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Separation and characterization of phytochemicals from of white mangrove *Avicennia Marina*

T Kumaran[1], S Sujithra[2], D Beula Shiny[3], J Vijila Jasmin[4], M Jenifer Tamizharasi[5]
[1-5] PG and Research, Department of Zoology, Muslim Arts College, Affiliated to Manonmaniam Sundaranar University Thiruvithan, Kanya Kumari, Tamil Nadu, India

Abstract
The plant investigation has opened up a new respective biopharma research. Mangroves are specific group of salt tolerant plants that grow within coast regions of tropic and sub-tropic along the coastlines. Mangroves have been used in folk medicine for treatment of several. Compounds isolated from marine source have the chemical structures which are not commonly found in terrestrial counter parts. In recent years an increasing number of marine natural products have been reported to display antimicrobial, compounds. The knowledge of chemical constituents of mangrove plants is desirable to understand herbal drugs and their preparation. In the present study suggests that the *Avicennia marina* extract, significant implications in food and pharmaceutical industry as a source of basic material in the preparation of nutrient supplement products and fine chemical synthesis. The findings of this study revealed that the mangrove plant *Avicennia marina* leaf extracts could be used as a potential alternative for development of bioactive leads in the treatment of infections.

Keywords: mangrove, *Avicennia marina*, bioactive, treatment, pharmaceutical

1. Introduction
Plants produce a diverse range of bioactive molecules, making them rich sources of different types of medicine. Most of the drugs today are obtained from natural sources or semi synthetic derivatives of natural products and used in the traditional systems of medicine. Mangrove plants living in an ecological hostile condition enumerated by different stress conditions such as salinity, high temperature, water logging, low oxygen and light are reported to be rich in antioxidant compounds. These compounds include phytochemicals such as cinnamic acids, coumarins, diterpenes, flavonoids, lignans, monoterpenes, phenylpropanoids, tannins and triterpenes (Bandaranayake 2002 [2]; Thatoi *et al*. 2014) [12].
Phytochemical studies on *A. marina* leaf extract have revealed the presence of secondary metabolites such as alkaloids, flavonoids, triterpenoids and steroids, which could also be expected to be responsible for its bioactivity. These compounds with antioxidant activity can inhibit mutation and cancer because they scavenge free radicals or induce antioxidant, enzymes (Abeysinghe *et al*., 2006) [1]. Naphthoquinones isolated from *A. marina* have been shown to exhibit marked inhibitory effect on mouse skin tumour promotion.
Many mangrove plants have been used in folklore medicine worldwide, recently extracts from mangroves and mangrovedependent species have proven to possess antimicrobial activities against human, animal and plant pathogens.Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new bio-pesticidal and pharmaceutical agents. Plants and Animals in the sea produce a great variety of compounds, often unique, that serve to protect against its natural enemies or act as chemical cues for reproduction or preying. A large number and variety of herbivores, ranging from highly mobile macro grazers (mammals, fish, sea urchin and large crustaceans and gastropods) to smaller more sedentary meso-grazers (small gastropods, amphipods, isopods and polychaetes) consume equally large numbers and variety of marine macro algae and produce a variety of secondary metabolites that serve to protect against pathogens and grazers.
The present study aimed to utilize the mangrove plant which is one of the important sources of mangrove. They are widespread in tropical and subtropical regions, growing in the saline intertidal zones of sheltered coast lines (Chelliah, 2001[3]; Tariq *et al.*, 2007) [11]. Mangrove species are woody, seed bearing and highly specialized plants (Duke *et al.*, 1998) [5] found coast lines of estuaries and lagoon. Because of their unique adaptations mangroves thrive well in the environment where other plants cannot grow (Shanmugapriya *et al.*, 2012) [10]. Mangroves are salt tolerant plants. The specific regions where plants occur are termed as mangrove ecosystem (Chelliah, 2001) [3].
Avicennia marina is commonly called white mangrove belongs to the family Avicenniaceae. It is a small medium sized tree (3 – 11 meter) with many branches. Extensive underground root system with "Pencil root" (Pneumatophores or breaking roots) up to 90 mm long. 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. Mangrove plants are used in many traditional medicine for the treatment of severe diseases. The mangrove plants have also been proved for antibacterial properties

2. Materials and Methods
2.1 Sample collection
The mangrove *Avicennia marina* was collected from the intertidal region of the manakudy, Kanyakumari district, Tamilnadu. The mangrove samples were washed thoroughly in fresh water to remove salt and other unwanted materials

and shade dried. The dried mangroves were cut into smaller pieces, grind into powder and stored at 4 °C until analysis.

2.2. Preparation of Extracts
The *A. marina* sample after drying were weighed and then chopped. The chopped samples were finely powdered using mixer grinder. The finely powdered samples were weighed and 5 grams of it were dissolved in various organic solvents, such as 80% ethanol, methanol and chloroform. It was kept for 48 hours at room temperature and mixed at regular intervals. After 48 hours the sample dissolved in each solvent was filtered using Whatman No1 filter paper to separate the filtrate for further use.

2.3. Phytochemical screening
Test for tannins
About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for saponins
0.5 g of extract was added 5 ml of distilled water in a test tube. The solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for steroids
One ml of the extracts was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids.

Test for terpenoids (Salkowski test)
To 0.5 g each of the extract was added 2 ml of chloroform. Concentrated H_2S0_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for triterpenoids
Ten mg of the extract was dissolved in 1 ml of chloroform; 1 ml of acetic anhydride was added following the addition of 2 ml of Conc. H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for anthraquinones
0.5g of the extract was boiled with 10 ml of sulphuric acid (H_2SO_4) and filtered while hot. The filtrate was shaken with 5 ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for flavonoids
Dilute ammonia (5 ml) was added to a portion of an aqueous filtrate of the extract. Concentrated sulphuric acid (1 ml) was added. A yellow coloration that disappears on standing indicates the presence of flavonoids.

Test for alkaloids
0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into two portions. Mayer's reagent was added to one portion and Draggendorff's reagent to the other. The formation of a cream (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent) was regarded as positive for the presence of alkaloids.

Test for cardiac glycosides (Keller-Killiani test)
To 0.5 g of extract diluted to 5 ml in water was added 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayed with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

2.4 Fourier Transform Infra-Red (FTIR) Spectroscopic Analysis
The selected one hot water extract such as *Avicennia marina* were analyzed qualitatively for the active compounds by Fourier transform infra-Red (FTIR) method. Fourier Transform Spectrometer is simply a technical variant, of a common infra-red spectrometer which yield an intensity signal as a function of wavelength or spectral colour. The setup differs from a classical grating or prism spectrometer in that way that does not record the spectral intensity directly as a function of wavelength but an interferogram is taken insisted.

Antimicrobial activity of A. *marina*
Extract of A. *marina* was tested for antibacterial activity by Muller-Hinton agar diffusion method. Broth bacterial cultures, 24 hrs old, grown on nutrient agar plates were used. An aliquot (0.05 ml) of inoculums was introduced to the molten agar medium was poured into a Petri-dish by pour plate technique. After solidification, the appropriate wells were made on agar plate using sterile cork-borer, 6.0 mm diameter wells were punched over the agar plates using sterile gel puncher. In agar, 0.1ml of each extract was introduced serially. Incubation period of 24-48 hrs at 28°C for fungal growth and 30°C for bacteria were maintained for observation of antimicrobial activity.

4. Results
4.1. *Avicenna marina* Collection
Plant Material for the species *Avicennia marina* was collected from coastal region in Tamilnadu from manakudy mangrove forest which is located at the Kanyakumari coast, during December 2019. *Avicennia marina* is a multipurpose tree, it is harvested from the wild for use as an insect repellent, dye plant, source of tannins, timber etc. This species is widespread and common throughout its range. It is a fast growing and fast regenerating, hardy species. Research has shown that several medically active components are present in the plant including iridoid glucosides, flavonoids and naphthoquinone derivatives.

4.2. Phytochemical analysis
This study has revealed the presence of phytochemicals considered as active medicinal chemical constituents. Important medicinal phytochemicals including alkaloids, terpenoids, flavonoids and anthraquinones were present in

the samples. The result of the phytochemical analysis showed that the mangrove leaf *Avicennia marina* had the presence of alkaloids, saponin, terpenoids, flavonoids and anthraquinones were present in the extract. (Table 1).

4.3. Functional group analysis

Fourier Transform Infrared Spectroscopy analysis for the antibacterial extracts' active fractions are given in the figure 3. The active fraction of hot water extracts of *Avicennia marina* gave the following peaks in the I-R spectrum. The peaks represented the various functional groups in the molecule. The broad peak around 3458.34 cm^{-1} may be the N-H stretching specific context RNH_2, R_2NH $RCONH_2$, RCONHR' and one peak at 2995.81 cm^{-1} may be due to C – H N-H stretching C_{sp}3-H specific context RNH_3^+, $R_2NH_2^+$, R_3NH^+. The peak at 2854.43 cm^{-1} stretching C – H N-H specific context may be due to C_{sp}3-H RNH_3^+, $R_2NH_2^+$, R_3 NH^+. The peak at 1625.24cm^{-1} stretching C-C N-O specific context may be due to C_{sp}3-H, RNH_3^+, $R_2NH_2^+$, R_3 NH^+. The peak at 1512.96 cm^{-1} stretching N-O specific context may be due RNO_2 RN=O. The peak at 1201.30 cm^{-1} stretching C-N, C-O, C-X specific context may be due C-N, C-O, C-F. The peak at 933.40 cm^{-1} stretching N-O specific context may be due C=N-OH. The observation revealed that it may be inferred that the compound is alkenes. Thus the extract may contain a free carbonyl group where the OH group is hydrogen bonded. The extract is also suspected to contain a carbonyl species in conjugation with O= bond (Fig 1)

4.4 Antibacterial activity of *A. marina*

The antibacterial activities of the selected extract (Zone of inhibition of mm diameter) were given in the Table 5 and Fig 6a to 6b. Among the different concentration (5 mg, 10 mg and 15 mg) of the extract, *A. marina* controlled the pathogen of 2.1, 3.4 and 6.1 mm of zone of inhibition in *S. aureus* respectively

Table 1: Phytochemical Analysis of selected mangrove leaf by standard protocol

S.No	Phytochemical Compounds	*Avicennia marina*
1.	Alkaloids	Positive
2.	Tannins	Negative
3.	Saponins	Positive
4.	Steroids	Negative
5.	Terpenoids	Positive
6.	Titerpenoids	Negative
7.	Flavonoids	Positive
8.	Anthraquinones	Positive
9.	Cardiac glycosides	Negative

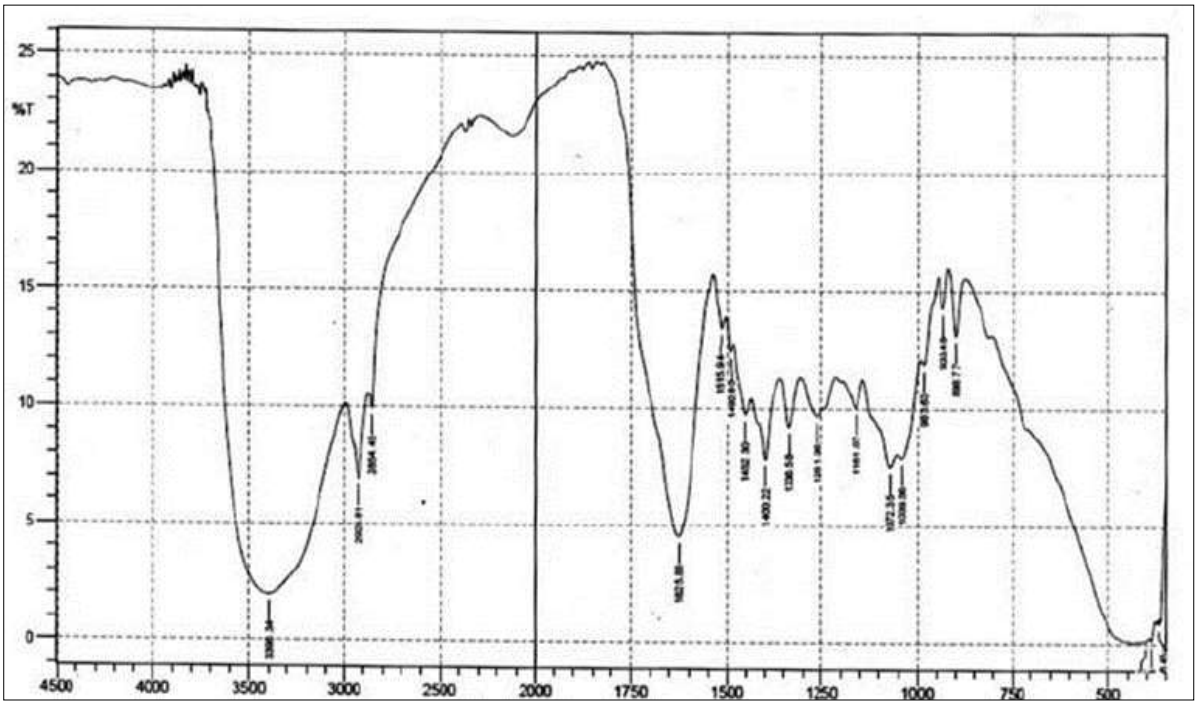

Fig 1: Molecular stretches of active principles isolated from the extract of *Avicennia marina* through FTIR Spectroscopic analysis

Table 2: Peak and Bond Type of the active principles isolated from the hot water extracts of *Avicennia marina* through FTIR spectroscopic analysis

S.No	Peak	Bond type	Specific context	V_1 cm^{-1}
1	800.77		C-Cl	800 – 850
		C-X	RO-N=O	750 – 815
		N-O	R2C=CHR	790 – 840
			m-	750 – 810
		Alkenes	1,2,3,4	800 – 810
2	933.40	N-O	C=N-OH	930 – 960
3	983.03	-	-	-
4	1030.80	C-N	C-N	1030 – 1230
		C-O	C-O	1020 – 1275
		C-X	C-F	1000 – 1350
5	1072.35	C-N	C-N	1030 – 1230

		C-O	C-O	1020 – 1275
		C-X	C-F	1000 – 1350
6	1161.07	C-N	C-N	1030 – 1230
		C-O	C-O	1020 – 1275
		C-X	C-F	1000 – 1350
		N-O	R-S(=O)$_2$-OR’	1145 – 1200
7	1201.30	C-N	C-N	1030 – 1230
		C-O	C-O	1020 – 1275
		C-X	C-F	1000 – 1350
8	1338.55	C-C	C-C	1250 – 1450
		C-X	C-F	1000 – 1350
		N-O	R$_2$S(=O)O	1310 – 1350
			R-S(=O)$_2$-OR’	1330 – 1420
9	1400.22		C-C	1250 – 1450
		C-C	RNO$_2$	1350 – 1560
		N-O	R-S(=O)$_2$-OR’	1330 – 1420
10	1457.30	-	-	-
11	1492.80	-	-	-
12	1512.96	N-O	RNO$_2$	1350 – 1560
			RN=O	1500 – 1600
13	1625.24	C-C	C=C	1600 – 1670
		N-O	RONO$_2$	1620 – 1640
			RO-N=O	1610 – 1680 (Two)
14	2854.43	C - H	C$_{sp}$3-H	2800 – 3000
		N-H	RNH$_3^+$, R$_2$NH$_2^+$, R$_3$NH$^+$	2250 – 3000
15	2995.81	C - H	C$_{sp}$3-H	2800 – 3000
		N-H	RNH$_3^+$, R$_2$NH$_2^+$, R$_3$NH$^+$	2250 – 3000
16	3458.34	N-H	RNH$_2$, R$_2$NH	3400 – 3500 (Two)
			RCONH$_2$, RCONHR’	3400 – 3500

Fig 2: Antibacterial activity of *A. marina* against *S. aureus*

Discussion

Plants have continuously been an exemplary source of medicines and lots of drugs available today, are directly or indirectly derived from them. Mangroves are a specialized group of salt tolerant plants that grow within the seacoast areas of tropic and sub tropic on the coastlines. Mangrove plants provide an important source for the search of novel drugs as they are stress tolerant plants and therefore synthesize a large number of phytochemicals having significant pharmacological properties like infectious diseases, diabetes and asthma.The bioactive compounds of mangrove plants may be used because of the potent source of modern drugs against various critical diseases.

The preliminary phytochemical analysis of these crude preparations was compared with the nature of the fluorescence emission under different conditions for clear understanding about the plants. The comparative analysis clearly showed a correlation between a compound present in it and their fluorescence behavior under different conditions (Packia Lincy *et al.*, 2013) [8].

The fluorescence analysis of the crude extract of *A.marina* exhibited clear fluorescence behavior at different radiations which can be taken as standard fluorescence pattern. Plants and their compounds have been provided as a good source of inspiration for novel drug compounds, so standardization of the plant material is need of the day. The preliminary qualitative phytochemical screening of *Avicennia marina* results were summarized in Table 1. Alkaloids, reducing sugar, tannins, saponins, flavonoids, phenolic group, carbohydrates are found to be present and steroids, pholobatanins, terpenoids, cardiac glycosides, amino acids, aromatic acids were absent in water extract of *A. marina*.

Recent strategies for developing novel medicines from unexplored natural resources recommended marine plants as an important source of potentially useful chemicals (Harvey 2000) [6]. The presence of phenolics in *A. marina* which are toxic to microbial pathogens especially shows the maximum activity against bacterial pathogens (Patra *et al.*, 2009) [9]. Alkaloids present in this plant are used as basic drug agents for antispasmodic, analgesic and antibacterial effect (Okwu, 2004) [7]. Flavonoids found in this plants are preventing the oxidative cell damage and having strong anticancer activity (Okwu, 2004) [7]. Saponins present in these plants are considered to be antifungal agents and tannins prevent the growth of the microorganism by precipitating nutritional microbial proteins and also have the property of cholesterol binding and bitterness (Okwu, 2004) [7].

Among the phytochemical tested in *Avicennia marina* leaf extract, which contains high phenolic compounds followed by tannin, alkaloid, saponin and flavonoids. IR spectrum showed strong absorption band at 1635 cm-1 (N-H, Primary amine), 1492 cm-1 (C - C Aromatic), 858 cm-1 (C - H

Aromatic), 764 cm-1 (C - H Aromatic) (Figure1). This analysis showed that association of functional groups and fourteen effective peaks were obtained between 4000 cm-1 to 450 cm-1. This kind of peaks indicates the presence of some organic functional groups such as alcohol, phenols and alkyl group. The presence of these identified functional groups in medicinal compound can be responsible for their therapeutic function.

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