryptophan	+
4	Histidine	+

Table: 2 Quantitative biochemical screening of *I.biloba*

	Compounds	Contents
1	Protein (0.2ml)	0.357g/dl
2	Protein (0.5ml)	0.4286g/dl
3	Total Phenol	920µg/dl
4	Iron	320µg/dl
5	Calcium	10mg/dl
6	Amylase	137 caraways
7	Catalase	4.329×10^{-4} µmol/min
8	Lipid	82.39g

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The essence of all beings is earth,
The essence of earth is water,
The essence of water is plants,
The essence of plants is the human being.

-Chandogya, from the Upanishads

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