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Research Article

Antibacterial Activity Test of Pandan Wangi Leaf Extract (*Pandanus amaryllifolius* Roxb.) against Growth of the *Staphylococcus aureus* and *Escherichia coli*

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ABSTRACT

Antibiotic resistance has encouraged the exploration of alternative antibacterial agents from natural sources, including the leaves of pandan wangi (*Pandanus amaryllifolius* Roxb.), which contain bioactive compounds such as flavonoids, alkaloids, and saponins. This study aimed to determine the antibacterial activity of pandan wangi leaf extract against *Staphylococcus aureus* and *Escherichia coli*, to identify the most effective extract concentration, to compare the inhibitory activity between Gram-positive and Gram-negative bacteria, and evaluate the amylase enzyme activity of both bacteria. The antibacterial test was conducted using the disc diffusion method with extract concentrations of 25%, 50%, 75%, and 100%. Chloramphenicol and sterile distilled water were used as positive and negative controls, respectively. The results showed that pandan wangi leaf extract exhibited higher antibacterial activity against *E. coli* than *S. aureus*. The highest inhibition against *E. coli* was observed at 75% concentration with an inhibition zone diameter of 7.22 mm, categorized as moderate inhibition. In contrast, the inhibitory activity against *S. aureus* was very weak with the highest inhibition zone of 0.97 mm at 100% concentration. In the amylase activity test, both bacteria did not produce clear zones on starch medium after iodine application, indicating the absence of amylase enzyme activity. These findings suggest that pandan wangi leaf extract has potential as a natural antibacterial agent, particularly against Gram-negative bacteria.

Keywords: amylase, antibacterial activity, *Escherichia coli*, *Pandanus amaryllifolius, Staphylococcus aureus.*

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INTRODUCTION

Antibiotic resistance has become a global health crisis due to the excessive and inappropriate use of antibiotics, leading to the emergence of resistant bacterial strains and reducing the effectiveness of infection therapy (Yadav and Kapley, 2021). This condition increases morbidity, mortality, and healthcare costs worldwide. The World Health Organization (WHO) predicts that antibiotic resistance could cause up to 10 million deaths annually by 2050 if effective alternatives are not developed (Nurkhalika *et al*. 2021). Therefore, the exploration of natural antibacterial agents from plants has become increasingly important.

Pandan wangi (*Pandanus amaryllifolius* Roxb.) is a tropical plant widely used in Southeast Asia and known to contain various bioactive compounds such as flavonoids, alkaloids, saponins, polyphenols, and tannins that possess antibacterial properties (Kiyato *et al*. 2022). Previous studies reported that pandan wangi leaf extract inhibited the growth of several pathogenic bacteria, including *Staphylococcus aureus* (Dumoal, 2010) and *Escherichia coli* (Mardiyaningsih, 2014). In addition, pandan wangi has also been reported to exhibit antioxidant and other pharmacological activities (Dewanti and Sofian, 2017). However, studies regarding the antibacterial activity of pandan wangi leaf extract against both Gram-positive and Gram-negative bacteria at different extract concentrations are still limited.

Staphylococcus aureus is a Gram-positive bacterium that can cause various infections ranging from mild skin infections to severe systemic diseases (Foster, 2017). Meanwhile, *Escherichia coli* is a Gram-negative bacterium commonly associated with gastrointestinal and nosocomial infections (Eshrati *et al*., 2025). Differences in bacterial cell wall structure may influence the antibacterial effectiveness of plant extracts. Furthermore, bacterial metabolic activity can be evaluated through amylase enzyme production, which indicates the ability of bacteria to hydrolyze complex substrates such as starch. Therefore, this study aimed to determine the antibacterial activity of pandan wangi leaf extract against *S. aureus* and *E. coli*, identify the most effective extract concentration, compare its inhibitory effect on Gram-positive and Gram-negative bacteria, and evaluate the amylase enzyme activity of both bacteria.

METHODS

This study used a laboratory experimental design to evaluate the antibacterial activity of pandan wangi leaf extract (*Pandanus amaryllifolius* Roxb.) against *S. aureus* and *E. coli* using the disc diffusion method. The research was conducted from December 2025 to February 2026 at the Microbiology Laboratory and Botany Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Lampung.

Fresh pandan wangi leaves were collected and processed into extract using the maceration method with 96% ethanol. The extract was prepared into four concentrations, namely 25%, 50%, 75%, and 100%. Chloramphenicol was used as the positive control, while sterile distilled water was used as the negative control.

Bacterial suspensions of *S. aureus* and *E. coli* were prepared in 0.9% NaCl solution and adjusted to the 0.5 McFarland standard. Antibacterial activity was tested using the disc diffusion method on Muller Hinton Agar (MHA) media. Sterile paper discs soaked in each extract concentration were placed on inoculated media and incubated at 37 °C for 24 hours. The inhibition zone diameter was measured using a caliper.

Amylase activity was tested qualitatively on Nutrient Agar supplemented with 1% starch. After incubation at 37 °C for 24 hours, iodine solution was added to the media surface. The formation of a clear zone around bacterial colonies indicated positive amylase activity.

Data were analyzed descriptively by calculating the average diameter of inhibition zones from three replications for each treatment. The antibacterial activity was categorized according to Davis and Stout (1971) as very strong (>20 mm), strong (11-20 mm), moderate (5-10 mm), and weak (<5 mm).

RESULTS AND DISCUSSION

Results

Antibacterial activity

The antibacterial activity of pandan wangi leaf extract (*Pandanus amaryllifolius* Roxb.) against *S. aureus* and *E. coli* was evaluated using the disc diffusion method at extract concentrations of 25%, 50%, 75%, and 100%. Chloramphenicol was used as a positive control, while sterile distilled water was used as a negative control. Antibacterial activity was indicated by the formation of inhibition zones around the paper discs.

The inhibition zone data presented in Table 1 indicate that pandan wangi leaf extract exhibited different levels of antibacterial activity depending on bacterial type and extract concentration. Inhibition zones formed by pandan wangi leaf extract against *S. Aureus* and *E. coli* seen in Figure 1 and Figure 2.

Table 1. Inhibition zone diameter of pandan wangi leaf extract against *S. aureus* and *E. coli*.

Bacteria	Concentration	Replication			Mean (mm)	Category
		1	2	3		
S. aureus	Negative control	-	-	-	-	No inhibition zone
	25 %	-	-	-	-	No inhibition zone
	50 %	-	-	-	-	No inhibition zone
	75 %	0.03	-	0.03	0.02	Weak
	100 %	0.03	0.74	2.14	0.97	Weak

	Positive control	22.27	22.73	22.19	22.40	Very strong
E. coli	Negative control	-	-	-	-	No inhibition zone
	25 %	0.23	1.28	-	0.50	Weak
	50 %	1.61	1.19	2.54	1.78	Weak
	75 %	5.15	7.3	9.2	7.22	Moderate
	100 %	6.56	5.23	5.08	5.62	Moderate
	Positive control	22.61	20.83	20.23	21.22	Very strong

Note: (-) Negative: no inhibition zone was formed

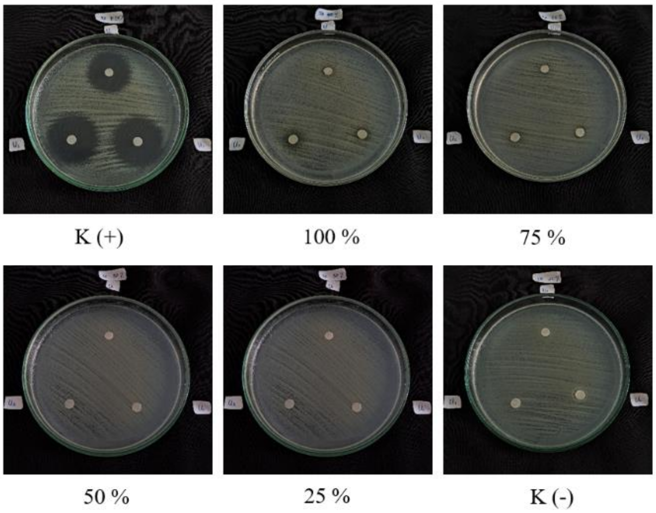

Figure. 1. Inhibition zones formed by pandan wangi leaf extract against *S. aureus*

Figure 1 shows the inhibition zones formed against *S. aureus* at different extract concentrations. The inhibition zones observed were relatively small, indicating weak antibacterial activity of pandan wangi leaf extract against *S. aureus*.

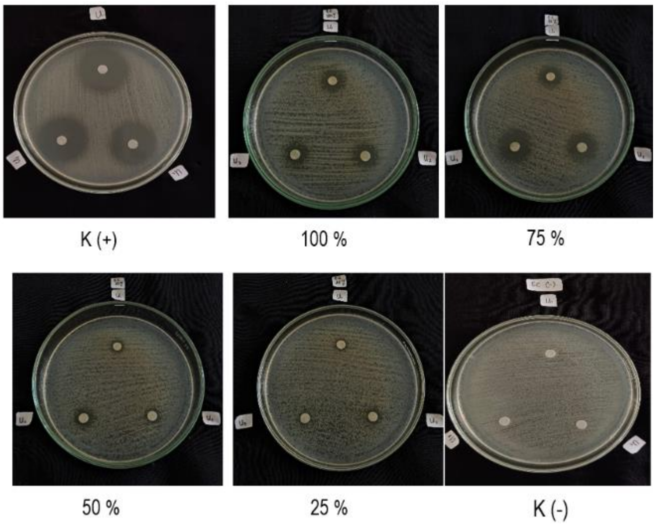

Figure. 2. Inhibition zones formed by pandan wangi leaf extract against *E. coli*

Figure 2 shows the inhibition zones formed against *E. coli* at different extract concentrations. Larger inhibition zones were observed at concentrations of 75% and 100%, indicating stronger antibacterial activity against *E. coli* compared to *S. aureus*.

Against *S. aureus*, inhibition zones were only observed at concentrations of 75% and 100%, with average diameters of 0.02 ± 0.02 mm and 0.97 ± 1.08 mm, respectively, which were categorized as weak inhibition according to Davis and Stout (1971). In contrast, the positive control chloramphenicol produced a strong inhibition zone of 22.40 ± 0.29 mm.

Meanwhile, the extract showed better antibacterial activity against *E. coli*. The inhibition zone increased from 0.50 ± 0.67 mm at 25% concentration to 7.22 ± 2.03 mm at 75% concentration. However, a slight decrease was observed at 100% concentration with an inhibition zone of 5.62 ± 0.82 mm. The positive control showed the highest inhibition zone with an average diameter of 21.22 ± 1.25 mm, while no inhibition zone was observed in the negative control.

Amylase activity

The amylase activity test results are presented in Figure 3. The amylase activity test showed that both *S. aureus* and *E. coli* did not produce clear zones on starch-containing media after iodine addition, indicating negative amylolytic activity. In contrast, the *Bacillus* sp. control produced a distinct clear zone, confirming positive amylase activity.

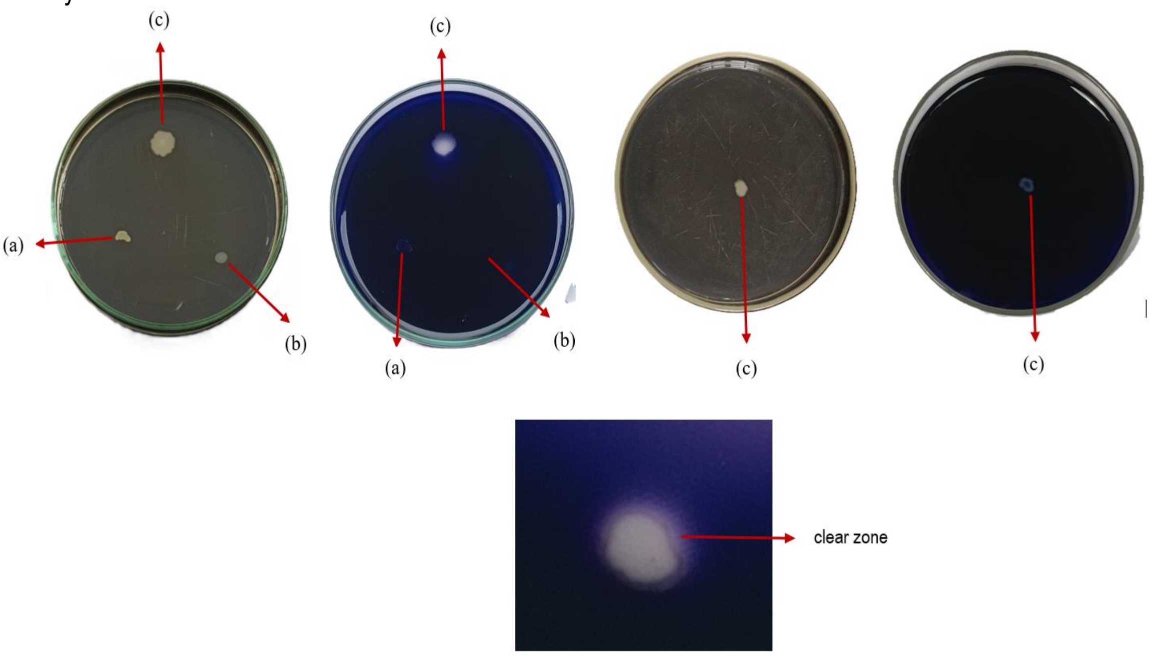

Figure 3. Amylase enzyme activity test of (a) *S. aureus*, (b) *E. coli*, and (c) *Bacillus* sp.

Discussion

Antibacterial activity

The increase in inhibition zone diameter from 25% to 75% concentration indicates that higher extract concentrations contained greater amounts of antibacterial compounds capable of inhibiting bacterial growth. However, the reduction in inhibition zone at 100% concentration may be related to the higher viscosity of the extract, which could reduce the diffusion rate of active compounds through the agar medium. Similar findings were reported by Dewi *et al.* (2018), who stated that highly concentrated extracts tend to diffuse less effectively in agar media, resulting in smaller inhibition zones.

Against *S. aureus*, the antibacterial activity of pandan wangi leaf extract was relatively low, as inhibition zones were only observed at concentrations of 75% and 100% with very small diameters. The weaker activity against *S. aureus* compared to *E. coli* may be related to differences in bacterial cell wall structure. *S. aureus*, as a Gram-positive bacterium, possesses a thick peptidoglycan layer that can limit the penetration of antibacterial compounds into bacterial cells (Bubonja *et al.*, 2020). In addition, differences in cell surface characteristics may reduce the interaction between bioactive compounds and bacterial cells, resulting in lower antibacterial effectiveness. These findings indicate that pandan wangi leaf extract was more effective against *E. coli* than against *S. aureus* under the conditions used in this study.

The inhibitory effect is associated with the presence of flavonoids, alkaloids, tannins, and saponins in pandan wangi leaves, which are known to interfere with cell membranes and protein synthesis. However, the overall effectiveness remains lower than that of standard antibiotics, as indicated by the relatively small inhibition zones.

Several factors may have influenced the relatively low antibacterial activity observed in this study. One important factor is the disc diffusion method itself, which depends on the ability of compounds to diffuse through agar media. Compounds with high molecular weight, high polarity, or increased viscosity may diffuse poorly, resulting in small inhibition zones despite possessing antibacterial potential (Zhydzetski *et al.*, 2024). In addition, the antibacterial activity of plant extracts can be influenced by extraction conditions, solvent type, environmental conditions during plant growth, plant age, and metabolite composition. Variations in these factors may affect the concentration and stability of active compounds extracted from pandan wangi leaves.

Amylase activity

The absence of clear zones suggests that *S. aureus* and *E. coli* were unable to hydrolyze starch under the test conditions. This finding indicates that these bacteria either do not produce extracellular amylase or produce the enzyme in very limited amounts. According to Madigan *et al*. (2018), both bacteria generally utilize simpler carbon sources such as glucose rather than complex carbohydrates like starch.

The negative amylase activity also suggests that enzymatic starch degradation did not contribute significantly to bacterial sensitivity toward pandan wangi leaf extract. Therefore, the inhibition observed in the antibacterial assay was more likely caused by the direct activity of bioactive compounds present in the extract rather than by interactions involving amylase production.

CONCLUSION

Pandan wangi (*Pandanus amaryllifolius* Roxb.) leaf extract exhibited antibacterial activity against both *E. coli* and *S. aureus*, with a more pronounced inhibitory effect on *E. coli*. The highest antibacterial activity against *E. coli* was achieved at a 75% concentration, yielding a 7.22 mm inhibition zone (moderate category), while the activity against *S. aureus* remained weak, reaching only 0.97 mm at 100% concentration. These findings confirm that the Gram-negative bacterium (*E. coli*) was more susceptible to the extract than the Gram-positive bacterium (*S. aureus*). Furthermore, both isolates demonstrated negative amylase activity, as evidenced by the absence of clear zones on starch media. This suggests that the observed antibacterial effects are primarily attributed to the bioactive compounds within the leaves rather than amylase production. Pandan wangi leaf extract can be used as an antibacterial agent.

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AUTHOR CONTRIBUTIONS

The author conducted the research, analyzed the data, and prepared the manuscript under the supervision and guidance of Sri Waluyo, Salman Farisi, and Mardi Santoso. The author has read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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