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Research Article

ANTIBACTERIAL ACTIVITY OF MANGROVE PLANT *RHIZOPHORA MUCRONATA* L. LEAVES

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Abstract

The mangrove plant of *Rhizophora mucronata* L. were collected from the Muthupet mangrove forest and used for the extraction of fresh and dried parts of leaves stem and root respectively. These plant materials were used to derive the ethanolic extracts and antibacterial activity of solvent extract were performed by the disc diffusion method, Agar well method and Minimal Inhibitory Concentration methods. In the dried plant sample the leaf part of *Rhizophora mucronata* is having higher inhibitory activity against the pathogenic bacteria compared than the fresh plant extracts of *Rhizophora mucronata*.L.

Keywords: Mangrove plants *Rhizophora mucronata* – solvent extraction- Antibacterial activity.

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1. Introduction

Mangrove is a woody plant, which lives between the sea and the land in areas, which are inundated to survive in salty soils that would kill most other kinds of plants. Majority of the mangrove plants posses the medicinal and commercial importance, such as *Rhizophora, Bruguiera, Excoecaria, Xylocarpus, Avicennia, etc.* are extensively used as firewood by the coastal people of tropical and subtropical countries. Mangroves serve as a nursery, feeding and spawning grounds for commercial fishes and shellfishes, provide detritus for the coastal ecosystem, reduce cyclone and wind damage and prevent soil erosion (Lawton *et al.*, 1981). It is also serves as a nesting and feeding ground for a variety of wild life and as a coastal pollutants sink or trap (Jagtap, 1987). They have been exploited by coastal people for their numerous products like fodder, fuel, wood, pulp vegetable tannins, poles for building and medicine (UNEP,1988). Numerous medicines are derived from mangrove. They are used for curing elephantiasis, abdominal troubles, and skin diseases.

They also cure sores, Leprosy, head-aches, rheumatism, snakebites, and boils, ulcers, diarrhoea and haemorrhages (Selvaraj et al., 1995).. As a potential source of mosquito repellents, larvicides and for antiviral drug formulations especially against AIDS and jaundice. Majority of the seaweeds and sea grasses already used to study their medicinal and economical activities, have been done by various researches. But no more works done by the previous workers in the mangrove plants related to anti microbial activity. The aim of the present study is to explore the potential of antibacterial substances occurring in the *Rhizophora mucronata* extracting with ethanol against five bacterial pathogens.

2. Materials and Methods

Mangrove plants and bacterial pathogens

The mangrove plants of *Rhizophora mucronata* were collected from the Muthupettai mangrove forest of Thiruvarur district of Tamilnadu, India. Totally, five different bacterial pathogens like*Vibrio cholerae, Salmonella typhi, Staphylococcus aureus, Proteus* sp. and *Enterobacter* sp. were used for the study.
Soxhelet extraction of fresh samples

2 gm of fresh sample of leaves of *Rhizophora mucronata* were separately taken.20 ml of Ethanol was added to the sample and the samples were ground by mortar and pestle. The ground samples were used for the extraction filtered through the whatman no 1 filter paper. The filtrate was used to assay for the antibacterial activity. For Dried sample, 15 gm coarsely powdered samples of leaf, stem and root of *Rhizophora mucronata* were taken.100 ml of Ethanol was added in the powdered samples. The extract was obtained from the powder by using the Soxhelet apparatus. Various concentrated ethanol extracts dried by air drying for 10 minutes.

Preparation of plant extract discs

The discs were prepared from Whatmann No 1 filter paper that is about 2 mm in diameter. The plant extracts (25 up to 200µl and 10 to 50 µl) were incorporated in to sterile discs. Each disc was placed individually in the Muller Hinton agar. This can be achieved by adding small quantities of the extracts and the discs were allowed to dry in laminar airflow.

Assay of antibacterial activity

Assay of antibacterial activity of the plant extract were done by disc diffusion technique, Agar well method and Minimal inhibitory concentration method.

Disc diffusion method

The Muller Hinton agar plates were prepared and the test bacterial strain was smeared on the agar surface using sterile cotton swab. The antibiotic disc loaded with plant extracts were placed on the surface of the agar plates. Controls were maintained by loading ethanol on discs. Then the plates were incubated at 37°C for 12-18 hours. The inhibition zone formation was observed and recorded.

Agar well method

Sterile Muller Hinton agar plates were prepared and wells were made by using cork porer. Then the bacterial cultures were inoculated into the plates with the help of cotton swab. Then the different concentrations of ethanolic extracts of the plant samples of stem, root, and leaves were added to the well. Then all the plates were incubated at 37°C for 24 hours. After incubation the zone of inhibition was observed and measured. Then the zones are compared with standard.

Minimal inhibitory concentration method

MH broth was prepared aseptically in the test tubes.1 ml of bacterial culture was added to the series of tube containing MH broth. The different concentrations of plant extract of root, stem, and leaf samples were added. Then all the test tubes were incubated at 37°C for 24 hours. After the incubation period the OD values were taken at 600 nm and compared with control.

Results

The mangrove plant parts of *Rhizophora mucronata* were tested for their antibacterial activity against certain opportunistic pathogen like S*taphylococcus aureus, Salmonella typhi, Vibrio cholerae, Proteus* sp. *Enterobacter* sp. (both gram positive and gram negative) using solvent extract of E*xcoecaria agallocha.*

Fresh leaf samples of *Rhizophora mucronata* L.

The results of disc method showed that the higher antibacterial activity for *Enterobacter* species (25 mm), *Staphylococcus aureus (*24 mm) and moderate activity were showed by *Salmonella typhi* (20mm) *Vibrio cholerae*(19mm) and *Proteus* sp. with 22 mm. Minimum inhibitory was observed in *Proteus* sp. (8 mm) and *S .typhi* (9 mm). In agar well method, the extract showed higher activity for P*roteus* sp. with 22 mm and *Vibrio cholerae* and 20 mm and minimum activity was observed in *S. aureus* (5 mm) and *Proteus* sp. (6 mm). In Broth culture method, the ethanol extract of sample were showed higher activity in *Salmonella typhi* (0.311) and *Vibrio cholerae* (0.232) and minimum with 0.056 in *Enterobacter* sp. and *S. aureus* with 0.096

Dried leaf samples of *Rhizophora mucronata* L.

Maximum anti bacterial activity was found to be *S. typhi* (26 mm), *V. cholerae* (24 mm) followed by *S. aureus* (23mm) and minimum inhibitory was observed in *Proteus* sp. (9 mm) and *S .aureus* (11 mm).

Extract of *E. agallocha* was showed higher activity for *Enterobacter* sp. (25 mm) and *S. aureus* (22 mm) and minimum activity was observed in *V. cholerae* (10 mm) and *S. typhi* and *Proteus* sp. (11 mm) using agar well method. the ethanol extract of sample were showed higher activity, *S. aureus* (0.369) and *S. typhi* (0.298) and minimum with 0.098 in *Proteus* sp. and *Enterobacter* sp. (0.123) in broth culture method.

Discussion

Blasco (1997) estimated the total mangrove forest in India in our study the plant *Excoecaria agallocha* was selected from the Muthupet Mangrove forest area. Srinivas *et al.* (1995) worked antimicrobial activity of the extract of *Azadirachta india* and *Nacegamia allata*. Present work investigated on antibacterial activity of the extract of *Excoecaria agallocha* various organic solvents were used for the extraction of the bio-active compounds of the plants (Saha *et al.*, 1995). Traditionally, it has been used for the treatment of rheumatoid arthritis. It is also reported to have hepatoprotective (Avadhoot and Rana, 1001). The leaves of *Vitex negundo* have been reported to used for the treatments of eye diseases, tooth hacks, rheumatoid arthritis, etc.,(Das and Das, 1994). In our work the organic solvent ethanol was used to extract the bioactive compounds of various parts of the plant. The bioactive components from the plant samples can be extracted by using soxhelet apparatus *(Tanira et al.,* 1994). Mangrove forests have played an important role in the Socio – economics life of the people. The mangrove forests have several valuable medicinal plants that are used in medicinal fields (Azariah *et. al.,* 1987). The *Excoecaria agallocha* is also a medicinal plant of mangroves and it is utilized for various purposes in this medical field. Several authors have shown that the antibacterial activities of many plants against pathogenic bacterial communities

Table. 1 Antibacterial activity of ethanol extract of fresh leaf sample of Rhizophora mucronata L.

Sl.No	Bacterial pathogens	Zone of inhibition (mm) in different concentrations					
		25 µl	50 µl	100 µl	150 µl	200 µl	Std antibiotic (Chloromphinicol)
1	Disc method *Staphylococcus aureus*	12	16	16	18	24	31
2	*Salmonella typhi*	9	13	15	17	20	30
3	*Vibrio cholerae*	10	10	13	17	19	31
4	*Proteus sp*	8	11	15	19	22	31
5	*Enterobacter sp.*	10	13	17	18	25	29
	Agar well method	10 µl	20 µl	30 µl	40 µl	50 µl	Std antibiotic (Chloromphinicol
	Staphylococcus aureus	5	8	9	11	15	29
	Salmonella typhi	9	10	13	15	18	29
	Vibrio cholerae	8	11	14	16	20	30
	Proteus sp	6	10	13	19	22	29
	Enterobacter sp.	7	9	11	13	18	29
	Broth Culture method	25 µl	50 µl	100 µl	150 µl	200 µl	Control
	Staphylococcus aureus	0.136	0.121	0.116	0.112	0.096	0.401
	Salmonella typhi	0.311	0.293	0.289	0.271	0.256	0.561
	Vibrio cholerae	0.232	0.210	0.172	0.145	0.113	0.396
	Proteus sp	0.192	0.156	0.135	0.120	0.082	0.278
	Enterobacter sp.	0.163	0.141	0.123	0.107	0.056	0.292

Table 2 Anti bacterial activity of dried leaf sample of Rhizophora mucronata L.

Sl.no	Test organisms	Zone of inhibition (mm) in different concentrations					
		25 µl	50 µl	100 µl	150 µl	200 µl	Std antibiotic (Chloromphinicol)
1	Disc method *Staphylococcus aureus*	11	13	18	20	23	30
2	*Salmonella typhi*	15	19	20	23	26	31
3	*Vibrio cholerae*	13	15	18	21	24	31
4	*Proteus sp*	9	12	13	15	20	31
5	*Enterobacter sp.*	11	13	15	17	19	30
	Agar well method	10 µl	20 µl	30 µl	40 µl	50 µl	Std antibiotic (Chloromphinicol
	Staphylococcus aureus	13	16	18	19	22	30
	Salmonella typhi	11	15	18	20	22	30
	Vibrio cholerae	10	13	15	18	18	31
	Proteus sp	11	13	16	18	20	30
	Enterobacter sp.	15	18	19	23	25	31
	Broth culture method	25 µl	50 µl	100 µl	150 µl	200 µl	Control
	Staphylococcus aureus	0.369	0.306	0.278	0.251	0.193	0.401
	Salmonella typhi	0.298	0.278	0.253	0.236	0.199	0.561
	Vibrio cholerae	0.263	0.256	0.192	0.180	0.160	0.396
	Proteus sp	0.158	0.149	0.136	0.107	0.098	0.278
	Enterobacter sp.	0.216	0.203	0.201	0.176	0.123	0.292

(Thomas *et al.*, 1996; Das and Das, 1994). This study has revealed the antibacterial activity of mangrove plants and can be suggested that the bioactive contents of the mangrove plants are promising natural antimicrobial agents that can be harnessed as potential antibacterial and fungal toxicants. Further, extensive studies are recommended for these mangrove plants samples to actually identify the bioactive compounds responsible for their antimicrobial activities.

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