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Antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis*

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ABSTRACT

Objective: To explore antibacterial activity and characterization of secondary metabolites isolated from mangrove plant *Avicennia officinalis (A. officinalis)*. **Methods:** In the present study the leaf extracts of *A. officinalis* were examined for its antibacterial potential using five different solvents against some reference strains of human pathogenic bacteria for the crude extract. Maximum activity was observed for ethyl acetate and hence different concentrations like 15 μL, 25 μL, and 50 μL of ethyl extracts was checked for its antibacterial activity. Partial purification of crude extract was carried by column chromatography and fractions were analyzed using gas chromatography-mass spectrometry (GC-MS) to identify compounds. **Results:** The crude ethyl acetate extracts of *A. officinalis* showed remarkable antibacterial activity with zones of inhibition of 13 mm against *Eschericia coli* (*E. coli*) and 11 mm against *Staphylococcus aureus* (*S. aureus*). Fraction 13 (ethyl acetate : methanol= 8 : 2) as the most potent one against with the minimal inhibitory concentration of 30 mm against *E. coli* and 25 mm against *S. aureus*. The GC-MS resultsof active column fraction (F13) revealed that the active principals were a mixture of hydroxy- 4 methoxybenzoic acid, diethyl phthalate, oleic acid. **Conclusions:** The leaf extracts with proven antibacterial effects can clearly be directed towards cancer treatment as to inhibiting cancer cell growth. The limited number of test organisms owes to a constraint of resource. So, the effect of strong bursts of leaf extracts on human pathogenic bacteria should further be tested on a wide range of test organisms.

1. Introduction

Mangroves have long been a source of astonishment for the layman and of interest for scientist and are biochemically unique, producing a wide array of novel natural products. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds. They provide a rich source of steroids, triterpenes, saponins, flavonoids, alkaloids and tannins. Therefore, it is worth to screen mangrove plants for the presence of new antibacterial compounds to combat the pathogenic bacteria. Antimicrobial activities of plant constituents such as phenol, quinines, flavones, flavanoids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors[1,2]. There is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and reemerging infectious diseases[3]. In addition to absorbing the effects of storm, these 'rainforests by the sea' have been considered a healthy source of life. To the best of our knowledge, medicinal plants are relied upon by 80% of the world's population and in India the use of mangroves for therapeutic purpose remains an important compound of traditional medicinal system. In light of this, the present study was initiated to investigate the leaf extracts of *Avicennia officinalis (A. officinalis)* collected from Parangipettai, Chidambaram District for its biological activity against certain bacteria that play havoc on human health.

2. Materials and methods

2.1. Sample collection and extraction of mangrove bioactive

Leaves of *A. officinalis* were collected from mangrove forest of Parangipettai, Chidambaram located in Tamilnadu. Prior to the extraction, the leaves of respective species were

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washed with sterile water to remove any associated debris., shade dried in order to prevent photolysis and thermal degradation, chopped into small pieces and ground coarsely to powder form in a mechanical grinder. For extraction of crude bioactives, 50 g of powered mangrove material were exhaustively extracted with 200 mL of various solvents like ethanol, petroleum ether, acetone, methanol and ethyl acetate using soxhlet apparatus. The extracts were further concentrated by recovering excess solvents to a thick oily natured crude in a rotary evaporator at reduced pressure. The extract was stored at 4 ℃ in air- tight plastic vials for further studies.

2.2. Antibacterial assay

Antibacterial activity was carried out against gram negative bacterium [*Eschericia coli (E. coli)*, ATCC 25922] and a gram positive bacterium [*Staphylococcus aureus (S. aureus)*, ATCC 29213] by agar well diffusion method. The assay system was prepared with gel punctured Muller Hinton Agar plates. Test culture was swabbed aseptically and inoculated on the surface of the Muller Hinton Agar so as to make a lawn and left to dry for the wells to be perfect. The leaf extract was dissolved in 1 mL of the solvent from which various (15 μL, 25 μL, 50 μL/well) concentrations were taken and loaded in the well using micropipette and one well was loaded with the respective solvent as control. Plates were incubated for 16 to 18 hrs at 37 ℃. The percentage of mortality was determined by observing the zones of inhibition.

2.3. Column chromatography and gas chromatography-mass spectrometry (GC-MS) analysis

The ethyl acetate extract of *A. officinalis* (1 gm) was loaded on a silica gel column packed with hexane and eluted with hexane and chloroform (9 : 1 to 1 : 9 & 100% chloroform) followed by ethyl acetate and methanol (9 : 1 to 1 : 9 & 100% methanol) to yield 26 fractions. Individual fractions when collected and tested revealed that the 13th fraction that was eluted using ethyl acetate and methanol (8 : 2) had maximum activity. The absorbance of the fractions eluted from the column chromatography was measured at a resolution from 200-800 nm using UV-Visible spectrophotometer and the readings were recorded. The fractions that were eluted in column chromatography using chloroform and ethyl acetate (2 : 8) exhibiting activity was subjected to GC-MS equipped with Agilent 5975 inert XL MSD to find out the active principle of the extracts.

3. Results

The plant material was subjected to an extraction process with solvents like ethanol, petroleum ether, acetone, methanol and ethyl acetate. The crude ethyl acetate extracts of *A. officinalis* showed remarkable antibacterial activity with zones of inhibition of 13 mm against *E. coli* and 11 mm against *S. aureus* (Table 1).

Bioactivity guided fractionation is another best method to seek the active compound by separating mixture compounds from targeting extracts. Elution of individual fractions of foliar extracts of *A. officinalis* by column chromatography and their absorbance at 200 to 300 nm in a UV visible spectrum revealed fraction 13 (Figure 1) (ethyl acetate: methanol= 8 : 2) as the most potent one against with the minimal inhibitory concentration (MIC) of 30 mm against *E. coli* and 25 mm against *S. aureus* (Table 1).

The GC-MS resultsof active column fraction (F13) revealed that the active principals were a mixture of hydroxy- 4 methoxybenzoic acid, diethyl phthalate, oleic acid (Figure 2).

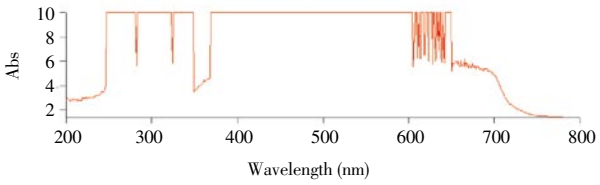

Figure 1. UV-visible spectrum of the extracts eluted from column chromatography (F13 fraction).

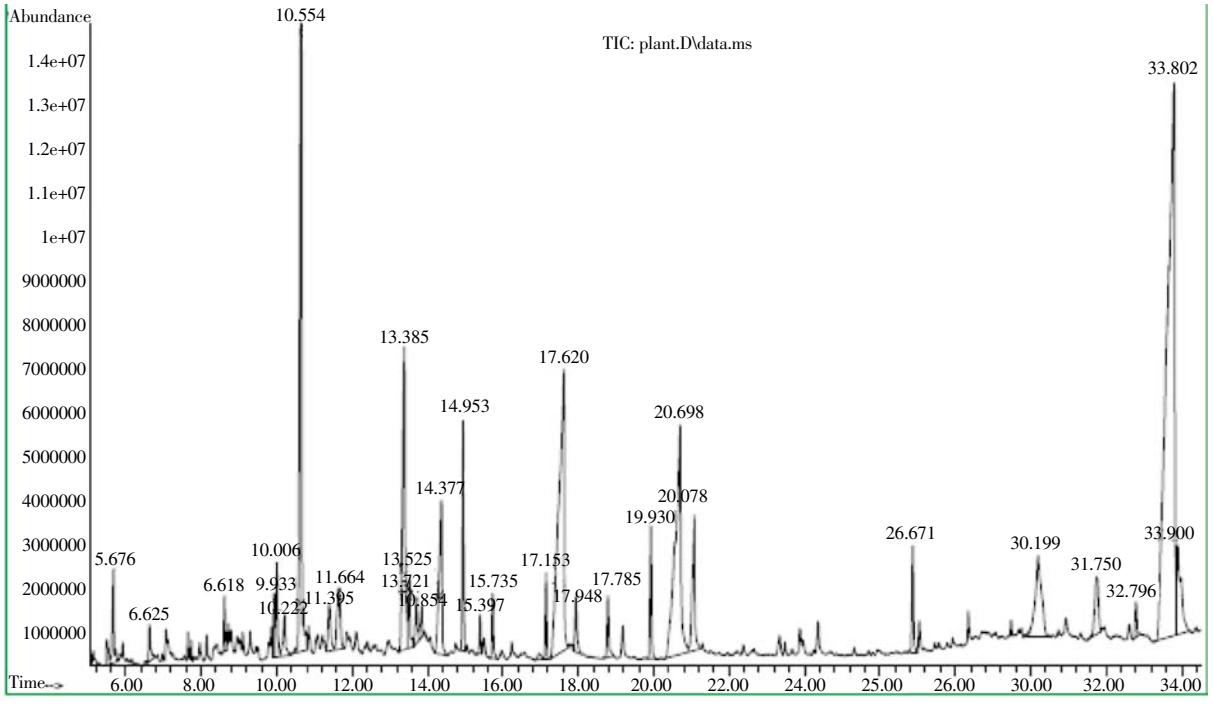

Figure 2. GC - MS spectrum of *A. officinalis*.
Abundance: hydroxy- 4 methoxybenzoic acid, diethyl phthalate, oleic acid.

Table 1
Antibactetrial activity of *A. officinalis*.

S.No	Organism	Sample	Zone of inhibition (mm)			MIC
			15 μL	25 μL	50 μL	
1	Crude extract	*E. coli*	10	10	13	10
2		*S. aureus*	8	9	11	8
3	Fraction eluted from column chromatography	*E. coli*	11	22	30	11
4		*S. aureus*	12	22	25	12

4. Discussion

The test organisms are based on the choice of their ubiquitous presence. *E. coli* is one of the causes of cholecystitis, bacteremia, cholangitis, neonatal meningitis, pneumonia and travellers diarrhea. And *S. aureus* has its notorious reputation for causing cellulites, boils, impetigo and scalded skin syndrome. The best of diagnosis, clinicians and treatment fail to treat the affected, as the antibiotic resistance in some strains are on the rise. This study also provides us hope to overcome failures of drug resistance by development of new drugs. Many researches in previous studies have also used a small number of test microorganisms[4].

The sensitivity of ethyl acetate to all of the mangroves extracts could be attributed due to the presence of common bioactive compounds that had inhibitory effects on the microorganisms. In comparison to our study, antibacterial activity of mangroves against fish pathogens had already been studied by many authors. Some scientist reported the root extracts of *Avicennia marina* against fish pathogens[5].

Future extension of this study includes bioassay against human cell lines especially cancer cells. Jones *et al* reported 3 chlordeoxylapachol, a 20 metabolite obtained from the chloroform extract of mangrove tree was active against K B Human cancer cells in the murine hallow fibre antitumour model[6].

Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoids, alkaloids, have variously been reported to have antimicrobial activity[7].

In our study, the crude extracts failed to exhibit the desired response, where as the fractions showed broad-spectrum activity against a few test organisms. This is believed to be a masking of antibacterial activity by the presence of some inhibitory compounds or factors in the extracts or may be a case of synergism too. Natarajan *et al* studied the antibacterial activity of methanolic leaf extracts of mangrove plants collected from Pichavaram and Thondi[8].

The studies of Powar *et al* that included screening the methanolic extracts of barks of eight mangrove species for antibacterial activity against Bacillus megateriumn and *S. aureus* did prompt us to study the leaf extracts for antibacterial effects[9].

With traditional knowledge taking a backseat, there is a grave danger of mangroves disappearing. So, documenting the scientific knowledge through research and development is important for the conservation and utilization of mangrove resources.

This is one of the best-designed studies to prove the antibacterial activity of leaf extracts of *A. officialis*. These results not only give a base for further research but also are useful for drug development in the present and future. This study can further be extended to test the clinical efficacy of the extracts.

The results do have immediate goals if further perusal with a larger motive is carried out. The leaf extracts with proven antibacterial effects can clearly be directed towards cancer treatment as to inhibiting cancer cell growth. The limited number of test organisms owes to a constraint of resource. So, the effect of strong bursts of leaf extracts on human pathogenic bacteria should further be tested on a wide range of test organisms. This study also means a natural alternative to antibiotics, which is an exciting and potentially extreme area of research.

Conflict of interest statement

We declare that we have no conflict of interest.

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