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Application of n-hexane extract from muli banana (*Musa acuminata* Colla.) roots as an antibacterial and antiseptic agent

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Abstract

Antimicrobial resistance (AMR) continues to pose a severe threat to global health, driving the urgent need for novel, plant-derived antimicrobial agents. This study evaluated the antibacterial potential of the n-hexane extract of muli banana (*Musa acuminata* Colla.) roots against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) to determine its viability as an alternative natural antibiotic and topical antiseptic. Following maceration in n-hexane, the extract underwent phytochemical screening and antibacterial evaluation using the agar well diffusion method at concentrations ranging from 60% to 100%. Phytochemical analysis revealed the presence of lipophilic alkaloids, flavonoids, and terpenoids. The extract exhibited significant, dose-dependent antibacterial activity against both tested pathogens ($p < 0.05$). The highest efficacy was observed at the 100% concentration, which yielded maximum inhibition zones of 26.56 mm against *E. coli* and 17.49 mm against *S. aureus*. These findings suggest that the lipophilic secondary metabolites present in the muli banana root extract hold considerable promise as effective natural antimicrobial agents capable of targeting and disrupting complex bacterial cell membranes.

Keywords: *Musa acuminata* Colla.; Lipophilic metabolites; Natural antibiotic; *Staphylococcus aureus*; *Escherichia coli*

1. Introduction

Antimicrobial resistance (AMR) has emerged as a severe and escalating threat to global human, animal, and environmental health. Consequently, the World Health Organization (WHO) has designated AMR as one of the ten greatest public health threats facing humanity. This phenomenon occurs when microorganisms develop genetic or physiological adaptations that render conventional antimicrobial drugs ineffective, thereby increasing disease duration, healthcare costs, and mortality rates, particularly within developing nations [1]. The irrational and widespread use of antibiotics continues to accelerate the development of resistance, critically narrowing available therapeutic options.

Pathogens such as *Staphylococcus aureus* and *Escherichia coli* are notorious for causing a wide array of serious clinical infections. Specifically, Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains are responsible for severe skin and systemic infections, often forming robust biofilms that further enhance their antibiotic resistance. Concurrently, certain *Escherichia coli* strains produce Extended-Spectrum Beta-Lactamase (ESBL) enzymes, significantly reducing the efficacy of β-lactam antibiotics [2]. The persistent proliferation of these resistant strains underscores the urgent demand for the development of safe and effective alternative antimicrobial agents.

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Plant-derived compounds offer a highly promising avenue for exploration. Banana plants are known to contain bioactive constituents such as flavonoids, saponins, tannins, and alkaloids, which exhibit recognized antibacterial potential [3]. These compounds work by inhibiting cell wall synthesis, disrupting membrane permeability, and inactivating microbial enzymes. Kepok banana stem extract can inhibit the growth of *Staphylococcus aureus* and *Escherichia coli* [4]. However, research on the roots is still limited, requiring further exploration. Plant roots, which interact directly with the soil environment, have the potential to produce secondary metabolites as a natural defense mechanism against pathogenic microorganisms. This study aims to evaluate the potential of the n-hexane extract of muli banana (*Musa acuminata* Colla.) roots as an antibiotic against *Escherichia coli* and as an antiseptic against *Staphylococcus aureus*. The results of this study are expected to contribute scientifically to the development of effective, safe, and sustainable natural antimicrobial sources as an alternative to addressing the problem of antimicrobial resistance.

2. Materials and Methods

2.1. Materials

The primary sample used was the roots of the muli banana (*Musa acuminata* Colla.). Solvents and reagents included n-hexane, Dragendorff's reagent, hydrochloric acid (HCl), concentrated sulfuric acid (H2SO4), glacial acetic acid (CH3COOH), magnesium metal powder, and iron (III) chloride (FeCl3). Microbiological assays utilized Nutrient Agar (NA) media, 0.9% NaCl solution, chloramphenicol (300 ppm), Dettol antiseptic, 70% alcohol, and distilled water. Bacterial isolates of *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922) were used as test organisms.

2.2. Instruments

The equipment used included an analytical balance, rotary evaporator, Biological Safety Cabinet (BSC), autoclave, incubator (37±1°C), magnetic stirrer, vortex mixer, water bath, hot plate, pH meter, Bunsen burner, vernier caliper, and microscope. Standard laboratory glassware and consumables (Petri dishes, test tubes, Erlenmeyer flasks, micropipettes, sterile cotton swabs, and inoculation loops) were also utilized.

2.3. Sample Preparation

Fresh roots of the muli banana (6 kg) were collected from healthy, dry plants that had ceased fruiting in the Gisting area, Tanggamus, Lampung Province. The roots were washed thoroughly under running water to remove soil and debris, cut into 2–3 cm pieces, and air-dried at room temperature for one week. The samples were subsequently oven-dried at 37 °C until completely dehydrated. The dried roots were ground using a blender to produce a fine powder (simplicia). Approximately 400 g of the dried powder was obtained and stored in a tightly sealed, dry container protected from light prior to extraction [5].

2.4. Preparation of Muli Banana Root Extract

The extraction was performed using the maceration method [6]. A total of 400 g of the dried root powder was macerated in n-hexane at a ratio of 1:10 (w/v) in a sealed container for 3 x 24 hours at room temperature, with occasional stirring. The mixture was filtered using filter paper, and the residue was re-macerated using the same solvent volume to ensure maximum extraction of active compounds. All filtrates were combined and concentrated using a rotary evaporator at 40–50°C until a viscous extract was obtained. This process yielded approximately 8 mL of crude extract, which was stored in a sealed glass bottle at 4 °C until use.

2.5. Phytochemical Screening

The crude extract was subjected to standard phytochemical screening to identify the presence of secondary metabolites, including alkaloids (Dragendorff's test), flavonoids (Magnesium and HCl test), terpenoids (Liebermann-Burchard test / glacial acetic acid and H2SO4), saponins (froth test), and tannins (FeCl3 test) [7].

2.5.1. Alkaloid Test

1 mL of the extract was placed into a test tube, followed by the addition of 1 mL of hydrochloric acid (HCl) and 2-3 drops of Dragendorff's reagent. The formation of brown-orange precipitates indicates the presence of alkaloids.

2.5.2. Flavonoid Test

1 mL of the extract was placed into a test tube, and 0.5 mL of concentrated hydrochloric acid (HCl) along with a small amount of magnesium powder (Mg) were added. The mixture was gently shaken, and the color change was observed. The appearance of red, orange, or purple color indicates the presence of flavonoids.

2.5.3. Terpenoid Test

1 mL of extract was mixed with 10 drops of acetic anhydride ($(CH3CO)_2O$) and 3 drops of concentrated H_2SO_4. The mixture was gently shaken and allowed to stand for 5-10 minutes. The formation of a red, purple, or blue-green color indicated the presence of terpenoids.

2.5.4. Saponin Test

A 0.5 mL aliquot of the extract was mixed with 5 mL of hot distilled water in a test tube. After cooling, the mixture was shaken vigorously for 10 seconds. The formation of stable foam (1-3 cm in height) that persisted for more than 10 minutes and did not disappear upon the addition of 2 N HCl indicated the presence of saponins.

2.5.5. Tannin Test

1 mL of extract was mixed with 10 mL of hot distilled water, heated for 5 minutes and filtered. The filtrate (1 mL) was mixed with 2-3 drops of 1% $FeCl_3$ solution. The formation of a dark blue, green, or blackish-green color indicated the presence of tannins.

2.6. Determination of Antibacterial Activity

Antibacterial activity was evaluated using the agar well diffusion method on Nutrient Agar (NA) against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923).

2.6.1. Inoculum Preparation

Pure bacterial cultures were streaked onto NA slants and incubated at 37°C for 24 hours. The bacterial suspension was prepared by suspending one inoculation loop of the isolate into 9 mL of sterile 0.9% NaCl solution, homogenized until the turbidity matched the 0.5 McFarland standard (equivalent to approximately 1.5 × 10^8 CFU/mL) [8].

2.6.2. Antibacterial Assay

The bacterial suspension was evenly swabbed onto the surface of the NA plates. Wells were punched into the agar, and 0.1 mL of the banana root extract at varying concentrations (60%, 70%, 80%, 90%, and 100% diluted in n-hexane) was introduced into each well. Chloramphenicol (300 ppm) and Dettol were used as positive controls for antibiotic and antiseptic activity, respectively, while pure n-hexane served as the negative control. The plates were incubated at 37°C for 24 hours. The diameter of the zone of inhibition formed around each well was measured using a vernier caliper [8].

2.7. Statistical Analysis

Data analysis was performed using IBM SPSS Statistics 29.0.2. The normality of the data was assessed using the Shapiro-Wilk test. Normally distributed data ($p > 0.05$) were analyzed using a One-Way ANOVA, followed by a Tukey HSD post hoc test to determine significant differences between treatment groups. Non-normally distributed data ($p < 0.05$) were analyzed using the Kruskal-Wallis test followed by the Mann-Whitney test [9].

3. Results and Discussion

3.1. Phytochemical Profile of Muli Banana Root Extract

Table 1 Phytochemical test results of n-hexane extract of muli banana root (*Musa acuminata* Colla.)

Compound	Result	Observation	Method/Reagent
Alkaloids	Positive (+)	Dark orange precipitate ring	Dragendorff
Flavonoids	Positive (+)	Reddish-orange color	Mg + HCl
Terpenoids	Positive (+)	Dark brownish-red color	Liebermann-Burchard

Phytochemical screening was conducted to identify the secondary metabolites present in the muli banana root (*Musa acuminata* Colla.) extract. The qualitative analysis revealed that the n-hexane extract tested positive for the presence of alkaloids, flavonoids, and terpenoids, while saponins and tannins were not detected. The documentation of these tests is presented in Figure 1, and the results are summarized in Table 1.

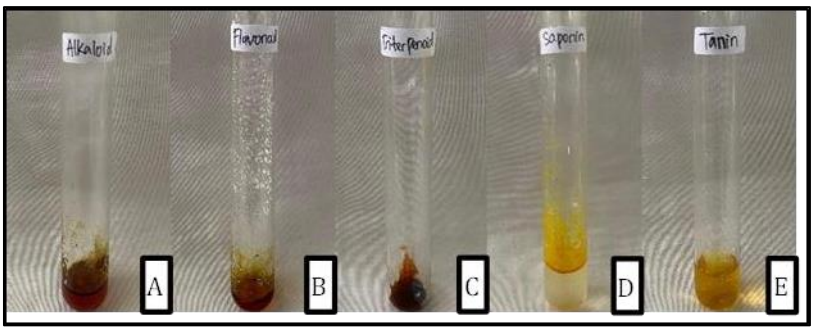

Figure 1 Phytochemical test results of n-hexane extract of muli banana roots (*Musa acuminata* Colla.) (A) Alkaloids (B) Flavonoids, (C) Terpenoids, (D) Saponins, (E) Tannins

Recent pharmacological evaluations emphasize that various parts of the Musa genus, particularly the roots and pseudostems, are rich reservoirs of bioactive secondary metabolites. These include phenolic acids, flavonoids, alkaloids, and terpenoids, which contribute significantly to their well-documented therapeutic and antimicrobial properties [10, 11]. However, the specific metabolic profile recovered from the plant matrix is fundamentally dictated by the choice of the extraction solvent [12]. In this study, n-hexane was intentionally utilized as a non-polar solvent. This ensures that the extracted alkaloids, flavonoids, and terpenoids are predominantly lipophilic (fat-soluble) in nature.

This specific solvent affinity is a crucial finding. While highly polar solvents like methanol or ethanol typically extract a broad spectrum of hydrophilic compounds, such as tannins and saponins, which were expectedly absent in our hexane extract, as non-polar solvents selectively isolate lipophilic constituents [13]. The presence of these specific non-polar compounds provides a strong theoretical basis for the extract's profound antibacterial potential. Lipophilic secondary metabolites exhibit a high structural affinity for the lipid-rich domains of bacterial cell membranes. Once they partition into the lipid bilayer, they are known to disrupt membrane fluidity and induce the lethal leakage of intracellular components, which serves as a primary mechanism for bacterial growth inhibition [14].

3.2. Antibacterial and Antiseptic Efficacy

The biological activity of the extract was evaluated against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 using the agar well diffusion method. The extract demonstrated notable inhibitory effects against both pathogens, characterized by the formation of distinct, clear zones of inhibition around the wells across all tested concentrations (60% to 100%), as visually depicted in Figure 2 and Figure 3. This robust antibacterial response is highly consistent with recent bioprospecting studies from 2020 to 2025, which increasingly highlight various parts of the *Musa acuminata* plant, particularly its underutilized biomass as potent sources of broad-spectrum antibacterial agents [11, 15].

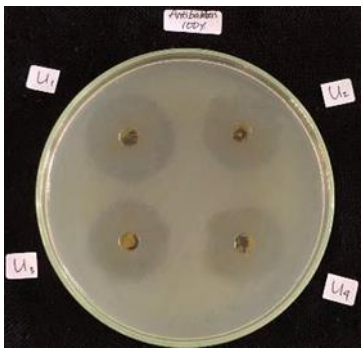

Figure 2 Zones of inhibition of n-hexane muli banana root extract against *E. coli*

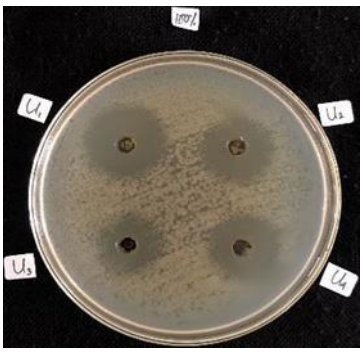

Figure 3 Zones of inhibition of n-hexane muli banana root extract against *S. aureus*

Statistical evaluation using a One-Way ANOVA followed by a Tukey HSD post hoc test confirmed the significance of these findings. For the antibiotic test against *Escherichia coli*, the negative control showed a significant difference ($p < 0.05$) compared to all variations of the muli banana root extract. The inhibitory power increased in a clear dose-dependent manner, with the 100% concentration yielding the most substantial average inhibition zone diameter of 26.56 mm (Table 2). Recent literature corroborates this strong dose-dependent efficacy; for instance, comprehensive screenings by Sa'diyah et al. [16] and other recent evaluations have demonstrated that Musa extracts effectively inhibit *Escherichia coli* growth due to the synergistic action of their secondary metabolites, which are capable of overcoming the resistance typically provided by the Gram-negative outer membrane [17].

A similar significant trend was observed in the antiseptic evaluation against the Gram-positive pathogen, *Staphylococcus aureus*. The post hoc analysis revealed that all extract concentrations exhibited significant antibacterial activity ($p < 0.05$) relative to the negative control. While the lower concentrations (60%, 70%, and 80%) did not differ significantly from one another ($p > 0.05$), the 100% concentration was significantly more effective, producing a maximum inhibition zone of 17.49 mm (Table 2). Pure n-hexane (negative control) did not produce any zone of inhibition around the well, visually confirming that the observed antibacterial activity was entirely attributable to the active secondary metabolites extracted from the muli banana roots.

Table 2 Antibacterial and antiseptic activity of n-hexane muli banana root extract

Treatments	**Concentration (%)**	**Zone of Inhibition against *E. coli* (mm)**	**Zone of Inhibition against *S. aureus* (mm)**
Negative control	n-hexane	0.00 ± 0.00	0.00 ± 0.00
Banana root extract	60	16.56 ± 2.66[a]	8.74 ± 0.62[a]
	70	17.25 ± 2.87[a]	11.66 ± 1.23[a]
	80	17.63 ± 2.65[b]	13.31 ± 1.04[a]
	90	19.16 ± 2.72[b]	14.21 ± 1.99[a]
	100	26.56 ± 7.59[b]	17.49 ± 3.78[b]
Positive control	Chloramphenicol (300 ppm)	29.00 ± 5.38[c]	-
	Dettol antiseptic	-	31.09 ± 3.03[b]

Notes: Table 2 shows the measurement results of inhibition zone diameters in the antibiotic and antiseptic tests. Statistical analysis was performed using One-Way ANOVA followed by Tukey HSD test at a significance level of $p < 0.05$. Significant differences are indicated by different letter notations.

This high efficacy against *Staphylococcus aureus* aligns perfectly with significant clinical findings from recent studies. These evaluations demonstrate that *Musa acuminata* extracts not only inhibit standard *Staphylococcus aureus* strains but also exhibit significant activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) [14, 15, 18]. This highlights the immense potential of muli banana root extract not just as a standard antibacterial, but specifically for advanced clinical antiseptic applications to combat resistant dermal infections.

3.3. Antibacterial Mechanisms of Lipophilic Compounds

The profound antibacterial efficacy of the muli banana root extract is intrinsically linked to the lipophilic nature of its secondary metabolites. Extracted via n-hexane, the isolated alkaloids, flavonoids, and terpenoids exhibit a high partition coefficient, allowing them to rapidly penetrate the lipid bilayer of bacterial membranes [19]. As conceptually illustrated in Figure 4, once intercalated into the membrane, these lipophilic molecules initiate a cascade of structural disruptions. Recent molecular and microbiological studies confirm that terpenoids physically accumulate within the phospholipid bilayer, causing structural expansion, a significant reduction in membrane fluidity, and subsequent membrane depolarization [20]. Concurrently, lipophilic flavonoids bind to and alter the conformation of bacterial surface proteins, while non-polar alkaloids disrupt molecular transport processes and impede cellular respiration. These synergistic interactions drastically increase membrane permeability, triggering the fatal leakage of essential cytosolic ions, intracellular proteins, and ATP, which eventually culminates in bacterial cell lysis.

Interestingly, the extract exhibited a remarkably larger zone of inhibition against the Gram-negative *Escherichia coli* (26.56 mm) compared to the Gram-positive *Staphylococcus aureus* (17.49 mm) at the highest concentration. This

differential susceptibility is strongly governed by the distinct architectural barriers of their respective cell walls (Figure 4). *Escherichia coli* possesses a complex outer membrane heavily fortified with a lipopolysaccharide (LPS) layer. While this LPS layer serves as an impenetrable barrier to many conventional hydrophilic antibiotics, the predominantly lipophilic metabolites in the n-hexane extract are exceptionally adept at dissolving into the lipid-rich domains of this outer membrane [21, 22]. By destabilizing the LPS layer and altering porin functionality, the active compounds rapidly compromise the outer barrier and subsequently degrade the inner cytoplasmic membrane.

Conversely, *Staphylococcus aureus* relies on a thick, cross-linked peptidoglycan layer rather than an outer lipid membrane. While this layer provides structural rigidity, the matrix is inherently porous. The lipophilic secondary metabolites can easily permeate through these peptidoglycan pores to directly target and interact with the underlying lipid-rich cytoplasmic membrane [23]. This direct interaction disrupts the proton motive force and crucial enzymatic activities situated in the plasma membrane, thereby confirming the extract's robust potential as an effective topical antiseptic agent against resistant Gram-positive pathogens.

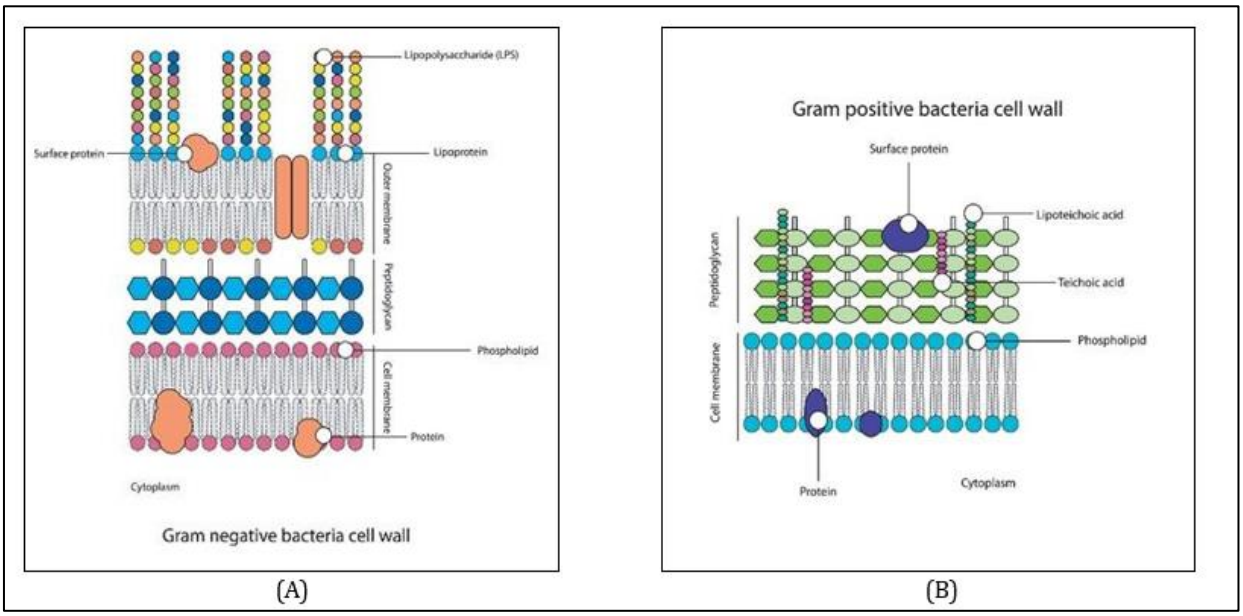

Figure 4 Schematic representation of the proposed antibacterial mechanism of lipophilic secondary metabolites from muli banana root extract against the complex outer membrane of Gram-negative E. coli (A) and the porous peptidoglycan layer of Gram-positive *S. aureus* (B) [24]

4. Conclusion

This study demonstrates that the n-hexane extract of muli banana (*Musa acuminata* Colla.) roots is rich in lipophilic secondary metabolites, specifically alkaloids, flavonoids, and terpenoids, which drive its strong antibacterial properties. The extract displayed substantial, dose-dependent efficacy against both Gram-negative and Gram-positive pathogens. At the optimal concentration of 100%, it produced pronounced inhibition zones of 26.56 mm against *Escherichia coli* and 17.49 mm against *Staphylococcus aureus*. These compelling results substantiate the extract's potential for development as a natural antibiotic and an effective topical antiseptic. Future in vivo studies and expanded screening against diverse, drug-resistant clinical isolates are highly recommended to further establish its broad-spectrum therapeutic applications.

Compliance with ethical standards

Acknowledgments

The authors declare that they have no conflict of interest.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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