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Evaluation of the Antibacterial Activity of Rhizophora Mucronata Mangrove Leaf ExtractAgainst Antibiotic Resistant Pathogens

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ABSTRACT

Introduction: *Rhizophora mucronata* is a plant of marine origin commonly referred to as asiatic mangrove, red mangrove or loop root mangrove . *R. mucronata* species are utilized traditionally for treating various health conditions like haematoma, hepatitis, hematuria, ulcers, elephantiasis and so on.

Aim: This study aims at evaluating the antibacterial activity of *Rhizophora mucronata* mangrove leaf extract against the antibiotic resistant pathogens.

Materials and Methods: The fresh leaves of *Rhizophora mucronata* were collected, washed, shade dried for 2-3weeks and turned into a fine powder. For extract preparation, 10g of powdered sample mixed with 100 ml methanol for 24 hours and passed through Whatman filter paper. It was centrifuged at 3000 rpm and further filtered by 0.45 µm syringe micro filter and evaporated via rotary evaporator. Drug resistant *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* collected were cultured in Muller Hinton broth for 24 hours to prepare the bacterial suspension. The concentration of microbial suspension was fixed at 108 CFU/ml. 1ml of suspension was spread over on Muller Hinton agar plate and incubated for 24hrs at ambient temperature. Whatman filter paper discs (5mm) were impregnated with various concentrations (50, 100, 150, 200, 250 & 300 µg/ml) of leaf extract , inhibition zones around the discs were measured. The MIC of the leaf extract was determined in 5 concentrations (10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml and 50 µg/ml) inoculated bacteria in test tubes are incubated for 24 hr and the results are noted as well growth (+) and inhibited (-).

Results: Comparing the zone of inhibition measured against the three bacteria, *Rhizophora mucronata* leaf extract showed the maximum zone of inhibition,32 ± 1.24 mm, at the maximum concentration (300 µg/ml) against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae Staphylococcus aureus* showed a maximum inhibition of 27± 1.22 and 23 ± 1.32 respectively.The Minimum Inhibitory Concentration (MIC) produced by *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* was found out to be 40 µg/ml, 30 µg/ml and 30 µg/ml respectively.

Conclusion: This study presented extensive information about the antibacterial activity of *Rhizophora mucronata* against antibiotic resistant pathogens. Infection with drug resistant microorganisms such as *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* may be treated with *Rhizophora mucronata* after further studies

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INTRODUCTION

The urinary bladder is a hollow organ located in the lower abdomen that stores urine from the kidneys (through the ureter) until urination. Urothelial cells, which line the urinary[1] bladder[1] and urinary tract, are specialized transitional epithelial cells that accommodate the volume. Smooth muscle lines the bladder, which may relax to accept larger quantities and contract[1] (under voluntary or reflex control)[1] to expel urine down the urethra[1] and out of the body (Zhuo et al, 2021).

One of the most prevalent urinary tract illnesses is cystitis. The word cystitis was developed to describe the infection's location, which termed to bladder infection, which is usually caused by bacteria from intestinal flora (Ki et al, 2017; Huether, 2019) *S.saprophyticus* was discovered to be the second most prevalent cause of cystitis, behind E. coli. It has unique urotropic and ecological characteristics that distinguish it from other staphylococci and *Escherichia coli* (Dolores et al, 2020).

KEYWORDS:
Antibacterial activity, Antibiotic resistance Mangrove plant, Natural source, *Rhizophora mucronate*

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The development of tumors and their transformation into malignancies is occasionallyunder the influence of a group of microorganisms. Bacteria, which are one of and in reducing global warming by its production of increasedamount of carbon sequestration.[(5)] Medicinally, *R. mucronata* species are utilized for treating various health conditions like haematoma, hepatitis, hematuria, ulcers, elephantiasis etc. traditionally.[(6)] In folk medicine, the leaves of *R. mucronata* are used in treating diarrhoea and related gastric motility disorders since it is popular for its anti diarrhoea properties.[(7)]

Antimicrobial resistance has evolved to become a serious challenge associated with a high rate of mortality. Drug resistance is seen in various gram positive and gram negative bacteria which when combined with the lack of preventive measures, effective therapy and new antibiotics are untreatable.[(8)] Studies regarding the antibacterial activity of *Rhizophora mucronata*[(9,10)] were carried out . Many other studies such as antidiabetic activity,[(1112)] bioremediation of marine plants,[(13,14)] isolation of alkaloids and melanin from marine sources,[(15,16)] anti-inflammatory activity,[(17)] antibacterial activity,[(18)] antihyperglycemic activity,[(19)] antioxidant activity (20), enzyme producing marine microbes[(21,22)] has been studied extensively in previous literature. Our team has extensive knowledge and research experience that has translated into high quality publications.[(23-28)] Thus, the aim of this study was to evaluate the antibacterial activity of *Rhizophora mucronata* mangrove leaf extract against the antibiotic resistant pathogens.

MATERIALS AND METHODS

Study setting: Marine Biomedical and Environmental Health Research Lab - Blue Lab,Saveetha dental College, Chennai , India.. Before the initiation of the study, ethical clearance was obtained and the ethical approval number IHEC/SDC/ UG-1996/21/94.

Collection of plant material and preparation

The fresh leaves of Rhizophora mucronata were collected from Muthupet mangrove forest area, Tamilnadu. The leaves were washed thoroughly with tap water then shade dried on table tissue paper for 2-3weeks and turned into a fine powder.

Preparation of extraction

10g of dried powdered mangrove leaf sample was mixed with 100ml of methanol/Ethanol (V/V) and allowed to sit for 24 hours at ambient temperature. Then the mixture was passing through whatman filter paper (No.4) then the filtrate was centrifuged at 3000rpm for 10min and further filtered by 0.45µm syringe micro filter. At last, the solvents are evaporated via vacuum rotary evaporator until samples are obtained in powder form. Then the sample was stored in a shadowy aluminum container at 4ºC for further analysis.

Bacterial Suspension

Drug resistant gram positive *Staphylococcus aureus* and gram negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were collected from the Department of Microbiology, Saveetha Medical College and Hospital, Tamilnadu. The bacterial pathogens were cultured in Muller –Hinton Broth for 24 hours at room temperature. From this, the bacterial suspension was prepared with saline and the optical density was measured at 600 nm. The concentration of microbial suspension was fixed as 108 CFU/ml. 1ml of suspension was spread over on Muller Hinton agar plate and incubated for 24 hours at an ambient temperature.

Antibacterial activity

The antibacterial activity of mangrove leaf extract was performed with disc diffusion method. Whatman filter paper discs (5mm) were impregnated with various concentrations (50, 100, 150, 200, 250 & 300 µg/ml) of leaf extract. The inoculated plates were incubated for 24 hours at room temperature and the inhibition zones around the discs were measured. The results were expressed from an average of three with standard deviation.

Minimum Inhibitory Concentration

Minimal Inhibition Concentration of mangrove leaf extractwas determined in 5 concentrations (10 -50 µg/ml) with blank (extract in Muller Hinton broth). The inoculated bacteria in test tubes are incubated for 24hr in ambient temperature. The results are noted as well growth (+) and inhibited (-)

RESULTS

The antibacterial activity of *Rhizophora mucronata* leaf extract was estimated against one gram positive *Staphylococcus aureus* and two gram negative bacteria, *Klebsiella pneumoniae and Pseudomonas aeruginosa*. The zone of inhibition formed against *Staphylococcus aureus* at 50 µg/ml was 3 ± 1.6, 100 µg/ml was 6 ± 1.4, 150 µg/ml was 10 ± 1.5, 200 µg/ml was 13 ± 0.94, 250 µg/ml was 17 ± 1.26 and at 300 µg/ml was 23 ± 1.32. The zone of inhibition formed against *Klebsiella pneumoniae* at 50 µg/ml was 6 ± 1.18, 100 µg/ml was 12 ± 1.25, 150 µg/ ml was 15 ± 1.19, 200 µg/ml was 20 ± 1.23, 250 µg/ml was 23 ± 1.26, 300 µg/ml was 27 ± 1.22. The zone of inhibition formed against *Pseudomonas aeruginosa* at 50 µg/ml was 5 ± 1.23, 100 µg/ml 9 ± 1.31, 150 µg/ml was 15 ± 1.20, 200 µg/ml was 21 ± 1.34, 250 µg/ml was 26 ± 1.28 and 300 µg/ml was 32 ± 1.24. (Table 1)

Inferring from the results obtained, the bacteria against which the *Rhizophora mucronata* leaf extract produced the maximum zone of inhibition at the maximum concentration (300 µg/ ml) was *Pseudomonas aeruginosa* (Table 1) and the least MIC was offered by the positive control, Tetracycline (Table 2). The Minimum Inhibitory Concentration (MIC) produced by *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* was found out to be 40 µg/ml, 30 µg/ ml and 30 µg/ml respectively. The MIC of the positive control, Tetracycline, was found to be 20 µg/ml which was lesser whencompared to the MIC of the three bacteria. (Table 2)

Table 1: Table represents the zone of inhibition provided by *Rhizophora mucronata* leaf extract against *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa.*

µg / ml	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*
0	0	0	0
50	3 ±1.6	6 ± 1.18	5 ± 1.23
100	6 ± 1.4	12 ± 1.25	9 ± 1.31
150	10 ± 1.5	15 ± 1.19	15 ± 1.20
200	13 ± 0.94	20 ± 1.23	21 ± 1.34
250	17 ± 1.26	23 ± 1.26	26 ± 1.28
300	23 ± 1.32	27 ± 1.22	32 ± 1.24

Table 2: Table representing the Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* compared with the positive control, Tetracycline.

MIC	*0*	*10*	*20*	*30*	*40*	*50*	*MIC(µg/ml)*
Staphylococcus aureus	+	+	+	+	-	-	40
P. control	+	+	-	-	-	-	20
Klebsiella pneumoniae	+	+	±	-	-	-	30
P. control	+	+	-	-	-	-	20
Pseudomonas aeruginosa	+	+	+	-	-	-	30
P. control	+	+	-	-	-	-	20

DISCUSSION

The antibacterial activity of *Rhizophora mucronat*a leaf extract were reported by Sahoo et al., resulted that the maximum zone of inhibition was provided against *Staphylococcus aureus* measuring about 16 mm followed by 12.3 mm zone of inhibition against *Streptococcus* sp. and the minimum zoneof inhibition was against P. mirabilis measuring about 11 mm. The antibacterial activity of *Rhizophora mucronata* leaf extracts was reported by Sahoo et al 2012, concluded that the ethanol extract showed activity against all the pathogens tested but the aqueous extract was found to be active only against *P. vulgaris* (6.7 mm).[29]

Saragih *et al.*, conducted a study regarding the antibacterial activity of methanol extract of *Rhizophora mucronata* mangrove plant and concluded that the zone of inhibition provided by *R. mucronata* against *Staphylococcus aureus* was fond to be 6.1 mm and that of *E. coli w*as found to be 6.4 mm.[30] S Gurudeeban *et al.*, conducted a study on the antibacterial activity of the alkaloid rich fraction of *R. Mucronata* and concluded that the anti microbial activity tests resulted in maximum zone of inhibition of 19.56 ± 0.19 against Staphylococcus aureus when compared to other varieties of bacteria that were isolated from diabetic foot ulcer. The study concluded that an alkaloid-rich fraction of *R. mucronata* is a very good source providing natural antibacterial and antioxidant activity.[31]

An *in vitro* study conducted by Baskaran *et al.*, stated that the Chloroform leaf extracts of *Rhizophora mucronata* resulted in proving to be the most active against drug resistant *Vibrio* spp. which were isolated from marine water Lobster's larvae

hatcheries. The study also evaluated the significant antibacterial activity of *Rhizophora mucronata* against *V. harveyi* and *V. campbelli*.[32] When comparing the antibacterial and antioxidant potential of Indian mangroves proving *Rhizophora mucronata* species to be highly capable of providing good antibacterial activity against antimicrobial resistant pathogens.[33] Previous studies have been done by our team on various topics regarding the benefits of marine plants [34 - 50]

This study screened the antibacterial activity of *Rhizophora mucronata* mangrove leaf extracts against antibacterial resistant pathogens and resulted in favour for *Rhizophora mucronata* extracts providing maximum zone of inhibition against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*. The limitation of this present study was that it is conducted only *in vitro* level. Hence, study can be extended in animal models before utilizing it for human purposes.

CONCLUSION

This study presented extensive information about the antibacterial activity of *Rhizophora mucronata* against antibiotic resistant pathogens such as *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa*. The Minimum Inhibitory Concentration (MIC) produced by *Staphylococcus aureus, Klebsiella pneumoniae and Pseudomonas aeruginosa* was found out to be 40 µg/ml, 30 µg/ml and 30 µg/ml respectively. Hence, it can be used for the management of drug resistant infections with these pathogens.\

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