RESEARCH
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CYTOTOXIC EFFECT OF MANGROVE EXTRACT FROM *AVICENNIA MARINA* LEAVES ON STAPHYLOCOCCUS SP

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Abstract

INTRODUCTION:
Staphylococcus sp are a group of Gram Positive bacteria that commonly reside on human skin and mucous membranes.Staphylococcus sp have emerged as significant pathogens, causing a wide range of infections, from mild skin infections to life threatening diseases like endocarditis. *Avicennia marina*, commonly known as gray mangrove or white mangrove, is a species of mangrove tree classified in the plant family Acanthaceae. The objective of this study is to investigate the cytotoxic effect of mangrove extract against *Staphylococcus sp* and This study aims to Evaluate the cytotoxic effect of *Avicennia marina e*xtract against Staphylococcus sp focusing on its potential antimicrobial activity.

MATERIALS AND METHODS:
Clinical isolates of *Staphylococcus* species were obtained and stored appropriately. *Avicennia marina* leaves were collected, air-dried, and extracted with methanol. *Staphylococcus* sp. Biofilms were formed on Baird Parker broth base using coverslip method.Biofilms were stained with acridine orange and propidium iodide. Cell viability analysis was conducted using the MTT assay after exposing the *Staphylococcus sp* to *Avicennia marina* extract. The extract was administered every 24 hours for 96 hours to evaluate antimicrobial activity through changes in biofilm staining and confocal imaging analysis.

RESULTS:
The extract of *Avicennia marina* demonstrated remarkable inhibitory effects on the growth of *Staphylococcus* species. When the bacteria were treated with 1 ml/mg of the extract every 24 hours for a total duration of 96 hours, the extract consistently exhibited the ability to suppress the growth of *Staphylococcus*, indicating its potential as a potent natural antimicrobial agent.

CONCLUSION:
In conclusion, the extract consistently showed the ability to suppress the growth of Staphylococcus, suggesting its potential as a natural antimicrobial agent. These findings highlight the promising antimicrobial properties of Avicennia marina extract, which may have significant implications for the development of novel therapeutics to combat Staphylococcus infection.

KEYWORDS:
*Avicennia marina,*Cytotoxic effect,Leaf Extract,Mangrove,*Staphylococcus sp.*

INTRODUCTION:

Mangroves, the unique ecosystems found in tropical and subtropical regions, have been recognized as a rich source of bioactive compounds with diverse pharmacological properties. These coastal forests are home to a wide range of plant and animal species, including various halophytic plants that have evolved to adapt to harsh saline environments. Over the years, extensive research has been conducted to explore the potential medicinal properties of mangrove plants and their extracts(1).Mangroves contain several secondary metabolites, according to phytochemical analysis, which may have antibacterial properties. Therefore, the mixtures of secondary products present in plants may be the cause of the positive medical effects of plant materials(1,2).

The Acanthaceae (Avicenniaceae) family includes *Avicennia marina*. *A. marina* is named for the esteemed doctor Avicenna, also known as Ibn Sina. *A. marina* is a little evergreen tree that can reach heights of more than 10 meters. The plant's common names include gray mangrove and white mangrove.The plant's range includes subtropical China, southeastern Australia, Fiji in Polynesia, and East Africa.The plant is primarily found in tropical areas, such as Southwestern and Southeastern Asia, along the Arabian gulf and Bay of Bengal shores(3).*Avicennia marina*'s bark, leaves, and fruits are used to cure skin conditions in traditional medicine. From *A. marina*, iridoid glucosides, fatty acids, sterols, and hydrocarbons have previously been extracted.*A. marina* was also reported to have cytotoxicity and in vitro antimalarial activity(4).*A. marina*'s ability to survive under harsh environmental circumstances is due to the presence of many phytochemicals. This plant is a strong candidate for the treatment of a variety of illnesses due to the presence of numerous types of phytochemicals. For instance, leaves are used to cure rheumatism, ulcers, and abscesses. Additionally, the leaf extract is utilized to treat food poisoning and malarial fever(5).

Staphylococcus sp. are clump-forming Gram-positive cocci(6). *S.aureus* inhabits the axillae and nasal passages, but *S.epidermidis* is a frequent commensal of human skin. *S.aureus* produces a wide range of possible virulence factors, such as surface proteins that facilitate colonization of host tissues and Inhibitors of phagocytosis that harm host cells and result in illness symptoms. Coagulase-negative staphylococci often exhibit fewer virulence factors and are less pathogenic(7). *S.aureus* causes superficial skin lesions, localized abscesses, deep-seated infections like osteomyelitis and endocarditis, dangerous skin infections like furunculosis, and nosocomial infections of surgical wounds. *S.aureus* and *S.epidermidis* together induce infections connected to ingested medical equipment. In addition, *S. aureus* causes toxic shock syndrome by secreting superantigens into the bloodstream and food poisoning by the release of enterotoxins. Urinary tract infections are brought on by *S.saprophyticus*, particularly in females. Other species like *S.lugdunensis, S.haemolyticus, S.warneri, S.schleiferi,* and *S.intermedius* are infrequent pathogens, contributing to the captivating diversity of staphylococci's infectious performances(8).The emergence of antibiotic-resistant strains, such as methicillin-resistant Staphylococcus aureus (MRSA), has further complicated the treatment of staphylococcal infections, highlighting the urgent need for alternative therapeutic strategies(9).

The exponential rise in bacteria that are resistant to multiple drugs (MDR) including antibiotics, is a serious problem in today's healthcare environment. The failure of the treatments and the increasing mortality rate can be attributed in part to this rise in MDR.Therefore, it is important that antibacterial agents be created that can effectively combat infectious disease and antibiotic resistance.In recent years, there has been growing interest in exploring natural products as potential sources of antimicrobial agents. Mangrove plants have gained considerable attention due to their unique ability to thrive in challenging environments and their rich chemical diversity. The extract derived from mangrove plants has been reported to exhibit a broad spectrum of bioactivities, including Antimicrobial, Antioxidant, Anti-inflammatory, and Anticancer properties.The cytotoxic effect refers to the ability of a substance to cause cell death or damage. Several studies have investigated the cytotoxic properties of mangrove extracts against various cancer cell lines, demonstrating their potential as natural anticancer agents.

However, the evaluation of their antimicrobial activities, particularly against bacteria such as *Staphylococcus sp* remains relatively unexplored.The objective of this study is to investigate the cytotoxic effect of mangrove extract against *Staphylococcus sp* and the findings of this study will contribute to our understanding of the antimicrobial potential of mangrove extracts and their possible application as alternative or complementary therapies against *Staphylococcus sp* infections.This study aims to Evaluate the cytotoxic effect of *Avicennia marina e*xtract against Staphylococcus sp. focusing on its potential antimicrobial activity.

MATERIALS AND METHODS:

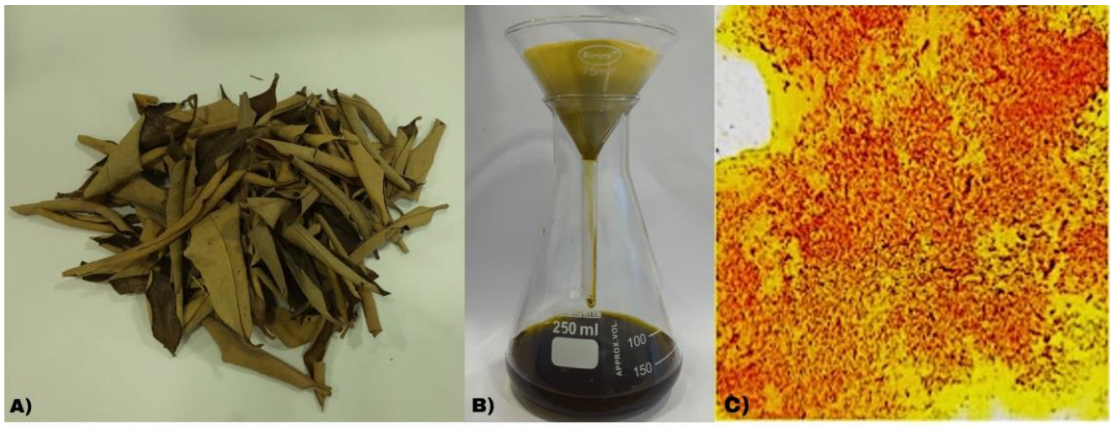


Figure 1 A)Avicennia marina leaves B) Crude extract C) biofilm Culture

The current study is conducted under the department of forensic odontology in Saveetha Dental College and Hospital.The duration of the study is 3 months.

Microorganisms and Culture: Clinical isolates of *Staphylococcus sp.* were obtained from Saveetha Medical College and stored in airtight containers with appropriate labeling, including isolate names, storage dates, culture medium names, and preservation solution details. All microbes were identified following the guidelines of Bergey's Manual of Determinative Bacteriology (1923).

Chemicals: Acridine orange and propidium iodide dyes were used for the biofilm study, providing valuable insights into the biofilm formation and inhibition processes.

Preparation of *Avicennia marina* Extract: Leaves of *Avicennia marina* were collected, thoroughly washed, and air-dried. The dried leaves were then finely ground to a powder form. To obtain the extract, the powdered leaves were subjected to methanol extraction. Briefly, 50 grams of the dried powder was soaked in 500 mL of methanol for 72 hours at room temperature, with occasional shaking. After the extraction period, the methanol extract was filtered through filter paper, and the solvent was evaporated using a rotary evaporator under reduced pressure. The resulting *Avicennia marina* methanol extract was then stored in airtight containers for further use.

Staphylococcus sp. Culture: *Staphylococcus sp.* was cultured on Baird Parker Agar for general growth purposes. To study biofilm formation, the bacteria were cultivated on Baird Parker broth base using coverslip methods, allowing the assessment of potential biofilm inhibition.

Cell Viability Analysis: The cell viability of *Staphylococcus sp.* was analyzed using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay, as per the method described by Peter and Toshima (2023). In brief, log-phase cultures of *Staphylococcus sp.* were prepared in nutrient-rich broth. The bacterial cells were then exposed to varying concentrations of the *Avicennia marina* methanol extract for specified time intervals. After the exposure period, the MTT reagent was added to each sample, and the absorbance was measured spectrophotometrically at a specific wavelength. The reduction in cell viability was determined by comparing the absorbance values of treated and untreated (control) samples.

Biofilm Study: The formation of biofilms was studied using the methodology outlined by Rawaf (2023). In brief, the clinical isolates of *Staphylococcus sp.* were inoculated in microtiter plates, and 1 ml/mg of *Avicennia marina* was introduced. The plates were then incubated at room temperature for 24 hours, and the resulting biofilm was quantified using acridine orange and propidium iodide staining. The coverslip method was also utilized for observing biofilm inhibition, where the pathogens were cultivated in conical flasks with coverslips and incubated for 24 hours. After incubation, the coverslips were stained with acridine orange and propidium iodide for further assessment.Confocal laser scanning microscopy was employed to visualize the intricate three-dimensional structure of the biofilm. This allowed us to assess the effects of the *Avicennia marina* methanol extract on biofilm formation and inhibition, providing valuable insights into the antimicrobial potential of the extract against *Staphylococcus sp.*

RESULTS:

The extract of *Avicennia marina* demonstrated remarkable inhibitory effects on the growth of *Staphylococcus* species. When the bacteria were treated with 1 ml/mg of the extract every 24 hours for a total duration of 96 hours, the extract consistently exhibited the ability to suppress the growth of *Staphylococcus*, indicating its potential as a potent natural antimicrobial agent.

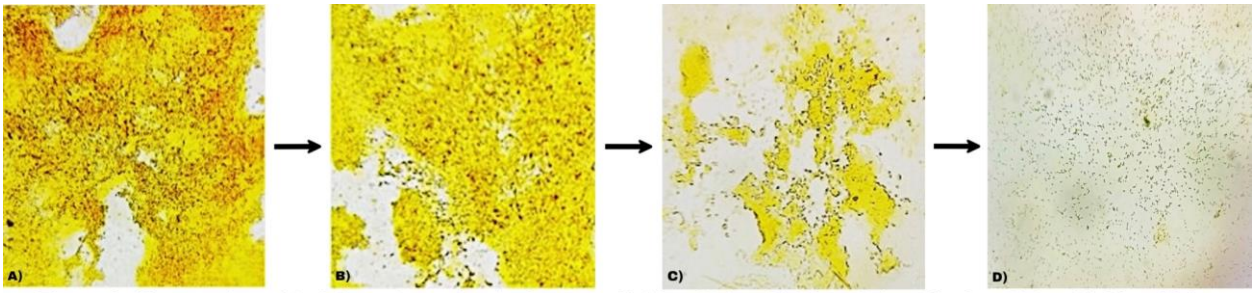
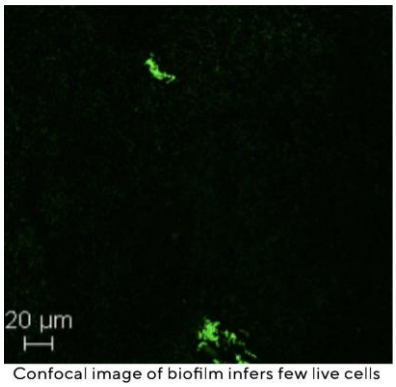


Figure 2-After treating with Avicennia marina leaf extract A) After 24 hrs B) After 42 hrs C) after 72 hrs D) After 96 hrs

During the initial staining with acridine orange, the biofilm exhibited a prominent orange color, indicating the presence of active bacterial growth. However, after treating the biofilm with 1 ml/mg of the *Avicennia marina* extract every 24 hours for a total of 96 hours, the orange color visibly reduced, suggesting a significant inhibition of *Staphylococcus* sp. This observation further supports the potent antimicrobial activity of the *Avicennia marina* extract, which effectively targeted the biofilm-forming *Staphylococcus* bacteria.

The confocal imaging results corroborated the findings from the staining with acridine orange. In the confocal images, the green color represented bacterial growth within the biofilm. In our study, the green color intensity was notably reduced, indicating a substantial inhibition of bacterial growth in the presence of the *Avicennia marina* extract. These confocal imaging results further emphasize the antimicrobial potential of the extract and its ability to disrupt the formation and growth of *Staphylococcus* biofilms.



Confocal image of biofilm infers few live cells

The results of this research shed light on the potential applications of *Avicennia marina* extract as an alternative treatment strategy to combat *Staphylococcus* infections, especially in cases of drug resistance. Utilizing natural sources such as *Avicennia marina* for antimicrobial development presents an environmentally friendly and sustainable approach towards future therapeutics.These findings encourage further investigations into the specific bioactive compounds within the *Avicennia marina* extract responsible for its antimicrobial effects. Understanding the mechanisms of action can provide valuable insights for potential drug development and optimization.

DISCUSSION:

A significant issue in medical research is the growing bacterial and fungal resistance to many antimicrobial synthetic medicines. The plant's biologically active antibacterial components have been proven through ethnopharmacological studies. The results of the current investigation demonstrated that *A. marina* leaf extracts had antibacterial properties.These results are supported by a previous research which concluded that The root extract acted most effective against the bacterial strains *P. aeruginosa, B. subtilis, S. aureus,* and *E. coli,* as well as the fungal strains *Aspergillus fumigatus* and *Candida albicans* and they also concluded that *S. aureus* and *E. coli* were significantly resistant to leaf extract of *Avicennia marina* in ethyl acetate. Fruit and seed ethanolic extracts have also been proven to have antifungal properties. As a result, this plant can be utilized to treat a variety of illnesses brought on by microbes resistant to antibiotics. There are numerous ways that this mangrove plant may be utilized to treat diseases brought on by bacteria that are resistant to antibiotics(5).

In a previous study on Antibacterial Activity of Chosen Mangrove Plants Against Bacterial Specific Pathogens, various plant extracts were tested for their antibacterial properties. Among them, the leaf extract of *A. marina* exhibited the highest antibacterial activity against *P. aeruginosa*, followed by *B. cylindrica* and *C. decandra*. Additionally, *A. marina* leaf extract showed substantial inhibition against eye pathogens, including *S. epidermidis*, *Acinetobacter*, and *E. coli*. The Minimum Inhibitory Concentration (MIC) values for *A. marina* extract were found to be lower against *P. aeruginosa*, *Acinetobacter sp*, and *E. coli* compared to *Klebsiella pneumoniae* and *Staphylococcus epidermidis*. These findings not only reaffirm the antimicrobial potential of *A. marina*, but also align with and support the results of our own research, where we observed promising antimicrobial activity of the *Avicennia marina* extract against *Staphylococcus* species, including biofilm inhibition. The collective evidence underscores the significance of *A. marina* as a valuable natural resource in combating increasing resistance of bacteria to synthetic antimicrobial drugs(10).

In this study, we compared the antimicrobial potential of Avicennia marina extract against Staphylococcus species and its effect on biofilm inhibition. Our findings demonstrated promising antimicrobial activity, with the Avicennia marina extract consistently suppressing the growth of Staphylococcus, particularly in biofilm form. These observations align with a previous study focusing on the medical potentialities of A. marina, where culturable fungal endophytes associated with A. marina leaves exhibited activity against microorganisms(11).

In comparison to the previous study evaluating antimicrobial, antioxidant, and cytotoxic activities of *A. marina*, *P. australis*, and *M. oleifera* ethanolic plant extracts, our study focused specifically on the antimicrobial potential of *Avicennia marina* extract against *Staphylococcus* species and its effect on biofilm inhibition. Similar to the previous study, our findings also indicate promising antimicrobial activity of the *Avicennia marina* extract. The significant inhibitory effects observed on *Staphylococcus* biofilm formation are in line with the cytotoxic activities demonstrated by *M. oleifera* extract against HepG2 cell lines in their study, where phenolic ingredients were attributed to play a major role in the cytotoxic effects. The correlation between antioxidant activities and phenolic contents, as reported in the previous study, may also contribute to the biofilm inhibition properties of the *Avicennia marina* extract(11).

In agreement with the findings of our study, a prior investigation into the antifungal and antibacterial potentials of Avicennia marina extracts came to the conclusion that A. marina has noteworthy antimicrobial characteristics. Our research also showed that Avicennia marina extract has strong antibacterial properties against Staphylococcus species, particularly when it forms biofilms. Together, the two investigations support the antibacterial potential of A. marina as a source of bioactive chemicals in nature. The earlier study highlighted A. marina's potential as a fresh supply of antibacterial agents with potential uses in the pharmaceutical and cosmetic industries. Together with those of the prior study, our findings demonstrate the potential of A. marina as a practical and long-lasting natural remedy for treating a variety of diseases, particularly those brought on by bacteria resistant to antibiotics(12).

A study on Antibacterial activity of some medicinal mangroves against antibiotic-resistant pathogenic bacteria, it was found that all plant extracts displayed greater inhibition against *S. aureus* compared to *Proteus sp*. Notably, the plant extracts from *L. racemosa* and *A. marina* exhibited the highest antibacterial activity against both bacterial strains among all the tested mangrove plants. These findings highlight the potential of *L. racemosa* and *A. marina* extracts as effective natural agents in combating antibiotic-resistant bacterial infections, particularly those caused by *S. aureus* and *Proteus sp(2)*.

Avicennia marina plant extracts exhibit strong antimicrobial activity against a certain set of microorganisms, and the extent of this activity is dependent on both the plant component and the extraction solvent. As a result, this plant may be utilized to treat a variety of illnesses brought on by germs resistant to antibiotics(5).A variety of mangrove plant extracts of methanol, ethanol, and water showed antibacterial efficacy against pathogenic isolates as well as antibiotic-resistant bacteria, according to preliminary research and studies by others.Most

plant extracts reduced the growth of S. aureus, and several plant extracts hindered the growth of Proteus species. As a result, the plant extracts from these specific mangrove plant species can be used as a source to create medications that would help treat infections brought on by these bacteria(2).As a future direction, quantification and purification of the *Avicennia marina* extract should be pursued to determine the complete chemical characterization and active ingredients, which could further elucidate the underlying mechanisms of its potent antimicrobial and biofilm inhibition effects. This chemical characterization could potentially pave the way for the development of novel therapeutics based on natural antimicrobial agents derived from *Avicennia marina* to combat *Staphylococcus* infections, including drug-resistant strains(11).

CONCLUSION:

In conclusion, the extract of Avicennia marina demonstrated inhibitory effects on the growth of Staphylococcus species.After treating the bacteria with 1 ml/mg of the extract every 24 hours for a total duration of 96 hours, the extract consistently showed the ability to suppress the growth of Staphylococcus, suggesting its potential as a natural antimicrobial agent. These findings highlight the promising antimicrobial properties of Avicennia marina extract, which may have significant implications for the development of novel therapeutics to combat Staphylococcus infection.

CONFLICT OF INTEREST:

The author reported the conflict of interest while performing this study to be nil.

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ETHICAL CLEARANCE:

Since it is in vitro study ethical clearance is not needed.

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