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Isolation of endophytic bacteria and evaluation of fermented avicennia marina fruit filtrate as natural antimicrobials against staphylococcus aureus

Galih Dwiki Ramanda[1*], Anggraini Putri Utami[1], Yuyun Nisaul Khairillah[1], Nur Jati Jagad[1], M. Fiqri Baihaqi[1], Annisa Nasyawa Fahira[1]

[1]Institut Teknologi dan Kesehatan Muhammadiyah Kalimantan Barat, Jl. Sungai Raya Dalam, Kabupaten Kubu Raya, Kalimantan Barat, Indonesia

Corresponding Author E-mail: galih@itekesmukalbar.ac.id*

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ABSTRACT

Antimicrobial resistance (AMR) has become a major global health challenge, necessitating the exploration of alternative antimicrobial sources from natural products. This study aimed to determine the optimum fermentation time, characterize the colony morphology and Gram-staining characteristics of endophytic bacterial isolates, and evaluate their antimicrobial activity against Staphylococcus aureus. This study employed a quantitative laboratory experimental approach. Endophytic bacteria were isolated from Avicennia marina fruit through serial dilution and characterized based on colony morphology and Gram-staining characteristics. Antimicrobial activity was evaluated using the disk diffusion method. Five endophytic bacterial isolates (AM41–AM45) were successfully obtained, consisting of three Gram-positive and two Gram-negative isolates. Among them, isolate AM41 exhibited the highest inhibitory activity against S. aureus (19.71 mm), followed by AM45 (8.83 mm) and AM42 (8.00 mm), whereas the remaining isolates showed no inhibitory activity. Differences in antimicrobial activity were observed among the fermentation times tested. The largest inhibition zone was recorded after 72 h of fermentation (21.67 mm), followed by 96 h (20.07 mm), while shorter fermentation periods produced lower inhibitory activity. These findings indicate that endophytic bacterial isolates and fermented filtrates of A. marina fruit possess antimicrobial potential against S. aureus. Under the conditions tested, the highest antimicrobial activity of the fermented filtrate was observed after 72 h of fermentation.

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1. INTRODUCTION

Bacterial infections resistant to conventional antimicrobials represent one of the major challenges in global health today. Antimicrobial resistance (AMR) has evolved into a worldwide health crisis, with more than 1.27 million deaths annually directly attributed to resistant bacterial infections, along with projections of a significant increase in the coming decades (Marino et al., 2025; WHO, 2022). This rise in resistance is largely driven by the irrational use of antibiotics, such as inappropriate dosing and premature discontinuation of therapy, which promotes microbial adaptation and resistance development (Ventola, 2015). In addition, factors such as easy access to antibiotics, lack of regulatory control, and inappropriate therapeutic selection further accelerate the spread of bacterial resistance (Rather et al., 2017).

One of the priority pathogens in antimicrobial resistance is Staphylococcus aureus, a major cause of both community-acquired and nosocomial infections. This bacterium exhibits a high capacity for adaptation and increasing resistance to various antibiotics, including the emergence of Methicillin-resistant Staphylococcus aureus (MRSA), which has become a global concern (Tong et al., 2015). Globally, S. aureus is reported as one of the leading causes of bloodstream infections, with continuously increasing levels of antibiotic resistance (Lee et al., 2018). Furthermore, MRSA has been classified as a high-priority pathogen by the World Health Organization due to its significant disease burden and mortality rate (Tacconelli et al., 2018). These conditions highlight the urgent need to develop new and effective antimicrobial sources to combat increasingly difficult-to-treat infections.

One promising approach is the exploration of natural products, particularly from marine and coastal ecosystems with high biodiversity. Marine natural products have been reported as a potential source for the development of novel antimicrobial compounds, especially against resistant bacteria such as Staphylococcus aureus (Carroll et al., 2019; Ramadhanty & Lunggani, 2021; Tangapo et al., 2022).

Fermentation is one method that can enhance the biological activity of natural materials through the production of secondary metabolites by microorganisms. This process can increase the concentration of active compounds such as organic acids, polyphenols, and antimicrobial peptides, which play a role in inhibiting pathogenic bacterial growth (Marco et al., 2017; Swain et al., 2014). However, the effectiveness of antimicrobial activity is highly influenced by process parameters, such as filtrate concentration and fermentation time, which must be optimized to achieve maximum efficacy. Mangrove ecosystems represent a unique environment rich in bioactive compound-producing organisms, including Avicennia marina. This plant is known to contain various bioactive secondary metabolites, including flavonoids, phenolic compounds, terpenoids, alkaloids, tannins, and other phytochemicals with potential antimicrobial activity (Annas et al., 2023; Rozirwan et al., 2023; Kumari et al., 2021). Flavonoids and phenolic compounds can inhibit bacterial growth through membrane disruption, interference with nucleic acid synthesis, and inhibition of essential enzymes, whereas terpenoids and alkaloids may alter cell membrane permeability and disrupt cellular metabolism. In addition, endophytic microorganisms associated with A. marina may produce antimicrobial peptides and other bioactive metabolites that contribute to the antimicrobial properties of the host plant. These phytochemicals and microbial metabolites provide a strong chemical basis for the development of natural antimicrobial agents (Cerri et al., 2022).

Several studies have reported the antimicrobial potential of Avicennia marina and its associated endophytic microorganisms. Ramadhanty and Lunggani (2021) demonstrated the antibacterial activity of Avicennia marina extracts; however, the study did not investigate the influence of fermentation on antimicrobial efficacy. Likewise, Tangapo et al. (2022) evaluated the antimicrobial properties of mangrove-associated microorganisms but did not optimize fermentation parameters that may affect secondary metabolite production. Previous studies have primarily focused on plant extracts or microbial isolates, while information regarding the optimization of fermentation time and its relationship with antimicrobial activity remains limited. Moreover, studies integrating the characterization of endophytic bacterial isolates with antimicrobial activity evaluation against Staphylococcus aureus are still scarce. Therefore, this study addresses these gaps by combining the characterization of endophytic bacteria isolated from A. marina fruit with the evaluation of antimicrobial activity and optimization of fermentation time against Staphylococcus aureus.

Table 1. Comparison of Previous Studies and the Novelty of the Present Study

Study	Research Object	Research Focus	Limitation
Ramadhanty et al. (2021)	Endophytic bacteria from *Avicennia marina*	Isolation of endophytic bacteria and evaluation of antimicrobial activity against *Staphylococcus aureus* and *Salmonella typhi*	Did not investigate fermentation processes or optimize fermentation time.
Tangapo et al. (2022)	Endophytic fungi from *Avicennia marina*	Isolation of endophytic fungi and optimization of fermentation time for antibacterial activity	Focused on fungal endophytes and did not characterize bacterial endophytes from *A. marina* fruit.
Trivedi & Thumar (2023)	Endophytic fungi from *Avicennia marina* roots	Characterization and antibacterial activity of endophytic fungi	Did not evaluate fermentation-time optimization and focused exclusively on fungal endophytes.
Present study	Endophytic bacteria and fermented *Avicennia marina* fruit filtrates	Morphological and Gram characterization of bacterial endophytes, antimicrobial evaluation, and optimization of fermentation time against *Staphylococcus aureus*	Provides integrated information on bacterial endophytes isolated from *A. marina* fruit and the optimum fermentation time for antimicrobial activity.

Therefore, this study addresses these gaps by combining the characterization of endophytic bacteria isolated from A. marina fruit with the evaluation of antimicrobial activity and optimization of fermentation time against S. aureus. The novelty of this study lies in the integration of endophytic bacterial characterization and fermentation-time optimization of A. marina fruit filtrates to enhance antimicrobial activity against S. aureus, which has received limited attention in previous studies.

Although numerous studies have explored the potential of plants as antimicrobial sources, research on the optimization of filtrate concentration and fermentation time of Avicennia marina fruit, particularly from the mangrove ecosystem of Desa Pasir, Mempawah Regency, West Kalimantan, remains limited. Furthermore, studies integrating the identification of endophytic bacterial morphology with antimicrobial activity testing against Staphylococcus aureus are still scarce. Therefore, this study is important as an effort to explore local natural resources with the potential to be developed as sustainable natural antimicrobial alternatives.

Based on the role of fermentation in enhancing microbial secondary metabolite production, it is hypothesized that different fermentation times significantly affect the antimicrobial activity of Avicennia marina fruit filtrates against Staphylococcus aureus. Longer fermentation periods are expected to increase the production of bioactive metabolites, resulting in greater antimicrobial activity up to an optimum fermentation time.

This study aims to determine the optimal filtrate concentration and fermentation time to produce antimicrobial activity, to identify the morphology of endophytic bacteria resulting from the fermentation of Avicennia marina fruit, and to evaluate antimicrobial activity against Staphylococcus aureus using disk diffusion and well diffusion methods. The results of this study are expected to contribute scientifically to the development of new antimicrobial sources that are more effective, environmentally friendly, and relevant in addressing the global issue of antimicrobial resistance.

2. METHODS

A. Research Design

This study employed a quantitative laboratory experimental approach using a Completely Randomized Design (CRD). The treatment factor consisted of five fermentation times of Avicennia marina fruit, namely 36, 48, 60, 72, and 96 hours. The response variable was the antimicrobial activity against Staphylococcus aureus, measured as the diameter of the inhibition zone (mm). Each treatment was tested using three replicate discs, and the mean inhibition zone diameter was used for statistical analysis.

B. Time and Location on Study

Mature fruits of Avicennia marina were collected from the mangrove ecosystem of Mempawah Mangrove Park, Pasir Village, Mempawah Hilir District, Mempawah Regency, West Kalimantan, Indonesia. The fruits were washed thoroughly with running water to remove adhering debris and contaminants. The samples were then divided into two portions: one portion was used for the isolation of endophytic bacteria, while the other portion was used for fermentation, antimicrobial activity testing, phytochemical screening, and FTIR characterization.

C. Research Procedures

a. Sample Collection and Preparation

Mature fruits of Avicennia marina were collected from the mangrove ecosystem of Mempawah Mangrove Park, Pasir Village, Mempawah Hilir District, Mempawah Regency, West Kalimantan, Indonesia. The fruits were washed thoroughly with running water to remove adhering debris. The collected samples were then divided into two portions: one portion was used for the isolation of endophytic bacteria, while the other portion was used for fermentation and antimicrobial activity testing of the fermented filtrate.

b. Isolation and Characterization of Endophytic Bacteria

The surface of Avicennia marina fruit samples was sterilized sequentially using 70% ethanol for 10 minutes, 2.5% sodium hypochlorite (NaOCl) for 10 minutes, followed by 70% ethanol for 10 minutes, and rinsed three times with sterile distilled water. The samples were then aseptically crushed using a mortar inside a Laminar Air Flow (LAF) cabinet. One gram of the sample was homogenized in 9 mL of sterile 0.85% NaCl solution, followed by serial dilution up to 10^{-5} (Utami et al., 2025). Suspensions from the 10^{-4} and 10^{-5} dilutions were inoculated onto Nutrient Agar (NA) using the spread plate method and incubated at 28–30°C for 2–7 days. Colonies showing different morphological characteristics were purified using the streak plate method. Characterization was conducted based on colony morphology, including shape, color, margin, elevation, and texture. Gram staining was performed to determine cell morphology and Gram characteristics.

c. *Antimicrobial Activity Test of Endophytic Bacterial Isolates*

Purified endophytic bacterial isolates were inoculated into Nutrient Broth (NB) and incubated at 28°C for 24 hours with shaking at 120 rpm. The cultures were centrifuged to obtain cell-free supernatants containing bioactive compounds. A suspension of Staphylococcus aureus was spread evenly on Nutrient Agar (NA) plates. Sterile paper discs (6 mm diameter) impregnated with 20 µL of bacterial supernatant were placed on the agar surface. Chloramphenicol (100 ppm) and sterile distilled water were used as positive and negative controls, respectively. The plates were incubated at 37°C for 24 hours, and inhibition zones were measured in millimeters (mm).

d. *Fermentation of Avicennia marina Fruit and Antimicrobial Activity Test of Fermented Filtrate*

A total of 500 g of mature Avicennia marina fruits were peeled and homogenized. The homogenized material was wrapped in sterilized banana leaves that had been previously cleaned with 70% ethanol and allowed to dry. The samples were then placed in covered plastic containers provided with small ventilation holes and subjected to spontaneous fermentation without the addition of any starter culture. Fermentation relied on indigenous microorganisms naturally associated with the fruit material. The process was conducted at ambient room temperature (approximately 27–30°C) for 36, 48, 60, 72, and 96 hours. At the end of each fermentation period, the filtrate was collected for antimicrobial activity testing.

e. *Antimicrobial Activity Test of Fermented Filtrate*

The filtrate obtained from the fermentation of Avicennia marina fruit was centrifuged at 1000 rpm for 15 minutes to separate the supernatant. A volume of 20 µL of the supernatant was applied onto sterile paper discs. A suspension of Staphylococcus aureus (1 mL) was inoculated into Nutrient Agar (NA), poured into petri dishes, and allowed to solidify. Paper discs containing the filtrate, positive control (chloramphenicol 100 ppm), and negative control (sterile distilled water) were placed on the agar surface. The plates were incubated at 37°C for 24 hours. After incubation, the diameter of the inhibition zones formed around the discs was measured using a ruler or caliper. The size of the inhibition zone was used as an indicator of the antimicrobial activity of the fermented filtrate at each fermentation time variation.

D. Statistical Analysis

The inhibition zone data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) to determine the effect of fermentation time on antimicrobial activity against Staphylococcus aureus. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were conducted using Microsoft Excel and SPSS software.

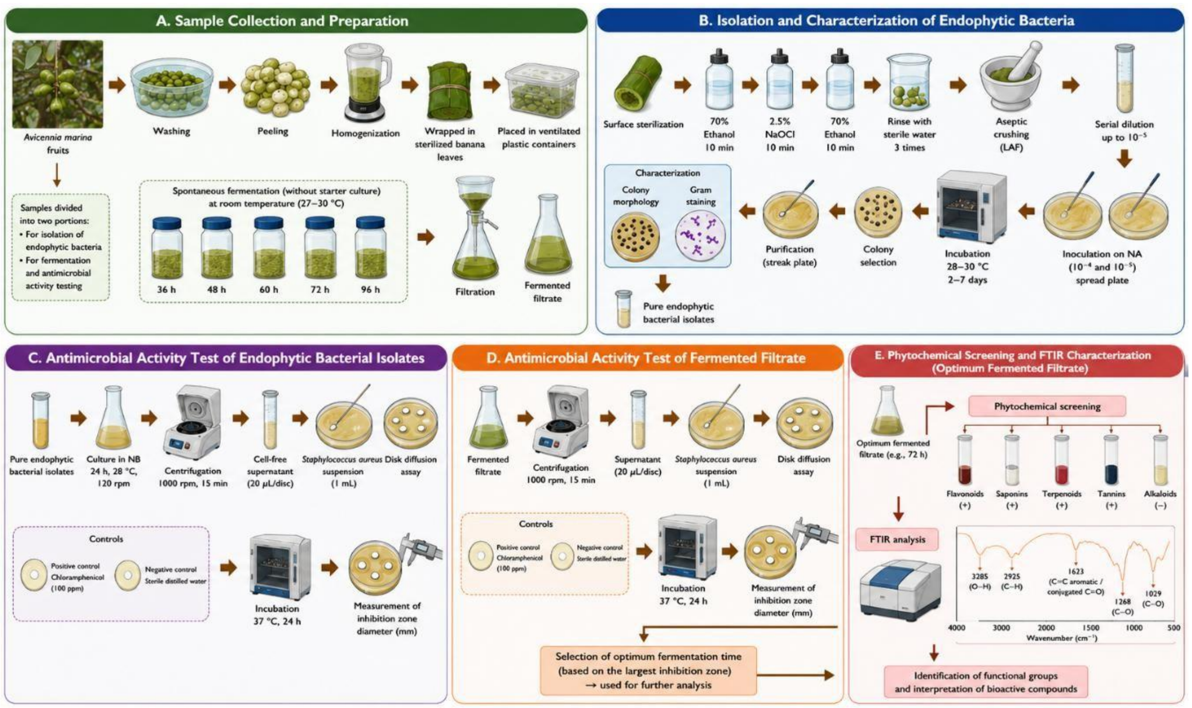

Figure 1. Scematic Research

3. RESULTS AND DISCUSSIONS

The isolation results indicate that Avicennia marina fruit serves as a rich source of endophytic bacteria with diverse morphological characteristics. The successful recovery of five isolates from the 10^{-4} dilution confirms that the microbial density within the fruit tissue remains sufficiently high to support the growth of representative colonies. This finding supports previous national studies reporting that mangrove-associated endophytic bacteria exhibit high diversity and adaptability due to the unique environmental pressures of coastal ecosystems, such as salinity and fluctuating nutrient availability (Handayani et al., 2023; Sukarno et al., 2022). Variations in colony morphology, including shape, margin, elevation, and pigmentation, further indicate phenotypic diversity that may reflect different metabolic capabilities and ecological roles within plant tissues.

A. Isolation and Characterization of Endophytic Bacterial Isolates

The isolation results of endophytic bacteria from Avicennia marina fruit showed that five bacterial isolates (AM41, AM42, AM43, AM44, and AM45) were successfully obtained and purified through serial dilution up to 10^{-5}. These isolates were recovered from the 10^{-4} dilution, which empirically provided an optimal number of colonies for purification compared to the 10^{-5} dilution, where bacterial growth was very limited. This indicates that the microbial density at the 10^{-4} dilution was within an ideal range to allow the isolation of representative single colonies.

Figure 2. Isolate Dilution 10^{-4}

Macroscopically, the isolates exhibited relatively similar colony characteristics with some specific variations. The colonies were generally irregular in shape, with serrated or uneven margins and convex elevation. The dominant colony color was yellow, suggesting the production of pigments as part of bacterial secondary metabolism. These morphological variations indicate phenotypic diversity among the isolates and suggest possible taxonomic diversity as well as adaptation to different microhabitats within plant tissues. However, definitive taxonomic identification cannot be established based solely on colony morphology and Gram-staining characteristics.

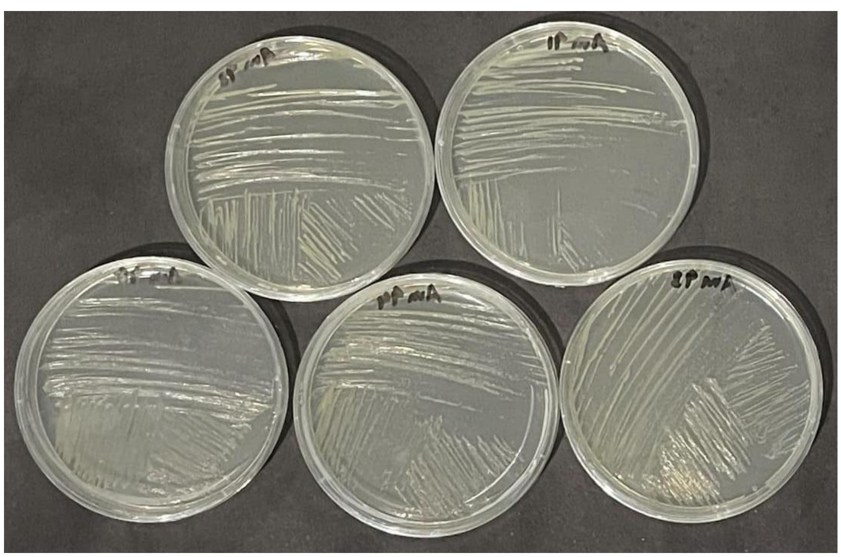

Figure 3. Endophytic Bacterial Isolates of Avicennia Marina Fruit

The diversity in colony morphology reflects differences in bacterial growth patterns on the culture medium, which are influenced by environmental factors such as nutrient availability and humidity. These isolates also exhibit variation in bacterial cell types based on microscopic observations. Some isolates display different cell shapes, including cocci (spherical) and bacilli (rod-shaped), with cell arrangements varying from single cells, pairs, chains, to clusters. Microscopic observation using Gram staining provides further information regarding the bacterial cell wall type, distinguishing between Gram-positive and Gram-negative bacteria, and offers a clearer understanding of cellular morphology. Microscopic observation using Gram staining revealed that three isolates (AM41, AM42, and AM45) were classified as Gram-positive bacteria, while two isolates (AM43 and AM44) were Gram-negative. This distinction reflects differences in bacterial cell wall structure, which may influence their adaptability and ability to produce bioactive metabolites. In addition, observed cell shapes included cocci and bacilli, with various arrangements such as single cells, pairs, chains, and clusters, which are detailed in Table 2.

Gram staining results revealed a predominance of Gram-positive bacteria over Gram-negative bacteria. This observation is consistent with studies on endophytic bacteria from coastal plants, which report that Gram-positive bacteria are more resilient to environmental stress due to their thicker peptidoglycan layer (Juma, 2025). Moreover, Gram-positive bacteria are often associated with higher potential in producing secondary metabolites, including antimicrobial compounds, which are crucial in microbial competition within the host environment.

The diversity in morphological characteristics suggests that Avicennia marina fruit serves as a suitable habitat for a wide range of endophytic bacteria with diverse biological potentials. This variability provides an important basis for selecting isolates with potential as antimicrobial-producing agents.

Table 2. Morphological and Gram-Staining Characteristics of Endophytic Bacterial Isolates of Avicennia marina Fruit

Isolate	Shape	Margin	Elevation	Color	Gram Reaction
AM41	Irregular, elongated	Serrated	Convex	Yellow	Positive
AM42	Irregular	Undulated	Convex	Yellow	Positive
AM43	Irregular	Serrated	Convex	Yellow	Negative
AM44	Irregular	Undulated	Convex	Yellow	Negative
AM45	Irregular, small	Smooth	Convex	Yellow	Positive

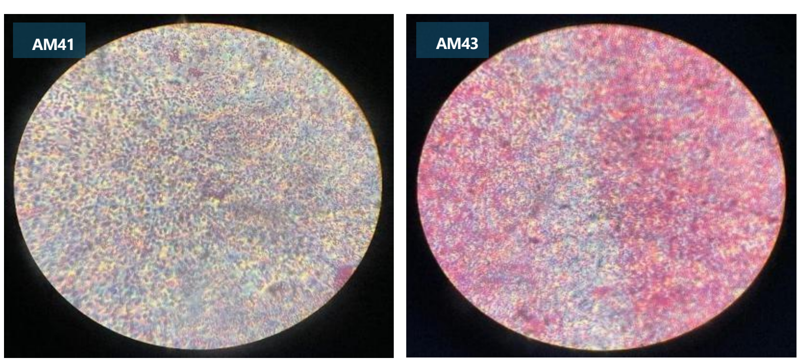

Figure 4. Micrographs of Isolates in Antimicrobial Activity Test

B. Antimicrobial Activity of Endophytic Bacteria

The antimicrobial activity of the bacteria was tested using the disk diffusion method. In this method, sterile disks impregnated with bacterial suspensions from the isolates were placed on agar media that had been inoculated with the test bacteria. If the antimicrobial compounds were effective in inhibiting microbial growth, a clear zone would form around the disks on the Petri dish. This clear zone indicates the area where the growth of microorganisms is inhibited or absent.

The results of antimicrobial activity testing against Staphylococcus aureus using the disk diffusion method demonstrated variations in inhibitory ability among the tested endophytic bacterial isolates. Of the five isolates obtained, three isolates (AM41, AM42, and AM45) exhibited antimicrobial activity, indicated by the formation of inhibition zones around the disks, while two isolates (AM43 and AM44) showed no inhibitory activity, which are detailed in Table 3.

Table 3. Antimicrobial Activity Test with pathogenic bacteria Staphylococcus aureus

No.	Isolate Code	Inhibition Zone Diameter (mm)
1	AM41	19.71
2	AM42	8.00
3	AM43	–
4	AM44	–
5	AM45	8.83

The antimicrobial assay results showed that only three out of five isolates exhibited inhibitory activity against Staphylococcus aureus, with isolate AM41 demonstrating the highest activity. This aligns with previous findings that antimicrobial activity among endophytic bacteria is highly selective, depending on the presence of specific biosynthetic gene clusters responsible for metabolite production (Amelia et al., 2021; Hidayati et al., 2017). The variability in inhibition zone diameter suggests differences in both the type and concentration of bioactive compounds produced. This result directly supports the issue highlighted in the introduction regarding the urgent need for new antimicrobial sources, as not all microorganisms possess equal capacity to combat pathogenic bacteria.

Isolate AM41 exhibited the strongest inhibitory activity against Staphylococcus aureus. The larger inhibition zone may indicate a greater ability to produce or release antimicrobial metabolites under the conditions tested. However, the specific compounds responsible for this activity were not determined in the present study. Meanwhile, isolates AM42 and AM45 showed moderate activity, possibly due to limited metabolite production or narrower antimicrobial spectra. The absence of activity in isolates AM43 and AM44 indicates that these isolates either do not express antimicrobial compounds under the tested conditions or produce compounds ineffective against Staphylococcus aureus. Similar patterns have been reported in studies of endophytic bacteria from tropical plants, where only a subset of isolates demonstrated antibacterial activity (Beiranvand et al., 2017).

Isolate AM41 exhibited the highest antimicrobial activity, with an inhibition zone diameter of 19.71 mm, which falls into the strong inhibition category. Meanwhile, isolates AM45 and AM42 produced inhibition zones of 8.83 mm and 8 mm, respectively, which are categorized as moderate to weak inhibition. These differences indicate variation in the ability of each isolate to produce antimicrobial compounds.

The absence of inhibition zones in isolates AM43 and AM44 suggests that these isolates may not produce antibacterial compounds under the test conditions, or they produce them in concentrations too low to exert a detectable inhibitory effect. This may also be influenced by physiological factors such as bacterial growth phase, culture conditions, and the expression of genes responsible for secondary metabolite production.

Overall, these findings indicate that not all endophytic bacteria possess significant antimicrobial activity; however, certain isolates demonstrate strong potential to be further developed as natural antibacterial agents.

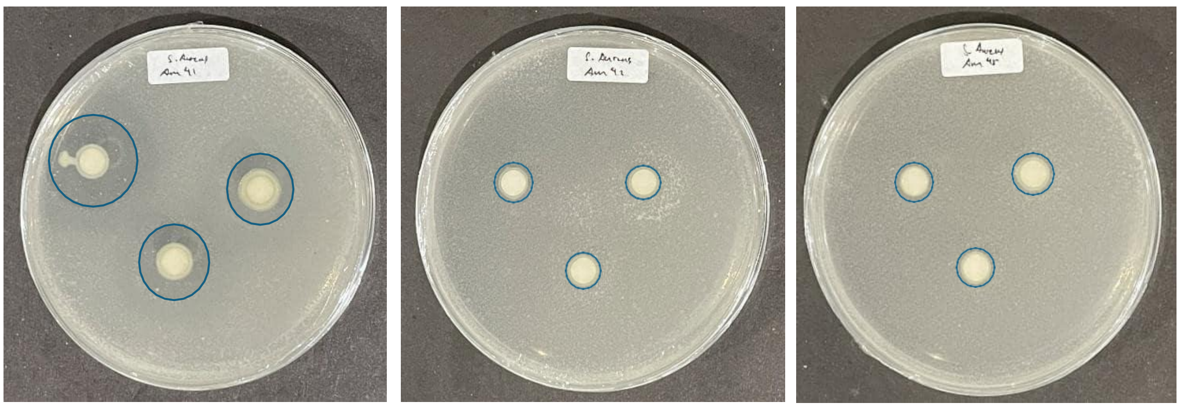

Figure 5. Clear zone isolates endophytic bacteria from Avicennia marina fruit

C. Antimicrobial Activity of Fermented Filtrate

The antimicrobial activity of fermented Avicennia marina fruit filtrates against Staphylococcus aureus varied according to fermentation time. Antimicrobial activity was evaluated based on the diameter of the inhibition zones formed around the discs on the test medium. At 36 h of fermentation, the mean inhibition zone was 4.67 ± 0.06 mm, indicating relatively weak antibacterial activity. The inhibition zone increased to 5.10 ± 0.10 mm at 48 h and further increased to 7.80 ± 0.00 mm at 60 h. The highest antimicrobial activity was observed at 72 h of fermentation, with a mean inhibition zone diameter of 21.67 ± 0.58 mm. At 96 h, a slight decrease was observed, with an inhibition zone of 20.07 ± 0.12 mm, although antibacterial activity remained relatively high. The complete results are presented in Table 4.

Table 4. Effect of Fermentation Time on Antimicrobial Activity Against Staphylococcus aureus

Fermentation Time (hours)	Inhibition Zone (mm)	Inhibition Zone (mm)	Inhibition Zone (mm)	Mean (mm)	Standard Deviation (mm)
36	4.7	4.6	4.7	4.67	0.058
48	5.1	5.0	5.2	5.1	0.1
60	7.8	7.8	7.8	7.8	0.0
72	22.0	21.0	22.0	21.67	0.58
96	20.2	20.0	20.0	20.07	0.12

One-way ANOVA demonstrated that fermentation time significantly affected the antimicrobial activity of fermented *A. marina* fruit filtrates against *S. aureus* ($p < 0.05$). The increase in inhibition zone diameter from 36 h to 72 h indicates that fermentation time plays an important role in determining the antibacterial effectiveness of the fermented filtrate. The highest activity observed at 72 h suggests that this fermentation period provided the most favorable conditions for generating antibacterial activity under the conditions tested.

In the fermentation filtrate assay, antimicrobial activity increased significantly with fermentation time, reaching its peak at 72 h. This observation may be associated with the formation, release, or transformation of bioactive compounds during fermentation. Previous studies have reported that fermentation can enhance the biological activity of plant-derived materials through microbial metabolic processes and biochemical transformations that increase the availability of bioactive constituents (Hyeon et al., 2023). The slight decline in antibacterial activity observed at 96 h may indicate changes in metabolite stability or composition during prolonged fermentation. Similar trends have been reported in fermented natural products, where antimicrobial activity reaches an optimum level before gradually decreasing due to the degradation or transformation of active compounds (Rusmalina et al., 2024).

To better understand the chemical constituents responsible for the observed antibacterial activity, phytochemical screening was performed on the fermented filtrate. The results revealed the presence of flavonoids, saponins, terpenoids, and tannins, whereas alkaloids were not detected Table 5.

Table 5. Phytochemical Screening Results of Fermented *Avicennia marina* Fruit Filtrate

Phytochemical Compound	Indicator of Positive Reaction	Observation	Result
Alkaloids	Formation of precipitate or turbidity after addition of Mayer's reagent	Clear pink solution observed, no precipitate formed	Negative (-)
Flavonoids	Development of red, yellow, or orange coloration	Red coloration observed	Positive (+)
Saponins	Formation of stable foam persisting for approximately 7 min	Stable foam formed	Positive (+)
Terpenoids	Formation of red or purple coloration	Red coloration observed	Positive (+)
Tannins	Formation of dark blue, greenish-black, or black coloration after $FeCl_3$ addition	Dark blue-black coloration observed	Positive (+)

The phytochemical profile obtained in this study is consistent with previous reports on A. marina. Annas et al. (2023) reported the presence of flavonoids, tannins, and terpenoids in A. marina extracts, while Rozirwan et al. (2023) identified phenolic and flavonoid compounds associated with antibacterial activity. Similar findings were reported by Cerri et al. (2022) and Kumari et al. (2021), who demonstrated that A. marina contains various secondary metabolites with antimicrobial and antioxidant properties. The agreement between the present findings and previous studies strengthens the evidence that A. marina is a rich source of bioactive compounds with potential antimicrobial applications.

The detected secondary metabolites may contribute to the antibacterial activity observed against S. aureus. Flavonoids are known to interfere with bacterial cell membrane permeability and nucleic acid synthesis, while tannins can precipitate proteins and inhibit bacterial enzymes. Saponins may increase membrane permeability by interacting with membrane sterols, whereas terpenoids can disrupt membrane integrity and induce leakage of intracellular components. The synergistic action of these compounds may explain the antibacterial activity observed in the fermented filtrate.

The phytochemical findings were further supported by Fourier Transform Infrared (FTIR) spectroscopy analysis. The FTIR spectrum revealed several characteristic absorption bands associated with bioactive secondary metabolites. A broad absorption band at 3285 cm^{-1} corresponded to O–H stretching vibrations, indicating the presence of hydroxyl groups commonly found in phenolic compounds and flavonoids. The absorption peak at 2925 cm^{-1} was attributed to aliphatic C–H stretching vibrations. A peak observed at 1623 cm^{-1} suggested aromatic C=C or conjugated carbonyl groups, while peaks at 1268 cm^{-1} and 1029 cm^{-1} were assigned to C–O stretching vibrations associated with phenolic compounds, flavonoids, and other oxygenated metabolites.

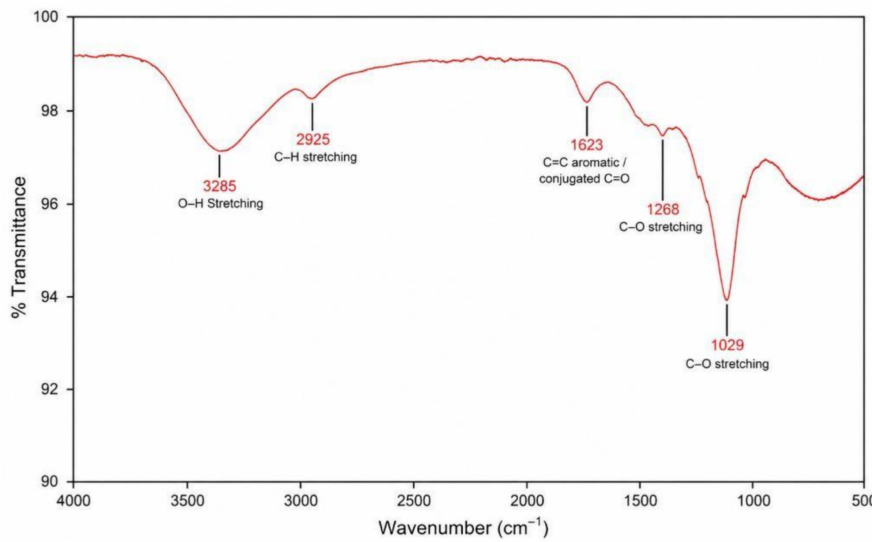

Figure 6. FTIR Spectrum of Avicennia marina

The FTIR results support the phytochemical screening findings by confirming the presence of hydroxyl, aromatic, and oxygen-containing functional groups commonly associated with antimicrobial compounds. The occurrence of O–H and C–O functional groups is particularly consistent with phenolic compounds and flavonoids detected during phytochemical screening. These functional groups have been reported to contribute to antibacterial activity through interactions with bacterial cell walls, membranes, proteins, and intracellular metabolic pathways.

Collectively, the antimicrobial assay, phytochemical screening, and FTIR characterization suggest that fermented A. marina fruit contains bioactive metabolites capable of inhibiting the growth of Staphylococcus aureus. The optimum fermentation time was determined to be 72 h, producing the highest antibacterial activity among the tested treatments. These findings support the potential utilization of fermented A. marina fruit as a natural antimicrobial source and reinforce the importance of mangrove ecosystems as reservoirs of biologically active compounds with potential applications in addressing antimicrobial resistance.

These findings provide important implications for the development of natural antimicrobial agents derived from mangrove ecosystems, which are known for their rich biodiversity and untapped bioactive potential. However, despite the promising results, several limitations should be acknowledged. The identification of bacterial isolates was limited to morphological characterization without molecular confirmation, and the specific bioactive compounds responsible for the antimicrobial activity were not chemically characterized. Additionally, the antimicrobial assays were conducted against a single test organism, and the study was performed exclusively under laboratory conditions, which may not fully represent real-world applications.

Therefore, further research is recommended to conduct molecular identification using 16S rRNA gene analysis to accurately determine bacterial taxonomy, as well as to perform phytochemical or chromatographic analyses to identify and characterize the active compounds. Future studies should also evaluate the antimicrobial efficacy against a broader range of pathogenic microorganisms and explore large-scale and field-based applications to better assess the practical potential of fermented Avicennia marina as a sustainable antimicrobial resource.

The results of this study strongly support the background discussed in the introduction regarding the global challenge of AMR and the urgent need to explore alternative antimicrobial sources. The antimicrobial activity of endophytic bacteria and fermented Avicennia marina fruit highlights the potential of mangrove ecosystems as reservoirs of novel bioactive compounds. This reinforces the relevance of natural-product-based approaches as sustainable and environmentally friendly alternatives to conventional antibiotics, particularly in addressing resistant pathogens such as Staphylococcus aureus.

4. CONCLUSIONS

This study successfully isolated five endophytic bacterial isolates (AM41–AM45) from Avicennia marina fruit, exhibiting diverse morphological characteristics and varying Gram properties. Among these, three isolates demonstrated antimicrobial activity against Staphylococcus aureus, with isolate AM41 showing the highest inhibitory effect. In addition, the fermentation filtrate of Avicennia marina fruit exhibited significant antimicrobial activity influenced by fermentation time, with optimal activity observed at 72 hours. These findings indicate that both endophytic bacteria and fermented filtrate of Avicennia marina have potential as natural antimicrobial sources. Therefore, this study provides a scientific basis for the development of environmentally friendly and sustainable antimicrobial agents derived from natural resources.

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