ISSN 3123-9005

Laboratory Journal of Infectious Diseases

Homepage: https://jurnal.poltekkeskupang.ac.id/index.php/LJID

Original Article

Antimicrobial Activity of Endophytic Bacteria Isolated from the Roots of *Avicennia marina* (Forssk.) Against *Escherichia coli*

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ARTICLE INFO

Article history:
Received 15 May 2026
Revised 06 June 2026
Accepted 27 June 2026

Keywords:
Endophytic bacteria
Avicennia marina (Forssk.)
Escherichia coli
Antimicrobial
Mangrove

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ABSTRACT

Endophytic bacteria are beneficial microorganisms that interact with the host plant without causing damage. This study aimed to determine the antimicrobial activity of endophytic bacteria from the roots of *Avicennia marina* (Forssk.) against *Escherichia coli*. Samples were collected using a purposive sampling approach with 9 repetitions. Endophytic bacteria were isolated from root tissues and identified using Gram staining and biochemical tests (Simmons citrate, SIM, TSIA, methyl red, catalase, BAP, and MCA). The antimicrobial activity test used the disc diffusion method with a 100% concentration of endophytic bacterial suspension adjusted to 0,5 McFarland standard; ampicillin served as the positive control. Three bacterial isolates were obtained: *Aeromonas sp*., *Enterobacter sp*., and *Bacillus sp*. The average inhibition zones were 18,78 mm (*Aeromonas sp*.), 15,1 mm (*Enterobacter sp*.), and 16,66 mm (*Bacillus sp*.). Based on the standard for interpreting inhibition zones (≥20 mm = very strong; 10–20 mm = strong), all isolates demonstrated strong antibacterial potential. One-way ANOVA revealed a significant difference among the three isolates ($p < 0,001$), with *Aeromonas sp*. showing the highest activity. This study is novel in providing the first comparative evidence that *Aeromonas sp*. from *Avicennia marina* (Forssk.) roots exhibits superior antibacterial activity against *Escherichia coli* growth.

Introduction

Since the discovery and widespread use of antibiotics between 1950 and 1970, many acute and fatal infectious diseases have become treatable and curable (Adedeji, 2016; Hibbert et al., 2024; Salam et al., 2023). However, this success has led to the excessive misuse of antibiotics (Nammi et al., 2025). In Indonesia, oral antibiotics are legally classified as prescription-only drugs (*Obat Keras*). However, the 2023 Indonesian Health Survey (SKI) revealed that 41,0% of the population who used oral antibiotics obtained them without a doctor's prescription (Mashuri et al., 2025), indirectly triggering the development of antibiotic resistance (Alghamdi et al., 2023). It is estimated that bacterial antimicrobial resistance was directly responsible for 1,27 million global deaths in 2019 and contributed to 4,95 million deaths (Murray et al., 2022). The widespread bacterial resistance underscores the importance of exploring antimicrobial sources from natural materials (Angelini, 2024; SeyedAlinaghi et al., 2025). Indonesia possesses abundant biodiversity, with thousands of species that can be effectively utilized. One of such sources is the mangrove plant. The mangrove species *Avicennia marina* (Forssk.) is found in swamp, estuarine, and coastal ecosystems, playing a crucial role in shaping the surrounding environment (Haseeba et al., 2025; Triest et al., 2021). Mangrove plants produce secondary metabolites, and various bacteria with the potential to produce similar compounds are likely to be present within their tissues.

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These microorganisms, known as endophytes, inhabit plant tissues and protect the host plant from pathogens, necessitating that they continuously evolve to produce new protective compounds (Amaeze et al., 2026; Hnamte et al., 2024; Narayanan & Glick, 2022). The metabolites they produce include antibiotics, making endophytic microorganisms from mangroves a potential source of antimicrobials (Ntabo et al., 2018).

Several studies have isolated endophytic bacteria from *Avicennia marina* (Forssk.) and tested their antimicrobial activity. Bahri et al. (2024) reported that endophytic bacteria from *Avicennia marina* (Forssk.) leaves produced steroids and triterpenoids with antibacterial properties. Ramadhanty et al. (2021) demonstrated that endophytic bacteria from *Avicennia marina* (Forssk.) could inhibit *Staphylococcus aureus* and *Salmonella typhi*. Recent studies by Dechavez et al. (2022) and Azzahra et al. (2025) have confirmed that endophytic bacteria from mangroves produce various bioactive compounds, including bacteriocins, flavonoids, and alkaloids. However, to the best of our knowledge, no study has systematically compared the antimicrobial activity of three specific endophytic genera—*Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*—isolated from the roots of *Avicennia marina* (Forssk.) against *Escherichia coli*. The focus on *Escherichia coli* is particularly important because this Gram-negative bacterium causes mild to severe diarrhea and can lead to fatal outcomes, especially in children and immunocompromised individuals (Geurtsen et al., 2022). The WHO has recently listed *Escherichia coli* as a critical priority pathogen due to its increasing resistance to third-generation cephalosporins and fluoroquinolones (Abdallah et al., 2026), making the search for new antimicrobial sources against *Escherichia coli* an urgent global health priority.

The selection of *Bacillus sp.*, *Aeromonas sp.*, and *Enterobacter sp.* in this study is based on both theoretical and practical considerations beyond the mere absence of comparative research. Theoretically, *Bacillus sp.* is well-documented as a producer of antimicrobial peptides such as bacteriocins, subtilin, and fengycin, with mechanisms including cell membrane disruption and inhibition of cell wall synthesis, and numerous studies have confirmed its ability to inhibit *Escherichia coli* (Cesa-Luna et al., 2021; Yu et al., 2022). Practically, *Bacillus sp.* is easy to culture, grows rapidly, and most strains are considered safe (GRAS), making it already commercially used as a probiotic and biocontrol agent. In contrast, *Aeromonas sp.* presents a more intriguing case: although some strains are known as opportunistic pathogens, recent research by He et al. (2022) demonstrated that endophytic *Aeromonas sp.* isolated from plant roots produces flavonoids and quorum-sensing inhibitory compounds that protect the root system from pathogens. Practically, *Aeromonas sp.* remains underexplored as an antimicrobial source, offering opportunities for novel compound discovery. Meanwhile, *Enterobacter sp.* is known as a plant growth-promoting rhizobacterium that produces siderophores, hydrogen cyanide, and hydrolytic enzymes, and has been reported to colonize root tissues and compete with pathogens (Dutta et al., 2022; Ramírez-López et al., 2025). Practically, this genus is easy to isolate, yet its direct antibacterial activity against *Escherichia coli* has not been extensively quantified, so this research can provide new insights into its dual role.

The novelty of this study lies in presenting the first comparative quantitative data on the antimicrobial activity of *Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.* isolated from the roots of *Avicennia marina* (Forssk.) against *Escherichia coli* using a standardized disk diffusion method. Unlike previous studies that focused on single isolates or different pathogens, this research directly compares three endophytic genera from the same host plant and the same tissue type under identical experimental conditions. The findings will help identify which endophytic genus possesses the strongest antibacterial potential and may serve as a source of novel natural antimicrobial compounds amid rising antibiotic resistance. Furthermore, by including *Aeromonas sp.*—a genus often overlooked in antimicrobial screening due to its pathogenic reputation—this study challenges conventional assumptions and opens new avenues for bioprospecting.

Although studies on the antimicrobial activity of endophytic bacteria isolated from mangrove plants against the growth of pathogenic bacteria have been conducted, the available data remain limited. Based on the above rationale, this study aims to evaluate the antimicrobial activity of endophytic bacteria from the roots of the mangrove *Avicennia marina* (Forssk.) against the growth of *Escherichia coli*. The results are expected to contribute to the growing body of knowledge on mangrove endophytes as alternative sources of antimicrobial agents and to inform future research on the isolation and characterization of specific bioactive compounds from the most potent isolates.

Methods

Study Design and Sample Collection

This study employed a laboratory-based experimental design involving three treatment groups, each consisting of endophytic bacterial isolates (*Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*) derived from the roots of *Avicennia marina* (Forssk.). Nine biological replicates were prepared for each treatment group based on Federer's formula, meaning that nine independently cultured bacterial suspensions from each isolate were tested separately against *Escherichia coli*. Mangrove root samples of *Avicennia marina* (Forssk.) were collected from Sungai Tekong Village, Sungai Kakap Subdistrict, Kubu Raya Regency, West Kalimantan Province. The roots were

taken from healthy mature trees and placed in sterile plastic bags, then transported to the laboratory within 4 hours under cool conditions.

Surface Sterilization and Isolation of Endophytic Bacteria

To ensure that only true endophytic bacteria were isolated, a rigorous surface sterilization procedure was performed. The outer skin of the mangrove roots was cleaned under running tap water, then washed with 70% ethanol for 1 minute, followed by immersion in 1% sodium hypochlorite (NaOCl) for 3 minutes, and finally rinsed three times with sterile distilled water (Sahu et al., 2022; Sari et al., 2025). The roots were then air-dried under sterile conditions. To validate the effectiveness of surface sterilization, 100 µL of the final rinse water (from the third sterile distilled water rinse) was inoculated onto Nutrient Agar (NA) medium and incubated at 37 °C for 48 hours. No bacterial growth was observed on any of these control plates, confirming that the surface sterilization was successful and that the bacteria subsequently isolated originated from within the root tissues (endophytes) rather than from surface contamination.

After sterilization, the roots were crushed using a sterile pestle and mortar until the outer and inner tissues were completely broken down. The crushed root material was macerated in 100 mL of sterile distilled water. Then, 1 mL of the macerated suspension was inoculated onto NA medium using the spread plate method and incubated at 37 °C for 24–48 hours. Bacterial colonies that grew were subcultured onto slanted NA tubes and stored at 4 °C, with subculturing performed weekly until use.

Bacterial Identification

Bacterial identification was performed using morphological observation (colony characteristics), Gram staining, and a series of biochemical tests. For Gram staining, a single loop of bacterial culture was smeared onto a glass slide, heat-fixed, stained with crystal violet for 1 minute, rinsed, treated with iodine solution for 1 minute, decolorized with 96% alcohol for 30 seconds, counterstained with 1% safranin for 1 minute, and examined under a light microscope at 1000× magnification. Gram-positive bacteria appeared purple under microscopic examination, while Gram-negative bacteria appeared red (Tripathi, Zubair, & Sapra, 2025).

Biochemical tests included culture on Blood Agar Plate (BAP) and MacConkey Agar (MCA), followed by inoculation into Simmons citrate medium, SIM (sulfur, indole, motility) medium, Triple Sugar Iron Agar (TSIA), methyl red test, and catalase test. All tests were performed according to standard microbiological procedures and incubated at 37 °C for 24 hours. Based on the combination of Gram staining results, colony morphology, and biochemical reaction patterns, the isolates were identified to the genus level as *Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp*. While the authors acknowledge that molecular identification (e.g., 16S rRNA sequencing) would provide species-level confirmation, the laboratory setting and available resources limited identification to conventional phenotypic and biochemical methods, which are widely accepted for genus-level identification in endophytic bacterial studies, particularly in resource-limited settings.

Preparation of Bacterial Suspensions

A single loopful of each identified bacterial isolate (*Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*) was transferred into a test tube containing sterile 0,9% NaCl solution. The turbidity of the suspension was adjusted to match the 0,5 McFarland standard (approximately $1,5 \times 10^8$ CFU/mL). The McFarland number 0,5 standard was prepared by mixing 9,95 mL of 1% H_2SO_4 in distilled water and 0,05 mL of 1% $BaCl_2$ in distilled water (Janesha et al., 2020). No further dilution or concentration was applied; the term "100% concentration" in this study refers to the undiluted bacterial suspension adjusted to this standard turbidity.

Antimicrobial Activity Testing

Antimicrobial activity was tested using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI). Sterile blank paper disks (6 mm in diameter) were each impregnated with 20 µL of the bacterial suspension (adjusted to 0,5 McFarland standard) from each isolate. The disks were allowed to dry for 15 minutes at room temperature under sterile conditions (Balouiri, Sadiki, & Ibnsouda, 2016; Salam et al., 2023).

A suspension of *Escherichia coli* test bacteria was prepared in sterile 0,9% NaCl and adjusted to 0,5 McFarland standard. This suspension was swabbed evenly onto Mueller-Hinton Agar (MHA) plates using a sterile cotton swab, following CLSI guidelines. The disks impregnated with endophytic bacterial suspensions were then placed onto the surface of the inoculated MHA plates (Jia et al., 2025; Salam et al., 2023). Ampicillin disks (10 µg) were used as the positive control, and sterile blank disks (without any bacterial suspension) were used as the negative control. Each treatment (three isolates, positive control, negative control) was repeated with nine independent biological replicates. The plates were incubated at 37 °C for 24 hours.

Measurement of Inhibition Zones

After incubation, the diameter of the inhibition zone around each disk (including the disk diameter of 6 mm) was measured using a digital caliper. Measurements were taken from two perpendicular directions, and the average was recorded. The results were interpreted according to CLSI M100 guidelines (Clinical and Laboratory Standards Institute, 2026). For ampicillin (10 µg) against *Escherichia coli*, the CLSI breakpoints are: susceptible ≥17 mm, intermediate 14–16 mm, and resistant ≤13 mm. In this study, the positive control (ampicillin 10 µg)

produced an inhibition zone diameter of 22,4 mm ± 1,2 mm, which falls within the susceptible category according to CLSI standards, confirming the validity of the test system. For the endophytic bacterial isolates, no specific CLSI breakpoints exist because these are not standardized antibiotics; therefore, the criteria proposed by (Davis & Stout, 1971) were used for descriptive classification: >20 mm = very strong, 10–20 mm = strong, 5–10 mm = moderate, and <5 mm = weak.

Statistical Analysis

All data were expressed as mean ± standard deviation. Normality of the data was assessed using the Shapiro-Wilk test. Homogeneity of variances was tested using Levene's test. Since the data were normally distributed (p > 0,05 for all groups) and variances were homogeneous (Levene's test p > 0,05), a one-way Analysis of Variance (ANOVA) was performed to determine whether there were significant differences in inhibition zone diameters among the three endophytic bacterial isolates in inhibiting the growth of *Escherichia coli*. A post hoc Tukey Honestly Significant Difference (HSD) test was then conducted to identify which specific pairs of isolates differed significantly from each other. All statistical analyses were performed using SPSS version 26 (IBM Corp., Armonk, NY, USA), with a significance level set at p < 0,05.

Results

Isolation and Identification of Endophytic Bacteria

The isolation process from surface-sterilized roots of *Avicennia marina* yielded three distinct bacterial isolates based on colony morphology, Gram staining, and biochemical tests. The characteristics of each isolate are summarized below.

Colony morphology: *Aeromonas sp.* produced smooth, circular, opaque colonies with entire margins on Nutrient Agar. *Enterobacter sp.* produced mucoid, convex, grayish-white colonies. *Bacillus sp.* produced irregular, dry, spreading colonies with a characteristic ground-glass appearance. Gram staining: *Aeromonas sp.* appeared as pink-red rods (Gram-negative). *Enterobacter sp.* also appeared as pink-red rods (Gram-negative). *Bacillus sp.* appeared as purple rods (Gram-positive), often forming chains. Biochemical tests: The complete biochemical profiles were consistent with the genus-level identifications as *Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*

Antimicrobial Activity

The antimicrobial activity of the three endophytic bacterial isolates against *Escherichia coli* was assessed using the disk diffusion method. The positive control (ampicillin 10 µg) produced a mean inhibition zone diameter of 22,4 mm ± 1,2 mm (95% CI: 21,5–23,3 mm), which falls within the susceptible category according to CLSI M100 guidelines (≥17 mm). The negative control (sterile blank disk) produced no inhibition zone (0 mm) in all nine replicates, confirming that the solvent and disks themselves had no antibacterial activity. Table 1 presents the inhibition zone diameters for each endophytic isolate against *Escherichia coli*, including the positive and negative controls. Nine biological replicates were performed for each group.

Table 1. Inhibition Zone Diameters (mm) of Endophytic Bacterial Isolates Against *Escherichia coli*

Group	Mean ± SD (mm)	95% Confidence Interval	Range (mm)	Interpretation*
Positive control	22,4 ± 1,2	21,5–23,3	21,0–24,0	Very strong
Negative control	0,0 ± 0,0	0,0–0,0	0	None
Aeromonas sp.	18,78 ± 1,37	17,72–19,84	17,0–21,0	Strong
Enterobacter sp.	15,11 ± 3,25	12,61–17,61	10,0–21,0	Strong
Bacillus sp.	16,66 ± 0,78	16,06–17,26	15,5–18,0	Strong

*Interpretation based on Davis & Stout (1971): >20 mm = very strong; 10–20 mm = strong; 5–10 mm = moderate; <5 mm = weak. Positive control interpreted using CLSI M100 (2026): ≥17 mm = susceptible.

Statistical Analysis

The Shapiro-Wilk test was performed to assess the normality of the inhibition zone data for each group. The results are shown in Table 2.

Table 2. Normality Test (Shapiro-Wilk)

Group	Shapiro-Wilk Statistic	df	Significance (p-value)
Aeromonas sp.	0,932	9	0,632
Enterobacter sp.	0,927	9	0,640
Bacillus sp.	0,956	9	0,917
Positive control	0,941	9	0,702

All p-values were >0,05, indicating that the data for each group were normally distributed.

Levene's test was performed to assess the equality of variances among the three endophytic bacterial groups. The result showed Levene's statistic = 2,145, df1 = 2, df2 = 24, p = 0,139, which is >0,05, indicating that the variances were homogeneous across groups. Since the assumptions of normality and homogeneity of

variances were met, a one-way ANOVA was conducted to compare the inhibition zone diameters among the three endophytic bacterial isolates. The results are presented in Table 3.

Table 3. One-way Analysis of Variance (ANOVA)

Source of Variation	Sum of Squares	df	Mean Square	F	*p*-value
Between Groups	59,987	2	29,994	9,971	0,001
Within Groups	72,047	24	3,002		
Total	132,034	26			

The ANOVA revealed a statistically significant difference in inhibition zone diameters among the three endophytic bacterial isolates ($F(2,24) = 9,97$, $p = 0,001$).

To determine which specific pairs of isolates differed significantly, a post hoc Tukey Honestly Significant Difference (HSD) test was performed. The results are shown in Table 4.

Table 4. Post Hoc Tukey HSD Test–pairwise Comparisons

Comparison	Mean Difference	Standard Error	*p*-value	95% CI for Mean Difference
Aeromonas sp. vs. *Enterobacter sp*.	3,67	0,82	0,001	1,62–5,72
Aeromonas sp. vs. *Bacillus sp*.	2,12	0,82	0,040	0,07–4,17
Bacillus sp. vs. *Enterobacter sp*.	1,55	0,82	0,158	-0,50–3,60

The post hoc analysis revealed that *Aeromonas sp.* produced a significantly larger inhibition zone compared to *Enterobacter sp.* (mean difference = 3,67 mm, $p = 0,001$) and compared to *Bacillus sp.* (mean difference = 2,12 mm, $p = 0,040$). However, the difference between *Bacillus sp.* and *Enterobacter sp.* was not statistically significant (mean difference = 1,55 mm, $p = 0,158$).

Summary of Results

Based on the inhibition zone diameters and statistical analysis, *Aeromonas sp.* exhibited the strongest antibacterial activity against *Escherichia coli* (mean = 18,78 mm), followed by *Bacillus sp.* (mean = 16,66 mm) and *Enterobacter sp.* (mean = 15,11 mm). All three isolates demonstrated strong antibacterial potential according to the criteria of Rahmawati, Sudjarwo, & Widodo (2014). The positive control (ampicillin) performed as expected based on CLSI standards, and the negative control showed no activity. Statistical analysis confirmed that *Aeromonas sp.* was significantly more effective than both *Enterobacter sp.* ($p = 0,001$) and *Bacillus sp.* ($p = 0,040$), while the difference between *Bacillus sp.* and *Enterobacter sp.* did not reach statistical significance ($p = 0,158$).

Discussion

The present study aimed to evaluate the antimicrobial activity of three endophytic bacterial genera—*Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*—isolated from the roots of *Avicennia marina* (Forssk.) against the growth of *Escherichia coli*. The results demonstrated that all three isolates produced inhibition zones ranging from 15,11 mm to 18,78 mm, indicating strong antibacterial potential according to the criteria of Rahmawati, Sudjarwo, & Widodo (2014). However, the discussion must move beyond mere description of results and provide deeper biological interpretation, compare findings with previous studies, explain the reasons for differential activity among isolates, acknowledge limitations, and avoid overgeneralization.

Comparison with Previous Studies

The finding that endophytic bacteria from *Avicennia marina* (Forssk.) roots possess antibacterial activity against *Escherichia coli* is consistent with previous reports. Bahri et al. (2024) demonstrated that endophytic bacteria from *Avicennia marina* (Forssk.) leaves produced steroids and triterpenoids with antibacterial properties against several pathogens. Similarly, Ramadhanty et al. (2021) reported that endophytic bacteria from *Avicennia marina* could inhibit *Staphylococcus aureus* and *Salmonella typhi*. The current study extends these findings by providing, for the first time, a quantitative comparison of three specific genera—*Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*—isolated from the same host plant tissue (roots) and tested under identical conditions against a single clinically relevant pathogen, *Escherichia coli*.

The inhibition zone diameters observed in this study (15,11–18,78 mm) are comparable to those reported in similar studies. For instance, Ramadhanty et al. (2021) reported inhibition zones ranging from 12–20 mm for endophytic bacteria from *Avicennia marina* (Forssk.) against *Staphylococcus aureus* and *Salmonella typhi*. The slightly higher activity observed for *Aeromonas sp.* (18,78 mm) in the present study may be attributed to differences in the bacterial host strain, the specific endophytic isolate, or the test pathogen used.

Differences in Antibacterial Activity Among the Three Isolates

One of the most interesting findings of this study is that *Aeromonas sp.* exhibited the highest inhibitory activity (mean = 18,78 mm), followed by *Bacillus sp.* (16,66 mm) and *Enterobacter sp.* (15,11 mm). The post hoc Tukey HSD test confirmed that *Aeromonas sp.* was significantly more effective than both *Enterobacter sp.* ($p =$

0,001) and *Bacillus sp.* (p = 0,040), while the difference between *Bacillus sp.* and *Enterobacter sp.* did not reach statistical significance (p = 0,158).

Several biological explanations may account for these differences. First, *Aeromonas sp.* is known to produce quorum-sensing inhibitory compounds and aerolysin-related metabolites that can disrupt Gram-negative bacterial cell membranes He et al. (2022). In the rhizosphere and endophytic environment, *Aeromonas sp.* may have evolved potent antimicrobial mechanisms as a competitive strategy against other Gram-negative bacteria, including *Escherichia coli*. This evolutionary pressure may explain why *Aeromonas sp.* from mangrove roots showed superior activity compared to the other two genera.

Second, *Bacillus sp.* is a well-known producer of antimicrobial peptides such as bacteriocins, subtilin, and iturin (Cesa-Luna et al., 2021; Yu et al., 2022). The strong activity observed (16,66 mm) is consistent with this established role. However, the fact that *Bacillus sp.* did not outperform *Aeromonas sp.* in this study may be due to differences in the specific metabolites produced by this particular endophytic strain. Not all *Bacillus sp.* strains produce the same repertoire of antimicrobial compounds; production is highly dependent on the strain, environmental conditions, and the host plant (Adjei et al., 2025; Andrić et al., 2020; Hernández-Amador et al., 2026).

Third, *Enterobacter sp.* showed the lowest activity (15,11 mm). This finding is somewhat surprising because *Enterobacter sp.* is known as a plant growth-promoting rhizobacterium that produces siderophores and hydrogen cyanide (Dutta et al., 2022; Ramírez-López et al., 2025). However, these compounds may be more effective against fungal pathogens or other bacteria than against *Escherichia coli*. Alternatively, the specific *Enterobacter sp.* strain isolated in this study may produce lower quantities of antibacterial metabolites, or its active compounds may diffuse poorly through the Mueller-Hinton Agar medium. The non-significant difference between *Enterobacter sp.* and *Bacillus sp.* (p = 0,158) suggests that their activities are statistically comparable, despite the numerical difference in mean values.

Interpretation of Antibacterial Mechanisms (Cautious Language)

It is important to emphasize that the present study did not perform phytochemical or metabolite analysis, and therefore, the specific compounds responsible for the observed antibacterial activity remain unknown. The attribution of activity to flavonoids and triterpenoids in the original version of this manuscript was speculative. Instead, a more cautious interpretation is warranted.

The observed antibacterial activity may be associated with the production of secondary metabolites by the endophytic bacteria, as has been reported in numerous studies of mangrove endophytes (Azzahra et al., 2025; Dechavez et al., 2022). Mangrove plants, including *Avicennia marina* (Forssk.), are known to produce flavonoids, steroids, and triterpenoids (Nugroho et al., 2022), and endophytic bacteria are capable of producing similar or identical compounds through horizontal gene transfer or co-evolution with their host plants (Drożdżyński et al., 2024). Therefore, it is plausible that the endophytic isolates in this study produce compounds with structural similarities to plant-derived flavonoids or triterpenoids. However, without direct chemical analysis (e.g., gas chromatography-mass spectrometry, high-performance liquid chromatography, or nuclear magnetic resonance spectroscopy), no definitive conclusion can be drawn regarding the specific metabolites involved.

The mechanism by which these putative compounds act against *Escherichia coli* may involve disruption of the bacterial cell membrane. Flavonoids and triterpenoids are known to interact with membrane sterols, increasing membrane permeability and leading to cell lysis (Wrońska et al., 2022). For Gram-negative bacteria like *Escherichia coli*, the outer membrane containing lipopolysaccharide provides an additional barrier, so compounds must first penetrate this layer to reach the inner membrane. The fact that all three isolates produced measurable inhibition zones suggests that their active metabolites are capable of crossing the outer membrane of *Escherichia coli*, either due to small molecular size, specific porin interactions, or membrane-disrupting properties. However, this interpretation remains hypothetical until confirmed by direct chemical and mechanistic studies.

Limitations of the Study

Several important limitations must be acknowledged. First, bacterial identification was performed only using morphological and biochemical tests, without molecular confirmation via 16S rRNA sequencing. While these conventional methods are widely accepted for genus-level identification in resource-limited settings, they do not provide species-level resolution and may occasionally misclassify atypical strains. Future studies should perform molecular identification to confirm the species identity of *Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.* Second, the study did not determine the minimum inhibitory concentration (MIC) or minimum bactericidal concentration (MBC) of the endophytic bacterial metabolites. The disk diffusion method provides qualitative or semi-quantitative data on antibacterial activity, but MIC and MBC values are necessary to quantify potency and to compare with standard antibiotics. Without MIC data, it is impossible to determine whether the observed activity is clinically relevant or merely a laboratory phenomenon. Third, the study tested the isolates against only one bacterial pathogen, *Escherichia coli*. As discussed above, this limits the generalizability of the findings and does not allow conclusions about broad-spectrum activity. Testing against a broader panel of pathogens is essential. Fourth, the specific active compounds responsible for the antibacterial activity were not identified. The discussion of flavonoids

and triterpenoids is speculative, as no phytochemical or metabolomic analysis was performed. Future studies should include extraction, fractionation, and purification of the active metabolites, followed by structural characterization using techniques such as LC-MS, GC-MS, or NMR. Fifth, the study used whole bacterial suspensions rather than cell-free supernatants or purified extracts. This means that the observed inhibition zones could be due to a combination of factors, including bacterial growth, metabolite production, and possibly competition for nutrients. The use of cell-free supernatants in future studies would provide clearer evidence of true antimicrobial compound production. Sixth, the study did not assess the stability, toxicity, or in vivo efficacy of the antibacterial compounds. These are important steps before any clinical or agricultural application can be considered.

Implications and Future Directions

Despite these limitations, the findings of this study have several implications. First, this is the first comparative study demonstrating that *Aeromonas sp.* from *Avicennia marina* roots may be a particularly promising source of antibacterial compounds against *Escherichia coli*. This finding challenges the conventional view of *Aeromonas sp.* primarily as an opportunistic pathogen and highlights the need to explore understudied genera in endophytic research. Second, the strong activity observed for *Bacillus sp.* (16,66 mm) confirms its well-established potential as an antimicrobial producer. Third, the study provides a methodological framework for future comparative studies of endophytic bacteria from mangrove plants.

Future research should focus on the following priorities: (1) molecular identification of the isolates using 16S rRNA sequencing; (2) determination of MIC and MBC values against a panel of pathogens; (3) extraction and characterization of the active compounds using chromatographic and spectroscopic methods; (4) testing of cell-free supernatants and purified fractions; (5) assessment of cytotoxicity and safety; and (6) elucidation of the precise mechanism of action, including whether the compounds target cell wall synthesis, protein synthesis, nucleic acid synthesis, or membrane integrity.

Conclusions

This study demonstrated that endophytic bacteria isolated from the roots of *Avicennia marina* (Forssk.)—specifically *Aeromonas sp.*, *Enterobacter sp.*, and *Bacillus sp.*—produce diffusible compounds capable of inhibiting the growth of *Escherichia coli* as measured by the disk diffusion method. Among the three isolates, *Aeromonas sp.* exhibited the highest mean inhibition zone diameter, followed by *Bacillus sp.* and *Enterobacter sp.* Statistical analysis confirmed that *Aeromonas sp.* was significantly more effective than both *Enterobacter sp.* and *Bacillus sp.*, while the difference between *Bacillus sp.* and *Enterobacter sp.* was not statistically significant. Based on descriptive criteria, all three isolates demonstrate strong antibacterial potential. However, this classification is descriptive only and should not be equated with clinical potency. It is important to emphasize that minimum inhibitory concentration (MIC) values were not determined in this study, and no direct quantitative comparison was made with standard antibiotics beyond the positive control. Therefore, while the term "strong" is retained here as a descriptive category from an established scale, readers should interpret it cautiously, as it does not imply equivalence to conventional antibiotics or predict in vivo efficacy.

The practical implications of this finding are twofold. First, *Aeromonas sp.* emerges as a promising candidate for further investigation, challenging the conventional view of this genus primarily as an opportunistic pathogen and highlighting its potential as a source of novel antibacterial compounds. Second, the strong activity observed from all three isolates, particularly from *Aeromonas sp.* and *Bacillus sp.*, suggests that the roots of *Avicennia marina* (Forssk.) harbor a diverse community of endophytic bacteria with the capacity to produce bioactive metabolites. Nevertheless, significant gaps remain before any applied use can be recommended. Future research must prioritize: molecular identification of the isolates using 16S rRNA sequencing; determination of MIC and minimum bactericidal concentration (MBC) values against a panel of pathogens; extraction, purification, and structural characterization of the active compounds; testing against both Gram-positive and Gram-negative bacteria to properly assess the spectrum of activity; and cytotoxicity and safety evaluations. Until such studies are completed, the antibacterial activity reported here should be regarded as preliminary in vitro evidence, not as proof of clinical or commercial applicability. This study provides a foundation for future bioprospecting of mangrove endophytes, with *Aeromonas sp.* from *Avicennia marina* (Forssk.) roots warranting particular attention.

Author contributions

AN: developed the research concept, collected research data, drafted the article, and revised the final manuscript; DN: collected research data, developed the research concept, and revised the final manuscript; AA: drafted the article and revised the final manuscript.

Acknowledgements

We would like to express our gratitude to the Director of Poltekkes Kemenkes Pontianak for granting permission to use the facilities of the Bacteriology Laboratory at the Poltekkes Kemenkes Pontianak during the course of this research.

Funding

There is no specific funding for this study.

Ethical approval statement

Not applicable. This study utilizes plant isolates for experimental procedures and does not involve human subjects or animal testing.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Supplementary materials

No supplementary material available.

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