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Phytochemical Screening, GC-MS Analysis and Antibacterial Evaluation of Ethanolic Leaves Extract of *Avicennia marina*

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ABSTRACT

Medicinal plants were used to treat diseases traditionally since ancient times. The present work aims to investigate the bioactive constituents through GC MS analysis of ethanolic leaf extracts of *A.marina*. Phytochemical screening confirmed the presence of Alkaloids, Flavonoids, Phenols, Reducing sugars, Saponins, Tannin, Glycoside, Triterpenoids and Carbohydrate in *A.marina*. The characterization of the compounds by Gas Chromatography – Mass Spectrometry (GC-MS) technique has reported the presence of thirty compounds in *A.marina* leaves. These compounds possess different pharmacological properties like anti-microbial, antioxidant, anti-inflammatory and hepatoprotective properties.

Keywords: *Avicennia marina,* GC-MS, Bioactive compounds, Antibacterial activity.

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INTRODUCTION

Plants play a significant role in the prevention and treatment of diseases and can even prevent and reduce the adverse effects of conventional treatments [1]. They can be a source of chemical compounds of biological and pharmacological importance. History revealed that plants are vital sources of many successful drugs, and they are important for screening of new lead compounds [2]. Mangrove plants are used in many traditional medicine for the treatment of severe diseases. The mangrove plants have also been proved for antiviral, antibacterial and antiulcer properties [3-4]. Mangroves have been a source of several bioactive compounds and they have been used in folklore medicines and extracts have proven activity against human, animal and plant pathogens. Secondary metabolites like alkaloids, flavonoids, steroids, phenolics and terpenoids have been characterized from mangrove plants and possess toxicological, pharmacological and ecological importance [5-6].

Avicennia marina one of the common tree species of mangrove forest ecosystem belonging to the family Verbenaceae, is a cosmopolitan species widely distributed along tropical and subtropical coastlines. The bark, leaves and fruits of *A. marina* have reported as antibacterial, antifungal, antiviral agents and also possess anticancer, antiplasmodial, antitumor, and antiulcer properties [7-12]. The determination of phytocompounds is largely performed by relatively cost and frequent laborious techniques such as gas (GC) and liquid (LC) chromatography united with specific detection schemes [13]. Analysis of chemicals in small amount has become easier and much more cost-effective due to the development of hyphenated chromatographic techniques such as GC or LC-MS. GC-MS analysis can identify pure compounds present at less than 1gm [14]. However, simple and cost-effective tests are necessary to detect the phytocomponents.

Gas Chromatography – Mass Spectrum (GC-MS) technique has been increasingly employed to analyze the secondary metabolites present in the medicinal plants, as this technique has been proved to be a best valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds [15]. The biotechnological industries shows interest on medicinal plants and as well as most of the drug industries depend on plant parts for the future production of pharmaceutical compounds. With this background the present study was

aimed to identify the phytoconstituents present in *Avicennia marina*.

METHODS

Collection and extraction of mangrove plant leaves

Fresh and Healthy leaves of *Avicennia marina* were collected from their natural habitat of Muthupet mangrove in Thiruvarur district, Tamil Nadu, India and authenticated by professionals in the Department of Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India. The herbarium number of the plant is SK001. After washing with distilled water, the leaves were shade dried, powdered and extracted separately in ethanol. Plant powder (20 gm) was taken and soaked in 100 ml of solvent and kept in shaker for 24 hrs. After centrifugation at 5000 rpm, the solvent phase was separated and evaporated. The crude was stored at 40º C and used for further studies.

Phytochemical Qualitative Analysis

The ethanolic leaves extracts were assessed for the existence of the phytochemical analysis by using the standard methods [16-19].

Gas Chromatography-Mass spectrometry (GC-MS) analysis

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 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110°C, and then maintained at the same 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 per minute and maintained for 9 min. The temperature of injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected gradually in split mode as 10:1. The range of mass spectrum 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

Interpretation of mass spectrum of GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The unknown component's spectrum was compared with the spectrum of the known components stored in the NIST library. The structure, name and molecular weight of the components of the test materials was ascertained.

Evaluation of extract's antibacterial activity

The antibacterial activity of the mangrove leaf extract was evaluated using disc diffusion method. One loop of each bacterial stock culture was sub-cultured on Mueller-Hinton agar, then the paper discs (Whatman filter paper, 6mm diameter), which were dipped in different extract concentrations, were laid on the surface of the medium. The extract concentrations (5, 10, 15, 20, 25, 30, 35, 40 mg/ml) were prepared using sterile distilled water. All the culture mediums were incubated for 24 h at 37 °C, then the diameter of the growth inhibition zone was carefully measured using a ruler [20]. All experiments were performed in triplicate.

RESULTS

Table 1: Qualitative phytochemical analysis of *Avicennia marina*

S.No	Tests	Appearance	Results
1.	Alkaloids	Pale precipitate	+
2.	Flavonoids	Dirty brown color	+
3.	Cardiac glycosides	Brown ring formation	+
4.	Steroids	Violet to blue color	+
5.	Terpenoids	Reddish brown color	+
6.	Tannins	Yellow precipitate	+
7.	Anthraquinones	Red color	+
8.	Protein	Absence of pink red color	-
9.	Saponins	Absence of honey comb like froth	-

Table 2: GC-MS analysis of ethanolic extract of *Avicennia marina* leaves

S. No.	Peak Name	Retention time	Peak area	% Peak area
1.	Name: 4-Penten-2-ol Formula: $C_5H_{10}O$ MW: 86	7.08	4992661	0.9804
2.	Name: 2-Butene, 2-methyl- Formula: C_5H_{10} MW: 70	10.92	1317081	0.2586
3.	Name: Triquinacene Formula: $C_{10}H_{10}$ MW: 130	11.59	736375	0.1446
4.	Name: Nonanoic acid Formula: $C_9H_{18}O_2$ MW: 158	13.75	1902114	0.3735
5.	Name: 2(R),3(S)-1,2,3,4-Butanetetrol Formula: $C_4H_{10}O_4$ MW: 122	14.88	108862904	21.3770
6.	Name: 2-Hexanone, 3-cyclohexylidene-4-ethyl- Formula: $C_{14}H_{24}O$ MW: 208	15.82	976629	0.1918
7.	Name: Nona-3,5-dien-2-ol Formula: $C_9H_{16}O$ MW: 140	16.54	768598	0.1509
8.	Name: 2,2,6,8,12-Pentamethyl-7,9,10-trioxa-tricyclo[6.2.2.0(1,6)]dodec-11-ene Formula: $C_{14}H_{22}O_3$ MW: 238	17.80	2488335	0.4886
9.	Name: D-Allose Formula: $C_6H_{12}O_6$ MW: 180	18.33	3060763	0.6010
10.	Name: Dodecanoic acid Formula: $C_{12}H_{24}O_2$ MW: 200	18.55	11052012	2.1702
11.	Name: Nonanoic acid, 3-methylbutyl ester Formula: $C_{14}H_{28}O_2$ MW: 228	18.85	2957382	0.5807
12.	Name: Heptanoic acid, 3,5,5-triethyl- Formula: $C_{13}H_{26}O_2$ MW: 214	19.20	5154689	1.0122
13.	Name: 3-Cyclohexen-1-carboxaldehyde, 3-methyl- Formula: $C_8H_{12}O$ MW: 124	19.39	8454506	1.6602
14.	Name: 2-Naphthalenemethanol, α-methyl-, (±)- Formula: $C_{12}H_{12}O$ MW: 172	19.59	712455	0.1399
15.	Name: Benzonitrile, 4-ethenyl- Formula: C_9H_7N MW: 129	20.47	975220	0.1915
16.	Name: Phenol, 2,6-dimethoxy-4-(2-propenyl)- Formula: $C_{11}H_{14}O_3$ MW: 194	20.71	1472950	0.2892
17.	Name: Benzoic acid, 3,4,5-trimethoxy- Formula: $C_{10}H_{12}O_5$ MW: 212	21.78	4779990	0.9386
18.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	22.18	34397904	6.7546
19.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$ MW: 268	22.35	2539396	0.4987
20.	Name: Butanoic acid, 3-methyl-, 3,7-dimethyl-2,6-octadienyl ester	22.57	7233219	1.4204

	Formula: $C_{15}H_{26}O_2$ MW: 238			
21.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$ MW: 256	24.88	56945332	11.1822
22.	Name: Hexadecanoic acid, ethyl ester Formula: $C_{18}H_{36}O_2$ MW: 284	25.06	44265320	8.6922
23.	Name: 4-(3,5-Di-tert-butyl-4-hydroxyphenyl) butyl acrylate Formula: $C_{21}H_{32}O_3$ MW: 332	25.41	4549989	0.8935
24.	Name: 4-Oxazolecarboxylic acid, 4,5-dihydro-2-phenyl-, 1-methylethyl ester Formula: $C_{13}H_{15}NO_3$ MW: 233	27.28	2513853	0.4936
25.	Name: Phytol Formula: $C_{20}H_{40}O$ MW: 296	28.03	21068804	4.1372
26.	Name: (E)-9-Octadecenoic acid ethyl ester Formula: $C_{20}H_{38}O_2$ MW: 310	29.04	23578426	4.6300
27.	Name: Octadecanoic acid, 2-methyl-, methyl ester Formula: $C_{20}H_{40}O_2$ MW: 312	29.50	9422720	1.8503
28.	Name: cis-9-Hexadecenal Formula: $C_{16}H_{30}O$ MW: 238	32.96	7569641	1.4864
29.	Name: 2-Hydroxy-2-methyl-but-3-enyl 2-methyl-2(Z)-butenoate Formula: $C_{10}H_{16}O_3$ MW: 184	32.43	7045837	1.3836
30.	Name: Squalene Formula: $C_{30}H_{50}$ MW: 410	41.30	127456712	25.0282

Figure 1: GC-MS CHROMATOGRAM OF *A. marina* leaves

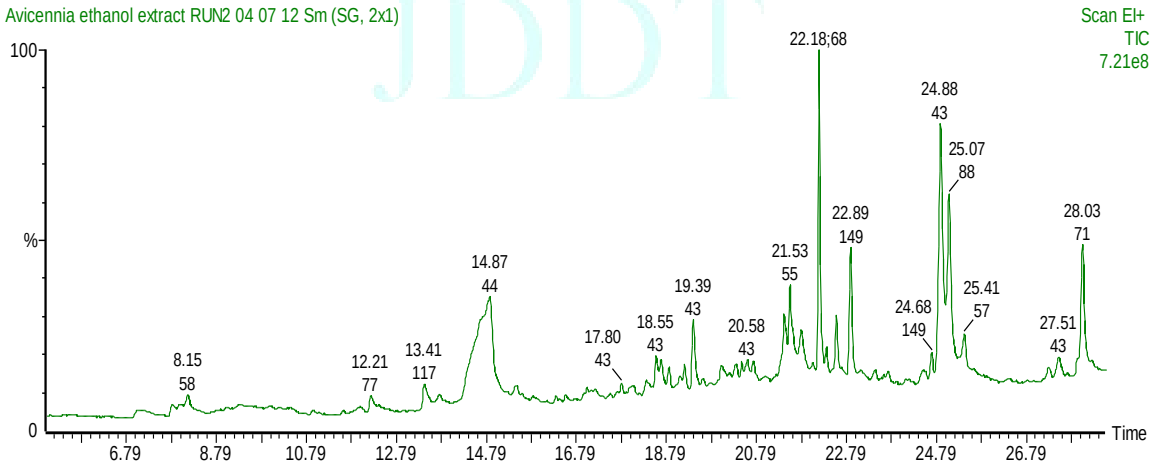
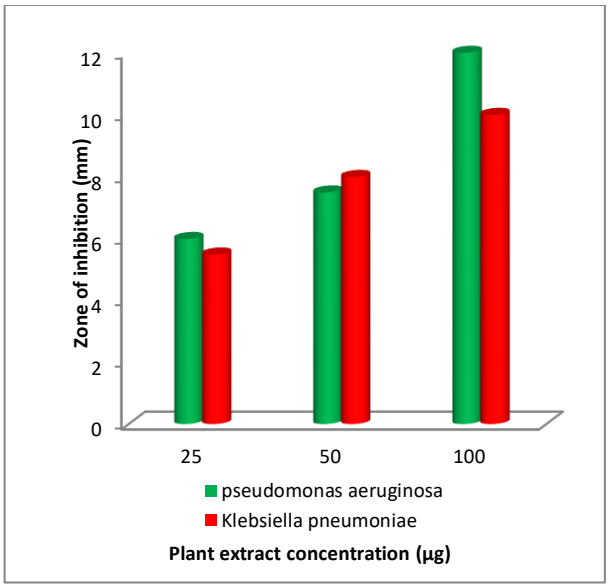


Figure 2: Antibacterial activity of *A. marina* leaves



DISCUSSION

Phytochemicals are responsible for medicinal activities of the plants. Based on this fundamental knowledge several pharmaceutical industries are established. The phytochemical constituents that are playing a significant role in medicines can be identified using crude extracts/drugs of the plants [21]. Nowadays the organic compounds from plants have been studied and their activity has increased. The combination of GC and MS, which are best separation technique and best identification technique respectively made GC-MS as an ideal technique for volatile and semi-volatile bioactive compound's qualitative analysis [22]. The ethanolic extract of *A.marina* was analyzed by GCMS to detect various compounds with the help of NIST library.

The GC-MS analysis revealed 30 chemical compounds. Squalene (41.30 RT) is the highest retention time chemical compound and 4-Penten-2-ol (7.08) is the lowest retention time chemical compound. The compound Nonanoic acid is a C9 straight-chain saturated fatty acid which occurs naturally as esters of the oil of pelargonium which has antifungal properties, and is also used as a herbicide. It is also used in the preparation of plasticisers and lacquers [23]. The compound n-Hexadecanoic acid, Hexadecanoic acid, methyl ester, Benzoic acid, D-Allose showed pharmacological activity as reported in the plant *Evolvulus alsinoides* [24]. Phytol is an acyclic diterpene alcohol that can be used as a precursor for the manufacture of synthetic forms of vitamin E and vitamin K1 [25-26]. Phytol have been reported in previous studies, including its activity against Mycobacteria, anticonvulsant, antispasmodic and anticancer activities.[27-30]. Squalene is a natural compound, a linear triterpene synthesized in plants [31]. It is a natural antioxidant molecule that protects cells from oxidative damage by exposure to ultraviolet light and other external sources. This molecule participates as a defense mechanism for the internal and external tissues of the skin in the human body [32].

Anti-bacterial activity showed that the inhibition zones were found increased considerably when the concentration rate increased. The results of antibacterial activity revealed that ethanolic leaves extract of *A.marina* have significant activity against tested pathogen and maximum growth inhibition was observed against *Pseudomonas aeruginosa* (12 mm) followed by *Klebsiella pneumoniae* (10 mm). Similar results were described by Tambekar, who reported that the antibacterial potential of *Dashmula churna* against *S. aureus, S. epidermidis, P. vulgaris, S. typhi, B. subtilis, E. coli, K. pneumoniae, E. aerogenes* and *P. aeruginosa* and its usefulness in treatment of the bacterial infections [33]. The compounds identified by the initial qualitative analysis and GCMS analysis have many uses in medical field. Each compounds that are identified in the extract have their unique character to treat a variety of diseases. Further studies are required to reveal its significance in specific field to treat the diseases properly.

CONCLUSION

The presence of various bioactive compounds in the *A.marina* justifies the use of whole plant for various ailments by traditional practitioners. However the isolation of individual phytochemical compound and analyzing their biological activity will definitely yield productive results. The results of this study offer a base of using *A.marina* as herbal alternative for the synthesis of antimicrobial agents. From the results, it could be concluded that *A.marina* contains various bioactive compounds. Hence, it can be recommended as a plant having phytopharmaceutical importance.

CONFLICT OF INTEREST STATEMENT

We declare that we have no conflict of interest.

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