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Antibacterial Effects of Leaf Extracts of Four Mangrove Plant Species on *Staphylococcus aureus* and *Streptococcus pneumoniae*

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Antibiotic resistance is a significant global health concern, with bacteria developing resistance to commonly used antibiotics. Evaluating anti bacterial properties of natural extracts may offer alternative treatments to combat resistant bacterial strains. In this current investigation, the antibacterial activity of leaves of *Avicennia marina*, *Avicennia officinalis*, *Bruguiera cylindrica* and *Rhizophora apiculata* was evaluated against human pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. Ethanol extracts of mature leaves of these mangrove plants were prepared and tested for antibacterial activity using agar disc diffusion method. *Streptococcus*

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aureus was inhibited by leaf extracts of *A.marina*, *A.officinalis* and *B.cylindrica*. In this study, the effectiveness of charcoal treated and charcoal untreated leaf extracts were also compared. Charcoal treated leaf extracts of *Avicennia marina*, *Avicennia officinalis*, *Bruguiera cylindrica* and *Rhizophora apiculata* were able to inhibit the growth of both tested pathogenic bacteria more than that of untreated leaf extracts. The leaf extract of *Avicennia marina* exhibited the highest antibacterial activity against *S.pneumoniae* and *S.aureus* compared to other extracts. The results suggested that these extracts offer enormous promise for producing novel antibiotics and could be used as an alternative source for treatment of infections caused by these tested pathogenic bacteria.

Keywords: Anti-bacterial; Streptococcus; Staphylococcus; Avicennia marina; Avicennia officinalis; Bruguiera cylindrica; Rhizophora apiculata.

1. INTRODUCTION

Mangroves and mangrove associated plants contain certain biologically active antimicrobial compounds, including steroids, triterpenes, saponins, flavonoids, alkaloids carbohydrate, reducing sugar, combined reducing sugar, glycosides, proteins, terpenoids and tannins [1,2,3]. Many of these compounds have pharmacological and therapeutic effects, as they exhibit antioxidant, antibacterial, antifungal, anti-diabetic and anti-cancer activities [4].

Antibiotics are often used for the treatment of myriad of diseases. Along with the benefits of treating the disease, it also causes few harmful effects in humans. Diversity of gut microbes are their colonization is affected by the application of antibiotics [5]. Moreover, irrational use of antibiotics can do more harm than benefits [6].

The plant derived natural medicines, which are being used for centuries for the treatment of several human health issues, are relatively safer than the synthetic or artificial alternatives. The potential integration of mangrove plant extracts into pharmaceuticals or topical treatments could address the increasing challenges posed by resistant bacterial strains. As bacterial strains are becoming resistant to antibiotics rapidly, screening of antibacterial property of medicinal plants is very important.

Staphylococcus aureus, a Gram-positive spherical bacterium [7], commonly causes skin and soft tissue infections like impetigo, folliculitis, abscesses and necrotizing pneumonia [8], yet it is also a leading cause of severe conditions such as bacteremia and infective endocarditis [9]. *Streptococcus pneumoniae* is a Gram-positive bacterium. The commonly known diseases caused by *S. pneumoniae* are pneumonia, otitis media, meningitis, septicemia and bacterimia [10,11]. The respiratory tract infections caused by *S. pneumoniae*, remain a formidable problem [12].

The study opens avenues for future research, including the isolation, purification and identification of specific bioactive compounds responsible for antimicrobial activity. The present study made an attempt to determine the antibacterial activity of leaf extracts from *Avicennia marina*, *Avicennia officinalis*, *Rhizophora apiculata* and *Bruguiera cylindrica* against two human pathogenic bacteria – *Staphylococcus aureus* and *Streptococcus pneumoniae.*

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at Ayiramthengu mangrove forest (lat. 9° 02’- 9° 16’ N and long. 76° 20’ - 76° 32’ E), located in the Kollam district. The mangrove ecosystem is a part of Kayamkulam estuary (lat. 9° 07’ - 9° 16’ N and long. 76° 20’ - 76° 28’ E), a narrow stretch of tropical backwater on the west coast of Peninsular India.

Collection of plant samples: Leaves of *Avicennia marina, Avicennia officinalis, Rhizophora apiculata* and *Bruguiera cylindrica* was collected from Ayiramthengu mangrove forest. The mangrove species were identified using Identification Manual of ‘Mangroves in India’ by Botanical Survey of India and ‘Mangroves of Kerala’ [13]. The samples were washed thrice with sterile distilled water to remove epiphytes and other foreign particles and mopped using blotting sheets. The leaves were dried under shade for 20 days.

Preparation of leaf extracts: The leaves were chopped into small pieces and powdered using a pestle and mortar. A small amount of these powders (2.5 g) were soaked in 10mL of ethanol (1:4 ratio). The containers were sealed and stored for a period of 3 days at room temperature. The mixture was stirred at 24 hour interval. Each sample was then filtered through Whatman No. 1 filter paper and transferred into four beakers, which was then heated in a water bath at 40°C for 2 minutes for solvent evaporation. The extract was then centrifuged at 10,000 rpm for 2 minutes. The supernatant was maintained in eppendorf tubes at 4°C for later use [14].

Preparation of charcoal treated leaf extract: The leaf extracts of the four mangrove species, soaked in ethanol were filtered and transferred to four separate beakers. A small amount of charcoal (2-5 mg) was added. The mixture was then heated in a water bath at 40°C for 2 minutes and then centrifuged at 10,000 rpm for 2 minutes. The supernatent was then stored in eppendorf tubes at 4°C for later use [15].

Transfer of bacterial colonies to agar medium: Bacterial pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae* were obtained from Tropical Institute of Ecological Sciences (TIES), Kottayam. Bacterial colonies were transferred to nutrient agar medium through swab culture. The bacterial suspension of chosen bacterial pathogens was cultured over the surface of nutrient agar at 37± 2˚ C in an incubator, free from contamination.

Antibacterial activity of leaf extracts: 25µL ethanolic extract was impregnated on to each Whatmann filter paper No.1 disc (6 mm. diameter). Discs impregnated with the leaf extracts were placed on the solid agar medium by pressing slightly and incubated at 37±2°C for 18 - 24 h. After that, the zone of inhibition was measured and expressed in millimeter. The diameter of inhibition zone was considered as a measure of antibacterial activity.

3. RESULTS

In the present study, leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica and Rhizophora apiculata* were used to test antibacterial activity against human pathogenic species of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Eight different leaf extracts were prepared (untreated ethanol extract of *A. marina*, *A. officinalis*, *B. cylindrica* and *R. apiculata*, charcoal treated extract of *A. marina*, *A. officinalis*, *B. cylindrica* and *R. apiculata*) and their effects on each bacterium is mentioned in Table 1 and Table 2.

According to Table 1, the ethanol leaf extracts of *Avicennia marina* strongly inhibited the growth of the tested pathogenic bacteria more effectively than any other leaf extracts. The maximum zone of inhibition for *Streptococcus pneumoniae* was recorded against *A.marina* (8.83 mm) and *B. cylindrica* (8.83 mm) followed by *A. officinalis* (8.3 mm). The leaf extract of *R.apiculata* was unable to inhibit the growth of *S. pneumoniae*. The ethanolic leaf extracts of *A. marina* was more effective against *Staphylococcus aureus* (12 mm), followed by *A.officinalis* (8.67 mm). The ethanolic extract of *B. cylindrica* and *R. apiculata* were unable to inhibit the growth of *S.aureus*. Ethanol was taken as control. Controls did not exhibit any inhibitory effect on tested bacteria. These results agree with previous results [16,17].

Charcoal treated plant extracts strongly inhibited the growth of pathogenic bacteria compared to untreated extracts. Table 2 shows the result of charcoal treated leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata.* Charcoal treated ethanol extract of *A.marina* exhibited highest zone of inhibition compared to any other leaf extract. Specifically, the extract of *Avicennia marina* (15.33 mm) showed more inhibition than *A. officinalis* (13.67 mm), *B.cylindrica* (11.67 mm) and *R. apiculata* (11.33 mm) against *Streptococcus pneumoniae*. In the case of *S. aureus*, charcoal treated extract of *A. marina* had an inhibition zone of 16.33mm, followed by *A. officinalis* (15 mm), *B.cylindrica* (14.67 mm) and *R. apiculata* (9.67 mm).

The result of antibacterial activity of charcoal untreated and treated mangrove plant leaves against *S. aureus* and *S. pneumoniae* is illustrated in Fig. 3 and Fig. 4 respectively. These results suggest that charcoal treatment enhanced the antibacterial activity of the leaf extracts against *S. pneumoniae*. *Avicennia marina* exhibited an increase in the mean zone of inhibition from 8.83 mm (charcoal untreated) to 15.33 mm (charcoal treated). *Avicennia officinalis* showed an increase in the mean zone of inhibition from 8.3 mm to 13.67 mm with charcoal treatment. In case of *Bruguiera cylindrica*, the mean zone of inhibition increased from 8.83 mm to 11.67 mm, and for *Rhizophora* *apiculata*, mean zone of inhibition increased from 0 mm to 11.33 mm. Inhibition of *S. aureus* also showed significant increases with the charcoal-treated extracts. *Avicennia marina* exhibited an increase from 12 mm to 16.33 mm, while *A. officinalis* showed an increase from 8.67 mm to 15 mm. *B.cylindrica* displayed an increase in the zone of inhibition from 0 mm to 14.67 mm, and *R.apiculata* exhibited growth inhibition increase from 0 mm to 9.67 mm. The current study leads to the result that, mangrove plant species of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata* can be considered to produce new medicines for certain bacterial infections.

Table 1. The mean zone of inhibition of ethanolic mangrove leaf extracts on Streptococcus pneumoniae and Staphylococcus aureus. 0 indicates no zone of inhibition. The diameter of inhibition zone was considered as a measure of antibacterial activity

Bacteria	Mean zone of inhibition in diameter (mm)				
	Control	*Avicennia marina*	*Avicennia officinalis*	*Bruguiera cylindrica*	*Rhizophora apiculata*
Staphylococcus aureus	0	12	8.67	0	0
Streptococcus pneumonia	0	8.83	8.3	8.83	0

Table 2. Mean zone of inhibition of charcoal treated ethanolic mangrove leaf extracts on Staphylococcus aureus and Streptococcus pneumoniae. 0 indicates no zone of inhibition. The diameter of inhibition zone was considered as a measure of antibacterial activity

Bacteria	Mean zone of inhibition in diameter (mm)				
	Control	*Avicennia marina*	*Avicennia officinalis*	*Bruguiera cylindrica*	*Rhizophora apiculata*
Staphylococcus aureus	0	16.33	15	14.67	9.67
Streptococcus pneumonia	0	15.33	13.67	11.67	11.33

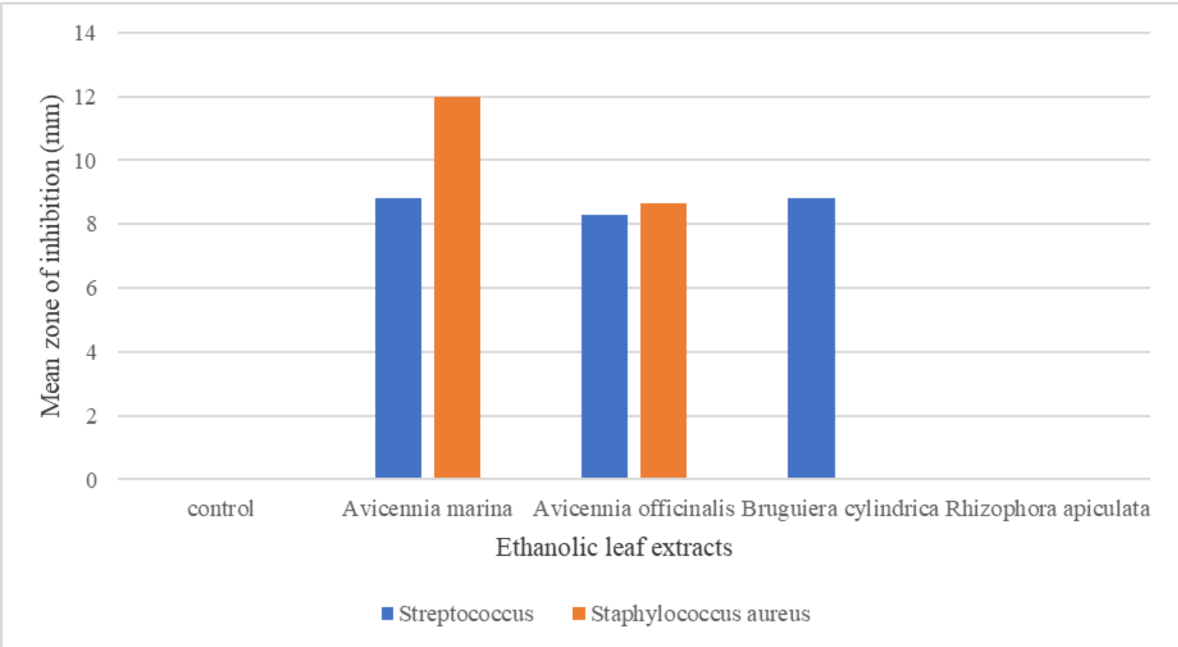

Fig. 1. Mean zone of inhibition of *Streptococcus pneumoniae* and *Staphylococcus aureus* by ethanolic leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata*

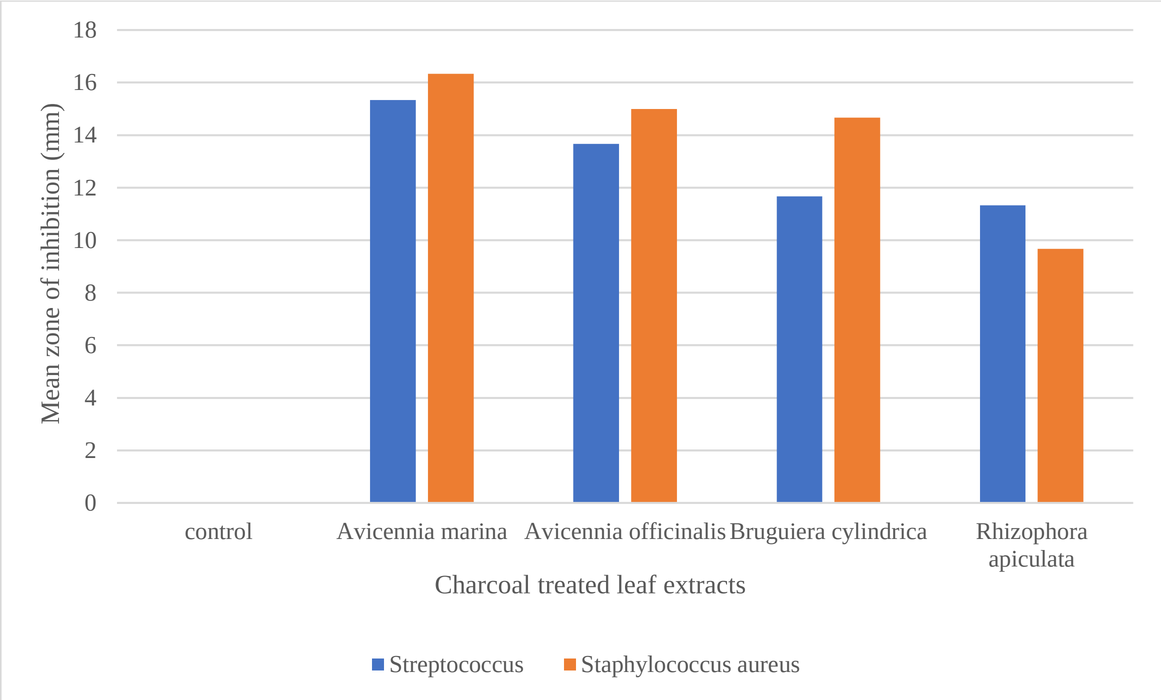

Fig. 2. Mean zone of inhibition of *Streptococcus pneumoniae* and *Staphylococcus aureus* by charcoal treated ethanolic leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata*

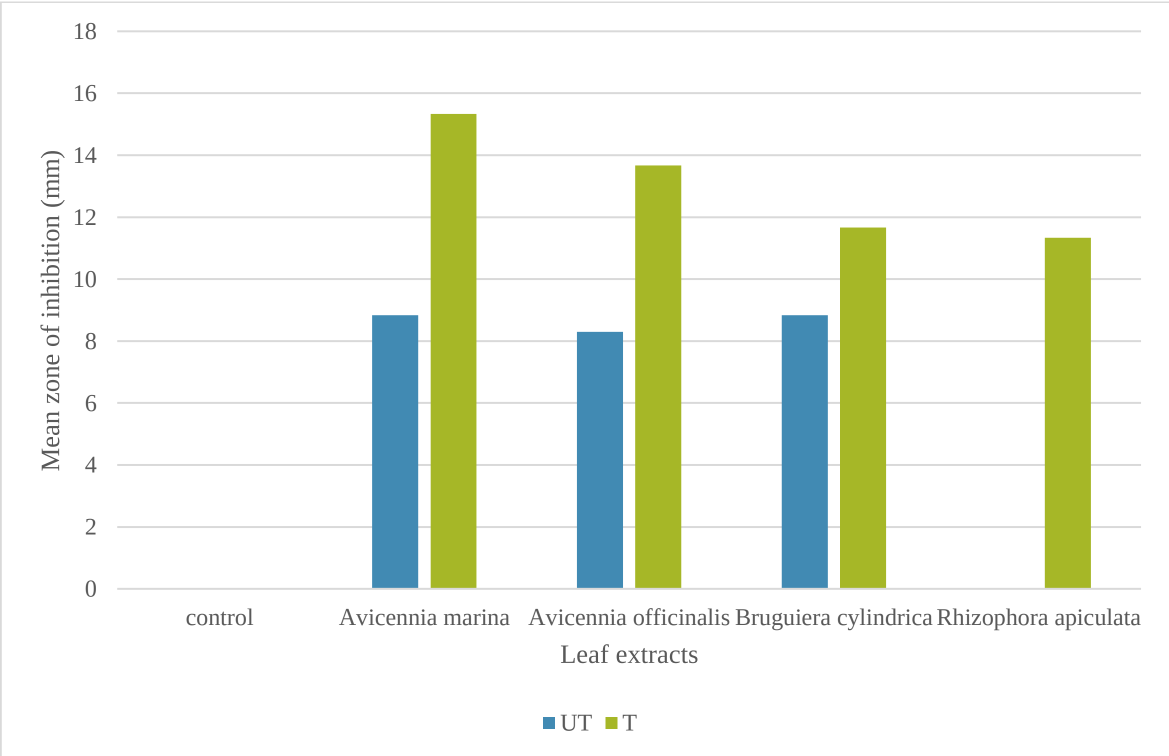

Fig. 3. Comparison of mean zone of inhibition of *Streptococcus pneumoniae* using charcoal treated and untreated leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata*. UT- Charcoal untreated, T-Charcoal treated

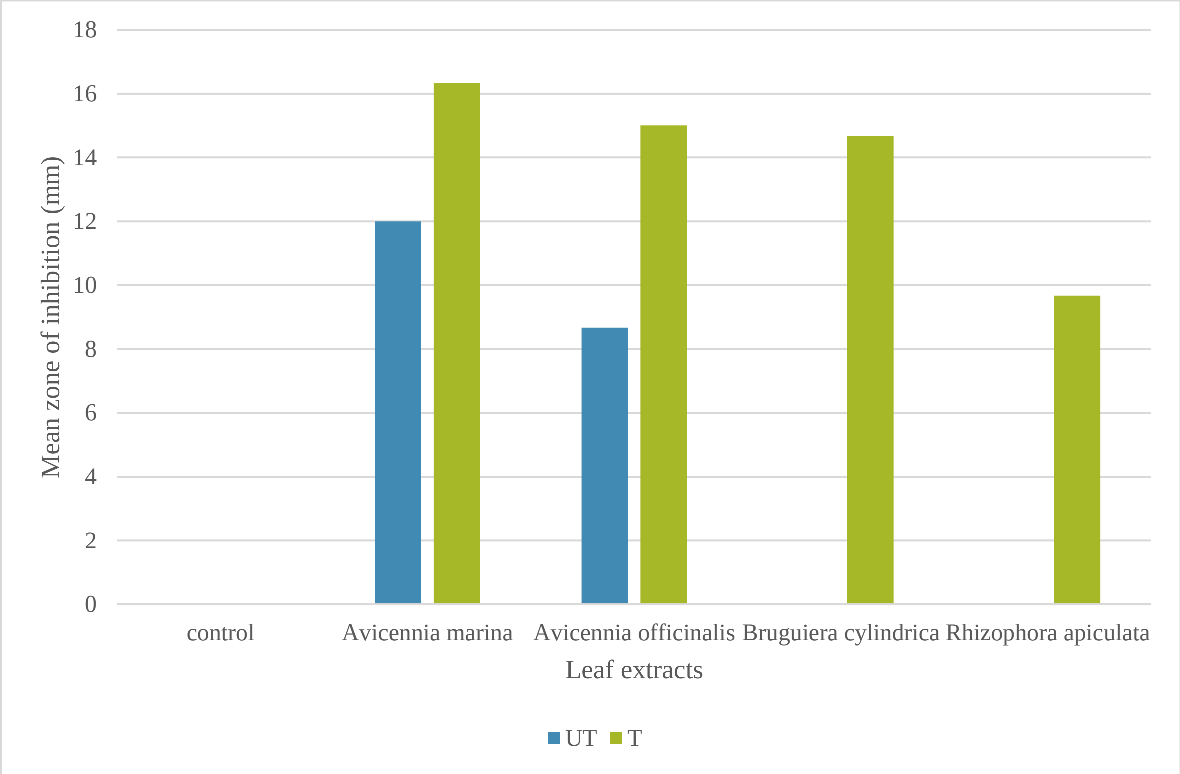

Fig. 4. Comparison of mean zone of inhibition of *Staphylococcus aureus* using charcoal treated and untreated leaf extracts of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata*. UT- Charcoal untreated, T-Charcoal treated

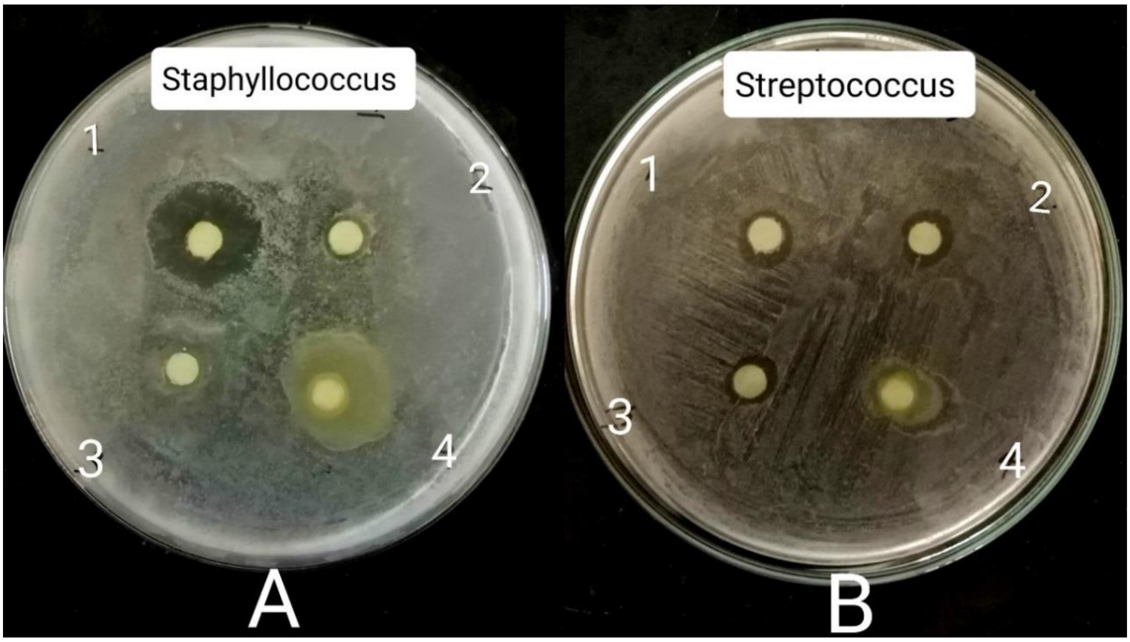

Fig. 5. The inhibition zone of untreated ethanolic extracts of Avicennia marina (1), Avicennia officinalis (2), Bruguiera cylindrica (3) and Rhizophora apiculata (4) against *Staphylococcus aureus* (A) and *Streptococcus pneumoniae* (B) at concentration of 25µL. (Zone of inhibition was measured by determining the diameter of the inhibition zone)

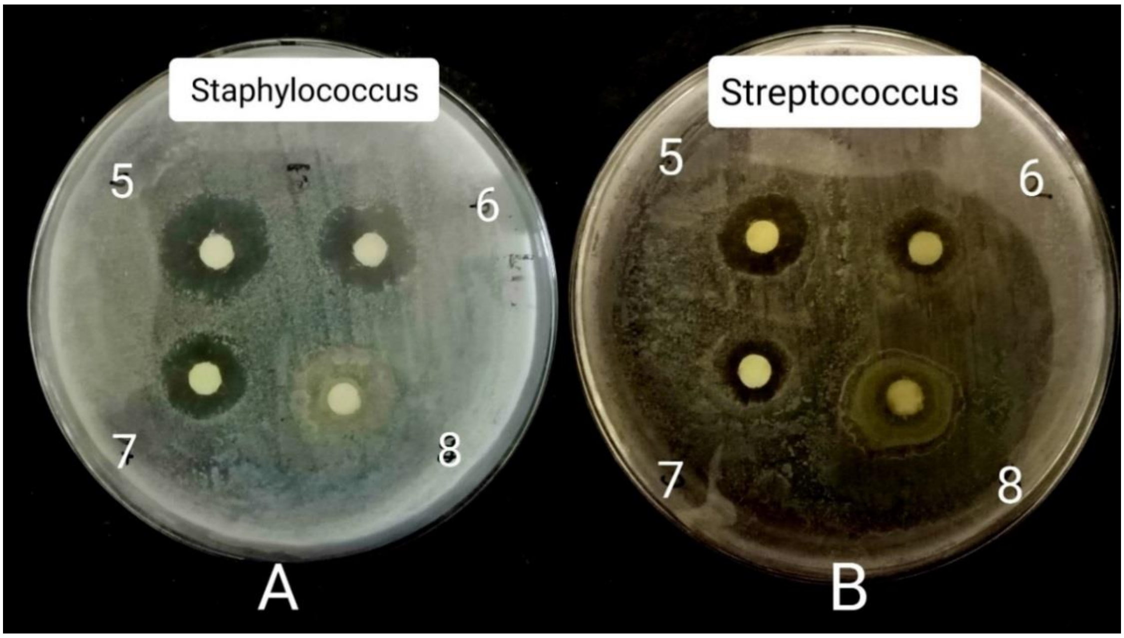

Fig. 6. The inhibition zone of charcoal treated ethanolic extracts of Avicennia marina (5), Avicennia officinalis (6), Bruguiera cylindrica (7) and Rhizophora apiculata (8) against *Staphylococcus aureus* (A) and *Streptococcus pneumoniae* (B) at concentration of 25µL. (Zone of inhibition was measured by determining the diameter of the inhibition zone)

4. DISCUSSION

Antibiotics are the conventional mode of medication in the treatment of bacterial diseases. As bacterial strains are becoming resistant to antibiotics rapidly, screening of antibacterial property of medicinal plants is very important. Mangroves are a great source of medicinal ingredients. Different parts of mangrove plants, in different solvents, exhibit the presence of some important chemical compounds.

According to phytochemical screening, mature leaves of *A. marina* contain alkaloids, steroids, triterpenoids, phenols, saponins, tannins, glycosides terpenoids and flavonoids [18,17]. *A. officinalis* is being used as an aphrodisiac, diuretic, hepatitis and leprosy. The leaf and bark extracts of *A. officinalis* are good sources of antioxidant, antimicrobial agents [19]. The bark, flower, fruit and leaf of *R.apiculata* is traditionally used for diarrhoea and is also used for treatment of nausea, vomiting, typhoid, hepatitis and as an antiseptic and mosquito larvicidal too [20]. The phytochemical screening of *Rhizophora apiculata* shows the presence triterpenes, phenols, saponins, steroids and the fruit, leaf and root extract of *B. cylindrica* is traditionally used to treat diarrhea, fever, and many ailments. *Bruguiera cylindrica* contains tannins, flavonoids, fatty acids, fatty acid amides, carboxylic acids, alkaloids and saponins [21].

Polarity of solvents used plays a key role in the outcome of bioactive compounds extracted. Ethanol has a higher degree of inhibition and effectiveness when compared to methanol, glycerol and aqueous plant extracts. It has the ability to bind with hydrophilic compounds such as flavonoid and phenolic compound. Ethanol could not influence the growth of bacteria as no zone of inhibition was observed in controls [16].

In this study, plant extracts of mature leaves of *A. marina*, *A. officinalis*, *R. apiculata* and *B. cylindrica* were used to test the antibacterial activity against human pathogenic species of *Streptococcus pneumoniae* and *Staphylococcus aureus*. Different plant extracts exhibited different degree of zone of inhibition. The present investigation revealed similar inhibition of ethanol extract of *Avicennia marina, Avicennia officinalis, Bruguiera cylindrica* and *Rhizophora apiculata* leaves on most of the tested bacteria when compared with the available literature [15,17].

Leaf extracts of *Avicennia marina* exhibited more pronounced inhibition against all bacterial species than other leaf extracts of *Avicennia officinalis, Rhizophora apiculata* and *Bruguiera cylindrica*. The growth of *S.aureus* and *S. pneumoniae* was strongly inhibited by the leaf extract of A.marina. The results correlated with previous studies [20,21]. Results of charcoal treated leaf extracts against the all bacterial strains were promising. The charcoal treated plant leaf extracts showed more zone of inhibition against all tested bacterial strains than untreated plant extracts. *Avicennia marina* showed highest zone of inhibition against the pathogenic bacteria when compared to other charcoal treated plant extracts. Overall, the examined mangrove leaves offer enormous promise for producing novel antibiotics that are effective against pathogenic bacteria [22,23].

The treatment of plant extracts with charcoal exhibited higher zone of inhibition than untreated plant extracts. In most studies, charcoal treated plant extracts were able to inhibit almost all bacterial strains more than those of untreated plant extracts [17]. Charcoal in itself has the property of being absorbent, neutralizing toxins, poisons, and noxious gases. The plant derived natural medicines, which are being used for centuries for the treatment of several human health issues, are relatively safer than the synthetic or artificial alternatives.

5. CONCLUSION

The evaluation of antibacterial property of mangrove leaf extract against *Streptococcus pneumoniae* and *Staphylococcus aureus* has revealed that the extracts exhibit significant inhibitory effects on both bacterial strains, suggesting its potential as an alternative antibacterial agent. These findings highlight the value of mangrove leaves as a natural resource for developing new antimicrobial treatments. Further research is recommended to isolate and characterize the active compounds responsible for the antibacterial activity and to evaluate their effectiveness. The antibacterial properties of mangrove leaf extract offer a promising avenue for the development of new antimicrobial therapies, particularly in the fight against antibiotic-resistant bacteria. This study contributes valuable insights into the potential applications of natural plant extracts in modern medicine, highlighting the importance of continuing research in this field.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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