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RESEARCH ARTICLE

PHYTOCHEMICAL PROFILE AND ANTIBACTERIAL ACTIVITY OF THE MANGROVE PLANT *AVICENNIA OFFICINALIS* L.

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ABSTRACT

Traditionally, mangrove plants have been used medicinally in diverse cases like to treat infections, relief pain and purify blood and as antioxidant. *Avicennia officinalis* is a folk medicinal plant used mainly against rheumatism, paralysis, asthma, snake-bites, skin disease, hepatitis, leprosy and ulcer. Phytochemical constituents from *Avicennia officinalis* leaf extracts were determined qualitatively. Crude extracts of the plants under study were screened for the presence of alkaloids, phenolics, flavonoids, tannins, diterpenes, triterpenes, sterols, saponins, glycosides, proteins, carbohydrates and reducing sugars. *Avicennia officinalis* was tested for bioactivity against seven bacteria.

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INTRODUCTION

Alkaloids, phenolic compounds and terpenoids produced by plants play a vital role in protecting the plants under biotic and abiotic stresses. Presence of such compounds confer some medicinal properties in plants. Plants remain the most common source of traditional health remedies (Safary *et al*., 2009). During the last century, however, the use of synthetic drugs led to a decline in the use of plant-derived compounds, so that at one time it was believed by many that the synthetic drugs would perhaps completely replace the use of traditional plant-derived medicines. However, in recent years, a resurgence of the use of herbal drugs has once again been witnessed, firstly because the synthetic drugs have been found to be hazardous in many cases, and secondly because there is growing awareness that the plant derived-medicines have none of the side effects that are so common in the case of synthetic drugs. Due to their stressful habitat mangrove plants produce a handsome amount of bioactive phytochemicals. Mangrove plants possess novel chemical compounds many of which are biologically active and have medicinal values (Bandaranayake, 2002). Extracts from different mangrove plants are reported to contain diverse medicinal properties (Agoramoorthy *et al*., 2007) and are active both against human pathogens and plant pathogens (Chandrasekaran *et al*., 2009).

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However, the majority of these plants have not yet undergone chemical, pharmacological and toxicological studies to investigate their bioactive compounds (Singh A. *et al*., 2009). Due to its tropical location and long coastline, India possesses a good amount of mangrove vegetation. *Avicennia officinalis* is a folk medicinal plant used mainly against rheumatism, paralysis, asthma, snake-bites, skin disease, hepatitis, leprosy and ulcer (Bandaranayake, 2002; Shanmugapriya *et al*., 2012). The fruits and leaves are also used as aphrodisiac and diuretic (Bandaranayake, 2002). A decoction of the plant with sugar candy and cumin is used in dyspepsia with acid eructation (Kathiresan 2001; Ramanathan, 2000). The fruits are plastered onto tumors in India. Unripe seeds are poulticed onto abscess, boils, and smallpox sores. Indochinese uses the bark for skin afflictions, especially scabies. A resinous substance exuded from the bark acts as a contraceptive, and apparently can be taken all year long without ill effects (Thirunavukkarasu *et al*., 2011). Plant extract shows some antimicrobial and antioxidant activity (Sharief and Rao, 2011; Thirunavukkarasu *et al*., 2011).

MATERIALS AND METHODS

Sample Collection Areas: The plant samples were collected from different sites of the mangrove forest of Indian Sundarban (between 21˚40'04"N - 22˚09'21"N latitude, and 88˚01'56"E - 89˚06'01"E longitude) located in the South 24-Paragnas District of West Bengal, India.

Plant material: Leaves of the plant mentioned in Table 1 were collected randomly from different parts of the above mentioned sample collection areas and were subsequently used for conducting tests.

Sample Collection and Processing: Fresh and healthy leaves of the plant mentioned in table1 were collected from the above mentioned sample collection areas and used for the present investigation. The leaves were then washed with sterile distilled water to make it free from dirt and dust, quickly mopped and dried on blotting sheets. These leaves were then shade dried at room temperature for 10 days. Shade dried leaves were then cut into small pieces and crushed to obtain powder with the help of a mechanical grinder.

Extraction: 50 grams of mangrove leaf powder was added to 250 ml. of methanol. After 48 h of incubation in the shaker, the supernatant was collected and freed from solvent by evaporation under reduced pressure. The residues (crude extracts) obtained were finally dried under vacuum, stored in sterile dark containers and were tested for their phytochemical profile and antimicrobial activities.

Preliminary Qualitative Phytochemical Analysis: Phytochemical constituents from the mangrove sample extracts were determined qualitatively. Crude extracts of the plants under study were screened for the presence of alkaloids, phenolics, flavonoids, tannins, diterpenes, triterpenes, sterols, saponins, glycosides, proteins, carbohydrates and reducing sugars using the methods adopted from different authors (Hanaa et al., 2008; Panda et al., 2012).

Evaluation of Antibacterial Activity

Microorganisms, culture media and incubating temperatures: All of the different extracts were individually tested against a panel of bacteria including Gram negative *Erwinia herbicola* (MTCC NO. 3609) incubated at 37^0C, *Escherichia coli* (MTCC-443) incubated at 37^0C, *Serratia marcescens* (MTCC NO. 7298) incubated at 30^0C, *Xanthomonas* sp. (MTCC NO. 7444) incubated at 30^0C and Gram positive *Arthrobacter chlorophenolicus* (MTCC NO. 3706) incubated at 28^0C, *Bacillus subtilis* (MTCC-441) incubated at 37^0C, *Staphylococcus aureus* (MTCC-96) incubated at 35^0C. All the bacterial strains were procured from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology (IMTECH), Chandigarh, India. The reference strains of bacteria were maintained on nutrient agar medium and LB medium slants at 4^0C with a subculture period of 30 days.

Preparation of McFarland standard: The turbidity standard was prepared by mixing 0.5 ml of 1.75% (w/v) $BaCl_2.2H_2O$ with 99.5 ml of 1% H_2SO_4, $BaSO_4$ (v/v). The standard was taken in screw cap test tube to compare the turbidity. The bacterial culture of selected strains were grown for 48- 72 hours and subsequently mixed with physiological saline. Turbidity was corrected by adding sterile saline until McFarland 0.5 $BaSO_4$ turbidity standard 10^8 Colony Forming Unit (CFU) per ml was achieved. These inoculates were used for seeding of the nutrient agar medium, LB medium respectively.

Disc diffusion assay: 1 mg of each sample was separately dissolved in 1 ml of propylene glycol and then the volume was adjusted to 10 ml by adding sterile water. The ultimate concentration reaches to 10^2 µg/ ml and sterilized by filtration (0.22 µm filter). From the solution of each concentrated sample(s) final concentrations were made from 500 µg/ ml to 100 µg/ ml by adding sterile double distilled water. The sterile paper discs (6 mm diameter) were saturated with 10 µl of the solution of the respective sample(s) at a concentration of 500 µg/ ml to 100 µg/ ml and placed on the inoculated agar of 10^8 CFU/ml. Antibacterial tests were then carried out by disc diffusion method (Sokmen et al., 2004) using 100 µl of suspension containing 10^8 CFU/ml of bacteria on nutrient agar medium, LB medium respectively. Negative controls were prepared using propylene glycol. Gentamicin (10 µg/ disc) was used as positive reference standards to determine the sensitivity of each bacterial species tested. The inoculated plates were incubated at 30^0 C, 37^0 C and 28^0 C respectively for 48 h, 24 h and 72 h. Antibacterial activity was evaluated by measuring the zone of inhibition and the diameters of these zones were measured in millimeters against the test organisms.

Determination of Minimum inhibitory concentration: The minimal inhibitory concentration (MIC) values were studied for the bacteria strains, being sensitive to the sample(s) in disc diffusion assay. The inoculates of the bacterial strains were prepared from 24-72 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The samples were dissolved in sterile propylene glycol, first diluted to the highest concentration (500 µg/ml) to be tested, and then serial dilutions were made in order to obtain a concentration range from 500 to 100 µg/ml in 10 ml sterile test tubes containing nutrient broth and LB broth medium respectively. MIC values of the sample(s) against bacterial strains were determined based on a micro well dilution method. The plate was covered with a sterile plate sealer and then incubated at appropriate temperatures for 24 - 72 h at 30^0 C, 37^0 C, 30^0 C and 28^0 C respectively. Bacterial growth was determined by absorbance at 600 nm and confirmed by plating 10 µl samples, forming clear wells on nutrient agar medium or LB medium respectively. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms. Each test in this study was repeated, at least, thrice.

RESULTS AND DISCUSSION

Results of Phytochemical Analysis and Discussions: The results of the preliminary phytochemical analysis of crude extract of the mangrove plant under study to reveal the presence or absence of alkaloids, phenolics, flavonoids, tannins, diterpenes, triterpenes, sterols, saponins, glycosides, proteins, carbohydrates and reducing sugars are summarized in Table 2. *Avicennia officinalis* extract contains alkaloids, phenolics, flavonoids, tannins, diterpenes, triterpenes, sterols, glycosides, carbohydrates and reducing sugars but lacks saponins and proteins. For many years the adaptive significance of most secondary metabolites was unknown. These compounds were thought to be simply functionless end product of metabolism, or metabolic wastes. Today we know that many secondary metabolites have ecological functions in plants. From the study, it is clear that *Avicennia officinalis* is phytochemically very diverse. These chemicals function as agents of plant-plant competition and plant microbe symbioses. They also attract pollinators and seed dispersers. Moreover, they protect plants against herbivores (mammals and insect) and against being infected by microbial pathogens.

Table 1. Some attributes of the plant species used in the study

Scientific Name	Family	Habit	Flowering Period	Fruiting Period	Local Name
Avicennia officinalis L.	Avicenniaceae	Tree	April to August	July to October	Jat Baen

Table 2. Qualitative phytochemical profile of the plant under study

Sl. No.	Phytochemicals screened	Tests done	Present(+) /Absent(-)
1	Alkaloids	a.Mayer's test	+
		b.Wagner's Test	+
		c. Hager's Test	+
		d. Dragendroff's test	+
2	Phenolics	.Ferric chloride test	+
3	Flavonoids	a.Ferric chloride test	+
		b.Lead acetate test	+
4	Tannins	a.Ferric chloride test	+
		b.Gelatin test	+
5	Diterpenes	a. Copper acetate test	+
6	Triterpenes	a.Salkowski test	+
		b. Lieberman Burchardt test	+
		c.Tschugajen test	+
7	Sterols	a .Salkowski Test	+
		b. Lieberman Burchardt test	+
8	Saponins	a. Foam test	−
		b. Haemolysis test	−
9	Glycosides	a.Sodium hydroxide reagent	+
		b.Kellar Killani's test	+
10	Protein	Millon’s test	-
11	Carbohydrate	Molisch’s test	+
12	Reducing sugar	Fehling’s test	+

Table 3. Antibacterial activity of the methanol extracts of *Avicennia officinalis* against the bacteria tested based on disc diffusion method

Name of the bacterium	Antibacterial potentiality of the crude extract
Arthrobacter chlorophenolicus	No
Bacillus subtilis	No
Staphylococcus aureus	Yes
Erwinia herbicola	No
Escherichia coli	No
Serratia marsescens	Yes
Xanthomonas campestris	No

Table 4. Assessment of antibacterial potentiality of the crude extract of *Avicennia officinalis*

Name of the bacterium	Concentration of extract (µg/ml)				
	100	200	300	400	500
Arthrobacter chlorophenolicus	No	No	No	No	No
Bacillus subtilis	No	No	No	No	No
Staphylococcus aureus	3.5mm*#	4.9mm*	5.4mm*	5.9mm*	6.2mm*
Erwinia herbicola	No	No	No	No	No
Escherichia coli	No	No	No	No	No
Serratia marsescens	4.3mm*#	7.1mm*	10.6mm*	13.1mm*	15.3mm*
Xanthomonas campestris	No	No	No	No	No

*Diameter of inhibition zone around the discs impregnated with extracts.No- means no antibacterial activity of the extract.
#the corresponding concentration of extract represents the minimal inhibitory concentration (MIC) value.

Figure 1. Leaves of *Avicennia officinalis* after seven days of shade drying

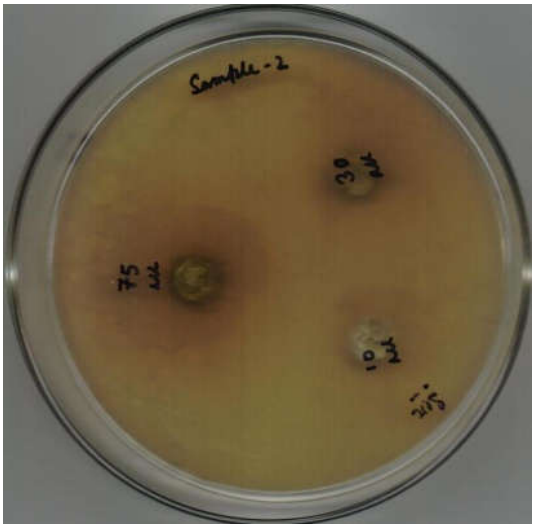

Figure 2. ***Avicennia officinalis*** **extract against** ***Staphylococcus aureus***

Figure 3. ***Avicennia officinalis*** **extract against** ***Serratia marsescens***

The ability of plants to compete and survive is therefore profoundly affected by these phytochemicals. Some of these chemicals protect plants from high UV radiation characteristics of their habitat. So they help the plants to adapt in harsh environment of mangrove habitat.

Results of Antibacterial Assay and Discussions: Different secondary metabolites from the groups namely terpenoids, phenolics and alkaloids are known for their antibacterial activity and the phytochemical analysis of extracts of the studied plant species showed the presence of one or the other group(s). So, we can expect some antibacterial properties in the extracts. This study revealed the preliminary evidence of antibacterial ability of leaf extracts in methanol. The details of antibacterial activity of the plant extracts are given in the following tables (Table 3 and Table 4) and Figure 2 & 3. Traditionally, mangrove plants have been used medicinally in diverse cases like to treat infections (TriSiam, 2011), relief pain and purify blood (Singh & Suttee, 2009) and as antioxidant (Chan et al., 2011). Alkaloids, terpenoids, sterols and phenolics were reported to exhibit different biological activities (Kubmarawa *et al*., 2008). For example, saponins are known to associate with hypercholesterolemia, hyperglycemia (Rupasinghe *et al*., 2003), anticancer, anti-inflammatory activities.

Plant steroids are known for their anti-inflammatory (Akindele and Adeyemi, 2007), analgesic (Malairajan *et al*., 2006), anti-microbial activities and ability to act on central nervous system (Argal and Pathak, 2006). Tannins have been widely recognized for their pharmacological properties. These are well studied for anti-diabetic, anti-inflammatory and anti-bacterial activities. Mangrove species with medicinal properties are harvested as herbal remedies by coastal communities in some countries including India. Antimicrobial activity of the leaf extract was tested against seven bacterial species used in the study. The plant extracts were unable to exhibit antibacterial activity against five of the tested bacteria. These bacteria may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the extract used may not contain sufficient amount of active principle(s). The extract of *Avicennia officinalis* showed broad-spectrum activity and was effective against two of the seven bacteria tested namely *Staphylococcus aureus* and *Serratia marsescens* with a MIC value of 100 µg/ml in both the cases.

The difference in sensitivity and MIC values (which is a measure of degree of effectiveness) may be due to the qualitative difference of the phytochemicals present and/or concentration of the active principles present in different plant extracts. Although the extract was active against only two of the tested bacteria, this result is very promising in that it was effective against both Gram positive and Gram negative bacteria. The results of the present study support the traditional use of the mangrove plant species under study as ethnomedicine. The profound chemical diversity within the mangroves provides an opportunity for the discovery of new drugs. The present work needs further extension to isolate and characterize the active anti-bacterial principles. These studies reveal that the plants under study can be considered as potential ethno pharmacological plants for the treatment of infections caused by Gram (+)ve as well as Gram (–)ve bacteria. Therefore the leaf extracts can be used to discover new bioactive natural products that may serve as natural antibiotics to replace or at least to supplement the synthetic pharmaceuticals to control microbial infection. The plant studied here can be used as a potential source of useful drugs. There is need for further studies on the plants parts in order to isolate, identify, characterize and elucidate the structure of antimicrobial bioactive compounds.

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