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Antibacterial activity of some Rhozophoraceae plant species

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Abstract

The present study was carried out to evaluate the antimicrobial properties of three mangrove plant species belonging to the family Rhizophoraceae. Three plant species *Rhizophora apiculata*, *Bruguiera gymnorrhiza* and *Ceriops decandra* are selected to understand the anti microbial properties using agar well diffusion method. The plant material stems with bark were extracted by using soxhlet extractor successively. The selected plant extracts showed more growth inhibition on gram negative bacteria than gram positive bacteria. Methanol extracts of *Bruguiera gymnorrhiza* and *Ceriops decandra* were found to show more antibacterial property than *Rhizophora apiculata*. Chloroform extracts of *Bruguiera gymnorrhiza* and *Ceriops decandra* exhibited considerable anti microbial activity. Hexane extracts of all the three plant species showed insignificant anti microbial activity. The results have given evidence on antibacterial property of mangrove plants and the scope to further studies on isolation of antimicrobial compounds from mangrove plant species.

Key words: Mangrove plants, Rhizophoraceae and Antimicrobial activity.

1. Introduction

Plants are good sources of numerous bioactive compounds which may have different biological activities like antimicrobial, anti allergic, anti-film, insecticidal etc. (Adebajo et al., 2006; Hintz et al., 2015). Plants have been used traditionally for several human and animal diseases in many parts of the world. As reports of Kim, 2005; Negi, 2012, 80% of developing countries' population have been traditional medicine for medicinal use. Nowadays pathogenic microorganisms become resistant to various antimicrobial drugs due to indiscriminate use of antimicrobial drugs on hosts (Cowman, 1999). So, there is a need to screening and indentify new antimicrobial agents from plants, because plants are potential source for natural antibiotics and those compounds have been using in synthesis of synthetic antibiotics as precursors (Yu et al., 2005; Alan et al., 2015). Mangroves are the one the important plants that grow in physiologically dry areas and show peculiar metabolism (Tomilson, 1986; Onuf et al., 1977). Because of their atypical nature the mangroves may provide us some extraordinary bioactive molecules (Kui Hong et al., 2009). So, the

present study carried out on evaluation of antimicrobial property of some mangrove plants belongs to the family Rhozophoraceae. The stem part of *Rhizophora apiculata*, *Bruguiera gymnorrhiza* and *Ceriops decandra* are selected for the present work.

2. Materials and Methods

2.1 Chemicals

All the solvents used in the present study were analytical grade. Standard drug ciprofloxacin from Apollo pharmacy (Manufacturer: Dr Reddy's Laboratories). Muller Hinton agar media was purchased from Sisco Research Laboratories Pvt Ltd., Mumbai.

2.2 Preparation of plants extracts

Three Mangrove plant species of Rhizophoraceae family have been collected from in and around Visakhapatnam and East Godavari districts, Andhra Pradesh, India (Table 1 and Fig 1). The fresh plant parts, stem with bark were collected to prepare the extracts for the study. The parts collected were washed with water to remove the soil and dust particles and then they were dried in completely shaded places. Then it is chopped into small pieces and coarsely powdered in willy mill.

Then the coarsely powdered material is weighed and extracted with Hexane, Chloroform and Methanol were used successively by soxhelt extractor for 8 to 12 hours at a temperature which is not exceeding the boiling point of the solvent.

Table 1. Selected plants for the antibacterial activity.

S No.	Name of the	Family	Part
	Plant Species		used
1	Rhizophora	Rhizophoraceae	Stem
	apiculata		with bark
2	Bruguiera	Rhizophoraceae	Stem
	gymnorrhiza	_	with bark
3	Ceriops	Rhizophoraceae	Stem
	decandra	_	with bark

2.3 Selected microorganisms

The microorganisms used for the experiments were procured from MTCC, IMTECH, Chandighar (Table 2).

Table 2. Bacterial strains for anti-bacterial activity.

S	Name of the bacterium	Type of the
No.		bacterium
1	Staphylococcus aureus	gram-positive
2	Streptococcus pneumoniae	gram-positive
3	Enterococcus faecalis	gram-positive
4	Listeria monocytogenes	gram-positive
5	Micrococcus luteus	gram-positive
6	Proteus mirabilis	Gram-negative
7	Shigella flexneri	Gram-negative
8	Acinetobacter baumannii	Gram-negative

2.4 Culture Media for bacterial culture and antibacterial activity

The Bacterial species were maintained in the nutrient broth medium on placing shaker in separate culture tubes for each species separately. For Anti bacterial activity Muller-Hinton Agar media was used.

2.5 Preparation of stock solutions

The stock solution of test extracts was prepared by dissolving the dried extracts at a concentration of $50\mu g/100\mu l$, $100 \mu g / 100 \mu l$ $500 \mu g / 100 \mu l$ $250 \mu g / 100 \mu l$, and in (DMSO) respectively. dimethylsulphoxide The stock solution of reference standards (Ciprofloxacin) was prepared at a concentration of 20μg/100μl in sterile water.

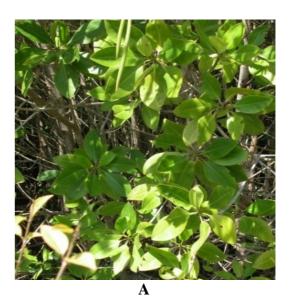






Fig 1. Selected plants for antimicrobial activity. A. *Ceriops decandra*; B. *Rhizophora apiculata*; C. *Bruguiera gymnorrhiza*

2.6 Antibacterial activity by agar well diffusion method

Agar well diffusion assay was used to screen for antimicrobial activity of the extracts of three different plant species. In agar well diffusion method the nutrient media was prepared in which for every 100ml of agar medium, Peptone-5g; Beef extract -10g; Sodium chloride - 5g; Agar -15g is maintained and the pH is adjusted between 7.2 and 7.4, then the agar nutrient medium is sterilized in an autoclave at 15 lb pressure for 30 min. The 20ml of sterilized Nutrient Agar was inoculated with 200µl of culture of bacteria at aseptic conditions and poured in 6" diameter Petri-dishes and allowed at room temperature for solidification and placed in refrigerator for 30min. Later than, with steel sterile borer (6mm) was used to make 5 uniform cups/wells in Petri-dishes. The cups/wells were filled with 40µl of the different concentrations of extracts and single dose of standard drug were allowed to diffuse plant extract into the medium for about 45 minutes. The plates was prepared were left for 2 hrs in refrigerator and then kept in an incubator at 37°C. After 24 hrs the agar plates were examined for inhibition zones, and the zones were measured in millimeters (Mounyr et al., 2016).

3. Results and Discussion

Antimicrobial activity selected extracts of the three extracts methanol, chloroform were studied in-vitro by agar well diffusion method eight bacterial strains. Results antimicrobial activity of the selected plants extracts showed in Tables 2. The methanol extracts of all the studied mangroves was more effective against tested bacteria. The significant antibacterial activity of the active extracts was compared with standard antibiotic ciprofloxacin. The extracts at lowest concentration 50µg/100µl do not showed any zone of inhibition on tested bacteria, chloroform extracts showed the moderate activity on microorganisms and the methanol extract at higher concentration 500µg/100µl showed maximum zone of inhibition. Different zone of inhibitions were observed for different extracts concentrations of selected mangrove plants and they showed more activity on gram negative bacteria than gram positive bacteria.

The plant *C. decandra* extracts were showed more activity on *S. pneumoniae* and the methanol extract showed the constant zone of inhibition on tested bacterial strains at $500\mu g/100\mu l$.

R. apiculata extracts were showed the very less antibacterial activity compared to other plants extracts and the methanol extract showed better activity compared to other extracts of it.

B. gymnorrhiza extracts were showed the activity as comparable to C. decandra and P. mirabilis. The lowest concentrations of all extracts do not show the any zone of inhibitions on microorganisms.

Antibacterial compounds from natural resources would be the alternative to overcome the microorganisms getting resistance problem. Hence, the present study has planned to find out the selected mangrove plants have antibacterial capacity from the most unexplored mangrove plants of Andhra Pradesh, India. The control or effect on bacterial strains of tested mangrove plants' extracts were may be due to presence of biologically active compounds like alkaloids, phenolics, steroids, flavanoids etc., (Fennel et al., 2004; Ravikumar et al., 2011). From the results of the present study confirm that the selected mangrove plants have the antibacterial capacity and when compared with the standard drug ciprofloxacin they showed little less activity. But, the isolated pure compounds may show the more activity as standard drugs. So, further research is needful in the isolation of pure compounds from selected mangrove plants extracts.

4. Conclusion

From the results of the present study, it may conclude that the mangrove plants have the antibacterial activity and they may also good source for the new antimicrobial compounds.

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Conflict of interest

We have none to declare.

Table 3. Antibacterial activity of selected mangrove plants.

Name of the plant	Name of the extract	Concentration of the Name of the bacterial strain								
		extract (µg/100µl)	S. a	S. p	E.f	L. m	M. l	<i>P. m</i>	S. f	A. l
Ceriops decandra	Hexane	50	-	-	-	-	-	-	-	-
		100	7	7	_	_	_	7	_	_
		250	8	10	-	-	7	8	7	7
		500	9	12	7	7	8	9	8	8
	Chloroform	50	-	-	-	-	-	-	-	-
		100	7	9	7	7	7	7	7	7
		250	8	10	9	8	8	9	8	8
		500	10	11	10	9	9.5	11	10	9
	Methanol	50	-	-	-	-	-	-	-	-
		100	7	10	-	7	7	-	7	-
		250	8	12 13	8	8	9	8	9	8
		500	9	13	10	10	10	9	11	10
Rhizophora apiculata	Hexane	50	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	-	-
		250	-	-	7	-	-	-	-	-
		500	_	_	8	-	_	-	7	_
	Chloroform	50	_	_	_	_	_	_	_	_
		100		_	6	_	_	_	_	_
		250	-	7	8		7		7	7
			-			-		-		
		500	-	7	9	8	9	-	8	9
	Methanol	50	-	-	-	-	-	-	-	-
		100	-	-	-	-	-	-	7	-
		250	-	7	-	7	8	-	8	-
		500	_	8	_	9	10	7	9	7
		50	_	_	_	_	_	_	_	_
Bruguiera gymnorrhiza	**	100	_	7	7	_	_	_	_	7
	Hexane	250	_	8	9	7	7	7	_	9
		500	7	9	10	9	8	9	7	11
	Chloroform	50	_	_	_	_	_	_	_	_
		100	6	7	7	-	-	7	7	-
		250	7	7	8	8	7	8	8	7
		500	7	8	9	10	8	10	9	8
	Methanol	50	-	-	-	-	-	-	-	-
		100	7	-	8	7	7	7	7.5	7
		250	9	7	9	8	8	9	9	8
G. 1 1		500	10	8	10	9	10	12	11	9
Standard drug	Ciprofloxacin	20	33	35	13	20	25	19	20	13
Control	DMSO	100	-	-	-	-	-	-	-	-

S. a: Staphylococcus aureus; S. p: Streptococcus pneumoniae; E. f: Enterococcus faecalis; L. m: Listeria monocytogenes; M. l:
Micrococcus luteus; P. m: Proteus mirabilis; S. f: Shigella flexneri; A. b: Acinetobacter baumannii.

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