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Antibacterial Activity of Mangrove Leaf and Bark Extracts Against Human Pathogens

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Abstract: The present study investigated the antibacterial activity of leaves and bark extracts of *Ceriops tagal* and *Pemphis acidula* using acetone, methanol, ethanol and water extract against human pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Vibrio parahaemolyticus, Staphylococcus aureus* and *Vibrio cholera*. The *Pempis acidula* possessed higher antibacterial potency than the *Ceriops tagal*, with which the highest activity was recorded in methanol extract of bark, more active against *S. aureus* (17.2 ± 0.1 mm). The minimum inhibitory concentration (MIC) value was obtained as 75 mg/mL for methanol extract of the same. The present preliminary results suggest a baseline idea for further isolation, identification, separation and characterization of active principle compound(s) from the effective mangrove plant, which might be useful for the controlling the drug resistant bacterial pathogens.

Key words: Antibacterial activity · *Ceriops tagal* · *Pemphis acidula* · Human pathogen MIC

INTRODUCTION

The marine world offers an extremely rich resource for important compounds of structurally novel and biologically active metabolites. It also represents a great challenge which requires inputs from various scientific areas to bring the marine chemical diversity up to its therapeutic potential. So far, many chemically unique compounds of marine origin, with different biological activities, have been isolated and a number of them are under investigation or development [1-3].

Mangrove plants have been used in folklore medicines and extracts from mangrove species have proven inhibitory activity against human, animal and plant pathogens. Several species of mangrove produce bioactive compounds that may control microbial growth [4-6]. Also, preliminary studies have demonstrated that the mangrove plant extracts have antibacterial activity against pathogenic bacterial strains; *Staphylococcus* sp., *E. coli* and *Pseudomonas* sp. and antibiotic resistant bacterial strains; *Staphylococcus* sp. and *Proteus* sp. [7-8]. Mangrove extracts can also be the possible sources of mosquito larvicides, antifungal, antiviral, anti-cancer and anti-diabetic compounds [9-13].

Secondary metabolites like alkaloids, phenolics, steroids and terpenoids have been characterized from mangroves and have toxicological, pharmacological and ecological importance [14, 15]. However, these studies are restricted to the mangroves of muddy region. Only few species like *Pemphis acidula* are growing only in coral sand substrates [16]. Studies on such species do not exist too much. Hence, the present investigation aimed to screen and compares the antibacterial potential of bark and leaves of sandy *Pemphis acidula* and muddy *Ceriops tagal* mangrove species collected from the Gulf of Mannar.

MATERIALS AND METHODS

Plant Materials: Leaves and bark of *Pemphis acidula* and *Ceriops tagal* were collected from Poomarichan Island Gulf of Mannar in January 2008.

Preparation of Plant Extracts: The collected plant materials were shade dried at room temperature until constant weight obtained and powdered. 100 gram of bark and leaf sample were then extracted individually with acetone, methanol, ethanol and water in a Soxhlet

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apparatus. The obtained extracts were concentrated to dryness by evaporating the solvents under reduced pressure using rotary evaporator [17].

Pathogenic Microorganisms: Bacterial pathogens such as *Pseudomonas aeruginosa, Klebsiella pneumoniae, Vibrio parahaemolyticus, Staphylococcus aureus* and *Vibrio cholera* were obtained from the Rajah Muthiah Medical College and Hospital (Annamalai University, Tamil Nadu, India). Cultures of the bacteria were maintained on nutrient agar slants at 4°C.

Antimicrobial Susceptibility Testing: The antibacterial assay was carried out by the agar well diffusion method [18] using Mueller Hinton agar medium (Himedia Pvt Ltd Mumbai). The microorganisms were activated by inoculating a loopful of the strain in nutrient broth (20 ml) in a 100 ml Erlenmeyer flask and incubated at 37°C on a rotary shaker for 24 h. Then, 0.1 ml of the fresh inoculum was spread onto the surface of sterile Mueller Hinton agar. Wells (4 mm diameter) were made on the seeded plates with the help of a sterilized cup-borer (4mm). The collected different extracts were further dissolved in DMSO (dimethyl sulfoxide, 5%, v/v). The diluted extracts (50µl) were dispended into the well and the plates were incubated aerobically at 37°C. In the same way, a negative and positive control wells were made with only DMSO and streptomycin (50µg/ml), respectively. The zones of inhibition (mm) of the different extracts were examined after 24 h. These studies were performed in triplicate.

Minimum Inhibitory Concentration (MIC): A broth micro dilution method was used to determine the minimum inhibitory concentration [19, 20]. All tests were performed in Mueller-Hinton medium (Himedia Pvt Ltd Mumbai). Serial double dilutions were prepared with a mixture of maximum active mangrove extracts: dimethylsulfoxide 95:5 in a 96- well microtiter plate over the range of 7-3,125 µl/l. Overnight broth culture of each strain was prepared and the final concentration of the microbe in each well was adjusted to 2×10^3 CFU/ml. Plates were incubated at 37°C for 24 h. The MIC was achieved at the lowest concentration of the mangrove extract at which the microorganism didn't demonstrate visible growth and the absorbance of each well was determined using an automatic ELISA tray reader adjusted at 630 nm (SLT Spectrophotometer). The samples were analyzed in duplicate and the assay was repeated twice. The antibiotic streptomycin was employed as positive control.

RESULT AND DISCUSSION

The results of antibacterial activity of the two mangrove plant parts extracted in various solvents are given in Table 1. The bark extract inhibited the growth of all the tested pathogens than the leaf extracts of both *Ceriops tagal* and *Pempis acidula,* in all the types of solvents used in the present study. Among the mangrove species, *Pempis acidula* exhibited maximum antibacterial activity, with a mean zone of inhibition of more than 17 mm. In contrast, the leaf extracts of the tested mangrove showed very less antibacterial activity as these produced a mean zone of inhibition less than 7 mm. Among various solvents, methanol extracts of *P. acidula* showed the maximum activity, which was highest against *S. aureus*.

The results of the present study clearly indicated that mangrove plant *Pempis acidula* extracts showed antimicrobial activity against tested pathogenic strains. The MIC value of two mangrove bark extract showed activity against all the tested pathogen as described in Table 2. Of the two mangrove species studied, *Pempis acidula* were effective against *S. aureus* at 75µg/ml. The methanol extracts of MIC values showed better inhibition than the other extracts. The present data are more or less consistent with the results obtained by [21] against *S. aureus, E. coli*, *P. aeruginosa, M. luteus* and *R. rhodochrous.* Certain other mangrove species such as *Rhizophora mucronata, R. lamarkii* and *Bruguiera cylindrica* were earlier reported to show antibacterial activity against methicillin resistant *Staphylococcus aureus* [22]. Similarly the aqueous extracts of *E. agallocha, L. racemosa and A. corniculatum* also showed antibacterial activity [23]. The antibacterial activity exhibited by the mangrove plant parts could be due to the presence of phytochemical like alkaloids, tannins, flavonoids and sugars present in the plant extracts [24]. However, some plant extracts were unable to exhibit antibacterial activity against tested bacterial strains. These bacterial strains may have some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the compound used may not be sufficient [25].

In almost all tests, crude methanolic extracts showed better inhibition against all tested bacterial strains, indicating that active ingredients in plant materials could be extracted into methanol. The chosen mangrove plants are to have reported are very heterogeneous mixtures of single substances which may act in a

Table 1: Antibacterial activity of *Pempis acidula* and *Ceriops tagal* extracts against the tested pathogens

		Inhibition zone (mm) 50 µg concentrations/well (4 mm)									
		Leaves				Bark					
Pathogens	Mangrove	Methanol	Aqueous	Acetone	Ethanol	Methanol	Aqueous	Acetone	Ethanol	DMSO	Streptomycin. 50 µg/ml
Pseudomonas aeruginosa	*Pempis acidula*	6.0±0.3	12.0±0.6	15.0±0.6	9.0±0.1	0.0±0.0	5.0±0.1	6.2±0.1	4.2±0.2	0.0±0.0	13.0±0.2
	Ceriops tagal	8.0±0.3	11.0±0.3	7.0±0.1	5.2±0.3	3.0±0.1	7.5±0.1	6.2±0.1	5.0±0.1		
Klebsiella pneumoniae	*Pempis acidula*	0.0±0.0	6.3±0.2	13.0±0.3	12.0±0.2	0.0±0.0	0.0±0.0	7.0±0.0	0.0±0.0	0.0±0.0	10.0±0.2
	Ceriops tagal	0.0±0.0	5.3±0.1	9.3±0.2	8.4±0.1	0.0±0.0	5.2±0.1	6.6±0.1	0.0±0.0		
Vibrio parahaemolyticus	*Pempis acidula*	5.2±0.3	8.0±0.2	12.5±0.2	10.2±0.1	0.0±0.0	4.2±0.3	6.3±0.3	0.0±0.0	0.0±0.0	18.0±0.2
	Ceriops tagal	0.0±0.0	4.0±0.1	10.0±0.1	7.5±0.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0		
Staphylococcus aureus	*Pempis acidula*	0.0±0.0	5.0±0.2	17.2±0.1	7.0±0.2	0.0±0.0	0.0±0.0	5.0±0.0	5.0±0.0	0.0±0.0	14.0±0.1
	Ceriops tagal	0.0±0.0	7.2±0.2	10.0±0.2	7.2±0.3	0.0±0.0	7.0±0.1	0.0±0.0	0.0±0.0		
Vibrio cholera	*Pempis acidula*	5.1±0.3	8.2±0.2	12.2±0.2	9.2±0.3	0.0±0.0	0.0±0.0	7.3±0.3	0.0±0.0	0.0±0.0	17.0±0.0
	Ceriops tagal	0.0±0.0	5.0±0.2	6.0±0.2	0.0±0.0	0.0±0.0	0.0±0.0	5.2±0.3	0.0±0.0		

Table 2: MIC (Minimum inhibitory concentration) of the maximum active *Pempis acidula* and *Ceriops tagal* extracts

Mangrove bark extracts MIC (µg/ml)[a]	*Pseudomonas aeruginosa*	*Klebsiella pneumoniae*	*Vibrio parahaemolyticus*	*Staphylococcus aureus*	*Vibrio cholera*
Methanolic extract of *Pempis acidula*	100	150	125	75	125
Methanolic extract of *Ceriops tagal*	200	200	150	150	200
Ethanolic extract of *Pempis acidula*	150	200	190	220	200
Ethanolic extract of *Ceriops tagal*	150	220	250	200	250
Water extract of *Pempis acidula*	220	150	200	220	210
Water extract of *Ceriops tagal*	250	210	220	220	-
Streptomycin	150	150	75	75	75

synergistic or antagonistic manner. [26] Reported the presence of galloyl, flavonol and glycosides from *Pempis acidula*. Mixtures of active constituents showed a broad spectrum of biological and pharmacological activity [27-28]. The present work thus proved that coral sand growing mangrove plant, *Pempis acidula* exhibit better biological activity. In order to tolerate the stressful conditions of the coral sand, the mangrove plants that are growing there may produce some unique chemicals which may have better biological activity. Further research is necessary for separation, purification and characterization of biologically active compound(s). Further studies are being carried out in order to separate the individual components that are present in plant extracts of *Pempis acidula*.

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