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PHYTOCHEMICAL PROFILING OF ETHANOLIC LEAVES EXTRACT OF *AVICENNIA GERMINANS*

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ABSTRACT Plants are a rich source of secondary metabolites with interesting biological activities. *Avicennia germinans* is a t raditional medicinal plant and the leaves have tremendous medicinal values. In the present study ethanolic leaf extract of *Avicennia germinans* was analyzed using Gas Chromatography–Mass Spectrometry (GC-MS) and the compound structures were identified with help of National Institute of Standards and Technology (NIST) library. GC-MS analysis of test plant revealed the presence of 12 bioactive compounds. Among them 3-dodecene, 1-hexodeconol, 9-eicosene and Tetratriacontane are important bioactive compounds which act as essential drugs for dangerous diseases and disorders and other compounds are used in antimicrobial, anti-inflammatory, antioxidant, cytotoxicity and cancer preventive activities.

KEYWORDS : *Avicennia germinans*, GC-MS, Bioactive compounds

INTRODUCTION

Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been isolated from natural sources, many of them based on their use in traditional medicine. Various medicinal plants have been used for daily life to treat disease all over the world. They have been used as a source of medicine. The widespread use of herbal remedies and healthcare preparations, such as those described in ancient texts like the Vedas and the Bible has been traced to the occurrence of natural products with medicinal properties. In fact plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry[1].

Medicinal plants are the source of many potent and powerful drugs. The plant derived drugs are healthier and safer alternate to the synthetic drugs[2]. Different parts of medicinal plants like root, stem, flower, fruit, seed etc. are used to obtain pharmacologically active constituents. Medicinal activities of plants can be attributed to the secondary metabolites such as alkaloids, flavonoids, glycosides, tannins, terpenoids and essential amino acids present in these plants. These active principles are isolated for direct use as drugs, lead compounds and or pharmacological agents[3]. Even today compounds from plants continue to play a major role in primary health care as therapeutic remedies in many developing countries[4].Standardization of plant materials is the need of the day. Several pharmacopoeia containing monographs of the plant materials describe only the physicochemical parameters. Hence the modern methods describing the identification and quantification of active constituents in the plant material can be useful for proper standardization of herbals and its formulations. Also the WHO has emphasized the need to ensure the quality of medicinal plants products using modern controlled technique and applying suitable standards[5]. Nowadays there were a number of dramatic advances in analytical techniques including TLC, UV, NMR and GC-MS that were powerful tools for separation identification and structure determination of Phytochemicals. In GC-MS used to identify the bioactive constituents of long chain hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds etc.,

Avicennia germinans L. is a mangrove plants belongs to the family Rhizophoraceae. Mangrove forest can decay into peat deposits because of fungal and bacterial processes as well as by the action of termites. It becomes peat in good geochemical, sedimentary and tectonic conditions. *Avicennia germinans* or black mangroves occupy different ecological niches and have slightly different chemical compositions so the carbon content various between the species as well as different tissues of the plant leaves and roots. The plant useful to diuretic, febrigual and anti-inflammatory effects, cure swellings of the skin, leprosy, laxative, sore eyes, sore throats leaves are used as human food, as medicine for infected wounds[6]. Hence the present study focused on Phytochemical profiling of Ethanolic Leaves Extract of *Avicennia germinans* using Gas chromatography and mass spectrometry.

MATERIALS AND METHODS

Collection and Authentication of Experimental Plant: The mangrove plant of *Avicennia germinans* leaves were collected from Muthupet mangrove, Tamil Nadu, South India. The leaves were identified with the help of flora of presidency, Tamil Nadu and Karnatic flora[7-8] and standard references[9].

Preparation of Extract: The dried and powdered leaves of *Avicennia germinans* (500 g) were extracted using soxhlet extractor by evaporating with 75% ethanol. The soxhlet extraction was carried out for 3 days and the extract was collected. The excess ethanol was evaporated by using vacuum evaporator. The sample is evaporated to dryness under boiling water bath at 55°C.

Phytochemical Analysis: The preliminary phytochemical evaluation of leaves was carried on extract prepared by successive extraction method in Soxhlet. The previously dried powdered (50 gm) were extracted in a Soxhlet apparatus with ethanol successively. The resultant extracts were evaporated to dryness under vacuum. These extract were subjected to chemical test for different phytoconstituents viz. alkaloids, carbohydrates, phenolics, flavonoids, proteins, amino acids, saponins, mucilage and resins etc. Chemical tests were identifying the phytochemicals as described[10-12]. Alkaloids, carbohydrates, tannins and phenols, flavonoides, gums and mucilage, fixed oils and fats and saponins were qualitatively analyzed.

Gas Chromatography-Mass spectrometry (GC-MS) analysis: The GC-MS analysis was carried out using a Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µmdf capillary column. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature was raised

upto 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Injection port temperature was ensured as 250°C and Helium flow rate as 1 ml/min. The ionization voltage was 70 eV. The samples were injected in split mode as 10:1. Mass Spectral scan range was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The fragmentation patterns of mass spectra were compared with those stored in the spectrometer database using National Institute of Standards and Technology Mass Spectral database (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds: The identity of the components in the extract was assigned by the comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with published literatures. National Institute of Standards and Technology library sources were also used for matching the identified components from the plant material.

RESULTS AND DISCUSSION

The result of phytochemical screening of the alcoholic extracts of *Avicennia germinans* revealed that the presence of alkaloids, flavanoids, phytosterols, tannins and phenols (Table 1). The plant extract of *Avicennia germinans* used for the present work was choosing on the basis of their medicinal values. Previous study in the naturally the ethanolic extracts of *Avicennia* spp. were subjected for phytochemical analysis. Phytochemical screening of the crude extract revealed that the presence of alkaloids, cardiac glycosides, terpenoids, saponins, tannin, flavonoids and steriods, but reducing sugars, carbonyl (aldehyde) and Phlobatanin show negative results[13].

This plants growing under natural conditions contain the spectrum of secondary metabolites such as phenols, flavanoids, quinones, coumarins, tannins and their glycosides, alkaloids, essential oils etc., the importance of these substance as microbial agents against the pathogen has been emphasized by several workers[10]. In the present study, it was clearly understood that the alcohol extracted maximum amount of the different type of metabolites present in the *A. germinans*. Boominathan and Ramamurthy1 reported that the phytochemical analysis of the *H. indicum* and *C. procumbens* extracts showed the presence of tannins, alkaloids, flavonoids and phenolic compounds. Tannins have been found to form irreversible complexes with proline-rich proteins.

GC-MS analysis of ethanolic leaves extract of *Avicennia germinans* clearly showed the presence of eleven compounds with their molecular weight (MW), molecular structure and biological activities are presented in Table 2. The GC-MS chromatogram of the fourteen peaks of the compounds detected was shown in Figure 1 and the components corresponding to the peaks were determined as follows: 3-dodecene, 1-hexodeconol, Phenol 2,4 bis(1,1dimethyl ethyl), Hexadecen1ol,trans 9, 9eicosene, 9,10 anthracenedione, tetracosane, 1,4 Benzenedicarboxylic acid, bis (2ethylhexyl) ester, 13Docosenamide, Tetratriacontane, Tetracosane 11 decyl etc.,

The differences between the compounds that we have found in the roots, stems and leaves of *Aristolochia clematitis* were studied by GC-FID. This study was performed on the alcoholic extracts of the three parts of the plant. From this study we have concluded that the compounds found in the root and steam are very similar. The aristolochic acid derivatives are present in both extracts, but in the leaves these derivatives are in very low concentration[14]. Ramamurthy[15] reported that the roots and leaves of *H. indicum* were studied by GC-MS. This study was performed on the alcoholic extracts of the two parts of the plant. From this study we have concluded that the compounds found in the root and leaves are very dissimilar. The organic acid derivatives are present in both extracts, but in the leaves these derivatives are in very high concentration.

Plants are integral part of human civilization. Medicinal plants are also been relied upon by over 80% of the world population for their basic health care needs. Drugs based on the Plants are of prime importance for several remedies in traditional and conventional medicine throughout the world and serves as a substitute for drug supply in modern medicine[16]. Medicinal plants with therapeutic properties are used for the treatment of many infectious diseases of humans as they contain many bioactive phytochemical constituents which are of curative effects. By consuming medicinal plants, can boost the immune system and increase antioxidant activity in humans. The high level of use as a medicinal plant due to easily available, cheap and relatively no side effects[17]. Tetracosane, also called tetrakosane, is an alkane hydrocarbon. Tetracosane showed some cytotoxic activity against AGS, MDA-MB-231, HT-29 and NIH 3T3 cells[18]. It also used as a good antibacterial activity.

1-hexodeconol is otherwise called as palmityl alcohol 1-hexadecanol is a fatty alcohol which are used in the cosmetic industry as an opacifier in shampoos or as an emulsifier or thickening agent in the manufacture of skin creams and lotions (pharmaceutical preparations) [4]. It is one of the active ingredients in some "liquid pool covers". Dioctyl terephthalate (bis 2-ethylhexyl) benzene-1, 4-dicarboxylate is an organic compound is a general purpose plasticizer that is considered safer than *ortho*-phthalate plasticizers due to its excellent toxicological profile. The terephthalates exhibit none of the peroxisome proliferation of liver enzymes that some *ortho*-phthalates have shown in several studies. It has uses in applications like extrusion, calendering, injection molding, rotational molding, dip molding, slush molding and coating.

The analytical methods used GC/MS is suitable for medicinal herbs organic compounds determination. The sample preparation method is rapid and precise. There is a difference between the compounds extracted from herb by infusion and tincture but the important thing is that the organic acid and fatty acids derivatives are present in both of them. On the other side the study shows that their concentration is higher in the roots and steams. The present study focused on identification of several constituents present in the ethanolic leaves extract of *Avicennia germinans*. This type of GC-MS analysis is the first step towards understanding nature of active compounds in this medicinal plant and helpful for the further detailed study. In this plant contains various bioactive compounds justifies the use of the whole plant for various ailments by traditional practitioners.

Fig 1. GC-MS CHROMATOGRAM OF *AVICENNIA GERMINANS*

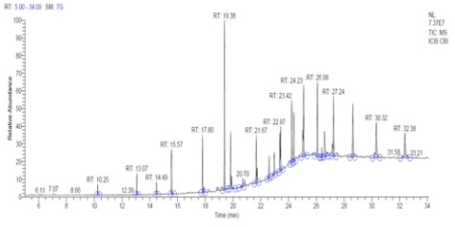

Table 1: Qualitative Phytochemical screening on extracts of *Avicennia germinans*

S. No	Name of Test	Test applied / Reagent used	Leaves extract
1	Alkaloids	A] Mayer's B] Wagner's C] Hagner's D]) Dragndorff's test	+ + + +
2	Flavanoids	HCl and magnesium turnings	+
3	Carbohydrate	Molisch's test	+
4	Tannins & Phenols	A] 10% Lead acetate B] Fecl3	+ +
5	Test for Steroids	A] Salkowski's Test B] Libermann-Burchard's Test	+ +

6	Gums & Mucilages	Alcoholic Precipitation	-
7	Fixed oil & Fats	Spot test	+
8	Saponins	Foam test	-
9	Phytosterols	LB test	+
10	Volatile oils	Hydro distillation method	+
11	Protein & free amino acids.	A] Biuret test B] Ninhydrin test C] Xanthoprotein test	+ + +

-, absents; +, present;

Table 2. GC-MS Analysis of *Avicennia germinans*

Name of the compound	Structure	Nature of the compound	Molecular Weight	Activity
3-dodecene		Alkene	168	Antibacterial
1-hexodeconol		Fatty alcohol	240	Antimicrobial, anti-inflammatory
Phenol 2,4 bis(1,1dimethyl ethyl)		Phenolic ester	206	Antibacterial, antioxidant
Hexadecen1ol,trans9		Fatty alcohol	240	Antimicrobial, anti-inflammatory
9eicosene		Long chain fatty acid	280	Antibacterial, cytotoxicity
9,10 anthracenedione		Aromatic organic compound	208	Antibacterial, antioxidant ,anticancer
tetracosane		alkane	338	Antibacterial, cytotoxicity
1,4Benzenedicarboxylic acid, bis(2ethylhexyl) ester		Dicarboxylic acid ester	390	Antimicrobial Antifouling
13Docosenamide, (Z)		amide	337	Antimicrobial
Tetratriacontane				
	alkane	478	Antimicrobial,antioxidant, Anticancer	
Tetracosane 11 decyl		alkane	478	Antimicrobial ,antioxidant

Source: - *Dr. Duke's Phytochemical and Ethno botanical Databases*

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