Antimicrobial activity of mangrove plants of Goa, India against human pathogenic bacteria

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Abstarct-Mangroves grow in saline coastal habitats in the tropical and subtropical regions. Mangrove ecosystem is of a great ecological and socioeconomic significance. Mandovi-Zuari estuarine complex in Goa are well known mangrove forests on the west coast of India. Different species of mangrove plants are found and are abundant along the Mandovi estuary of Goa. Mangrove plants are explored from ancient times as folklore medicine against different bacterial and fungal infections. In this study, three different species of mangrove plants, *Acanthus illicifolius*, *Ceriops tagal* and *Sonneretia caseolaris* were collected from three different stations along the Mandovi estuary at Divar, Ribandar and Old Goa. The solvent extracts of dried and powdered leaves of these mangrove plants were further investigated to evaluate the antimicrobial activity against human pathogenic bacteria, *Staphylococcus aureus*, *E.coli*, *Kliebsiella pneumoniae* and *Bacillus subtilis* using well diffusion method. The results suggest an idea for further characterization of active compound from the effective mangrove plant, which can be used to control the drug resistant pathogenic bacteria.

Keywords Antibacterial activity; *Acanthus illicifolius; Ceriops tagal*; *Sonneretia caseolaris;* human pathogens

Introduction

An estuary is a semi enclosed and coastal body of water with free communication to the ocean, and within which ocean water is diluted by freshwater derived from land (Levinson, 2010). The estuarine system makes a favorable and a good habitat for mangrove plant species (Pawar, 2012). Mangrove ecosystem is highly productive ecosystem, which yield commercial forest products, protect coastlines, and supports growth and reproduction of coastal fisheries and other aquatic organisms (Kumar and John, 2013). Among, more than 59 species of mangroves represented in India, 19 of these species dwell in the estuarine waters of Goa (Mohandas et al 2014). Moreover, mangroves of Mandovi-Zuari estuarine complex in Goa are among the best mangrove forests on the west coast of India (Giri et al 2014). Ayurvedic system of medicine involves study of different plants and its parts to cure infections and diseases which arise from pathogens. The whole idea of using mangrove plant leaf extract against bacterial pathogens came from ancient and coastal tribal people and the medicinal properties of these plants developed from informal experimentation. This knowledge was

passed over many generations. It is said that mangrove plants may have different properties like anti-fungal, anti-diabetic, anti-inflammatory and anti-cancerous to its acclaim (Arivuselvan et al 2011) but, there are very less evidences to adopt this knowledge. However, some of them have been reported in few research papers (Kumar and Choudhury, 2013; Bakshi and Chaudhuri., 2014; Arora et al 2014; Saranya et al 2015). One of the major properties is antimicrobial property against different pathogens. It has been reported in many research papers that the pathogenic load has been increasing in the Mandovi estuary with the increase of human activities (Nagvenkar and Ramaiah, 2009; Rodrigues et al 2011). As the pathogenic load increases, mangroves plants found in these marine waters could start developing anti-bacterial compounds. Some common human pathogens are *S. aureus*, *E. coli, Kliebsiella* sp. and *Bacillus* sp. *and Vibrio* sp. (Arivuselvan et al 2011). Antibiotic resistance of these pathogenic bacteria has increased in the recent years and is posing an ever increasing therapeutic problem (Sen and Batra, 2012). Hence, there is a continuous and urgent need to discover new antimicrobials with diverse chemical structures and novel mechanism of action for new and re-emerging infectious diseases (Rojas et al 2003). In marine waters, all types of microorganisms including pathogens are found which can also reside in the fish which is than consumed by humans. This study, made an attempt to bring forward the medicinal properties of the three different mangrove species found at different sites along the Mandovi estuary of Goa, India.

Methodology

The mangrove plants species were identified in the Department of Botany, Goa University, Goa- India as *Acanthus illicifolius* (Divar Island), *Ceriops tagal and Sonneretia caseolaris*. The *Ceriops tagal* (Ribandar) and *Sonneretia caseolarisare* (Old Goa) are characterized to be true mangroves, whereas, *Acanthus illicifolius*is as a mangrove associate (**Fig. 1**).The physical parameters like pH, temperature, dissolved oxygen (DO) and biological oxygen demands (BOD) were analyzed at the place of sample collection (**Table 1**).

Sample collection-Three sampling stations Divar, Ribandar and Old Goa were selected on the basis of distribution and availability of the mangrove plant species. Plants were chosen based on their traditional use against different infections, which were reported by the local people of the place. The plant samples were collected in sterile zip lock bags. Samples were preceded to the lab for further analysis. The mangrove plant identification was done in the Department of Botany at Goa University.

Culture and maintenance of pathogenic microragnisms-The four bacterial human pathogens were used in this study. Gram negative bacteria such as *Eschrerichia coli* (ATCC 113D) and *Klebsiella pneumoniae* (ATCC 1003) while gram positive bacteria used were *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633). Theses cultures were obtained from Department of Microbiology at Goa University. The bacterial cultures were maintained

by sub-culturing at regular intervals on nutrient agar medium (HiMedia) and stored at 4° C in refrigerator until used.

Plant exatraction-The mature and young leaves were separated in a sterile conical flask. Leaves were washed thoroughly in running tap water followed by distilled water. The plants were surface sterilized by using 70 % ethanol. The mature and young leaves separated were shade dried for 8-9 days at room temperature. As per the method described by Alo et al (2012), the dried leaves were grinded in to fine powder. Solvents used for extraction were ethanol 60 %, methanol 60 % and distilled water. 20 grams of the dried leaf powder was added in 100 ml of solvents. This was kept on shaker for 72 hours (3 days) at room temperature (37 °C). The mixtures were transferred to 50 mL sterile tubes and centrifuged for 10 min at 4,000 rpm at 25 °C (Mocan et al 2015). The supernatant was collected and filter sterilized using filter paper of pore size 0.22 µm. The filtrate was than stored at 4 °C in aliquots until used.

Antimicrobial assay by well diffusion method-Mangrove plant extracts was screened by using the agar-well diffusion method using Muller-Hinton agar. 100 µl of the pathogenic bacterial suspension was spread plated on the agar plates. A sterile cork borer of 6 mm diameter was used to bore wells on the agar medium. 100 µl of the various extract were added into each well using micropipette (Kumar and Shreya, 2011). The inoculated plates with extracts were allowed to diffuse into the agar by keeping the plates in the refrigerator for 1 hour (Pradhan et al 2012). The Mueller Hinton Agar plates were incubated at 37 °C for 24 hours. Antimicrobial activity was analyzed by measuring the diameter of zones of inhibition (cm) produced after incubation.

Results and Discussion

The results of antibacterial activity of the three mangrove plants extracted in various solvents are detailed in (**Table 2**). The aqueous extracts of the mangrove plant species did not show any inhibitory activity against the pathogens. The solvent and aqueous extracts of *Ceriops tagal* failed to show any activity against *K. pneumoniae,* while, highest inhibitory activity was shown by methanolic extracts of the *Acanthus illicifolius* (0.5cm) (**Fig. 2A**). The highest zone of inhibition, observed against *E.coli* was obtained by methanolic extracts of *Sonneretia caseolaris* (0.8 cm) followed by *Ceriops tagal* (0.5cm). The highest activity of ethanolic extracts of *Sonneretia caseolaris* against *E.coli* was (0.6 cm) (**Fig. 2B**). The highest inhibitory activity against *B. subtilis* was shown by methanolic extract of *Acanthus illicifolius* (0.9 cm) whereas, the ethanolic extract of *Acanthus illicifolius* showed highest inhibitory activity against *B. subtilis* (0.7cm) (**Fig. 2C**). The ethanolic and methanolic extracts of *Acanthus illicifolius* showed highest inhibitory activity against *S.aureus* (0.8 cm and 1.0 respectively) followed by *Ceriops tagal* (0.7cm and 0.8 respectively). However, both the solvent extracts of *Sonneretia caseolaris* failed to show any inhibitory activity against *S. aureus* (**Fig. 2D**).

Mangroves and mangrove associated plants are remarkable source of therapeutic agents having broad medicinal value (Bandaranayake, 2002). This rich property of mangrove plant extracts is demonstrated to be due to the presence of phytochemicals such as alkaloids, steroids, tannins, flavonoids and sugars in the extracts of this green vegetation (Poompozhil and Kumarasamy, 2014). For instance, the antibacterial activity of flavonoids is attributed to the phenolic structure possessing one carbonyl group that complexes with extracellular and soluble protein and, moreover, with bacterial cell wall (Cowan, 1999). These complexes assist in exhibiting the antibacterial activity. Interestingly, the organic extracts from mangrove plant exhibited a varying degree of bactericidal activity as revealed by different size of zone of growth inhibition. The maximum antibactericidal activity of methanolic extract that was extracted from *Acanthus illicifolius* showed maximum inhibitory activity against all the four pathogens. The effective solvent was methanol which gave maximum zone of inhibition as compared to ethanol. *B. subtilis and E.coli* were inhibited by ethanol and methanol extracts of all three species of mangrove plants.

Further, the highest inhibitory activity was observed for gram positive bacteria as compared to gram negative bacteria by extracts obtained from *Acanthus illicifolius*, and *Ceriops tagal*. This result corroborates to that of Abeysinghe (2010) which states that the presence of lipopolysaccharide layer along with proteins and phospholipids are the significant components of the outer membrane of gram negative bacteria as compared to gram positive bacteria that have only one outer peptidoglycan layer making the organism more susceptible to antimicrobicidal effect of organic extract of mangrove plant. Hence, the presence of outer lipopolysaccharide may restrict the access of antibacterial compound to the peptidogylycan layer of the cell wall. Consequently, this study reports for the first time the antibactericidal effect of methanolic extract of *Acanthus illicifolius* inhabiting Mandovi estuary of Goa, India against human gut pathogens and thus reveals it therapeutic potential in the treatment of infectious diseases caused by them.

Conclusion

The present study demonstrated the potential of mangrove plants *Acanthus illicifolius*, *Ceriops tagal* and *Sonneretia caseolaris* along the Mandovi estuary of Goa as a source of antibacterial compound of therapeutic significance.

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Figure 1 Mangrove plant species used in this study

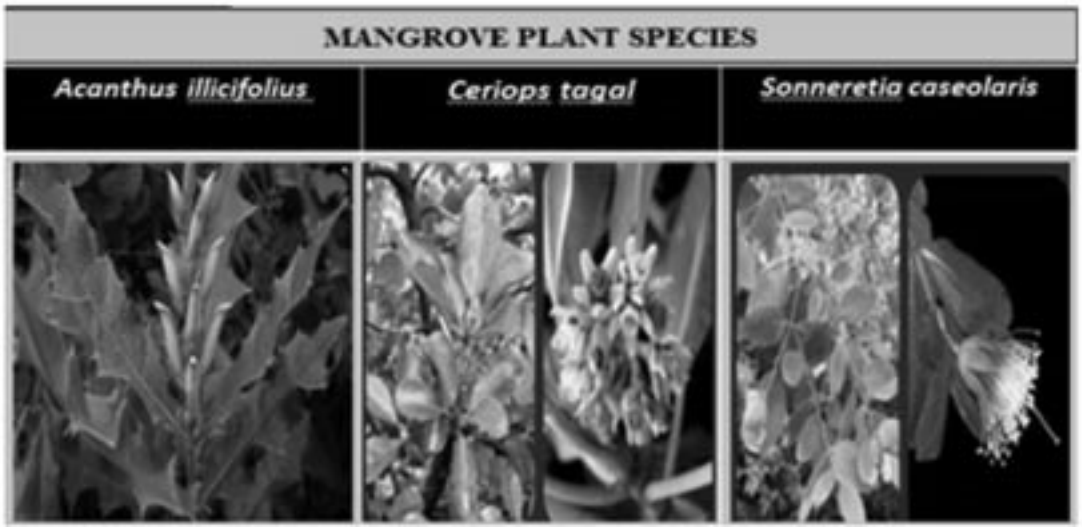

TABLE 1 Physical parameters recorded at the sampling sites of water sample.

Sr.no	Sampling Station	Temperature (°C)	pH	DO_1 (mg/ml)	DO_5 (mg/ml)	BOD (mg/ml)
1	Divar	27.2°C ± 0.3	7.6 ± 0.3	6.49	4.46	2.03
2	Old Goa	29.5°C ±0.2	6.9 ±0.3	8.51	6.49	2.02
3	Ribandar	28.6°C ± 0.3	7.1 ± 0.4	8.11	6.89	1.22

DO- Dissolved oxygen, DO_1–Initial dissolved oxygen, DO_5 – Dissolved oxygen value after 5 days of incubation, BOD- Biological oxygen demand

Table 2 Antimicrobial activity of mangrove plant leaf extracts against human pathogens

Bacteria	ZONE OF INHIBITION (cms)								
	Acanthus illicifolius			*Ceriops tagal*			*Sonneretia caseolaris*		
	DW	E	M	DW	E	M	DW	E	M
K. pneumonia	NI	0.4	0.5	NI	NI	NI	NI	0.2	0.3
E. coli	NI	0.3	0.3	NI	0.2	0.5	NI	0.6	0.8
B. subtilis	NI	0.7	0.9	NI	0.6	0.6	NI	0.1	0.2
S. aureus	NI	0.8	1.0	NI	0.7	0.8	NI	NI	NI

DW-Distilled Water; E- Ethanol; M-Methanol; NI- Not Inhibited

Figure 2 Antibacterial activities of mangrove plants against A. *K. pneumoniae* (ATCC 1003); B. *E. coli* (ATCC113D); C. *B. subtilis* (ATCC 6633); D. *S. aureus* (ATCC 6538).