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EXPLORING THE ANTIBACTERIAL ACTIVITY OF BANANA PEEL WASTE FROM AGUNG SEMERU VARIETY IN LUMAJANG AGAINTS GRAM POSITIVE AND GRAM-NEGATIVE

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ABSTRAK

Banana peels, a by-product of banana consumption, remain underutilized despite their promising bioactive potential. This study aims to assess the antibacterial properties of the peel extract from the Agung banana variety (*Musa paradisiaca* var. *sapientum* L.) against two pathogenic bacterial strains: *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The extract was prepared through a maceration method, and antibacterial activity was tested using the disk diffusion technique. A Completely Randomized Design (CRD) was employed, involving five concentration levels (0%, 25%, 50%, 75%, and 100%) with four replicates each. Data were analyzed using ANOVA, followed by Duncan's Multiple Range Test (DMRT) to determine significant differences between treatments. The results demonstrated that the Agung banana peel extract significantly inhibited the growth of both test bacteria. The 100% concentration yielded the largest inhibition zones, with mean diameters of 17.55 ± 3.39 mm for *P. aeruginosa* and 19.25 ± 0.91 mm for *S. aureus*. According to the classification of antibacterial strength, these results are considered strong. These findings suggest that the Agung banana peel has considerable potential as a natural and eco-friendly antibacterial agent.

Keywords: Agung Semeru Banana Peel, Gram-Positive Bacteria, Gram-Negative Bacteria,

INTRODUCTION

Banana peels, an abundant form of agricultural waste, hold untapped potential, particularly in the field of phytopharmaceuticals. Despite this, they are commonly discarded or used merely as animal feed. However, previous studies have revealed that banana peels are rich in bioactive compounds with significant pharmacological value, including phenolics, saponins, and alkaloids (Sari *et al*., 2018). The presence of these compounds highlights banana peels as a promising natural resource with potential for further development, particularly in the fields of health and herbal medicine. As the demand for eco-friendly, natural-based products continues to grow, utilizing banana peel waste as a source of antibacterial agents offers a compelling alternative to support sustainability in both the health and agricultural sector.

Earlier studies have indicated that water-based extracts derived from Ambon banana peels can effectively lower blood glucose concentrations (Safari *et al.*, 2022). This effect has been linked to the presence of bioactive compounds such as flavonoids, phenolic compounds, and saponins (Sari & Susilo, 2017), which are known for their antioxidant and antimicrobial activities (Sari *et al*., 2019) (Sari *et al*., 2020). These findings suggest that banana peels—particularly those from specific cultivars possess considerable yet underexplored therapeutic potential. The integration of such natural, plant-derived waste products into functional health applications not only supports the advancement of phytopharmaceutical research but also aligns with sustainable development goals by promoting the valorization of agricultural by-products. Therefore, expanding the pharmacological screening of banana peel extracts may contribute significantly to the discovery of novel, eco-friendly therapeutic agents.

Other studies have confirmed that banana peels contain a variety of biologically active phytochemicals that function as antimicrobial agents. Among these are flavonoid compounds such as 5,6,7,4'-tetrahydroxy-3,4-flavonediol (Atun *et al.,* 2007)(Hariningsih & Hartono, 2022), as well as phytochemicals like phlobatannins, anthraquinones, and quinones (Wijayanti & Susilowati, 2017). In addition, secondary metabolites such as phenols, terpenes, alkaloids, and triterpenoids found in banana peels have shown antifungal potential (Sari & Susilo, 2017). Therefore, the utilization of banana peels not only offers promising value in the medical and health fields but also aligns with the principles of environmentally conscious waste management.

One of the promising local banana cultivars with high development potential is the Agung variety, a regional specialty from Lumajang Regency, East Java. This variety has become a prominent agricultural commodity, with increasing production rates each year. Most of the harvested fruit is consumed fresh or processed into products such as banana chips, which consequently generate a large volume of peel waste. Research has shown that the peels of Agung bananas contain phenolic compounds, terpenoids/steroids, saponins, and alkaloids—bioactive constituents with known antibacterial properties (Sari & Susilo, 2017).

Meanwhile, the rising incidence of infections caused by pathogenic bacteria remains a major public health concern in Indonesia. *Staphylococcus aureus* (a Gram-positive bacterium) and *Pseudomonas aeruginosa* (a Gram-negative bacterium) are among the most common pathogens responsible for skin infections, respiratory illnesses, and wound complications (Hanina *et al*., 2022) (Untu, 2019), both species are exhibiting increasing resistance to synthetic antibiotics (Sukertiasih et al., 2021). The situation underscores the urgent need to identify safer and more sustainable therapeutic alternatives, particularly those derived from natural sources. So, this study aims to assess the antibacterial properties of the peel extract from the Agung banana variety (*Musa paradisiaca* var. *sapientum* L.) against two pathogenic bacterial strains: *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

MATERIALS AND METHODS

Extraction of Banana Peel

The initial step involved thoroughly washing the peels of the Agung banana to remove any adhering dirt or debris. These pieces were then air-dried at room temperature until completely dehydrated. Once dried, the banana peels were ground using a blender to obtain a fine powder, which served as the raw material for the maceration process (Sari & Susilo, 2017). A total of 100 grams of the powdered peel was macerated in 300 mL of distilled water for 24 hours. Following maceration, the mixture was filtered using a Buchner funnel connected to a vacuum pump to separate the filtrate from the residue. The resulting filtrate was concentrated using a rotary vacuum evaporator to obtain a thick extract. This entire procedure was repeated six times to ensure an adequate volume of extract was obtained (Rahmi *et al.*, 2022).

Antibacterial Activity Assay

The antibacterial activity of the Agung banana peel extract was evaluated using the disk diffusion method. A total of 0.5 mL of bacterial suspension was poured into a sterile Petri dish, followed by the addition of 10 mL of sterile Nutrient Agar (NA) medium. The mixture was gently swirled to ensure uniform distribution and then allowed to solidify. The extract, previously dissolved in distilled water, was prepared at a series of concentrations: 100, 50, 25, 12.5, 6.2, and 3.1 mg/mL. Each concentration was transferred into separate test tubes (Sari *et al.*, 2023). Three sterile paper disks (14.20 mm in diameter) were placed into each tube and soaked in the extract solution for 10 minutes to ensure complete absorption. The disks were then drained by adhering them to the inner wall of the tube for another 10 minutes using sterile forceps.

Once ready, the disks were aseptically placed onto the solidified media surface in the Petri dishes. A negative control was prepared using disks moistened with distilled water, while the positive control involved disks containing 30 µg of tetracycline. Each treatment was replicated three times (triplicate), and each Petri dish contained three test disks. All plates were incubated at 37°C for 18 to 24 hours. After incubation, antibacterial activity was determined by measuring the diameter of the inhibition zone (clear zone) surrounding each disk using a digital caliper, with results expressed in millimeters (mm) (Sari *et al.*, 2020).

Data Analysis

The data collected, in the form of inhibition zone diameters from the Agung banana peel extract, were quantitatively analyzed using SPSS software version 20. A one-way analysis of variance (ANOVA) was performed at a 5% significance level, followed by the Duncan Multiple Range Test (DMRT) to identify significant differences between treatments. Prior to the ANOVA, the data were subjected to normality and homogeneity tests to ensure the validity of the statistical analysis.

RESULTS AND DISCUSSION

This study aimed to assess the antibacterial effectiveness of Agung banana peel extract in inhibiting the growth of two clinically significant pathogenic bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial assay was conducted using five different concentration levels of the extract: 0%, 25%, 50%, 75%, and 100%. As illustrated in Table 1., a consistent and concentration-dependent trend was observed, where higher extract concentrations corresponded to broader zones of bacterial growth inhibition. This suggests that the antibacterial compounds present in the banana peel extract exert a dose-responsive effect on microbial activity. Notably, the 0% concentration (negative control) exhibited no inhibitory activity, reaffirming that the observed antibacterial effects were attributable solely to the active constituents within the extract.

Table 1. Statistical analysis was conducted using one-way ANOVA at a 5% significance level to evaluate the effect of Agung Semeru banana peel extract on the inhibition zones of *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
Diameter Zona Hambat Pertumbuhan Staphylococcus aureus	Between Groups	601,607	4	150,402	62,094	,000
	Within Groups	24,222	10	2,422		
	Total	625,828	14			
Diameter Zona Hambat Pertumbuhan Pseudomonas aeruginosa	Between Groups	557,426	4	139,357	52,897	,000
	Within Groups	26,345	10	2,635		
	Total	583,771	14			

Table 1. reveals that extract concentrations ranging from 25% to 100% produced clearly visible and measurable inhibition zones against both tested bacterial strains, indicating a dose-dependent antibacterial activity of the Agung banana peel extract. In contrast, the 0% concentration, which served as the negative control, showed no inhibition zone formation, thereby confirming the absence of inherent antibacterial activity in the solvent medium

(distilled water) used. Among the tested concentrations, the 100% extract demonstrated the most pronounced inhibitory effect, with average inhibition zone diameters reaching 17.55 mm for *Pseudomonas aeruginosa* and 19.25 mm for *Staphylococcus aureus*.

These findings indicate that the antibacterial potency of the extract increases proportionally with concentration, and also suggest that the bioactive compounds present in the extract are effective in suppressing the growth of both Gram-negative and Gram-positive bacteria. Such results support the potential application of banana peel extract as a natural antibacterial agent, particularly in the development of eco-friendly alternatives to synthetic antibiotics. These findings reinforce the hypothesis that the antibacterial potency of the extract is directly proportional to its concentration. This dose-dependent response supports the theory that the bioactive compounds present in banana peels exert greater effects at higher concentrations (Kholifah *et al.*, 2018;Zakiya *et al.*, 2018;Khoyriah *et al.*, 2017) (Table 2.).

Table 2. Mean inhibition zone diameters produced by Agung Semeru banana peel extract against the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* at various concentrations.

Concentration	P. aeruginosa (mm)	S. phylococcusaureus (mm)
0%	$0,00 \pm 0,00^{a}$	$0,00 \pm 0,00^{a}$
25%	$12,62 \pm 1,03^{b}$	$11,83 \pm 1,25^{b}$
50%	$14,02 \pm 0,40^{bc}$	$13,2 \pm 1,28^{b}$
75%	$14,62 \pm 0,68^{b}$	$13,8 \pm 2,84^{b}$
100%	$17,55 \pm 3,39^{a}$	$19,25 \pm 0,91^{c}$

Note: Means followed by the same letter are not significantly different based on Duncan's Multiple Range Test (DMRT) at the 5% significance level.

Overall, all treatments using extract concentrations of 25% and above exhibited consistent antibacterial activity, as indicated by the formation of clear inhibition zones around the paper disks. This antibacterial response was consistent across both bacterial species, with the strongest effect observed at the 100% concentration. These results are graphically illustrated in **Figure 1**, which presents the differences in inhibition zone diameters among the treatment groups. This finding further supports the assumption that secondary metabolites present in the Agung banana peel—such as phenols, saponins, alkaloids, and terpenoids/steroids—actively contribute to antimicrobial inhibition mechanisms (Sari & Susilo, 2017). The extract's ability to inhibit the growth of both Gram-positive and Gram-negative bacteria suggests that this banana peel variety possesses a broad-spectrum antibacterial potential. Therefore, it holds promise as a natural and eco-friendly antibacterial agent, with significant potential to contribute to local-based pharmaceutical sustainability.

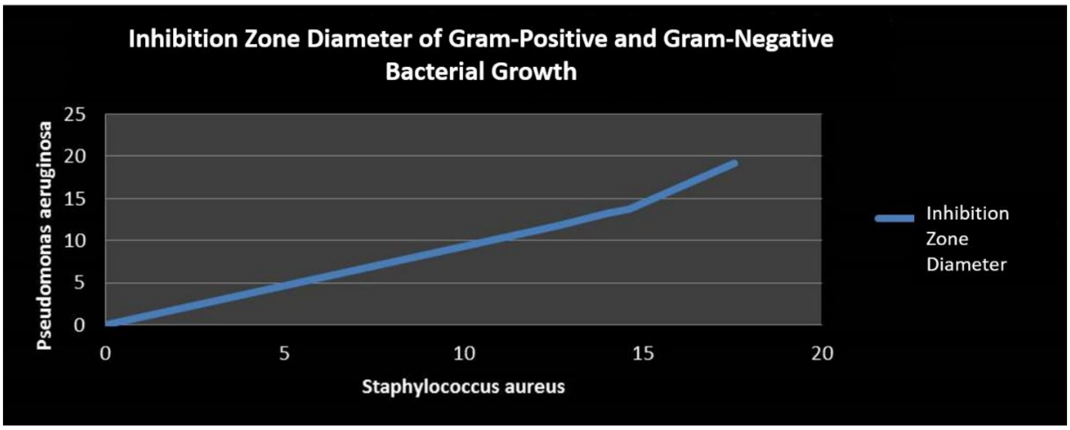

Figure 1. Correlation Graph between the Concentration of Agung Semeru Banana Peel Extract and The Diameter of Inhibition Zones (mm).

One-way ANOVA at a 5% significance level revealed that different extract concentrations had a significant effect on the size of the inhibition zones formed. This analysis was followed by Duncan's Multiple Range Test (DMRT) to compare the treatments more specifically. The results indicated significant differences among the concentration groups. The DMRT was used to classify the treatments based on their effectiveness and to identify which concentration exhibited the most substantial antibacterial activity.

Data analysis using one-way ANOVA at a 5% significance level demonstrated that variations in the concentration of Agung banana peel extract had a significant effect on the width of the inhibition zones formed around the discs. Further analysis using Duncan's Multiple Range Test (DMRT) revealed significant differences among the treatment groups, indicating that each concentration exhibited varying levels of effectiveness in inhibiting bacterial growth. This test is crucial for classifying treatments more specifically based on their effectiveness and for identifying which concentration had the greatest impact on antibacterial activity.

In assessing antibacterial efficacy, the classification standard proposed by Davis and Stout (1971) was used as a reference: inhibition zones <5 mm are considered weak, 5–10 mm moderate, 10–20 mm strong, and >20 mm very strong. Based on the inhibition zone diameters presented in Table 1, the antibacterial activity of the Agung banana peel extract falls into the "strong" category for both bacterial species, especially at the 100% concentration. These findings indicate that the bioactive compounds present in the extract hold promising potential for further development as natural antimicrobial agents (Sartika et al., 2019).

Moreover, a comparison between *Staphylococcus aureus* and *Pseudomonas aeruginosa* revealed that the Gram-positive bacterium (*S. aureus*) tended to exhibit larger inhibition zones than the Gram-negative (*P. aeruginosa*), particularly at higher extract concentrations. This suggests that the cell wall of Gram-positive bacteria is more susceptible to the active compounds in the extract. This observation aligns with the theory that differences in peptidoglycan structure and the presence of an outer membrane in Gram-negative bacteria influence their sensitivity to antibacterial agents (Yunita & Ratna Ayu P, 2020). It also supports the notion that the structure and composition of bacterial cell walls play a critical role in determining their vulnerability to antimicrobial compounds (Irawati *et al*., 2024).

CONCLUSION

Based on the results of this study, it can be concluded that the extract of *Agung Semeru* banana peel from Lumajang exhibits significant antibacterial activity against two tested bacterial strains: *Pseudomonas aeruginosa* (Gram-negative) and *Staphylococcus aureus* (Gram-positive). The application of the extract at various concentrations (0%, 25%, 50%, 75%, and 100%) produced measurable zones of inhibition, with the highest effectiveness observed at the 100% concentration level. At this concentration, the average inhibition zones were 17.55 ± 3.39 mm for *P. aeruginosa* and 19.25 ± 0.91 mm for *S. aureus*, making it the most effective in suppressing the growth of both bacterial species..

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