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AN INVITRO ANALYSIS OF ANTIMICROBIAL POTENTIAL OF RIZOPHORA (MANGROVE) SPECIES EXTRACTED USING ETHANOL SOLVENT

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ABSTRACT:

Background: A mangrove is a small tree or shrub that typically thrives in salty coastal waters. It refers to the unique vegetation found in tropical coastal regions, comprising specific species. Mangroves are prevalent across the globe, primarily in tropical and subtropical regions, typically within latitudes 25° N and 25° S. The global mangrove forest area covers 137,800 square kilometers, spread across 118 countries and territories. This research sought to assess the antibacterial properties of nanoparticles created from Rizophora extract.

Materials and Methods: The research was carried out at the Green lab of Saveetha Dental College, following approval from the Institutional Review Board of the university.

Leaves and barks of Rhizophora were gathered from the Gulf of Mannar Biosphere Reserve in Tamil Nadu. The gathered samples were first washed thoroughly with tap water and then air-dried in the shade on table tissue paper for a duration of four weeks. Afterwards, both the leaves and bark underwent a secondary wash under running tap water and were then dried in an incubator set at 40°C. Using an electric blender, the dried leaves and bark were finely crushed into uniform powders. These powders were subsequently soaked in three distinct solvents (95% ethanol, methanol, and chloroform) at room temperature in the absence of light for three days. Following this, each sample was filtered through Whatman® No. 1 filter paper (Whatman International, England), and the resulting filtered solutions were evaporated to dryness utilizing a water evaporator set at 40°C. The resultant plant extracts were dissolved in dimethyl sulfoxide (DMSO).

Results: The antibacterial efficacy of the Rhizophora extract was assessed through two distinct assays: the disc diffusion method and the Minimum Inhibitory Concentration (MIC) assay. In the disc diffusion test, various concentrations of the chosen clinical isolates (Klebsiella, Streptococcus, and Vibrio) were employed. Results indicated that S. aureus displayed the widest zone of inhibition, followed by Klebsiella and S. mutans.

Conclusion: This research investigated the Streptomyces species associated with the study and found that it demonstrated potent antibacterial properties. Consequently, it can be inferred that it possesses effective antibacterial activity against the targeted microorganisms (Klebsiella, Streptococcus, and Vibrio). This suggests potential for further exploration in future studies.

Keywords: Rizophora, Antimicrobial activity, Oral Pathogens, Amoxicillin consume, production, waste, natural resource, recycle, industrial ecology, sustainable design, supply chain, outsource, offshore, reuse, decarbonizer, decarbonize, carbon tax, carbon pricing, food waste, public procurement, fossil fuel subsidies

1. INTRODUCTION

Mangroves, small trees or shrubs mainly found in saline coastal waters, create vital ecosystems like mangrove swamps. (1) These swamps support diverse food webs relying on detritus and serve as crucial habitats for refuge, feeding, and nurturing. Marine invertebrates, such as ascidians, produce therapeutic compounds that show potential in treating various diseases by affecting membrane potential.(2) Moreover, mangroves play a role in nutrient transportation to neighboring marine habitats like seagrass beds and coral reefs.(3) Actinobacteria, particularly

of the Streptomyces type, are significant producers of bioactive compounds, although discovering new antimicrobials from them in conventional soil has become challenging.(4)

Research on marine actinobacteria, which produce valuable secondary metabolites for pharmaceutical purposes, is ongoing.(4,5) These substances possess unique chemical structures that could lead to the development of novel drugs to combat resistant diseases.(1) Recently, green synthesis and/or nanoparticle derivation have proven highly effective in demonstrating antibacterial properties with good biocompatibility.(6) This study aims to assess the antibacterial effects of nanoparticles synthesized from the Rhizophora plant against common oral pathogens.(7)

2. MATERIAL AND METHODS

Collection of Plant material and Preparation

Leaves and bark from Rizophora were collected from the Gulf of Mannar Biosphere Reserve in Tamil Nadu. The gathered samples were thoroughly washed with tap water and then air-dried in the shade on table tissue paper for a duration of 4 weeks (Figure 1). Following this, the leaves and bark underwent another wash under running tap water and were then dried in an incubator set at 40°C. Using an electric blender, the dried leaves and bark were finely ground into uniform powders. These powders were subsequently soaked in three different solvents (95% ethanol, methanol, and chloroform) at room temperature in the absence of light for three days (Figure 2). Afterward, each sample underwent filtration through Whatman® No. 1 filter paper (Whatman International, England), and the resulting filtered solutions were evaporated to dryness using a water evaporator at 40°C.(8) The resultant plant extracts were dissolved in dimethyl sulfoxide (DMSO).

Figure 1. Collected sample

Bacterial Suspension

The pathogenic bacteria Klebsiella pneumoniae, Streptococcus species, and Staphylococcus species were obtained from the Department of Microbiology, Saveetha Medical College and Hospital, Tamil Nadu. These bacterial pathogens were cultured in Muller–Hinton Broth for 24 hours at room temperature. A bacterial suspension was prepared from this culture using saline, and its optical density was measured at 600 nm (Figure 3). The concentration of the microbial suspension was standardized to 10^8 colony-forming units (CFU) per milliliter. One milliliter of this suspension was then spread evenly over Muller Hinton agar plates and incubated for 24 hours at room temperature.(2)

Figure 2. Preparation of the sample

Antibacterial Activity

The antibacterial activity of the actinobacterial extract was assessed using the disc diffusion method. Whatman filter paper discs (5mm) were saturated with different concentrations (0.5, 1, 1.5, 2, 2.5, and 3 mg/ml) of leaf extract dissolved in ethanol and methanol solvents (9). The plates inoculated with bacteria were then incubated for 24 hours at room temperature, and the diameter of the inhibition zones surrounding the discs was measured. Results were presented as the average of three replicates with standard deviation.

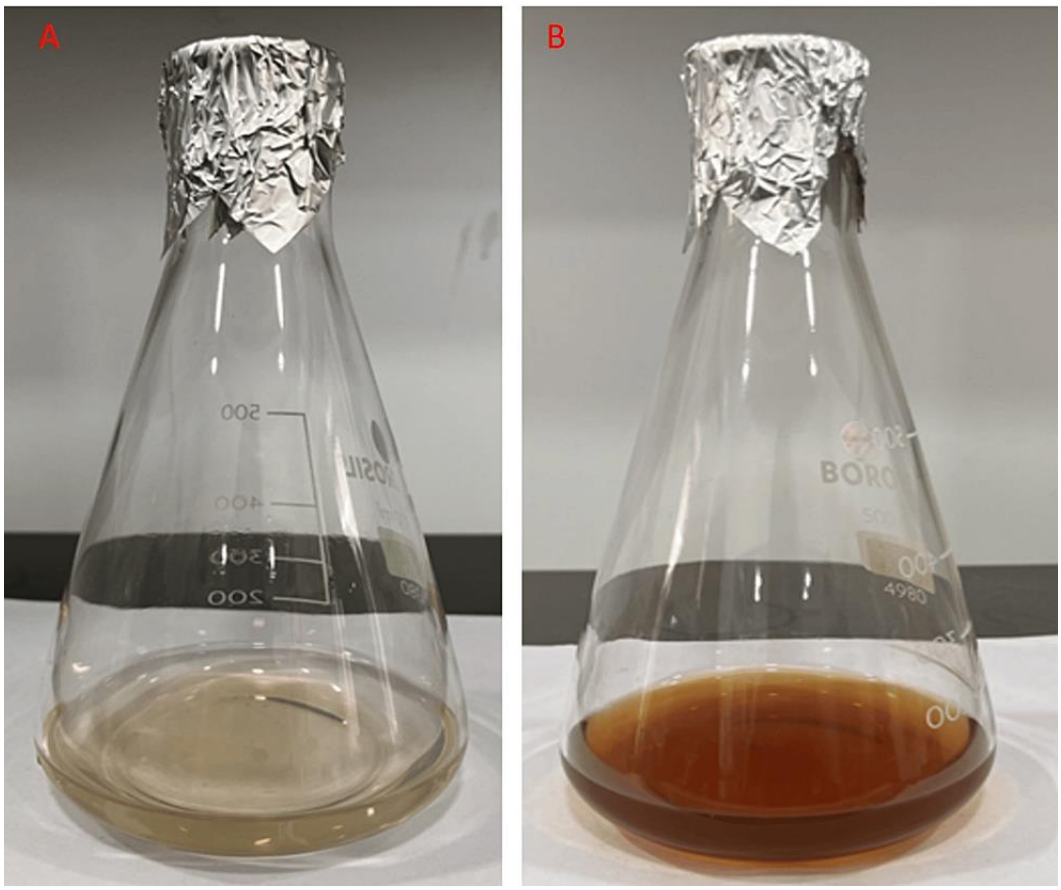

Figure 3. Prepared concentrate media

Minimum Inhibitory Concentration

The Minimum Inhibitory Concentration (MIC) of the actinobacterial extract in ethanol and methanol was determined across five concentrations ranging from 0 to 50 µg/ml (or equivalently, from 0.001 to 0.1 mg/ml), along with a blank control (extract in Muller Hinton broth). The bacterial cultures in test tubes were inoculated and then incubated for 24 hours at room temperature. Subsequently, the optical density of the cultures was observed.

3. RESULTS

The subsequent table presents the Zone of Inhibition observed for different concentrations of the extract against various pathogenic bacteria. (Table 1)

ug/ml	S.mutans	Klebsiella sp.	St.Er	P.aeruginosa	S.aureus
0	0	0	0	0	0
75	6	8	1.2	0	8
100	8	10	0.8	0	11
150	12	16	1.2	0	14

Table1. S.mutans- Streptococcus mutans; St.er- Streptococcus erysipelas; P.aeruginosa- Pseudomonas aeruginosa; S.aureus- Staphylococcus aureus

The ensuing table showcases the Maximum Inhibitory Concentration for oral pathogenic bacteria. (Table 2)

MIC	
Streptococcus Er.	30ug/ml
S. mutans	20ug/ml
Klebsiella	10ug/ml
P. aeruginosa	20ug/ml
S. aureus	30ug/ml

Table 2.S.mutans- Streptococcus mutans; St.er- Streptococcus erysipelas; P.aeruginosa- Pseudomonas aeruginosa; S.aureus- Staphylococcus aureus

The above table demonstrated that the nanoparticles synthesized using Rhizophora extract showed a positive effect on the antimicrobial activity and showed positive changes in the zone of inhibition for the various organisms with maximum effect concerning Streptococcus erysipelas (Figure 4).

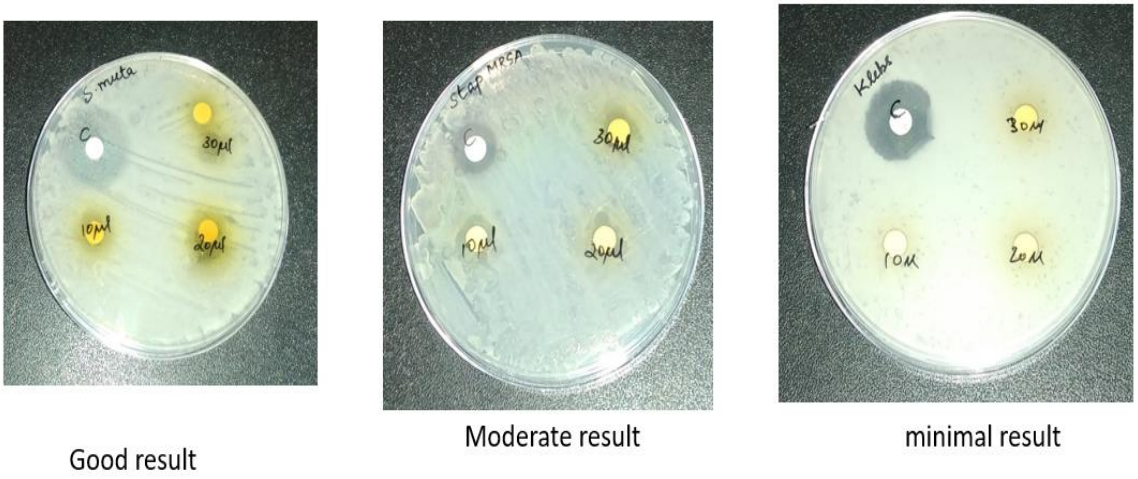

Figure 4. Zone of Inhibition

The nanoparticles demonstrated significant antibacterial effectiveness at two different concentrations, as indicated by the presence of inhibition zones. Specifically, when exposed to a concentration of 100 µg/ml, the inhibition zones for Streptococcus mutans, Streptococcus erysipelas, Pseudomonas aeruginosa, and Staphylococcus aureus were measured at 8±0.25 mm, 9.5±0.5 mm, and 9±1 mm, respectively. Similarly, at a concentration of 50 µg/ml, the inhibition zones for these bacteria were 7±0.2 mm, 8±0.5 mm, and 8±0.2 mm, respectively.

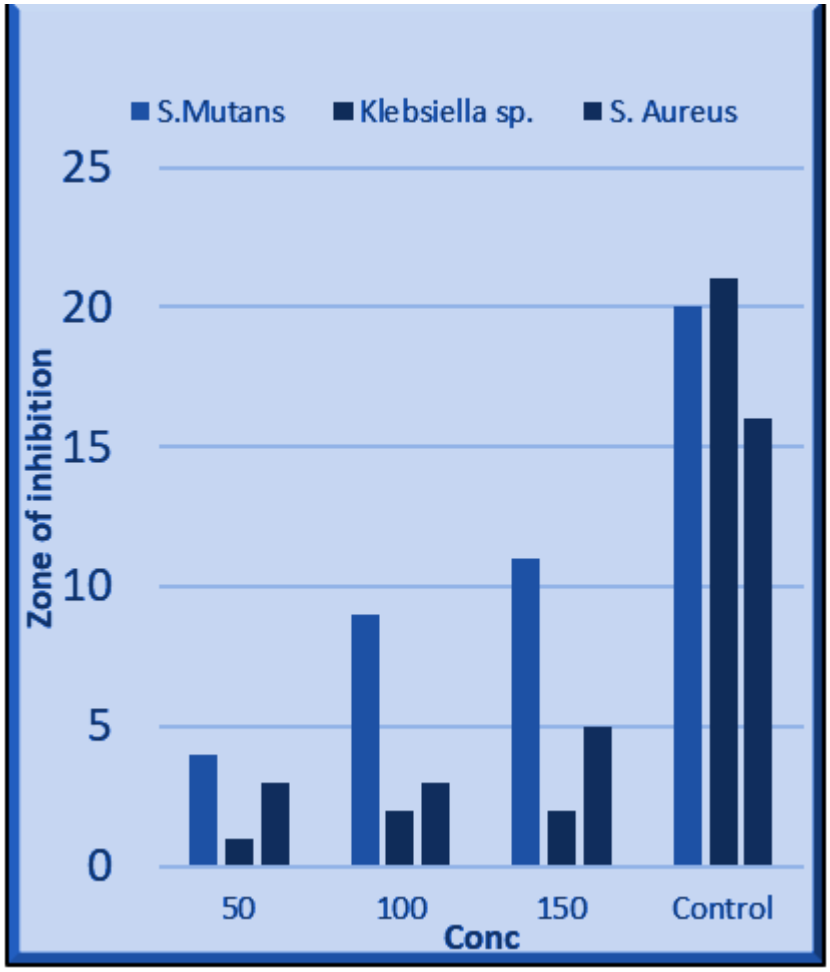

Figure 5. Graphical representation of ZOI at different concentrations
Figure 5 shows the Graphical representation of ZOI at different concentrations

4. DISCUSSION

Overall, gram-positive bacteria are generally considered more susceptible to various antimicrobial compounds due to differences in their cell wall structure. (10) However, our results demonstrate that the extracts are effective against both gram-positive and gram-negative bacteria.(11) Prior studies have highlighted the antibacterial properties of unsaturated fatty acid methyl esters in E. agallocha leaves.(3) Antimicrobial properties are valuable in combating undesired bacteria, particularly in infection treatment and food waste management.(12) Plant extracts' active constituents often disrupt microbial growth and metabolism.(13)

Our study revealed that Rhizophora extracts possess antibacterial activity against tested pathogenic strains, including antibiotic-resistant ones.(14) The presence of active compounds in the plant extracts likely contributes to growth inhibition, evident by clear patches surrounding the discs.(15) Despite gram-positive bacteria's perceived weakness due to their single peptidoglycan layer, and gram-negative bacteria's robust phospholipid membrane, our phytochemical content effectively inhibits the growth of these pathogenic strains.(16,17)

This antibacterial effect may be attributed to various active plant extract components, such as anthraquinones, terpenoids, flavonoids, saponins, phenolics, and alkaloids, which have known antibacterial properties(18). Pathogenic bacteria, including Klebsiella pneumoniae, Streptococcus species, and Staphylococcus species, were isolated and cultured for

experimentation. Minimum inhibitory concentration measurements showed a positive effect against these microorganisms when treated with synthesized nanoparticles.(4)

The methanolic extracts of different Rhizophora sections exhibit potential as antibacterial agents against oral pathogens.(17,18) Further study should focus on quantifying their phytochemical constituents for a deeper understanding.(5) The significant changes observed in the Zone of Inhibition compared to the control group indicate the clinically significant effect of these nanoparticles against common oral pathogens.(19)

5. CONCLUSION

Our study highlights the strong antibacterial activity of associated actinobacteria Streptomyces species. Future research could explore isolating and examining individual components for various properties such as antifungal, antioxidant, and insecticidal activity. These findings hold promise for developing Rhizophora extract-based nanoparticles to enhance antimicrobial activities in various products for medicinal purposes.

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