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Antibacterial potential of mangrove plant *Avicennia marina* against a clinical pathogen

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Abstract
Marine mangrove plant *Avicennia marina* (AM), commonly called the grey or white mangrove, is a species of mangrove trees belonging to the Acanthaceae family. Traditionally it is used in to cure skin diseases against fish stings, ringworms, sores, boils, skin ulcers and scabies. It has also been used as a contraceptive and in treating rheumatism. In the literature, AM has been reported to exhibit antifertility, anticancer, antimicrobial and antitumor activities. Phytochemically, AM has been found to contain a variety of natural product groups, including naphthalene derivatives, flavones, glucosides, phenylpropanoid glycosides, abietane ditrerpenoid glycosides, flavonoid terpenoids and steroids. In the present study, methanolic crude extracts of the gray mangrove Avicennia marina *leaf* was evaluated for its antimicrobial activity. The results have shown that the extract attempt to inhibit the growth of Bacterial species *Pseudomonas aeruginosa.* The methanol extract of leaf has significant effect on bacterial strains with inhibition zones ranging from 17 to 21 mm respectively.

Keywords: mangrove, avicennia marina, disc diffusion, *pseudomonas aeruginosa*

1. Introduction
Mangroves are perennial plants that grow in coastal wetlands of tropical regions. They are found in the marine environmental resources like inter-tidal zones of sheltered shores, estuaries, creeks, backwaters, lagoons, marshes and mud-flats. Mangrove forests are regarded as the wetlands on earth with biological diversity. Nearly 7% of the world mangroves are distributed in India [1]. They all are medicinal plants and known to produce certain bioactive molecules which react with other organisms in the environment; inhibiting bacterial or fungal growth. Antimicrobial properties of mangrove plants are being increasingly reported from different parts of the world. However, during the last decade screening of mangroves for bioactive active compounds, has received high interest as a potential bioresource for novel drug leads. Until now, more than 200 bioactive metabolites have been isolated from true mangroves like *Avicennia marina* in tropical and subtropical populations [2]. Mangroves are used in traditional medicine for the treatment of many diseases. The mangrove plants have also been proved for antiviral, antibacterial and antiulcer properties. 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 [3]. Secondary metabolites like alkaloids, phenolics, steroids, terpenoids have been characterized from mangroves and have toxicological, Pharmacological and ecological importance [4].
In the present study, bioactive metabolites of mangrove, obtained from Avicennia marina leaves were extracted and screened against clinically important multi-drug resistant bacterial strain like *Pseudomonas aeruginosa.*

2. Materials and methods
2.1 Plant collection
Avicennia marina leaves were collected from Pichavaram mangrove forest which is located in Cuddalore District, Tamil Nadu in southeast coast of India. Geographically it is located 11°24'- 11°27' North latitudes and 79° 46'-79° 48' East longitudes. Prior to the extraction laves of respective species were cleaned, shade dried in order to prevent photolysis and thermal degradation, The plant materials were taxonomically identified and authenticated by Department of Marine Biotechnology, Centre for Advanced Studies in Marine biology, Portonovo, Tamil Nadu, India.

2.2 Extract Preparation
Fresh Avicennia marina leaf sample were collected (2kg). The leaves washed in distilled water and shade dried at room temperature at 37°C and ground well in a manual Mill Shimdzu Blender [5]. 10 gram of the powder form of leaf material was stirred overnight in 70% methanol (100 ml), and then centrifuged at 10,000 rpm for 10 min at 4°C. The resultant supernatant was collected and the methanol removed by evaporation, yield the final product of extract in fine powder form. Then 0.5g of leaf extract was stirred well with 10 ml of boiling distilled water and used for antimicrobial assay.

2.3 Test microorganism
Pseudomonas aeruginosa was collected from Department of Microbiology, Bharathidasan University, Tiruchirappalli, Tamil Nadu used for the present study.

2.4 Antimicrobial Assays
Anti-bacterial activity of the *Avicennia marina* leaf extracts and their efficacy was quantitatively assessed using the Kirby-Bauers well diffusion method [6]. The inoculated plates were incubated at 37°C for 24 h and the inhibition zone was observed. All the experiments were carried out in triplicate. The following formula was used for comparison of the antimicrobial activity of the sample with that of the standard. By measuring the diameter of growth inhibition zone and the results were shown in plate (1), Table (1) and Figure (1) The aquous extract of *Avicennia marina* containing bioactive

compounds were used in the assay for anti-bacterial activity.

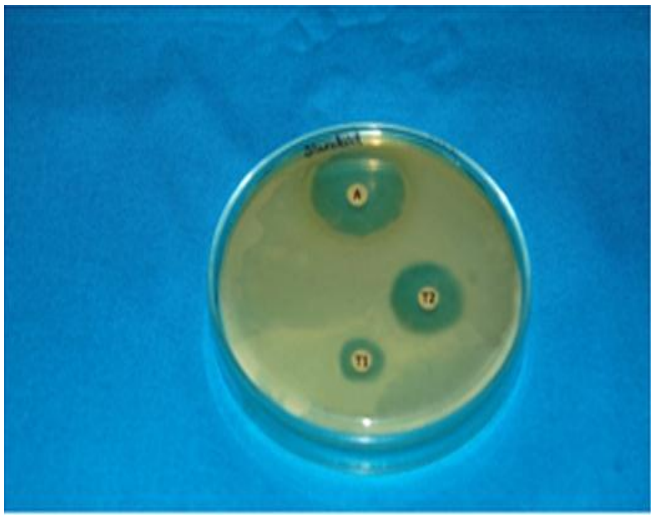

Plate 1: Kirby-Bauer's disc diffusion assay against P. aeruginosa

In the experimental wells only the aquous extract of the volume 100 μl was added by micro-pipette. The diameter of inhibition in *Avicennia marina* extract against *Pseudomonas aeruginosa* growth indicates the inhibitory effect (Plate 1) The diameter of inhibition in test extract (or) bioactive compounds or drug of *Avicennia marina* was 21 mm. The antibacterial effect of *Avicennia marina* shows positive result, when compared to regular standard antibiotics like penicillin and Tetracyclin. In solvent there is no inhibition. The diameter of inhibition of standard antibiotics shows; penicillin 17 mm and Tetracyclin (14 mm). The antibacterial activity of the aqueous extracts of *Avicennia marina* indicates greater efficiency than Tetracyclin and penicillin. In the present study; the percentage of inhibitory value in extracts of Avicennia marina is (70%) whereas in standard (table-1).

3. Results and Discussion

Antibacterial activity of aquous extract of *Avicennia marina* on *Pseudomonas aeruginosa* carried out by Kirby bauer well diffusion assay method. Similar findings were made by some researchers. Some reported the anti-bacterial activity of *Avicennia marina* against the microbes of gastro intestinal tract [7-9].

In the present study, methanol extract exhibited different degree of growth inhibition against tested bacterial strain *Pseudomonas aeruginosa* (Table 1).

According to Table 1, methanolic extracts of *A. marina* exhibited considerable antimicrobial activity against tested microbial strain and showed more inhibition than standard antibiotics (Table 1). The results of the present study clearly showed that mangrove plant *A. marina* extracts showed antimicrobial activity against tested pathogenic strains including antibiotic resistant strains. The effectiveness of the active compounds present in plant extracts cause the production of growth inhibition zones that appear as clear areas surrounding the wells. Antibacterial activity may be due to active components which are present in plant extracts [10, 11]. No inhibition was observed with controls, which proves that solvents could not act as antibacterial agents. In almost all tests, crude methanolic extracts showed better inhibition against the tested bacterial strain, indicating that active ingredients in plant materials could be extracted into methanol. According to the previous studies the presence of secondary metabolites such as alkaloids, flavonoids and steroids may exert antibacterial activity against tested bacterial strains [12, 13]. Further research is necessary for successful separation, purification and characterization of biologically active compounds using chromatographic methods and spectroscopic techniques.

Table 1

Test organism	Zone of inhibition in(mm)			
	Negative control (solvent only)	Positive control		Experimental
		Penicillin	Tetracycline	Methanol Extract
Pseudomonas aeruginosa	Nil	17 mm	14 mm	21mm
Percentage of Inhibition		68%	63%	71%

Negative control
It implies that the well contains only the solvent (acetone) devoid of bioactive compounds and authentic antibiotics.

Positive control
It indicates that the well contains authentic antibiotics penicillin, tetracycline in aqueous medium.

Experimental
It implies that the well contains only the bioactive compounds of the leaf extract obtained from *Avicennia marina*

Average
Mean value of 3 different assays.

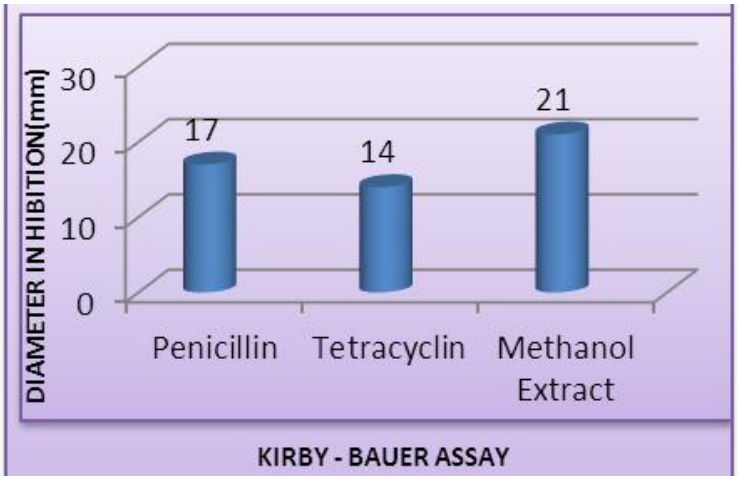

Fig 1

4. References

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