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Research Article

Utilization of Muli Banana plant roots (*Musa acuminata* Linn.) as an antibiotic and antiseptic

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ABSTRACT

Diseases caused by bacteria can usually be combated by administering antibiotics and using antiseptics. However, there are not a group of pathogen, such *Escherichia coli* as well as *Staphylococcus aureus*, which are highly resistant to the antibiotics, such as amoxicillin, penicillin, tetracycline and chloramphenicol. One of the efforts to control these bacteria is by using bioactive compounds derived from plants. Banana plants contain secondary metabolite compounds flavonoids, saponins, and tannins that can act as anti-infective agents, including muli banana (*Musa acuminata* Linn.). Banana plant roots as natural ingredients can be an alternative to antiseptics because they contain antibacterial compounds with milder side effects than long-term use of alcohol-based antiseptics that can reduce skin moisture. This study was conducted to determine the efficacy of banana muli root extract (*M. acuminata* Linn.) as an antibiotic and antiseptic. The hypothesis in this study is that the root extract at a certain concentration is thought to have effectiveness as an antibiotic (*E. coli*) and antiseptic (*S. aureus*). In this study, the Kirby-Bauer method was applied by agar diffusion and the results of the inhibition zone test were formed around the paper disc. The results showed that the optimum efficacy of muli banana root extract (*M. acuminata* Linn.) as an antibiotic (*E. coli* ATCC 25922) was at a concentration of 95% (2.96 mm) and as an antiseptic (*S. aureus* ATCC 25923) at a concentration of 75% (9.1 mm).

Keywords: antibiotics, antiseptics, *E. coli*, banana muli, *S. aureus*.

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INTRODUCTION

Banana is a crop that is widely distributed throughout Indonesia, with the largest production coming from East Java (29%), Lampung (20%), West Java (15%), and Central Java (9%) (Yuliastuti et al. 2020). The plant is easy to find as it is adaptable and does not require a specific habitat (Advinda et al. 2018). One of its varieties, banana muli, is typical of Lampung and has been registered with the Ministry of Agriculture (Diskominfotik Provinsi Lampung, 2023).

Plants produce secondary metabolites such as flavonoids, saponins and tannins, which act as anti-infectives and natural defence mechanisms (Holifah et al. 2020; Kanedi et al. 2021; Rumidatul et al. 2018). The production of these metabolites increases in response to environmental stress. Banana roots have the highest content of secondary metabolites with an antibacterial effect after the stem (Ningsih et al. 2013), as they act as the first defense organ against pathogens (Rumidatul et al. 2018). The effectiveness of antimicrobial compounds is influenced by physicochemical properties, characteristics of target microorganisms, and environmental conditions (Sumardi et al. 2013).

The mechanism action of these compounds varies. Flavonoids damage the bacterial cell membrane through protein denaturation, causing cell death. Saponins damage cell membranes by reducing surface tension, increasing permeability, and causing cell death. Tannins bind to proteins, causing shrinkage of the bacterial cell membrane and inhibiting its growth (Hidayatullah and Mourisa, 2023).

Two pathogenic bacteria that are often of concern are *S. aureus* and *E. coli*. *S. aureus* bacteria are Gram-positive bacteria that live saprophytes in the human body and can cause various infections such as wounds, meningitis, and pneumonia (Febrianasari, 2018). *E. coli* bacteria are Gram-negative bacteria that are commonly found in the human gut and can cause urinary tract infections or diarrhea (Firizki, 2013). Treatment of bacterial infections often uses antibiotics. However, misuse of antibiotics causes antibiotic resistance due to lack of knowledge and inappropriate doses (Pambudi& Utari, 2020). Genetic changes (mutations) in bacteria can make antibiotics ineffective (Dinas Kesehatan Kabupaten Jayapura, 2024). Some pathogenic bacteria such as *Pseudomonas* sp., *Klebsiella* sp., and *E. coli* show high levels of resistance to amoxicillin, tetracycline, Chloramphenicol, and penicillin (Refdanita et al. 2004).

Hand washing with soap or antiseptic is more effective in killing germs than water alone. Alcohol-containing antiseptics are efficient at killing germs, but continued use can lead to dry skin. Alternatively, banana plant roots can be utilized as a natural antibacterial agent thanks to their flavonoids, saponins and tannins content, with milder side effects (Holifah et al. 2020).

Previous research by Ningsih et al. (2013) showed a satisfactory inhibition zone of yellow kepok banana root extract (*M. paradisiaca* Linn.) against the growth of *S. aureus* and *E. coli*. However, the efficacy of muli banana root extract (*M. acuminata* Linn.) as an antibiotic (*E. coli*) and antiseptic (*S. aureus*) remains largely unknown. The research objective was therefore to identify the effectiveness of muli banana root extract as an antibiotic and antiseptic.

METHODS

Sample Preparation

Banana muli roots from Bandar Lampung that were not rotten or dry were cleaned, cut into small pieces (1-2 mm), dried for 1 week, and then oven dried at 37 °C. This dried sample was then processed into simplisia.

Extraction and Concentration Variation

Simplisia were soaked in 70% ethanol (ratio 1:3 or 1:5) for 3 days at room temperature, protected from light and shaken from time to time. The filtrate was then concentrated with a rotary evaporator and water bath to obtain a thick extract. The extract was then placed in a sterilised container in the refrigerator.

Preparation of Control Solution

Negative control: sterile distilled water was used to soak the paper discs for 24 hours. Positive control antibiotics: Chloramphenicol 250 mg powder with a concentration of 14,280 µg (1 g/70 mL) was homogenized and sterile paper discs were soaked in it. Antiseptic positive control: sterile paper disks were soaked in Dettol Antiseptic for 24 hours.

Preparation of NA (Nutrient Agar) Media

NA media was made by dissolving 7.4 g of media in 370 mL of distilled water, heating until dissolved, and then autoclaving for 30 minutes at 121°C and 2 atm pressure. The medium was then poured into plates for the antibiotic and antiseptic tests, each containing 7 sterile petri dishes.

Preparation of Test Bacterial Suspension

0.9% NaCl solution (0.0189 g NaCl in 21 mL sterile distilled water) was sterilised. To prepare the bacterial suspension, a bacterial culture (1 round ose) was taken and suspends in 10 mL of 0.9% NaCl until the turbidity meets 0.5 McFarland standard (equals to 1.5 x 10^8 CFU/mL) (Primadiamanti et al. 2022).

Antibiotic and Antiseptic Activity Test (Paper Disc Diffusion Method)

Antibiotic test: 0.1 ml of the bacterial suspension was added to a petri dish containing NA media (negative control sterile distilled water, root extract concentration 80%, 85%, 90%, 95%, 100%, and positive control chloramphenicol). Sterilised paper discs were placed on top of the media and all dishes incubated at 37°C for 24 hours. Inhibition zone diameter is measured for 5 days. Antiseptic activity test: Same procedure as the antibiotic test, but with a root extract concentration of 60%, 65%, 75%, 80%, 85%, and a positive control with the antiseptic Dettol. The inhibition zone was also measured for 5 days (Ningsih et al. 2013). Data analysis was conducted using SPSS Statistics 27.0.1 with the Kruskal-Wallis test (non-parametric test).

RESULTS AND DISCUSSION

Antibiotic Effectiveness of Muli Banana Root

Muli banana root extract showed inhibition against *E. coli* ATCC 25922 at all concentrations tested (80%, 85%, 90%, 95%, and100 %), characterised by the formation of a clear zone around the disc. The positive control Chloramphenicol also showed inhibition. The highest mean inhibition zone diameter for *E. coli* was found at 95% concentration (2.96 mm), followed by 85% (2.66 mm), 100% (1.15 mm), 90% (1.08 mm), and 80% (0.56 mm). The zone of inhibition of Chloramphenicol was 2.36 mm.

Antiseptic Effectiveness of Muli Banana Root

As an antiseptic, muli banana root extract only showed inhibition against *S. aureus* ATCC 25923 at 75% concentration, with an average inhibition zone diameter of 9.1 mm. Other concentrations (60%, 65%, 80%, and 85%) showed no inhibition. The positive control of Dettol Antiseptic showed a much larger inhibition zone of 39.33 mm. The results of the antibiotic inhibition zone test and antiseptic test can be seen in Figure 1 - Figure 6. The average diameter of the antibiotic test zone (*E. coli* ATCC 25922) and antiseptic test zone (*S. aureus* ATCC 25923) measured using a vernier caliper with an accuracy of 0.05 mm during 5 days of observation can be seen in Figure 7.

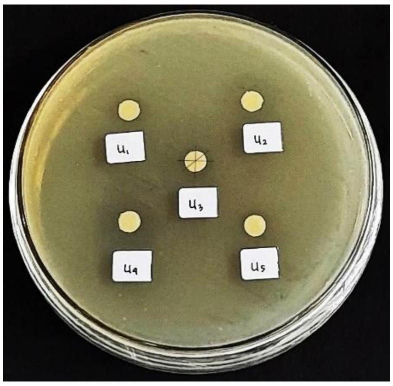

Figure 1. Positive control of antibiotic tests (chloramphenicol)

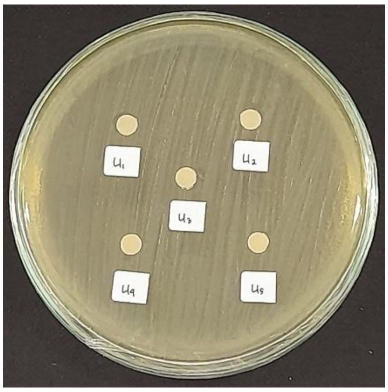

Figure 2. Negative control of antibiotic tests (sterile distilled water)

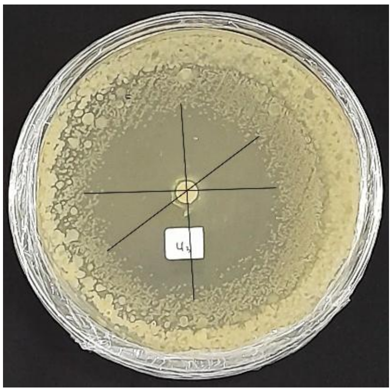

Figure 3. Positive control of antiseptic tests (Dettol antiseptik)

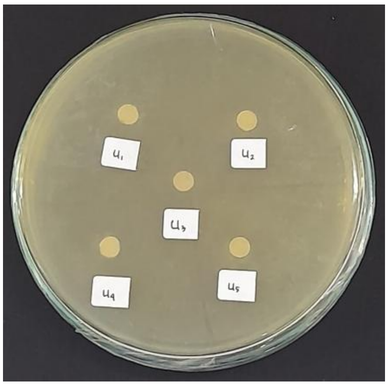

Figure 4. Negative control of antiseptic tests (sterile distilled water)

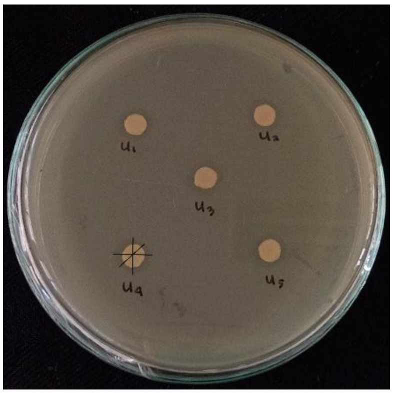

Figure 5. Antibiotics
95% extract concentration

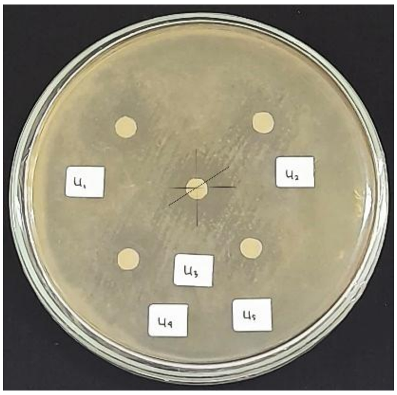

Figure 6. Antiseptic
75% extract concentration

Note: U1 = 1st retake; U2 = 2nd retake; U3 = 3rd retake; U4 = 4th retake; U5 = 5th retake

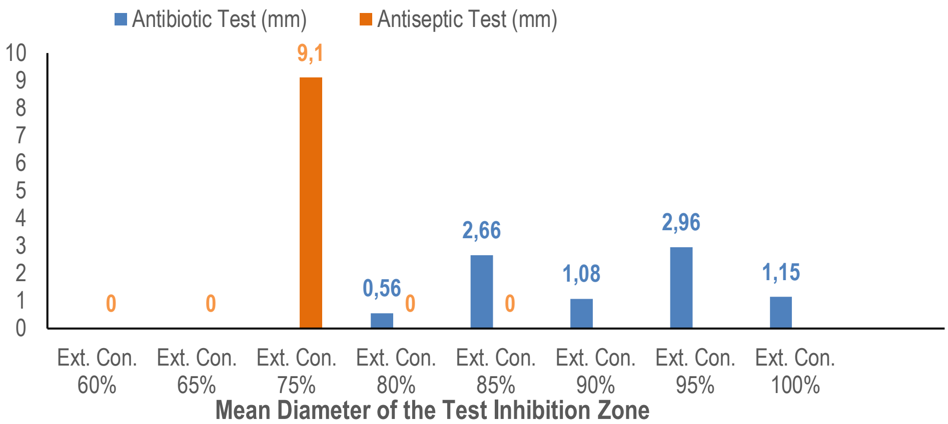

Figure 7. Mean diameter of the test inhibiton zone

Table 1. Mean diameter of the test inhibition zone

	Concentration (%)	Average Zone of Inhibition Test Diameter (mm)
Antibiotic Test (*E. coli* ATCC 25922)	80	0,56
	85	2,66
	90	1,08
	95	2,96
	100	1,15
Antiseptic Test (*S. aureus* ATCC 25923)	60	0
	65	0
	75	9,1
	80	0
	85	0

Table 2. Descriptive statistics test

	N	Mean	Std. Deviation
Treatment	70	7,50	4,060
Value	70	158,7620	715,02856
Valid N (listwise)	70		

Research Hypothesis Testing

Antibiotic Test

Based on mean rank analysis from Kruskal-Wallis test, 95% concentration of muli banana root extract showed initial indication as the optimal concentration in inhibiting *E. coli* ATCC 25922 bacteria. To statistically confirm the optimal effectiveness of the 95% concentration, a pairwise comparison test between treatments was conducted using Mann-Whitney analysis (with Bonferroni correction for significance adjustment). The Mann-Whitney test results showed: Negative control (sterile distilled water) was significantly different from 85% extract concentration ($p<0.05$, i.e. 0.005), 95% concentration ($p<0.05$, i.e. 0.002), and positive control Chloramphenicol ($p<0.05$, i.e. 0.002). Statistically, the 95% concentration was proven to provide optimal effectiveness as an antibiotic against *E. coli* ATCC 25922.

Table 3. Pairwise comparison of antibiotic tests: Mann-Whitney Analysis

Test Sample	Comparison Sample	Adj. Sig. Value (0,050)
Sterile distilled water (-)	Extract concentration 80%	1,000
	Extract concentration 90%	1,000
	Extract concentration 100%	0,635
	Extract concentration 85%	0,005*
	Extract concentration 95%	0,002*
	Chloramphenicol (+)	0,002*
Extract concentration 80%	Extract concentration 90%	1,000
	Extract concentration 100%	1,000
	Extract concentration 85%	0,112
	Extract concentration 95%	0,065
	Chloramphenicol (+)	0,059
Extract concentration 90%	Extract concentration 100%	1,000
	Extract concentration 85%	1,000
	Extract concentration 95%	0,770
	Chloramphenicol (+)	0,714
Extract concentration 100%	Extract concentration 85%	1,000
	Extract concentration 95%	1,000
	Chloramphenicol (+)	1,000
Extract concentration 85%	Extract concentration 95%	1,000
	Chloramphenicol (+)	1,000
Extract concentration 95%	Chloramphenicol (+)	1,000

Antiseptic Test

Mean rank analysis (Kruskal-Wallis test) on inhibition zone diameter data showed that 75% concentration of muli banana root extract was the initial optimal concentration as an antiseptic against *S. aureus* ATCC 25923 bacteria. To statistically confirm this optimality, a Mann-Whitney pairwise comparison test (with Bonferroni correction) was conducted. The results showed that. The positive control Dettol Antiseptic was significantly different from the negative control (sterile distilled water), as well as the extract concentrations of 60%, 65%, 80%, and 85% ($p<0.05$ for all comparisons). Based on data analysis of Mann-Whitney test comparison, 75% concentration statistically provides optimal effectiveness as an antiseptic against *S. aureus* ATCC 25923 bacteria.

Table 4. Pairwise comparison of antiseptic tests: Mann-Whitney Analysis

Test Sample	Comparison Sample	Adj. Sig. Value (0,050)
Sterile distilled water (-)	Extract concentration 60%	1,000
	Extract concentration 65%	1,000
	Extract concentration 80%	1,000
	Extract concentration 85%	1,000
	Extract concentration 75%	0,078
	Dettol Antiseptik (+)	0,002*
Extract concentration 60%	Extract concentration 65%	1,000
	Extract concentration 80%	1,000
	Extract concentration 85%	1,000
	Extract concentration 75%	0,078
	Dettol Antiseptik (+)	0,002*
Extract concentration 65%	Extract concentration 80%	1,000
	Extract concentration 85%	1,000
	Extract concentration 75%	0,078
	Dettol Antiseptik (+)	0,002*
Extract concentration 80%	Extract concentration 75%	0,078
	Extract concentration 85%	1,000
	Dettol Antiseptik (+)	0,002*
Extract concentration 85%	Extract concentration 75%	0,078
	Dettol Antiseptik (+)	0.002*
Extract concentration 75%	Dettol Antiseptik (+)	1,000

Secondary Metabolite Compounds of Muli Banana Root as Antibacterial

The roots of muli banana plants commonly contain flavonoids, saponins, and tannins, which are known as anti-infective agents. The inhibition of microorganisms by antimicrobial compounds can occur in various ways: rupture of the bacterial cell wall, increased permeability of the cell membrane so that cellular components can escape, inactivation of enzymes or damage to genetic material (Susanti & Thriandhany, 2024).

Flavonoids are one of the most abundant groups of secondary metabolite compounds found in plants and function as antimicrobials against bacteria, fungi, and viruses (Ningsih et al. 2023). Mechanisms include:1.) Inhibits cell membrane function; flavonoids form complexes with extracellular proteins, damage bacterial cell membranes, and disrupt intracellular compounds (Sujana et al. 2024). 2.) Disrupts bacterial energy metabolism; damage to cell membrane permeability causes leakage of cell components, resulting in lysis and death of bacterial cells (Brooks et al. 2007).

Saponins have an antibacterial effect, diffusing through external membranes and cell walls and then binding to the cytoplasm membrane. This disrupts and reduces the stability of the bacterial membrane, leading to leakage of cell fluid and ultimately causing death of the bacterial cell. Saponins are often used as antiseptics for infections of the skin, mucous membranes and wounds (Fatmawaty et al. 2017).

Tannins inhibit the growth of microorganisms. Their antibacterial mechanism includes: 1) Lysis of the bacterial cell; tannins target the polypeptide wall of the bacterial cell, which leads to incomplete cell wall formation and cell death. 2) Inactivation of bacterial enzymes (Hayon et al. 2023).

Concentration Relationship of Muli Banana Root Extract with Antibacterial Effectiveness

The zone of inhibition formed around the disc of muli banana root extract (which indicates the absence of bacterial growth) is caused by the content of secondary metabolite compounds such as flavonoids, saponins, and tannins (Putri et al. 2016). In generally, the highest the concentrations of the extract, the more ability the effective ingredient has to inhibit bacterial growth, indicating bacteriostatic properties (Triastinurmiatiningsih et al. 2015). However, this study shows that there are limitations to the relationship between concentration and effectiveness; in the antibiotic test, a concentration of 95% (zone of inhibition 2.96 mm) produced a zone of inhibition greater than 100% (1,15 mm). In the antiseptic test, a concentration of 75% (9.1 mm inhibition zone) was optimal, while 80% and 85% produced no inhibition zone at all.

This may be because too high extract concentrations can reduce the diffusion of active substances into the test medium. The more concentrated the extract, the less bioactive substances can diffuse, so the inhibitory effect is reduced. In addition, concentrations that are too low, such as 60% and 65% in the antiseptic test, also show no inhibition because they are not strong enough to change the physiological system of bacterial cells (Warokka et al. 2016). The formation and difference in the diameter of the inhibition zone are affected by several factors, including the amount and nature of the active substance, the characteristics and concentration of the bacteria, the viscosity of the medium, the diffusion ability of the antibacterial substance, and the environmental conditions of the test (Sari et al. 2014).

Differences in Bacterial Cell Wall Structure and Antibacterial Sensitivity

The difference in the inhibitory power of plantain root extract compared to *E. coli* (Gram negative) and *S. aureus* (Gram positive) is due to the different structure of the plantain root extract and cell wall composition of the bacteria, which affected the specific susceptibility to antimicrobial agents. The complex and nonpolar Gram negative cell wall is more difficult to penetrate, while the simpler and polar Gram positive cell wall is more easily penetrated by antibacterial compounds from muli banana root extract.

Gram Negative Bacteria (*E. coli* ATCC 25922)

E. coli has a complex cell wall with three layers: lipoproteins (outer), lipopolysaccharides (middle, serving as a barrier), and thin peptidoglycan (inner, lipid-rich) (Rini et al. 2017). The outer membrane is a bilayer of phospholipids and lipopolysaccharides that are nonpolar. This nonpolar nature makes it more difficult for antibacterial compounds from 70% ethanol extract to penetrate the cell, so *E. coli* is more resistant and requires a higher concentration of extract to show optimal antibacterial activity (Sudewi & Lolo, 2016).

Gram Positive Bacteria (*S. aureus* ATCC 25923)

S. aureus is generally more sensitive to antibacterial agents because its cell wall is more simple, consisting mostly of a thick layer of peptidoglycan (about 90%) and a thin layer of teichoic acid. The water-soluble pond acid showed that the cell wall of Gram-positive bacteria is polar. This polar nature makes it easier for polar compounds such as flavonoids and tannins from the extract to pass through the cell wall. So, *S. aureus* showed higher antibacterial activity even at lower extract concentrations, as seen in the test results of 75% extract.

CONCLUSION

From the results of research, the optimal effectiveness of muli banana root extract (*M. acuminata* Linn.) as an antibiotic (*E. coli* ATCC 25922) is found at a concentration of 95% (2,96 mm). Optimal effectiveness of muli banana root extract (*M. acuminata* Linn.) as an antiseptic (*S. aureus* ATCC 25923) is at a concentration of 75% (9.1 mm).

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AUTHOR CONTRIBUTIONS

All authors have read and approved the manuscript to be published.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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