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Full Length Research paper

Antibacterial potential of chosen mangrove plants against isolated urinary tract infectious bacterial pathogens

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Five Indian mangrove plants (*Rhizophora apiculata, Rhizophora mucronata, Bruguiera cylindrica, Ceriops decandra, Avicennia marina*) /parts (hypocotyls, bark, collar and flower) were investigated to evaluate the antibacterial activity against UTIs bacterial pathogens. Sixty nine bacterial strains were isolated from mid urine samples of 75 males and 75 females from Thondi coastal area, Ramanathapuram and were identified by conventional methods. *Escherichia coli* was predominant (41%) followed by *P. aeruginosa* (25%), *Klebsilla pneumonia* (22%), *Enterobacter* sp. (9%) and *Streptococcus aureus* (3%). The antibacterial activity of ethanolic extracts of mangrove plants/parts was evaluated by disk diffusion method. *R. mucronata* (28%) and *A. marina* (27%) exhibited antibacterial activity against isolated UTIs. The plant parts hypocotyls showed highest antibacterial activity (38%) against the UTIs pathogens. Preliminary phytochemical analysis of the plant parts revealed the presence of active compounds such as flavonoids, anthroquinone, phenolic group, alkaloids, and triterpenoids. The results provided evidence that, the studied plants might indeed be potential sources of anti UTIs bacterial pathogens

Key words: Antibacterial sensitivity, mangrove plants, phytochemicals, urinary tract infections (UTI), bacterial pathogens.

INTRODUCTION

Patients with non infectious disease who have stay in hospital have high risk to acquire nosocomial infection. It has been reported that, 10% hospital patients acquire this infection while staying in hospital (Asefzadeh, 2005). The common pathogenic bacteria which include *Escherichia coli, Klebsilla pneumoniae, Haemophilus influenza, Streptococcus pneumoniae* and *Proteus vulgaris* are the major causative agents of nosocomial infections (Saonuam et al., 2008; Nicholls et al., 1975). Generally, nosocomial infections develop in respiratory tract (Nicholls et al., 1975) and urinary tract (Saonuam et al., 2008) . Treatment with available antibiotics leads to resistance among pathogenic bacteria which leads to greater threat. Antimicrobials derived from the plants have been receiving increasing attention. Antimicrobial activities of plant constituents such as phenol, quinines, flavones, flavor- noids, tannins, terpenoids, essential oils and alkaloids have been reported by several authors (Weimann and Heinrich, 1997; Atindehou et al., 2002; Edeoga et al., 2005). 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 (Rojas et al., 2003). The present study made an attempt to find out the antibacterial activity and therapeutic properties of 12 mangrove plant parts against the 5 urinary tract infections bacterial pathogens isolates.

MATERIALS AND METHODS

Isolation of UTI bacterial pathogens

A total of 150 urine samples from 75 males and females patients

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Table 1. Traditional therapeutic properties of chosen mangrove plants.

Voucher specimen number	Plant species	Family	Parts used	Traditional therapeutic uses
AUCAS0003	*Rhizophora mucronata* Poir.	Rhizophoraceae	Hypocotyls, collar, bark	Antiviral, anti HIV activity, growth hormone tests on plants, biotoxicity on fingerlings of fish (Bandaranayake, 2002)
AUCAS0004	*Rhizophora apiculata* Blume	Rhizophoraceae	Hypocotyls, collar, bark	Antiviral, larvicidal, antifungal, antifeedant, antimicrobial activity, antiviral properties against human immuno deficiency (Bandaranayake, 2002)
AUCAS0005	*Bruguiera cylendrica* (L.) Bi	Rhizophoraceae	Hypocotyls, collar, bark	Treatment for hepatitis (Bandaranayake, 2002)
AUCAS0006	*Ceriops decandra* (Griff.) Ding Hou	Rhizophoraceae	Hypocotyls, collar, bark	Antiviral activity (Bandaranayake, 2002)
AUCAS0007	*Avicennia marina* (Forsk.) Vierh.	Avicenniaceae	Flower, bark	Analgesic, antiviral activity (Bandaranayake, 2002)

admitted in the hospitals as UTI problems were collected from different hospitals and laboratory localities along the coastal area of Thondi region, Palk Strait, India (9°44' N and 79° 10'E) in a separate sterile wide mouth bottle. Before collecting a sample, the women were instructed to swab the vulvae and men to retract the foreskin and cleanse the glans penis. Mid stream urine was collected in a sterile wide mouthed container. For the isolation of UTI bacterial strains, loop full of urine samples were streaked in to the nutrient agar, Mac Conkey agar, Blood agar and Choclate agar plates and incubated at 37 ± 2°C for 24 h. Next day individual colonies were selected and identified on the basis of morphological characteristics, gram staining, and biochemical characters (Thomas 1995, Chessbrough 2000).

Extraction of bioactive compounds

Fresh plant parts from 4 mangrove plants (Table 1) *viz., Rhizophora mucronata*, *Bruguiera cylindrica*, *Ceriops decandra* and *Avicennia marina* which were traditionally proved to have medicinal properties (Bandaranayake, 2002) were collected from Pichavaram mangrove forest, Pichavaram, Tamil Nadu, India (Lat 11°27'N; Lan79° 47E'). The taxonomic identities of these plants were confirmed by Prof. Dr. K. Kathiresan, CAS, Annamalai University, Parangipettai, Tamilnadu, India. The voucher specimens have been maintained in the school of Marine Sciences, Department of Oceanography and Coastal Area Studies, Alagappa University, Thondi, Tamilnadu, India. The shade dried leaves of about 500g were subjected for size reduction to coarse powdered. The powder was defatted with petroleum ether (60 - 80°C) and then extracted with 1 L of of 95% ethanol and water mixture by percolation method.

The ethanolic extract was concentrated under vacuum to get the fine residues. Whatmann filter paper No.1 disc (6 mm. diameter) impregnated with crude extracts (5 mg/ml) was prepared. Preliminary disc diffusion assay was performed to determine the antibacterial activity (Kim et al., 1995). Ethanol solvent without extracts was impregnated in Whatmann discs used as control. Bacterial suspension was spread over the surface of Mueller Hinton agar using sterile cotton swabs. Disks impregnated with the extracts were applied on the solid agar medium by pressing slightly and incubated at 37±2°C for 18 - 24 h. After that, the zone of inhibition was measured and expressed as millimeter in diameter. Phytochemical analysis of ethanolic extract of each plant parts was carried out by standard procedures (Kokate et al., 2003).

RESULTS

Out of the 150 midstream urine samples, 69 bacterial isolates were recovered and the biochemical tests revealed that, these isolates belong to 5 species (Table 2). Of these *E. coli* is the predominant one (41%); *P. aeruginosa* (25%), *K. pneumonia* (22%), *Enterobacter sp.* (9%) and *S. aureus* (3%) (Figure 1). Antibacterial activity of mangrove plant parts is represented in Table 3. Hypocotyls of *B. cylindrica* (8.3 ± 0.85 mm) showed highest zone of inhibition against *E. coli* followed by *C. decandra* hypocotyl (6.8 ± 0.64 mm) and *A. marina* flower (6.6 ± 0.35 mm). Bark extract of *A. marina* (17.0 ± 0.47 mm) showed highest zone of inhibition against *P. aeruginosa*, moreover *almost* all the plant parts showed inhibitory activity against *P. aeruginosa* except *R. apiculata* hypocotyl and collar. *R. mucronata* collar, *B. cylindrica* hypocotyl and bark extract of *A. marina* showed 6.0 ± 0.68 and 6.00 ± 0.87 mm zone of inhibition against K. *pneumoniae*. Hypocotyls of *R. mucronata* (6.0 ± 0.34 mm), *R. apiculata* (6.0 ± 0.36 mm), bark extract of *B. cylindrica* (6.0 ± 0.56 mm), *C. decandra* collar (6.0 ± 0.54 mm), flower (6.0 ± 0.51 mm) and bark (6.0 ± 0.54 mm) extract of *A. marina* showed maximum zone of inhibition against *Enterobacter sp.*

Bark extract of *A. marina* (22 ± 0.68 mm) showed maximum zone of inhibition and hypocotyl of *B. cylindrica* (7 ± 0.64) and *R. apiculata* (7 ± 0.68 mm) showed minimum zone of inhibition against *S. aureus.* The antibacterial activity of 5 different mangrove species showed that, *R. mucronata* (28%) showed greatest sensitivity against isolated UTIs bacteria, followed by *A. marina* (27%), *C. decandra* (18%), *B. cylindrica* (16%) and *R. apiculata* (11%) (Figure 2). However, hypocotyls (38%) showed highest antibacterial activity followed by bark (34%), collar (22%) and least activity was recorded in flower Figure 3).

Table 2. Biochemical characterization of isolated bacteria from UTI patients.

Characteristics	*P. aeruginosa*	*E. coli*	*K. pneumoniae*	*Enterobacter sp.*	*S. aureus*
Grams staining	-	-	-	-	+
TSI	Slant - Butt +	Slant - Butt -	Slant + Butt +	Slant + Butt -	-
Mannitol	Acid	Acid	Acid	Acid	-
motility	Motile	Motile	Non-Motile	Motile	Motile
Indole test	-	+	-	-	-
Methyl red test	+	+	-	-	-
V.P. test	-	-	+	+	-
Citrate test	-	-	+	+	-
Urease test	+	-	+	-	-
Oxidase test	+	-	-	-	-
Catalase test	+	-	-	-	+
H_2S production	-	-	-	-	-

'+': positive, '-': negative.

Table 3. Antibacterial activity of ethanolic extracts of chosen mangrove plants (Results in average mm).

Plant parts	*Rhizophora mucronata*			*Rhizophora apiculata*			*Bruguiera cylindrica*			*Ceriops decandra*			*Avicennia marina*	
Bacterial strains	**Collar**	**Hypocotyls**	**Bark**	**Collar**	**Hypocotyls**	**Bark**	**Collar**	**Hypocotyls**	**Bark**	**Collar**	**Hypocotyls**	**Bark**	**Flower**	**Bark**
Escherichia coli (n=29)	-	-	-	-	-	-	-	8.3±0.85 (n=8)	-	-	6.8±0.64 (n=7)	-	6.6±0.35 (n=16)	-
Pseudomonas aeruginosa (n=17)	6.0±0.51 (n=14)	10.8±0.64 (n=15)	10.0±0.87 (n=10)	7.0±0.24 (n=5)	-	-	6.0±0.92 (n=9)	6.0±0.65 (n=10)	7.0±0.38 (n=15)	8.2±0.86 (n=13)	8.4±0.52 (n=10)	6.4±0.68 (n=7)	6.0±0.24 (n=5)	17.0±0.47 (n=2)
Klebsiella pneumoniae (n=15)	6.0±0.69 (n=13)	-	-	-	-	-	-	6.0±0.87 (n=8)	-	-	-	-	-	6.0±0.68 (n=7)
Enterobacter sp. (n = 6)	-	6.0±0.34 (n=6)	-	-	6.0±0.36 (n=1)	-	-	-	6.0±0.56 (n=1)	6.0±0.54 (n=1)	-	-	6.0±0.54 (n=1)	6.0±0.54 (n=1)
Staphylococcus aureus (n = 2)	12±0.98 (n=1)	8±0.69 (n=2)	18±0.58 (n=2)	7±0.68 (n=1)	-	12±0.51 (n=2)	-	7±0.64 (n=1)	-	13±0.59 (n=1)	8±0.58 (n=1)	-	-	22±0.68 (n=2)
Total average zone of inhibition	8.0±0.72 (n=32)	8.2±0.55 (n=23)	9.0±0.72 (n=12)	7.0±0.46 (n=6)	6.0±0.36 (n=1)	12.0±0.51 (n=2)	6.0±0.92 (n=9)	6.8±0.75 (n=30)	6.5±0.47 (n=16)	9.0±0.66 (n=15)	7.7±0.58 (n=18)	6.4±0.68 (n=7)	6.2±0.37 (n=22)	12.7±0.59 (n=12)

n= number of isolates: '-'no sensitivity.

Table 4. Phytochemical constituents in mangrove plant parts.

Plant parts	*R. mucronata*			*R. apiculata*			*B. cylindrica*			*C. decandra*			*A. marina*	
Phyto-chemical Constituents	Collar	Hypocotyls	Bark	Collar	Hypocotyls	Bark	Collar	Hypocotyls	Bark	Collar	Hypocotyls	Bark	Flower	Bark
Sugar	+	+	+	+	+	-	-	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	-	+	+	+	+	-
Phenolicgroup	+	+	+	+	+	+	-	+	+	+	+	-	+	+
Alkaloid	+	+	+	+	+	-	-	-	-	+	+	+	+	-
Steroids	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Triterpenes	+	+	+	+	+	-	-	-	-	+	+	+	-	-
Flavonoids	+	+	+	+	+	-	-	-	-	+	+	-	-	-
Catachin	+	+	+	+	+	-	-	+	-	+	+	+	-	-
Tannin	+	+	+	+	+	+	+	-	+	+	+	-	+	+
Anthroquinone	+	-	+	+	-	-	-	-	-	+	+	-	-	-

+: presence; -: absence.

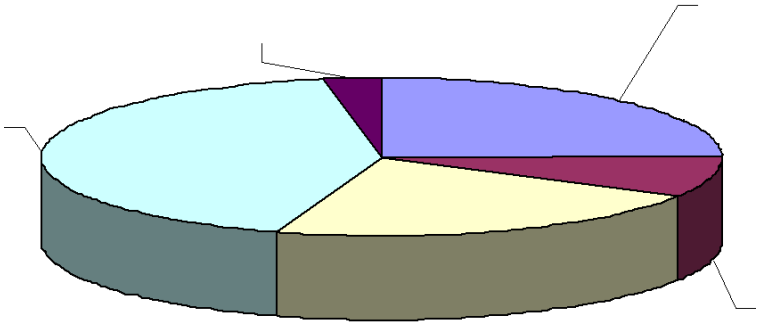

Figure 1. Percentage occurrence and distribution of bacterial pathogens in UTIs among the patients (n = 69).

The present study also revealed that, proteins and phenols were present in all the chosen plants. However the steroids were not presented in any parts. The other phytoconstituents that is sugar, alkaloids, triterpenoids, flavanoids, catachin, tannin and anthro-quinone are present in minute amounts discriminately reported among the plant parts chosen for the present study (Table 4).

DISCUSSION

Patients with non infectious diseases who have to stay in hospital for long period such as heart disease, cancer and other chronic diseases have high risk to get nosocomial infections (Nichollas et al., 1975; Asefzadeh, 2005; Saonuam et al., 2008). Martinez and Baquero, (2002) have reported from their research that, nosocomial infectious bacteria exhibited least susceptibility to antibiotics and some of these bacteria out rightly developed multi drug resistance to these antibiotics. Recently from a 3 year follow up study in USA, Dowzicky and Park (2008) reported that, UTI bacterial pathogens have exhibited decreased susceptibility rates to tigecycline over the years. Antibacterial compounds from natural resources would be the alternative to overcome the resistance problem.

Hence the present study has planned to find out the

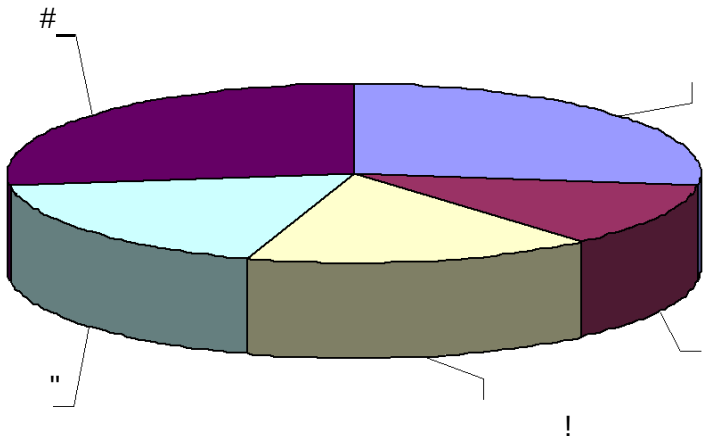

Figure 2. Percentage of antibacterial sensitivity of chosen plant species against UTI bacterial pathogens.

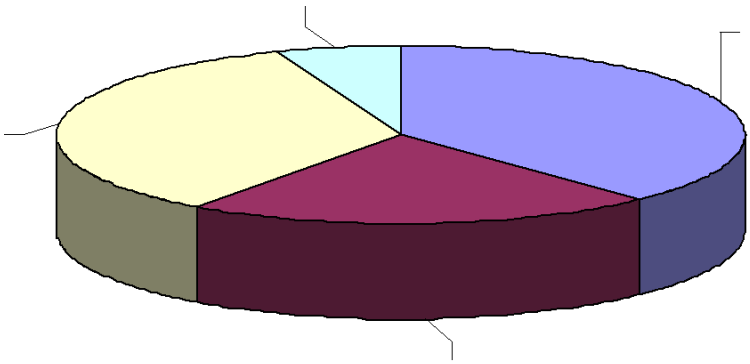

Figure 3. Percentage of antibacterial sensitivity of chosen plant parts against UTI bacterial pathogens.

newer antibacterial compounds from the most unexplored mangrove plants. In our present study, *E. coli* (41%) was found to predominant, this is similar to what Inabo and Obanibi, 2006; reports in their study. Despite the availability of antibiotics, UTIs remain the most common bacterial infections in human populations (Phillippon et al., 1989). Urinary tract infections occur more frequently in females (63%) than in males (Schaeffer et al., 2001). India has a great diversity of plants used in folk medicine and only few of these have been studied for antimicrobial studies (Ravikumar et al., 2005). 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 (Fennel et al., 2004). The hypocotyls of *R. apiculata, A. marina* and *R. mucronata* appear to have broad spectrum of antibacterial activity. Marine halophytes are already known for antimicrobial activity (Ravikumar et al., 2002; 2009) traceable to the presence of constituents unique to these groups of plants (Ravikumar et al., 1993). The chosen mangrove plants are to have reported are very heterogeneous mixtures of single substances which may act in a synergistic or antagonistic manner. Mixtures of active constituents showed a broad spectrum of biological and pharmacological activity (Robinson, 1967; Coelho-de-Souza et al., 1998; Atindehou et al., 2002). Tannins (Ravikumar et al., 1993) form irreversible com-plexes with proline rich proteins, resulting in the inhibition of cell

protein synthesis of bacteria (Scalbert, 1991). Flavonoids are phenolic structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial cell wall (Cowan, 1999), thus exhibits antibacterial activity through these complexes. All the plant extracts are having sugar compounds except the *R. apiculata* bark, it is possible that, the presence of sugar somehow facilitated the growth of the microor-ganisms and hence antagonizing the antibacterial activity of active compounds in the extracts (Arwa et al., 2008). Generally the gram positive bacteria are believed to be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier, where as, the gram negative bacteria possesses an outer phospholipidic membrane carrying the structural lipopolysaccharide compound. This makes the cell wall impermeable to drug constituents. Because of the presence of multilayered peptidoglycan and phospholi-pidic bilayer. In spite of the barriers the phytochemical constituents were effective in controlling the growth of these pathogenic strains (Scherrer and Gerhardt 1971). Based on the results, it is possible to conclude that, ethanolic extract of *Rhizophora mucronata* and *Avicenna marina* plant parts (hypocotyls, collar, bark and flower) had different level of antibacterial activity against the UTIs pathogens. The obtained results might be consi-dered sufficient to further studies for the isolation and identification of the active principles and to evaluate possible synergism among extract components for their antibacterial activity. Investigations are in progress to satisfy the standardization protocol as per WHO guidelines.

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