INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND DEVELOPMENT (IJPRD)
Platform for Pharmaceutical Researches & Innovative Ideas
www.ijprd.com

IN VITRO ANTIBACTERIAL ACTIVITY OF *AEGICERAS CORNICULATUM* AND *BRUGUIERA CYLINDRICA* AGAINST ISOLATED BACTERIAL URINARY TRACT INFECTIONS

GEEGI P G[1*],
ANITA P[2],ANTHONI SAMY A[3] and RAJ JS[1]

[1]Department of Biochemistry, St. Joseph's College (Autonomous), Tiruchirappalli -02.
[2]Department of Biochemistry, Shrimathi Indra Gandhi College for woman, Tiruchirappalli -02.
[3]Department of Biochemistry, Kurinji College of Arts and Science, Tiruchirappalli -02.

ABSTRACT

This study was conducted to investigate the bioactive potential of mangrove plants to develop alternative drug development for the treatment of bacterial Urinary tract infections (UTIs) which are frequent infections in the outpatient as well as in the nosocomial setting. Two mangrove plants / parts were investigated to evaluate the antibacterial activity against bacterial UTIs pathogens. Both of these plants, Aegiceras corniculatum and Bruguiera cylindrica exhibited excellent antibacterial activity against 5 bacterial pathogens viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus mirabilis and Staphylococcus aureus are isolated from urine samples.

Correspondence to Author

GEEGI P G

Assistant Professor**,** Department of Biochemistry, St. Joseph's College (Autonomous), Tiruchirappalli -02.

Email: geegi2009@gmail.com

Key words: *Antibacterial activity, Aegiceras corniculatum, Bruguiera cylindrica, phytochemicals, urinary tract infections (UTIs).*

INTRODUCTION

Urinary tract infections (UTIs) are among the most widespread microbial diseases and their financial encumbering on society is substantial. UTIs account for more than 7 million visits to physicians' offices and well over 1 million hospital admissions in the United States annually [1]. French epidemiologic studies evaluated its annual incidence at 53,000 diagnoses per million persons per year, which represents 1.05% to 2.10% of the activity of general practitioners. In the United States, the annual number of diagnoses of pyelonephritis in females was estimated to be 250,000 [2].

Acute simple pyelonephritis is a common form of upper UTI in females and results from the encounter of a parasite and a host. In the absence of urologic abnormality, this renal infection is mostly due to uropathogenic strains of bacteria [3,4], a majority of cases to community-acquired *E. coli*. 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 [5,6]. Generally, nosocomial infections develop in urinary tract [5] and respiratory tract [6]. In addition the pathogens are fully exposed to the nosocomial environment, including selective pressure by antibiotic or antiseptic substances. Therefore nosocomial UTIs comprise perhaps the largest institutional reservoir of nosocomial antibiotic resistant pathogens [7]. Many of the antibiotics and other synthetic drugs have sensitization reaction and other undesirable side effects in biological system and therefore, natural products are safer because they are more in harmony with the biological system [8].

The primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Mangroves are widespread in tropical and sub tropical regions, growing in the saline intertidal zones of sheltered coast lines and contain biologically active antimicrobial compounds [9]. Previous studies on mangrove plant parts and its major chemical classes displayed various level of biological activities such as antibacterial, antifungal, antiplasmodial, cytotoxic, antifouling, hepatoprotective, ichtyotoxic and free radical scavenging activities [10-14]. Mangrove plant extracts have been used for centuries as popular method for treating several health disorders. Numerous studies have been carried out on various natural products screening their antimicrobial activity [15-16]. In this regard the present study was made an attempt to find out the antibacterial and therapeutic properties of two mangrove plant parts against pathogenic bacterial strains.

MATERIALS AND METHODS

Plant materials

The Healthy Plant parts (leaf and bark) of *Bruguiera cylindrica* and *Aegiceras corniculatum* coastal medicinal plant species were collected from the muthupettai, South East Coast of India and used for study. The plants were identified and the voucher specimens were deposited in the herbarium cabinet facility sponsored by St.Joseph's College, Tiruchirappalli. Then these parts were segregated separately and dried in shade at room temperature and ground to powder. The powdered samples were used for extraction using ethanol.

Extraction of bioactive compounds

A known quantity [100g] of the dried powdered form of the plant material such as leaf, bark were taken in a soxhlet apparatus and soaked in 500ml ethanol and separately in bottles and closed. After 3 days extraction was collected and solvent were evaporated by keeping them in oven and the residue obtained was stored in a sterile container kept in refrigerator for further use. All the extracts were subjected to antibacterial activity assay and phytochemical analysis. The percentage of extraction from each sample was calculated using the following formula: Percentage of extraction (%) = Weight of the extract (g)/Weight of the plant material (g) ×100. The extracts of mangroves were screened for the presence of phytochemical constituents by following the method of [17, 18].

Isolation of microorganisms

Gram negative bacteria*, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* and Gram positive bacteria *Staphylococcus aureus, were* isolated from urine samples. The isolated bacteria were identified by following standard biochemical test [19].

Antibacterial assay

Filter paper disc method was used for testing of medicinal plant extract against 5 UTI pathogens. Whatman No.1 filter paper disc (6mm diameter) was impregnated with crude plant extracts (5 mg.disc^{-1}) was placed on Muller Hinton Agar (HIMEDIA, Mumbai) which was previously swabbed with UTI pathogens *viz. Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* and *Staphylococcus aureus.* The sterile disc impregnated with Dimethyl sulphoxide used as negative control whereas chloramphenicol impregnated disc is used as positive control. All the plates were incubated at 37^{O}C under static conditions. After 24 hrs, the zone

of inhibition appearing around the discs were measured and recorded in millimeter diameter.

Minimum Inhibitory Concentration Assay:

Minimum inhibitory concentration (MIC) was carried out with various concentration (3.25, 6, 12, 24.5, 50, 100, 500, 1000 µg.ml^{-1}) of extracts prepared with Dimethyl sulphoxide (DMSO) and mixed with 50 µl of 24 hrs old bacterial inoculum was mixed and allowed to grow overnight at 37°C for 48 hrs. Turbidity due to bacterial growth was observed in each concentration. The lowest concentration of the extracts which inhibits the growth of tested bacteria are observed and tabulated.

RESULTS

The isolated bacterial UTI pathogens were identified based on the morphological and biochemical characteristics [19]. The result of the present study revels that, the percentage of leaf and bark extract of *A. corniculatum* and B. cylindrica were estimated by 22.1%, 18.3% and 24.0%, 19.4% respectively. The extracts were tested for the antimicrobial activity against the UTI pathogens. The ethanolic extracts of coastal medicinal plants showed inhibitory effect against both gram positive and gram negative bacterial UTI pathogens. The leaf extract of *A. corniculatum* (14.35±0.13) showed highest zone of inhibition against *P. aeruginosa* and followed by *klebsiella sp.* and *proteus sp.* similarly bark extract of this plant has more effect over pseudomonas sp than other microorganisms. The ethanolic leaf extract of *Bruguiera cylindrica* shows maximum activity against *proteus mirablis* compare to other organisms. The bark extract of B. cylindrica (16.06±0.24) showed highest zone of inhibition against *S. aureus* (Table 1). The phytochemical studies reveals that the extracts of mangrove plants have variety of phytochemical constituents, namely, alkaloids, triterpenes, flavonoids, tannins, catachin, anthroquinone, phenols, sugars and proteins (Table 2).

Table 1: Antibacterial activity of ethanolic extracts of chosen mangrove plants against UTIs pathogens

S.NO.	UTIs pathogens	Antibiotic disc	*Aegiceras corniculatum*		*Bruguiera cylindrica*	
			Leaf	Bark	Leaf	Bark
		Zone of inhibition (mm in diameter)				
1	*Escherichia coli*	12.34±0.13	11.54±0.24	11.41±0.21	12.62±0.24	11.46±0.31
2	*Staphylococcus aureus*	14 .65±0.32	11.21±0.21	11.65±0.22	12.35±0.21	16.06±0.24
3	*Proteus mirabilis*	12.51±0.11	12.87±0.42	11.43±0.14	13.65±0.52	13.25±0.41
4	*Pseudomonas aeruginosa*	18.42±0.15	14.35±0.13	12.98±0.15	12.87±0.11	13.32±0.52
5	*Klebsiella pneumoniae*	14.67±0.32	13.65±0.12	11.14±0.62	12.13±0.17	13.56±0.62

Values are mean inhibition zone (mm) ± S.D of three replicates

Table - 2. Phytochemical constituents in *Aegiceras corniculatum* and *Bruguiera cylindrical* leaf and bark extract

Phyto constituents	*Aegiceras corniculatum*		*Bruguiera cylindrica*	
	Leaf	Bark	Leaf	Bark
Steroids	-	-	-	-
Sugar	+	+	+	+
Reducing Sugar	-	-	-	-
Alkaloids	++	-	-	++
Phenolic compounds	-	+	-	-
Catechins	-	-	-	-
Saponins	+	++	+	+
Anthroquinones	-	-	-	-
Tannins	+	+	+	+
Amino acids	+	+	+	+

" –" Absence. + - moderately present, ++ - Richly present

Table 3: Minimum Inhibitory Concentration (MIC) (µg.ml^{-1}) of the extracts from *Aegiceras corniculatum* and *Bruguiera clindrical* extract against UTI pathogens

S.NO.	UTI pathogens	*Aegiceras corniculatum*		*Bruguiera cylindrica*	
		Leaf	Bark	Leaf	Bark
		MIC(µg.ml^{-1})			
1	*Escherichia coli*	100	250	100	100
2	*Staphylococcus aureus*	100	100	50	50
3	*Proteus mirabilis*	100	250	100	250
4	*Pseudomonas aeruginosa*	50	100	100	50
5	*Klebsiella pneumoniae*	50	100	100	100

DISCUSSION

The results of the present study clearly showed that, extracts from *A. corniculatum* and B. cylindrica showed antimicrobial activity against tested pathogenic strains. The effectiveness of the active compounds present in the plant extracts showed growth inhibition which that appears as clear areas surrounding the disc. This antibacterial activity might be due to the active components which are present in plant extracts. However, some

plant extracts didn't exhibit the antibacterial activity against tested bacterial strains due to some kind of resistance mechanisms e.g. enzymatic inactivation, target sites modification and decrease intracellular drug accumulation [20] or the concentration of the compound used may not be sufficient. It can be concluded that, the plant extracts of *A. corniculatum* and *B. cylindrica* have greater potential as antimicrobial compounds against microorganisms and that they can be used for the treatment of infectious diseases caused by pathogenic microorganisms. 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 [21]. The Marine halophytes are already known for antimicrobial activity [22] traceable to the presence of constituents unique to these groups of plants [23]. The chosen mangrove plants are to have reported are very heterogeneous mixtures of single substances which may act in a synergistic or antagonistic manner. Some of the phytochemical compounds e.g. glycoside, saponin, tannin, flavonoids, terpenoid, alkaloids, have been reported to have antimicrobial activity [24, 25]. The phytochemical analysis reveals that the leaf extract of *A. corniculatum* and *B. cylindrica* richly posses total phenol flavanoid and phenol [23]. Mangrove plants like *B. cylindrica, R. apiculata, R. lamarkii* and *R. mucronata* are rich in polyphenols [26, 27]. Flavonoids are phenolic structure containing one carbonyl group complexes with extracellular and soluble protein [28], thus exhibits antibacterial activity through these complexes. Based on the results, it is possible to conclude that, ethanolic extract of *Bruguiera cylindrica* and *Aegiceras corniculatum* had different level of antibacterial activity against the UTIs pathogens. The present studies have to develop newer lead for better and safer chemotherapeutic agents from mangroves.

ACKNOWLEDGEMENTS

The authors are thankful to the authorities of St.Joseph' College, Tiruchirappalli, for providing required facilities.

Available online on www.ijprd.com

REFERENCES

1. Hooton TM, Stamm WE. Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am 1997;11:(3)551-581.
2. Kunin CM. Detection, prevention and management of UTIs. 5th edition. Lea & Febiger: Philadelphia, 1997.
3. Kahlmeter G; ECO.SENS. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO.SENS Project. J Antimicrob Chemother 2003; 51(1):69-76.
4. Hooton TM, Scholes D, Hughes JP, Winter C, Roberts PL, Stapleton AE, Stergachis A, Stamm WE. A prospective study of risk factors for symptomatic urinary tract infection in young women. N Engl J Med 1996;335(7):468-474.
5. Saonuam P, Hiransuthikul N, Suankratay C, Malathum K, Danchaivijitr S (2008). Risk factors for nosocomial infections caused by extended spectrum b- lactamase producing *Escherichia coli* or *Klebsiella pneumoniae* in Thailand. Asian Biomed 2: 485-491.
6. Nichollas AC, Pease PE, Gree ID (1975). Agglutinin response to bacterial l infection in acute exacerbations of chronic bronchitis. J. Clin. Pathol 28: 279-283.
7. Maki DG, Tambyah PA. Engineering out the risk for infection with urinary catheters. Emerg Infect Dis 2001; 7(2):342–7.
8. Atal CK (1985) Chemistry of some biological activity Indian medicinal plants. Proc Ind Nat Sci Acad 48:99-121.
9. Bhattacharjee, I., S.K. Chetterjee and S.N. Chetterjee, 2006. Antibacterial potentiality of Argemone mexicana solvent extracts against some pathogenic bacteria. Mem Ins Oswaldo Cruz, 101: 645-648.
10. Ravikumar, S., S. Nazar, A. Nural Shiefa and S. Abideen, 2005. Antibacterial activity of traditional therapeutic coastal medicinal plants against some pathogen. J. Environ. Biol., 26: 383-386.

11. D.H. Kuo and P.C. Shea, 2008. Evaluation of antioxidant polyphenols from selected mangrove plants of India. Asi. J. Chem., 20(2): 1311-1322.
12. Ravikumar, S., G. Ramanathan, S. Jacob Inbaneson and A. Ramu, 2010c. Antiplasmodial activity of two marine polyherbal preparaations from Cheatomorpha antennina and Aegiceras corniculatum against Plasmodium falciparum. Parasitol. Res., DOI 10.1007/s00436-010-2041-5.
13. Manilal, A., S. Sujith, G. Seghal Kiran and Joseph Selvin, 2009. Biopotentials of mangroves collected from the southwest coast of India. Glo. J. Biotechnol. Biochem., 4(1): 59-65.
14. Agoramoorthy, G., F.A. Chen, V. Venkatesalu, D.H. Kuo and P.C. Shea, 2008. Evaluation of antioxidant polyphenols from selected mangrove plants of India. Asi. J. Chem., 20(2): 1311-1322.
15. Ravikumar, S., S. Jacob Inbaneson, R. Sengottuvel and A. Ramu, 2010. Assessment of endophytic bacterial diversity among mangrove plants and their antibacterial activity against bacterial pathogens. Annl. Biolo. Resear., 1(4): 240-247.
16. Ravikumar, S., S. Jacob Inbaneson, M. Uthira Selvam, R. Kaleeswari, A. Ramu and B. Margaret Beula, 2011. Antibacterial Activity of Heterotrophic Endophytes from Karangakadu Mangrove Ecosystem, India.J.Pharm. Res., 4(1): 195-198.
17. Sofowara, A., 1982. Medicinal plants and traditional medicine in Africa. John Wiley and Sons Ltd.,
18. Kepam, W., 1986. Qualitative organic analysis (Spectrochemical techniques). Ed. II. McGraw Hill, London. pp: 40-58.
19. Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. and Williams, S. T. (1994). In: Bergy's Manual of Determinative Bacteriology, ninth ed. Williams and Wilkins Pub., MD, USA.
20. Schwarz, S. and W.C. Noble, 1999. Aspects of bacterial resistance to antimicrobials used in veterinary dermatological Practice, pp: 163-176.
21. Fennel CW, Lindsey KL, McGaw JL, Stafford GI, Elgorashi EE, Grace MO, Staden V (2004). Assessing African medicinal plants for efficacy and safety. Pharmacological screening and toxicology. J. Ethnopharmacol. 94: 205-217.
22. Ravikumar, S., L. Anburajan, G. Ramanathan and N. Kaliaperumal, 2002. Screening of seaweed extracts against antibiotic resistant post operative infectious pathogens. Seaweed Res. Utili., 24(1): 95-99.
23. Ravikumar, S. and K. Kathiresan, 1993. Influence of tannins, amino acids and sugar on fungi of marine halophytes. Mahasagar., 26(1): 21-25.
24. Okeke, M.I., C.U. Iroegbu, E.N. Eze, A.S. Okoli and C.O. Esimone, 2001. Evaluation of extracts of the root of Landolphia owerrience for antibacterial activity J. Ethnopharmacol., 78: 119- 127.
25. Ebi, G.C. and S.I. Ofoefule, 1997. Investigating into folkloric antimicrobial activities of Landolphia owerrience, Phyto. Res., 11: 149- 151.
26. K. Kathiresan and A.V. Ravi, 1990. *Indian Forest.*, 116, 390.
27. A.V. Ravi and K. Kathiresan, 1990. *Indian J. Marine Sci.*, 19, 224.
28. Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

*****