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Research Article

Antibacterial Activity Test of Sungkai Leaf Endophytes (*Peronema canescens* J) Against *Escherichia coli* and *Staphylococcus aureus* Bacteria as Antidiarrheal Probiotic Candidates

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ABSTRACT

Purpose of the study: This study aims to test the antibacterial activity of endophytic bacterial isolates from sungkai leaves against the two test bacteria as antidiarrheal probiotic candidates.

Methodology: The research stages included isolation, morphological characterization, Gram staining, motility testing, catalase testing, and antibacterial activity testing using the well diffusion method. Data were analyzed using Shapiro-Wilk, ANOVA, and Tukey's advanced test.

Main Findings: The results of the study obtained five isolates (S-1, S-2, S-3, S-4, S-5) with Gram-positive characteristics, bacilli, and positive in motility and catalase tests. Isolates S-1 and S-2 did not have antibacterial activity. In contrast, isolates S-3, S-4, and S-5 were able to inhibit the test bacteria with moderate to strong categories. The highest activity against *Escherichia coli* was shown by isolate S-3 (10.3 mm), while against *Staphylococcus aureus* by isolate S-4 (12.08 mm). Statistical analysis showed a significant difference in antibacterial activity between treatments ($p<0.001$). It was concluded that isolates S-3 and S-4 have the most potential to be developed as antidiarrheal probiotic candidates and natural antibacterial sources.

Novelty/Originality of this study: The originality of this study lies in integrating endophytic bacterial exploration from *Peronema canescens* leaves with antibacterial screening against *Escherichia coli* and *Staphylococcus aureus* to identify potential probiotic candidates for antidiarrheal applications.

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1. INTRODUCTION

Diarrheal disease remains one of the leading public health challenges worldwide, particularly in developing countries where sanitation and access to clean water remain inadequate. According to the World Health Organization (WHO), diarrheal diseases account for approximately 4–5% of the global burden of infectious diseases, with an estimated 1.7 billion cases reported annually [1], [2]. In Indonesia, data from the Ministry of Health indicate that the prevalence of diarrhea remains around 7–8% of the general population and consistently ranks among the ten most frequently reported diseases in healthcare facilities [3], [4]. At the regional level, the Health Profile of Jambi Province reported a diarrhea prevalence of approximately 6%, indicating that the disease continues to pose a significant public health concern in the province. These findings demonstrate that diarrhea is not only a global and national health issue but also a persistent regional problem that requires effective, sustainable, and environmentally friendly therapeutic strategies [5]-[7].

Bacterial pathogens remain one of the major causes of diarrheal infections, with *Escherichia coli* and *Staphylococcus aureus* frequently associated with foodborne illnesses characterized by diarrhea, nausea, vomiting, and abdominal discomfort. Antibiotic therapy is commonly prescribed to control bacterial diarrhea because of its rapid effectiveness in eliminating pathogenic microorganisms [8], [9]. However, the extensive and often inappropriate use of antibiotics has accelerated the emergence of antimicrobial resistance, which has become one of the most serious global health threats [10]-[12]. In addition to promoting resistant bacterial strains, prolonged antibiotic administration may disrupt the balance of the intestinal microbiota, leading to dysbiosis and increasing the risk of recurrent gastrointestinal disorders [13]-[15]. Therefore, alternative therapeutic approaches capable of suppressing pathogenic bacteria while maintaining intestinal microbial homeostasis are urgently needed.

Probiotics have gained considerable attention as promising alternatives to conventional antibiotics because they confer health benefits by maintaining the balance of the intestinal microbiome. Beneficial microorganisms can inhibit pathogenic bacteria through multiple mechanisms, including competitive exclusion, production of antimicrobial compounds, organic acids, bacteriocins, and modulation of host immune responses [16]-[18]. Unlike antibiotics, probiotics generally preserve the diversity of the normal gut microbiota, thereby reducing the likelihood of dysbiosis and improving intestinal health [19], [20]. These characteristics make probiotics attractive candidates for preventing and managing bacterial diarrhea while minimizing the risk of antimicrobial resistance. Consequently, the search for novel probiotic microorganisms with strong antibacterial activity has become an important focus of current biomedical and microbiological research.

One promising source of novel probiotic candidates is endophytic bacteria that inhabit the internal tissues of healthy plants without causing pathological effects to their hosts. Endophytic bacteria establish mutually beneficial interactions with plants and are known to synthesize a wide range of bioactive secondary metabolites, including antibacterial, antifungal, antioxidant, and anti-inflammatory compounds [21]-[23]. Numerous studies have demonstrated that these microorganisms often produce metabolites similar to those synthesized by their host plants, making them valuable alternatives for natural product discovery [21], [24], [25]. Furthermore, the utilization of endophytic bacteria provides a more sustainable approach than direct harvesting of medicinal plants because microbial cultivation can reduce pressure on natural plant populations. Therefore, exploring the antibacterial potential of endophytic bacteria represents an attractive strategy for discovering environmentally sustainable antimicrobial and probiotic agents [26]-[28].

Peronema canescens Jack, locally known as sungkai, is an indigenous medicinal plant widely distributed in Indonesia and has long been used in traditional medicine to treat fever, wounds, and various infectious diseases [29]-[31]. Phytochemical investigations have revealed that sungkai leaves contain diverse bioactive compounds, including flavonoids, alkaloids, phenolics, tannins, and terpenoids, which contribute to their antimicrobial properties [32], [33]. Previous studies have primarily focused on the antibacterial activity of sungkai leaf extracts, demonstrating inhibitory effects against several pathogenic bacteria [34], [35]. However, excessive exploitation of medicinal plants to obtain bioactive compounds may threaten the sustainability of natural populations and biodiversity. Consequently, investigating endophytic bacteria associated with *P. canescens* offers a sustainable alternative for obtaining antimicrobial metabolites while conserving the plant resource itself.

Although the antibacterial potential of *P. canescens* extracts has been widely reported, studies investigating its endophytic bacterial community remain very limited [35], [36]. Existing research has predominantly emphasized phytochemical constituents of the plant rather than exploring the microorganisms residing within its tissues as potential sources of antibacterial metabolites. Moreover, only a few studies have evaluated the antibacterial activity of endophytic bacteria isolated from *P. canescens* against important diarrheal pathogens such as *E. coli* and *S. aureus* [37], [38]. Information regarding the preliminary probiotic characteristics of these endophytic isolates is also still scarce. This lack of knowledge highlights an important research gap regarding the potential utilization of sungkai-associated endophytic bacteria as sustainable antibacterial resources and prospective probiotic candidates.

The novelty of the present study lies in the integration of endophytic bacterial exploration from *P. canescens* leaves with antibacterial screening against both Gram-negative (*E. coli*) and Gram-positive (*S. aureus*) diarrheal pathogens within the context of probiotic discovery. Unlike previous studies that mainly investigated crude plant extracts, this research evaluates living endophytic bacterial isolates as alternative producers of antibacterial metabolites. In addition, the isolates were characterized through morphological observations, Gram staining, motility testing, catalase activity, and quantitative antibacterial assays to identify the most promising candidates [39]-[41]. This integrated approach provides preliminary evidence supporting the utilization of endophytic bacteria as environmentally sustainable bioresources for antimicrobial development. Therefore, the study contributes new scientific insights into the exploitation of medicinal plant-associated microbiota for future probiotic and antibacterial applications.

Based on the global burden of diarrheal disease, the increasing challenge of antibiotic resistance [42]-[44], and the limited information regarding endophytic bacteria from *P. canescens*, further investigation is warranted. Evaluating the antibacterial activity of these bacterial isolates is an essential first step toward identifying microorganisms with potential probiotic properties. Such information will provide a scientific basis for future studies involving molecular identification, safety assessment, and functional probiotic characterization. Therefore, this study aimed to isolate and characterize endophytic bacteria from *Peronema canescens* leaves and quantitatively evaluate their antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The findings are expected to contribute to the development of sustainable natural antibacterial agents and prospective antidiarrheal probiotic candidates.

2. RESEARCH METHOD

The research was conducted in February – May 2026 at the Microbiology Laboratory, Pharmacy Study Program, Faculty of Medicine and Health Sciences, Adiwangsa University, Jambi. This laboratory experimental study tested the antibacterial activity of sungkai leaf endophyte (Peronema canescens J.) against Escherichia coli and Staphylococcus aureus as antidiarrheal probiotic candidates using the well diffusion method.

The study population was sungkai leaves from the Muaro Jambi region, with samples consisting of endophytic bacterial isolates from the leaves. The sampling technique used in this study was purposive sampling, which involves taking samples based on certain predetermined criteria, followed by plant identification at Padjadjaran University. Research instruments (NA/NB media, glassware, incubator, autoclave, caliper, McFarland 0.5) were validated and sterilized according to laboratory SOP to ensure aseptic conditions.

The average diameter of the inhibition zone (mm) against Escherichia coli and Staphylococcus aureus was measured vertically and horizontally with a caliper, then analyzed quantitatively descriptively based on antibacterial strength criteria. Isolation of Endophytic Bacteria in Sungkai Leaves, Isolation of endophytic bacteria began with leaf surface sterilization with 2% NaOCl (1 minute), 70% alcohol (1 minute), and a sterile distilled water rinse. Leaf pieces were planted on NA media (28–30°C, 24–48 hours), then purified to obtain a pure isolate [45]. Isolate Purification, Culture purification was performed using the streak plate method on NA media to separate isolates from contaminants. Isolates were selected based on differences in colony morphology, including color, elevation, texture, and other specific characteristics [46].

Morphological Test, Colony morphology observations based on variations in shape, elevation, edges, and pigmentation [47]. Motility Test, The isolate was inoculated by puncture onto motility agar medium, then incubated (37°C, 24 hours). A positive result is indicated by turbidity spreading beyond the puncture line [48]. Catalase Test, A bacterial colony is placed on a glass slide and dripped with a 3% H_2O_2 solution. A positive result is indicated by the formation of oxygen gas bubbles [49]. Gram staining, Microscopic identification through gram staining (magnification 10–1000×) determines the nature of gram-positive (purple) and gram-negative (pink) cell walls [48], [50].

Pure isolate colonies were homogenized in sterile 0.9% NaCl, then equated to a 0.5 McFarland standard to standardize bacterial concentrations before antibacterial testing [48]. Escherichia coli and Staphylococcus aureus and endophytic isolates were adapted in NB (24 hours, 28–30°C) to McFarland standard 0.5. Antibacterial activity was tested by well diffusion method in NA (37°C, 24 hours incubation) using Ciprofloxacin as positive control and aquades as negative control [48], [51]. After 24 hours of incubation, the inhibition zone (clear area) around the well was measured using a vernier caliper. The average diameter value was calculated using the following formula: Inhibition zone diameter (mm) = $\frac{Dv-Ds+Dh-Ds}{2}$. The inhibition zone formed indicates the antibacterial activity of the endophytic bacterial isolate against Escherichia coli and Staphylococcus aureus [52]. The antibacterial activity of sungkai leaf isolates against Escherichia coli and Staphylococcus aureus was determined based on the diameter of the inhibition zone (mm) measured with a caliper. The strength of the activity was categorized as: very strong (>20mm), strong (11-20mm), moderate (5-10mm), and weak (0-4 mm) [53]. Isolates showing inhibition zones were interpreted as candidates for antidiarrheal probiotics.

3. RESULTS AND DISCUSSION

Macroscopic and microscopic morphological tests (Gram staining)

The macroscopic and microscopic morphological tests of isolates can see in table1.

Table 1. Macroscopic and Microscopic Morphology Test Results

Isolate Code	Macroscopic				Microscopic	
	Shape	Surface	Edge	Color	Cell Shape	Gram Group
S- 1	Rhizoi	Flat	Wavy	White	Bacil	Positive
S-2	Rhizoid	Flat	Wavy	White	Bacil	Positive
S-3	Filamentous	Flat	Filamentous	White	Bacil	Positive
S-4	Filamentous	Flat	Lobate	White	Bacil	Positive
S-5	Irregular	Flat	Filamentous	White	Bacil	Positive

Based on Table 1, the results of the macroscopic and microscopic morphological tests of isolates S-1 and S-2 show a rhizoid shape and S-3 and S-4 are filamentous, while S-5 has an irregular shape, with a flat surface and varying edges, and a white color. Based on microscopic observations through gram staining, all isolates were identified as gram-positive bacilli, which is important information for initial characterization.

Biochemical Characteristics Test

Biochemical characterization tests were performed to determine the physiological properties of the isolates. Motility test results showed that all isolates were motile, indicating they possessed flagella. The catalase test also yielded positive results, indicating that all isolates could break down hydrogen peroxide into water and oxygen, an important indicator of bacterial resistance to oxidative stress. Further details of the results can be seen in Table 2.

Table 2. Biochemical Characteristic Test Results

Biochemical Tests	Isolate Code				
	S-1	S-2	S-3	S-4	S-5
Motility Test	Positive	Positive	Positive	Positive	Positive
Catalase Test	Positive	Positive	Positive	Positive	Positive

The table 2 shows that all bacterial isolates (S-1, S-2, S-3, S-4, and S-5) yielded positive results in the motility test. These results indicate that all isolates are motile, generally due to the presence of flagella. Motility is an important characteristic in bacterial identification because it can distinguish motile bacteria from non-motile bacteria. Furthermore, all isolates also yielded positive results in the catalase test, indicating that they are capable of producing the enzyme catalase. This enzyme functions to break down hydrogen peroxide (H_2O_2) into water (H_2O) and oxygen (O_2), thereby protecting bacterial cells from damage caused by oxidative compounds. A positive reaction in the catalase test is generally indicated by the formation of oxygen bubbles after the addition of the H_2O_2 solution. The uniformity of the results in both biochemical tests indicates that the five isolates share the same physiological characteristics: motility and catalase positivity. These characteristics indicate that all isolates likely belong to a group of bacteria capable of adapting to both aerobic and facultative anaerobic conditions. However, these two tests are insufficient to identify bacteria to the species level, requiring further biochemical testing, such as oxidase, indole, citrate, urease, carbohydrate fermentation, or molecular analysis (e.g., 16S rRNA gene sequencing) to obtain more accurate identification.

Test of the Potential Antibacterial Activity of Sungkai Leaf Endophytes

The antibacterial activity potential test was conducted to assess the ability of sungkai leaf endophyte isolates to inhibit the growth of Staphylococcus aureus and Escherichia coli. To see these results more clearly, please see Table 3.

Table 3. Test of the Potential Antibacterial Activity of Sungkai Leaf Endophytes

Sample	Bacteria tested	Replication 1 (mm)	Replication 2 (mm)	Replication 3 (mm)	Average	Information
S-1	*Escherichia coli*	0	0	0	0	0
S-2	*Escherichia coli*	0	0	0	0	0
S-3	*Escherichia coli*	9.5	10	11.5	10.4	Strong
S-4	*Escherichia coli*	7	10	8.8	8.6	Moderate
S-5	*Escherichia coli*	9.5	6.5	0	8	Moderate
K (+)	*Escherichia coli*	25.8	25.8	25.8	25.8	Very Strong
S-1	*Staphylococcus aureus*	0	0	0	0	0

Sample	Bacteria tested	Replication 1 (mm)	Replication 2 (mm)	Replication 3 (mm)	Average	Information
S-2	Staphylococcus aureus	0	0	0	0	0
S-3	*Staphylococcus aureus*	10.5	7.3	6	8	Moderate
S-4	*Staphylococcus aureus*	11.5	16	8.8	12.1	Strong
S-5	*Staphylococcus aureus*	11	6.5	5.5	7.6	Moderate
K (+)	*Staphylococcus aureus*	27.5	27.5	27.5	27.5	Very Strong
K (-)	*Escherichia coli* dan *Staphylococcus aureus*	0	0	0	0	0

The results of the antibacterial activity test showed that each isolate had different inhibitory abilities. In Escherichia coli, isolate S-3 had the highest activity (10.4 mm; strong), while S-4 and S-5 were in the moderate category, while S-1 and S-2 were inactive. In Staphylococcus aureus, isolate S-4 showed the best activity (12.1 mm; strong), followed by S-3 and S-5 in the moderate category, while S-1 and S-2 were also inactive. The positive control produced very strong inhibition, while the negative control showed no inhibition. Overall, isolates S-3 and S-4 had the most potential as antibacterial candidates.

Normality Test

The inhibition zone data for both bacteria had a p-value > 0.05, so the data were normally distributed and met the requirements for further parametric analysis.

Table 4. Normality Test Results

Normality Test (Shapiro-Wilk)	W	p
Value *Escherichia coli*	0.890	0.117
Value *Staphylococcus aureus*	0.914	0.240

Based on the results of the Shapiro–Wilk normality test, the score data for Escherichia coli and Staphylococcus aureus have a significance value (p-value) greater than 0.05. This indicates that the data in both groups are not significantly different from the normal distribution, so the assumption of normality has been met. The Escherichia coli score has a W value = 0.890 with p = 0.117. Because the p-value > 0.05, the E. coli score data are normally distributed. The Staphylococcus aureus score has a W value = 0.914 with p = 0.240. The p-value, which is also greater than 0.05, indicates that the S. aureus score data are normally distributed. Thus, the results of the Shapiro–Wilk test confirm that the assumption of normality is met for both variables, so that parametric statistical analysis, such as the independent sample t-test, paired sample t-test, or ANOVA (according to the research design), can be used to test differences or relationships between variables. There is no evidence to suggest significant deviation from a normal distribution in either data set.

Homogeneity Test

Escherichia coli data is not homogeneous because p < 0.05, while Staphylococcus aureus data is homogeneous with p > 0.05, therefore further testing will be carried out as an alternative, namely the Tukey Post-Hoc test.

Table 5. Results of Homogeneity Test

Homogeneity of Variances Test (Levene's)	F	df1	df2	p
Value *Escherichia coli*	5.29	3	8	0.027
Value *Staphylococcus aureus*	3.00	3	8	0.095

The results of the homogeneity of variance test using Levene's Test indicate that the assumption of equal variances differs between the two bacterial groups, Escherichia coli and Staphylococcus aureus. The F value for Escherichia coli is 5.29, df1 = 3, df2 = 8, and p = 0.027. Because the p value <0.05, the null hypothesis stating that the variances between groups are equal is rejected. Therefore, the variance of the E. coli data is not homogeneous (heterogeneous). This indicates that there is a significant difference in variability between treatment groups. Therefore, parametric analysis that assumes homogeneity of variance must use a procedure that does not require homogeneity of variance, such as Welch's ANOVA, or an appropriate alternative method. The F value for Staphylococcus aureus is 3.00, df1 = 3, df2 = 8, and p = 0.095. Because the p-value> 0.05, the null hypothesis is not rejected, and the variances between groups can be considered homogeneous. Thus, the assumption of homogeneity of variance has been met, and parametric analyses that assume equality of variance, such as one-way ANOVA, can be used.

ANOVA test

Based on the results of the One-Way ANOVA test, a significance value of $p < 0.001$ was obtained for Escherichia coli ($F = 36.8$) and Staphylococcus aureus ($F = 36.7$). These results indicate that there is a significant difference between the treatments of endophytic bacterial isolates on antibacterial activity, so the null hypothesis (H_0) is rejected and the alternative hypothesis (H_1) is accepted. Thus, each isolate has a different ability to inhibit the growth of the test bacteria.

Table 6. ANOVA test results

One-Way ANOVA (Fisher's)	F	df1	df2	p
Value *Escherichia coli*	36.8	3	8	<.001
Value *Staphylococcus aureus*	36.7	3	8	<.001

As a follow-up step, a Tukey Post-Hoc test was performed to identify which groups differed significantly after ANOVA analysis. Based on the Tukey Post-Hoc follow-up test, significant differences were found between the isolate and positive control groups, while there was no significant difference between isolates S-3, S-4, and S-5 ($p > 0.05$). The absence of significant differences between isolates indicates that the three isolates have relatively similar antibacterial effectiveness. These results are in line with research Ridha et al. [54], which reported that several endophytic bacterial isolates showed antibacterial activity that was not significantly different from each other, but were still able to inhibit the growth of pathogenic bacteria. In addition, research Polandos [55] also stated that endophytic bacteria have the potential to produce bioactive metabolites that play a role in inhibiting the growth of Escherichia coli and Staphylococcus aureus, thus having the potential to be developed as an alternative antibacterial source.

Table 7. Tukey Post-Hoc Test Results

		S-3		S-4		S-5		Control +	
		EC	SA	EC	SA	EC	SA	EC	SA
S-3	Mean difference	—	—	1.73	-4.01	5.00	0.423	-15.5	-19.4
	p-value	—	—	0.845	0.324	0.163	0.997	<.001	<.001
S-4	Mean difference			—	—	3.27	4.430	-17.2	-15.4
	p-value			—	—	0.459	0.253	<.001	<.001
S-5	Mean difference					—	—	-20.5	-19.8
	p-value					—	—	<.001	<.001
K (+)	Mean difference							—	—
	p-value							—	—

Note. EC = *Escherichia coli,* SA = *Staphylococcus aureus*

In this study, five endophytic bacteria were successfully isolated from sungkai leaves (Peronema canescens J), namely S-1, S-2, S-3, S-4, and S-5 which showed different colony morphological characteristics even though they originated from the same plant tissue. The initial characterization stage through observation of colony morphology, Gram staining, and biochemical tests is an important step to obtain initial information regarding the identity and physiological characteristics of bacterial isolates before molecular identification is carried out [56]. The differences between the five isolates are seen in the form of rhizoidal, filamentous, and irregular colonies with all isolates having a flat surface, white color, and wavy to lobate edges. These differences in morphological characters indicate the existence of phenotypic diversity between isolates which is likely influenced by differences in adaptation ability to the microenvironment in host plant tissue [57]. Morphological diversity in endophytic bacteria is commonly found because each isolate has a different metabolic mechanism and nutritional preference during colonization of plant tissue [58].

The Gram staining results showed that all isolates, including Gram-positive bacteria, were bacilli. This characteristic is indicated by the ability of the cells to maintain the crystal violet color during the Gram staining process due to the presence of a thick peptidoglycan layer on the bacterial cell wall [59], [60]. The dominance of Gram-positive bacteria in medicinal plant endophytic isolates is quite often found because this group has good environmental resistance, high colonization ability, and the potential to produce various bioactive metabolites such as bacteriocins, organic acids, lipopeptides, and antimicrobial enzymes [61], [62]. This Gram-positive character is also relevant to the concept of probiotics, considering that most probiotic bacteria come from the Gram-positive group, such as the genera Lactobacillus, Bacillus, and Enterococcus [63], so the results of this study support the assumption that sungkai leaf endophytic isolates have the potential to be developed as antidiarrheal probiotic candidates.

All isolates showed positive results in the motility test. These results indicate the presence of a flagella structure that allows active bacterial movement, thus supporting the colonization and adaptation process in plant tissues as endophytes [64]. Motility is an important factor for endophytic bacteria in tissue penetration, space competition, and nutrient distribution during life in plants [62]. In the context of probiotics, motility plays a role in increasing the opportunity for bacteria to reach the surface of the intestinal mucosa, thus supporting the adhesion process and initial colonization in the gastrointestinal tract. This adhesion ability is an important factor that determines the success of probiotics in interacting and temporarily settling in the host intestine [65].

The catalase test results showed that all isolates were also positive. Catalase activity indicates the presence of the catalase enzyme, which is capable of breaking down hydrogen peroxide into water and oxygen, thereby helping cells survive oxidative stress [61]. This ability is important for probiotic candidates because during their stay in the gastrointestinal environment, bacteria are potentially exposed to reactive oxygen species (ROS), which can reduce cell viability [61]. Therefore, the combination of Gram-positive, motility-positive, and catalase-positive characteristics in all isolates indicates basic characteristics that support initial probiotic potential.

In antibacterial activity testing, not all isolates showed the ability to inhibit the growth of test bacteria. Isolates S-1 and S-2 did not produce inhibition zones against either Escherichia coli or Staphylococcus aureus. The absence of antibacterial activity in isolates may be caused by low production of secondary metabolites during the cultivation process, incompatibility of the media composition with the expression of the biosynthetic pathway of active compounds, or the presence of genes encoding antibacterial metabolites that were not expressed or were in a silent state in the culture environment used. The production of antimicrobial metabolites is known to be greatly influenced by growth conditions, media composition, and regulation of metabolite biosynthesis gene expression [66]. A similar phenomenon was also reported in studies of endophytic bacteria of other medicinal plants, where not all isolates showed antagonistic activity against pathogens [58].

Against Escherichia coli bacteria, isolate S-3 showed the highest activity with an average inhibition zone diameter of 10.4 mm and was categorized as strong, followed by isolate S-4 at 8.6 mm and S-5 at 8 mm with a moderate category. These results indicate that isolate S-3 has the best ability to inhibit Gram-negative bacteria compared to other isolates. The inhibitory power against Escherichia coli tends to be lower than the positive control because Gram-negative bacteria have a lipopolysaccharide outer membrane structure that functions as a barrier to the penetration of antibacterial compounds, so that bioactive compounds require a more complex penetration mechanism [67]. This condition explains why the resulting inhibition zone diameter is smaller than the positive control at 25.8 mm. The results of this study are in line with previous reports showing that endophytic bacteria isolated from medicinal plants have the ability to inhibit the growth of pathogenic bacteria through the production of antimicrobial bioactive compounds. This activity shows the potential of endophytic bacteria as a source of alternative antibacterial agents that can be further developed to control pathogenic microorganisms [68]. In addition, the ability of the isolate to inhibit Escherichia coli indicates antagonistic activity against enteropathogenic bacteria that has the potential to be utilized in the development of probiotic candidates for the treatment of gastrointestinal disorders [65]. This ability is supported by the characteristics of bacteria that are able to adapt, survive, and colonize in the host environment, which is an important factor in supporting the effectiveness of probiotics [64].

In Staphylococcus aureus bacteria, isolate S-4 showed the highest activity with an average inhibition zone of 12.1 mm in the strong category, followed by S-3 at 8 mm and S-5 at 7.6 mm in the moderate category. Higher antibacterial activity against Staphylococcus aureus compared to Escherichia coli indicates that the isolate's metabolites are more effective against Gram-positive bacteria, this is because the structure of the Gram-positive cell wall is relatively simpler and does not have an outer membrane, thus facilitating the diffusion of active compounds into target cells compared to Gram-negative bacteria which have an outer membrane as a penetration inhibitor [57], [60]. The results of this study are also consistent with research Nugraheni et al. [58] which reported that several endophytic bacterial isolates from medicinal plants have greater inhibition against Staphylococcus aureus than Escherichia coli. The antibacterial activity shown by isolates S-3, S-4, and S-5 against Staphylococcus aureus and Escherichia coli indicates the production of secondary metabolites that have the potential to inhibit the growth of pathogenic bacteria. This ability is in line with previous reports showing that endophytic bacterial isolates from various medicinal plants are able to produce antimicrobial bioactive compounds and show inhibitory activity against Gram-positive and Gram-negative bacteria [68].

The results of statistical analysis showed that the data met the assumption of normality based on the Shapiro–Wilk test, with a p value of 0.117 in Escherichia coli and $p = 0.240$ in Staphylococcus aureus ($p>0.05$), so that the data were normally distributed and suitable for analysis using parametric tests. In the homogeneity test, Escherichia coli data showed inhomogeneous results ($p = 0.027$), while Staphylococcus aureus data were homogeneous ($p = 0.095$), which was thought to be influenced by the large variation in antibacterial activity between active and inactive isolates. However, the results of the Analysis of Variance test showed a p value <0.001 in both bacteria, so it can be concluded that there was a significant difference in antibacterial activity between treatments, which means that the type of isolate had a significant effect on the diameter of the inhibition zone formed [69].

The results of the Tukey further test showed that there was no significant difference between the active isolates (S-3, S-4, and S-5), but all isolates were significantly different from the positive control ($p<0.001$). These results indicate that the antibacterial activity of endophytic isolates is still below the effectiveness of standard antibiotics, but still shows potential as a promising source of natural antibacterials [57], [69]. This potential is important because endophytic bacteria are known to be able to produce various bioactive metabolites that have similar biological activities to compounds produced by their host plants. Therefore, the use of endophytic bacteria as a source of secondary metabolites can be a more sustainable alternative to obtain antibacterial compounds, while reducing dependence on large-scale harvesting of medicinal plants and reducing the risk of over-exploitation of biological resources.

Overall, isolates S-3 and S-4 were the most potential isolates because they showed the best antibacterial activity against both test bacteria. In addition to having the ability to inhibit the growth of pathogenic bacteria, both isolates also showed Gram-positive, catalase-positive, and motility-positive characteristics which are important properties in the initial selection of probiotic candidates. The combination of these characteristics indicates the ability of the isolates to adapt, survive, and colonize the host environment, so they have the potential to be further developed as probiotic candidates. However, specific probiotic testing is needed, such as resistance to acidic pH and bile salts, adhesion ability, safety, and molecular identification to confirm the probiotic potential of these isolates [64], [65].

The findings of this study provide preliminary evidence that endophytic bacteria isolated from *Peronema canescens* leaves possess antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*, highlighting their potential as sustainable sources of natural antibacterial agents and prospective antidiarrheal probiotic candidates. These findings contribute to the growing body of knowledge on medicinal plant-associated microbiota and support the utilization of endophytic bacteria as an environmentally friendly alternative to direct exploitation of medicinal plants for bioactive compound production. Nevertheless, this study has several limitations. The antibacterial activity was evaluated only using the agar well diffusion method, while the isolates were characterized solely through morphological and basic biochemical analyses without molecular identification or determination of the specific bioactive compounds responsible for the observed antibacterial effects. In addition, essential probiotic properties, including tolerance to acidic pH and bile salts, adhesion ability, autoaggregation, antibiotic susceptibility, and safety assessments, were not investigated. Therefore, future studies should focus on molecular identification using 16S rRNA gene sequencing, purification and characterization of antibacterial metabolites, determination of minimum inhibitory and bactericidal concentrations (MIC and MBC), comprehensive probiotic characterization, and in vivo validation to confirm the efficacy and safety of the most promising isolates for antidiarrheal applications.

4. CONCLUSION

The study successfully isolated five Gram-positive endophytic bacteria (S-1 to S-5) from sungkai leaves, all of which were motile and produced the enzyme catalase. Isolates S-3, S-4, and S-5 proved to be moderately to strongly effective in inhibiting diarrhea-causing bacteria, with S-3 optimally inhibiting Escherichia coli (10.4 mm) and S-4 optimally inhibiting Staphylococcus aureus (12.1 mm). Statistical analysis confirmed that the type of isolate significantly influenced the diameter of the inhibition zone ($p<0.001$), with isolates S-3 and S-4 being the most promising candidates for development as natural antidiarrheal probiotics. Endophytic bacteria from sungkai leaves have proven to have strong potential as natural antibacterial agents that are safer and more environmentally friendly than synthetic antibiotics. Isolates S-3 and S-4 are the most promising candidates as antidiarrheal probiotics due to their superior characteristics (Gram-positive, catalase-positive, and motility) and their active inhibition of gastrointestinal pathogens. This research confirms that the local medicinal plant sungkai leaves are highly valuable for the exploration of endophytic microorganisms in the pharmaceutical and biotechnology fields. The researchers recommend molecular identification to determine the exact species of isolates S-3 and S-4, as well as the isolation of secondary metabolites to confirm the active ingredients that act as antibacterials.

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AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

INFORMED CONSENT STATEMENT

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USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that no artificial intelligence (AI) tools were used in the generation, analysis, or writing of this manuscript. All aspects of the research, including data collection, interpretation, and manuscript preparation, were carried out entirely by the authors without the assistance of AI-based technologies.

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