



**IN VITRO ANTIBACTERIAL ACTIVITY OF AEGICERAS CORNICULATUM AND BRUGUIERA CYLINDRICA AGAINST
ISOLATED BACTERIAL URINARY TRACT INFECTIONS****GEEGI P G^{1*},**ANITA P², ANTHONI SAMY A³ and RAJ JS¹¹Department of Biochemistry, St. Joseph's College (Autonomous), Tiruchirappalli -02.²Department of Biochemistry, Shrimathi Indra Gandhi College for woman, Tiruchirappalli -02.³Department 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.

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

Correspondence to Author**GEEGI P G**Assistant Professor, Department of
Biochemistry, St. Joseph's College
(Autonomous), Tiruchirappalli -02.**Email:** geegi2009@gmail.com**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*, *Klebsiella pneumoniae*,

Haemophilus influenzae, *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, ichthyotoxic 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 Available online on www.ijprd.com

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°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 $\mu\text{g.ml}^{-1}$) 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 reveals 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 \pm 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 mirabilis* compare to other organisms. The bark extract of *B. cylindrica* (16.06 \pm 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	<i>Aegiceras corniculatum</i>		<i>Bruguiera cylindrica</i>	
			Leaf	Bark	Leaf	Bark
Zone of inhibition (mm in diameter)						
1	<i>Escherichia coli</i>	12.34 \pm 0.13	11.54 \pm 0.24	11.41 \pm 0.21	12.62 \pm 0.24	11.46 \pm 0.31
2	<i>Staphylococcus aureus</i>	14.65 \pm 0.32	11.21 \pm 0.21	11.65 \pm 0.22	12.35 \pm 0.21	16.06 \pm 0.24
3	<i>Proteus mirabilis</i>	12.51 \pm 0.11	12.87 \pm 0.42	11.43 \pm 0.14	13.65 \pm 0.52	13.25 \pm 0.41
4	<i>Pseudomonas aeruginosa</i>	18.42 \pm 0.15	14.35 \pm 0.13	12.98 \pm 0.15	12.87 \pm 0.11	13.32 \pm 0.52
5	<i>Klebsiella pneumoniae</i>	14.67 \pm 0.32	13.65 \pm 0.12	11.14 \pm 0.62	12.13 \pm 0.17	13.56 \pm 0.62

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

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

Phyto constituents	<i>Aegiceras corniculatum</i>		<i>Bruguiera cylindrical</i>	
	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) ($\mu\text{g}.\text{ml}^{-1}$) of the extracts from *Aegiceras corniculatum* and *Bruguiera clindrical* extract against UTI pathogens

S.NO.	UTI pathogens	<i>Aegiceras corniculatum</i>		<i>Bruguiera cylindrical</i>	
		Leaf	Bark	Leaf	Bark
MIC($\mu\text{g}.\text{ml}^{-1}$)					
1	<i>Escherichia coli</i>	100	250	100	100
2	<i>Staphylococcus aureus</i>	100	100	50	50
3	<i>Proteus mirabilis</i>	100	250	100	250
4	<i>Pseudomonas aeruginosa</i>	50	100	100	50
5	<i>Klebsiella pneumoniae</i>	50	100	100	100

DISCUSSION

The results of the present study clearly showed that, extracts from *A. corniculatum* and *B. cylindrical* 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 possess 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.

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