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Antibacterial Activity of Chosen Mangrove Plants Against Bacterial Specified Pathogens

[1]S. Ravikumar, [1]M. Syed Ali, [2]A. Ramu and [2]M. Ferosekhan

[1] Department of Oceanography and CAS, School of Marine Sciences,
Alagappa University, Thondi Campus, Thondi, Tamil Nadu, India
[2] Department of Inorganic Chemistry, School of Chemistry, Madurai Kamaraj University,
Madurai, Tamil Nadu, India

Abstract: Study of marine organisms for their bioactive potential, being an important part of marine ecosystem, has picked up the rhythm in recent years with the growing recognition of their importance in human life. The antibacterial activity of the mangrove leaves of *Avicennia marina, Ceriops decandra* and (non italic) *Bruguiera cylindrica* were tested against antibiotic resistant pathogens (ARB) *viz*. *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae , Pseudomonas aeruginosa* and eye pathogens *(EP) viz. E. coli, Proteus, Acinetobacter and Staphylococcus epidermidis.* Most of the plant extracts showed promising antibacterial activity against both the bacterial groups. However, Maximum antibacterial activity was observed with the leaf extract of *A. marina* with the zone of inhibition 7.12±0.11mm against *P. aeruginosa* followed by *B. cylindrica* (6.8±0.84mm) and *C. decandra* (6.02±0.02). Moreover, the leaf extract of *A. marina* (11.21±0.74mm) showed highest zone of inhibition against eye pathogens of *S. epidermidis* followed by and against *Acinetobacter* 9.21±0.94 and *E.coli* 8.23±0.86 respectively. The *A. marina* has Minimmum Inhibitory Concentartion value of 50μg.ml^{-1} as against *P. aeruginosa , Acinetobacter sp* and *E. coli* followed by MIC value of 100 μg ml^{-1} against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. The presence of active compounds such as flavonoids, anthroquinone, phenolic group, alkaloids and triterpenoids might be the responsible factors for bioactivity against chosen bacterial pathogens. The results provided evidence that, the chosen plants might indeed be the potential sources for the biological activity of antibiotic resistant bacteria and eye pathogens.

Key words: Antibacterial · Mangroves · MIC · MBC · Phytochemical

INTRODUCTION

Historically, plants have provided a source of inspiration for novel drug compounds, as plant derived medicines have made large contributions to human health and well-being. Their role is twofold in the development of new drugs: first they may become the base for the development of a medicine, a natural blueprint for the development of new drugs, or second: a phytomedicine to be used for the treatment of diseases. It is estimated that today, plant materials are present in, or have provided the models for 50% Western drugs [1]. 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 [2]. 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, cytotoxic and free radical scavenging activities [3-10]. 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 [11-13]. The present study made an attempt to find out the antibacterial and therapeutic properties of three mangrove plant parts against pathogenic and antibiotic resistant bacterial strains.

Corresponding Author: S. Ravikumar, School of Marine Sciences, Department Oceanography and CAS, Alagappa University, Thondi Campus, Thondi, Tamil Nadu, India.

MATERIALS AND METHODS

Plant and Extraction: Fresh elder leaf samples from three mangrove plants *viz. Avicennia marina* (AUOCASR1) *Ceriops decandra* (AUOCASR2) and *Bruguiera cylindrica* (AUOCASR3*)* were collected from Karangadu mangrove forest South East coast of India (Lat 9º 36'N and Long 78º 83'E,) and washed thrice with tape water and twice with distilled water to remove the adhering salts and other associated animals. The authentication of the plant species were done by Prof. K. Kathiresan, Faculty of Marine Sciences, Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India . Two hundred gram of dried mangrove leaves were chopped) in to small pieces and soaked in 500 ml of ethanol for 7 days for the extraction of bioactive compound (percolation). The coloured ethanol solvent was subjected for filtration and kept under rotary flash evaporator (Buchi, Japan) so as to get solid extract. The percentage of extraction was calculated using the following formula: Percentage of extraction (%) = Weight of the extract (g) / Weight of the dried plant material (g) x 100. The extracts of mangroves were screened for the presence of phytochemical constituents by following the method of [14, 15].

Test Organisms: The antibiotic resistant pathogens (ARB) *viz*. *Staphylococcus aureus, Streptococcus pneumonia, Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and eye pathogens (EP) *viz. E.coli, Proteus, Acinetobacter* and *Staphylococcus epidermidis* were used for the *in vitro* antibacterial assay.

Antibacterial Assay Test: The ethanolic extract ($1mg.ml^{-1}$) was impregnated on to a Whatmann filter paper No.1 disc (6 mm. diameter) Dimethyl sulphoxide solvent without the extract was used as control. Bacterial suspension (10^8 cell ml^{-1}) of chosen bacterial pathogens was spread over the surface of Mueller Hinton agar (HIMEDIA) using sterile cotton swabs. Disks impregnated with the disc were placed 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 [16].

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 hr 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. To avoid the possibility of misinterpretations due to the turbidity of insoluble compounds, the minimum bactericidal concentration (MBC) was determined by sub culturing the MIC dilutions on to the sterile agar plates. The lowest concentration of the extracts which inhibits the growth of tested bacteria are observed and tabulated.

RESULT

The result of the present study revels that, the percentage of leaf extract of *A. marina, B. cylindrica* and *C. decandra* was estimated by 20.6%, 14.6% and 10.6%. The leaf extract was tested for the antimicrobial activity against the antibiotic resistant pathogens and eye pathogens. The leaf extract of *A. marina* (7.12±0.11mm) showed highest zone of inhibition against *P. aeruginosa* followed by *B. cylindrica* (6.8±0.84mm) and *C. decandra* (6.02 ± 0.02mm) (Table 1). Moreover, the leaf extract of *A. marina* (11±0.74mm) showed highest zone of inhibition

Table 1: Antibacterial activity of ethanolic extracts of chosen mangrove plants against Antibiotic resistant bacteria and eye pathogens

S.No	Bacterial pathogens	*A. marina*	*C. decandra*	*B. cylindrica*
		----------Zone of inhibition (mm in diameter)----------		
	Antibiotic resistant bacteria Pathogens			
1	Staphylococcus aureus	-	-	-
2	Streptococcus pneumonia	-	-	-
3	Pseudomonas aeruginosa	7.12±0.11	6.02±0.02	6.8±0.84
4	Klebsiella pneumoniae	6.01±0.82	-	-
	Eye Pathogens			
5	E.coli	8.23±0.86	-	-
6	Proteus	-	-	-
7	Acinetobacter	9.21±0.94	6.02±0.09	-
8	Staphylococcus epidermidis	11.21±0.74	7.12±0.19	-

Values are mean inhibition zone (mm) ± S.D of three replicates, "–"No inhibition

Table 2: Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) (µg.ml^{-1}) of the extracts from *Avicennia marina* leaf extract against chosen antibiotic resistant bacteria and eye pathogens

	Avicennia marina leaf extract (µg.ml^{-1})	
Name of the Pathogens	MIC	MBC
Antibiotic resistant bacteria Pathogens		
Staphylococcus aureus	-	-
Streptococcus pneumonia	-	-
Pseudomonas aeruginosa	50	50
Klebsiella pneumoniae	100	100
Eye Pathogens		
Escherichia coli	50	50
Proteus sp.	-	-
Acinetobacter sp.	50	50
Staphylococcus epidermidis	100	100

(-), No Inhibition

In the antibacterial assay test the concentration of extract used was 1 mg/ml that is 1000 µg/ml. When this concentration was used *Avicennia marina* extract did not show any zone of inhibition that is no antibacterial activity against *Staphylococcus aureus Streptococcus pneumonia and Proteus sp.* Contrarily, in MIC assay the minimum inhibitory concentration for these species were found to be 1000, 500 and 500 µg/ml respectively. How it is possible…?? Justify. If the extract is not showing any antibacterial activity at a particular dose (1000 µg/ml) then how we can get that dose (or even lower 500 µg/ml dose) as a minimum inhibitory concentration…??

Query Reply

1. In the MIC assay results, there was as a typing mistake was occurred in the table 2. However, the corrected values have been entered in the appropriate place with hyphen (-) symbol. So kindly request you that, take necessary action according to the corrected galley proof.

Table 3: Phytochemical constituents in crude leaf extracts of mangrove leaves
" –" Absence. + - moderately present, ++ - Richly present

Mangrove extracts	Alkaloid	Terpenoid	Flavanoid	Phenol	Steroid	Saponin	Catachin	Poly phenol	Proanthocyanin	Steroidal glycosides	Tannin	Protein	Carbohydrate	Aminoacid
Avicennia marina	++	++	++	+	+	+	-	+	+	+	+	++	+	+
Ceriops decandra	+	+	+	-	+	-	-	-	-	-	+	+	++	+
Bruguiera cylindrica	++	+	+	+	+	-	-	-	-	-	+	+	+	+

against eye pathogens of *S. epidermidis* followed by 9.21± 0.94mm and 8.23±0.86mm against *Acinetobacter* and *E.coli* respectively. Likewise, *A. marina* has MIC value of 50µg ml^{-1} against *P. aeruginosa* , *Acinetobacter sp* and *E. coli* followed by MIC value of 100µg ml^{-1} against *Klebsiella pneumoniae* and *Staphylococcus epidermidis* (Table 2). The extract of *A. marina* showed MBC value of 50µg ml^{-1} against *P. aeruginosa* , *Acinetobacter sp* and *E. coli*. The leaf extract of *A. marina* showed MBC value of 100µg ml^{-1} against *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. 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 3).

DISCUSSION

The results of the present study clearly showed that, extracts from *A. marina* showed antimicrobial activity against tested pathogenic strains including antibiotic resistant 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 [17] or the concentration of the compound used may not be sufficient. It can be concluded that, the plant extracts of *A. marina* have greater potential as antimicrobial compounds against microorganisms and that they can be used for the treatment of infectious diseases caused by resistant pathogenic microorganisms. Marine halophytes are already known for antimicrobial activity [18, 3] traceable to the presence of constituents unique to these groups of plants [19]. 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 [20 , 21]. The phytochemical analysis reveals that, the leaf extract of *A. marina* richly posses total phenol, flavanoid and phenol [19] form irreversible complexes with

proline rich proteins, resulting in the inhibition of cell protein synthesis of bacteria [22]. Flavonoids are phenolic structure containing one carbonyl group complexes with extracellular and soluble protein [23], thus exhibits antibacterial activity through these complexes. 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 phospholipidic bilayer. In spite of the barriers the phytochemical constituents were effective in controlling the growth of these pathogenic strains [24]. Based on the results, it is possible to conclude that, ethanolic extract of *Avicenna marina* had different level of antibacterial activity against the antibiotic resistant bacteria and eye pathogens. The present studies have to develop newer lead for better and safer chemotherapeutic agents from mangroves. Further studies are needed to identify the pure compound and to establish the exact mechanism of action for antibacterial action of the plant extract.

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