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(RESEARCH ARTICLE)

Antimicrobial Activity of Ethyl Acetate Extract of *Musa acuminata* Colla. Roots Against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*

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Abstract

Bacterial and fungal infections remain a major health problem due to the increasing resistance of microorganisms to synthetic antimicrobial agents, highlighting the need for alternative natural antimicrobial sources. The roots of muli banana (*Musa acuminata* Colla.) are known to contain bioactive compounds with potential antimicrobial activity. This study aimed to identify the secondary metabolites and evaluate the antimicrobial activity of the ethyl acetate extract of muli banana roots against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. This laboratory experimental study employed the well diffusion method using five extract concentrations with four replications (n = 4). Antibacterial and anti-*Candida* activities were analyzed using one-way ANOVA followed by the Least Significant Difference (LSD) test, while antifungal activity against *Aspergillus niger* was analyzed using the Kruskal–Wallis and Mann–Whitney tests. Phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, and triterpenoids. The ethyl acetate extract of muli banana roots significantly inhibited the growth of *Escherichia coli* ($p < 0.001$), *Staphylococcus aureus* ($p < 0.001$), and *Candida albicans* ($p < 0.001$). The highest inhibitory activity was observed at 100% concentration, producing mean inhibition zone diameters of 4.42 mm against *Escherichia coli*, 12.96 mm against *Staphylococcus aureus*, and 9.60 mm against *Candida albicans*. In contrast, the extract did not inhibit the growth of *Aspergillus niger* at all tested concentrations. These findings suggest that the ethyl acetate extract of muli banana roots has the potential to be developed as a natural source for antibacterial and antifungal products.

Keywords: *Musa acuminata*; Antimicrobial activity; Ethyl acetate extract; Inhibition zone; Phytochemical screening

1. Introduction

Diseases caused by pathogenic microorganisms remain a global health problem. Bacteria such as *Escherichia coli* and *Staphylococcus aureus*, as well as fungi such as *Candida albicans* and *Aspergillus niger*, are known to cause various infections in humans, particularly among immunocompromised individuals [1,2]. The excessive use of antibiotics and synthetic antifungal agents has contributed to the emergence of antimicrobial resistance, including resistance among certain *Candida* species, thereby reducing treatment efficacy [3]. In addition, prolonged use of some synthetic antimicrobial agents may cause undesirable side effects, highlighting the need for safer and more effective alternatives derived from natural sources [4].

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Plants are known to produce a wide range of secondary metabolites, including alkaloids, saponins, flavonoids, tannins, terpenoids, and triterpenoids, many of which exhibit antimicrobial properties [5]. Plant roots represent a potential source of bioactive compounds because they interact directly with soil microorganisms and environmental stressors, which may stimulate the production of defensive metabolites [6]. One plant that has attracted attention as a potential source of natural antimicrobial compounds is the muli banana (*Musa acuminata* Colla.).

Previous studies have demonstrated the antimicrobial potential of various parts of *Musa* species, including the fruit peel, leaves, pseudostem, and rhizome [7,8]. However, information regarding the antimicrobial activity of muli banana roots remains limited. To date, few studies have investigated the antimicrobial properties of ethyl acetate extracts derived from muli banana roots, particularly against both bacterial and fungal pathogens. Moreover, data on their inhibitory activity against *Candida albicans* and *Aspergillus niger* are still scarce. Therefore, the antimicrobial efficacy of ethyl acetate extract of muli banana roots against representative Gram-positive bacteria, Gram-negative bacteria, yeasts, and filamentous fungi remains insufficiently explored, warranting further investigation [7,8].

Therefore, this study was conducted to identify the secondary metabolites present in the ethyl acetate extract of muli banana roots and to evaluate its antibacterial and antifungal activities against *Escherichia coli*, *Staphylococcus aureus*, *Candida albicans*, and *Aspergillus niger*. The hypothesis of this study was that the ethyl acetate extract of muli banana roots contains bioactive secondary metabolites capable of inhibiting the growth of the tested microorganisms.

2. Materials and Methods

2.1. Materials

The materials used include muli banana roots (*Musa acuminata* Colla.), ethyl acetate solvent, Dragendorff's reagent, hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), acetic anhydride, magnesium powder (Mg), and iron (III) chloride ($FeCl_3$). The media used consist of Mueller-Hinton Agar (MHA), Nutrient Agar (NA), Potato Dextrose Agar (PDA), 0.9% NaCl solution, distilled water, chloramphenicol, nystatin, Dettol, and ethyl acetate as a negative control as well as a solvent for diluting the extract. The test microorganisms used are *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Candida albicans*, and *Aspergillus niger*.

2.2. Instruments

The equipment used includes an analytical balance, rotary evaporator, biological safety cabinet (BSC), autoclave, incubator, magnetic stirrer, vortex mixer, water bath, hot plate, pH meter, spirit burner, and caliper. Laboratory glassware such as petri dishes, test tubes, erlenmeyer flasks, and micropipettes are also used.

2.3. Sample Preparation

The muli banana roots were washed under running water, cut into 2–3 cm lengths, and then air-dried at room temperature for one week. The samples were then oven-dried at 37°C until dry, then ground using a blender to obtain a powdered herb. The powdered herb was stored in a tightly closed, dry container, protected from light, until extraction was performed.

2.4. Making Ethyl Acetate Extract of Muli Banana Roots

Extraction was performed using the maceration method. The powdered simplicia was macerated with ethyl acetate solvent using a ratio of 1:10 (b/v) for 3 × 24 hours at room temperature with occasional stirring. The mixture was then filtered, and the residue was macerated again using the same volume of solvent. All filtrates were combined and evaporated using a rotary evaporator to obtain a thick extract, then stored in a closed glass bottle until used.

2.5. Phytochemical Test

The acetate extract of muli banana roots was qualitatively screened for the presence of alkaloids, flavonoids, saponins, terpenoids, and tannins using standard phytochemical procedures [9]. Alkaloids were detected using Dragendorff's reagent (brownish-orange precipitate). Flavonoids were identified using the Mg-HCl test (red/orange/purple coloration). Terpenoids were detected using the Liebermann-Burchard test (red or yellow color). Saponins were tested by foam formation (stable foam >10 minutes), and tannins were identified using 1% $FeCl_3$ (dark blue or blackish-green color).

2.6. Making Extract Concentration

The stock extract solution was prepared by mixing 20 mL of the concentrated extract with 35 mL of ethyl acetate and was designated as the 100% (v/v) concentration. Serial dilutions were prepared using ethyl acetate as the diluent to achieve the required concentrations. Concentrations of 20%, 25%, 30%, 35%, and 40% were used for the *Aspergillus niger* assay, whereas concentrations of 80%, 85%, 90%, 95%, and 100% were used for the *Candida albicans* assay. In addition, concentrations of 60%, 70%, 80%, 90%, and 100% were used for the antibacterial assays against *Escherichia coli* and *Staphylococcus aureus*.

Concentration dilution formula = M1 X V1 = M2 X V2

Information:

M1 = Concentration before dilution (%)
V1 = Volume before dilution (mL)
M2 = Concentration after dilution (%)
V2 = Volume after dilution (ml)

2.7. Antimicrobial Activity Testing

2.7.1. Preparation of test bacterial inoculum (Escherichia coli and Staphylococcus aureus)

Pure bacterial cultures were inoculated onto NA slants and incubated at 37°C for 24 hours. A bacterial suspension was prepared by adding one loop of the isolate to 9 mL of sterile 0.9% NaCl solution, then homogenized until the turbidity was equivalent to the McFarland 0.5 standard (1.5×10^8 CFU/mL).

2.7.2. Antibacterial activity test

The bacterial suspension was spread evenly on the surface of the Mueller Hinton Agar (MHA) medium using a sterile cotton bud. Next, wells were made on the surface of the medium, then each well was filled with 0.1 mL of ethyl acetate extract of muli banana roots with varying concentrations of 60%, 70%, 80%, 90%, and 100%. Chloramphenicol (300 ppm) and Dettol were used as positive controls to compare antibacterial activity, while ethyl acetate was used as a negative control to ensure that the solvent did not provide an antibacterial effect. Each treatment, including all variations in extract concentration, positive control, and negative control, was carried out 4 times (n = 4) to obtain more accurate and statistically reliable data. The medium was then incubated at 37°C for 24 hours. After the incubation period, antibacterial activity was observed by measuring the diameter of the inhibition zone formed using a vernier caliper [10].

2.7.3. Preparation of test fungal inoculum (Aspergillus niger and Candida albicans)

A pure culture of *Candida albicans* was inoculated onto Potato Dextrose Agar (PDA) slants, then incubated at 28°C for 48 hours until good colony growth was obtained. Next, a suspension of *Candida albicans* was prepared by taking one loop of pure colonies and placing it in 9 mL of sterile 0.9% NaCl solution, then homogenized until the turbidity level was equivalent to the McFarland 0.5 standard (1.5×10^8 CFU/mL).

Meanwhile, a pure culture of *Aspergillus niger* was inoculated onto Potato Dextrose Agar (PDA) media in a Petri dish using the spot method, then incubated at 28°C for 7 days until optimal spore growth occurred. The *Aspergillus niger* inoculum was then applied to a test medium using the midpoint method to test antifungal activity.

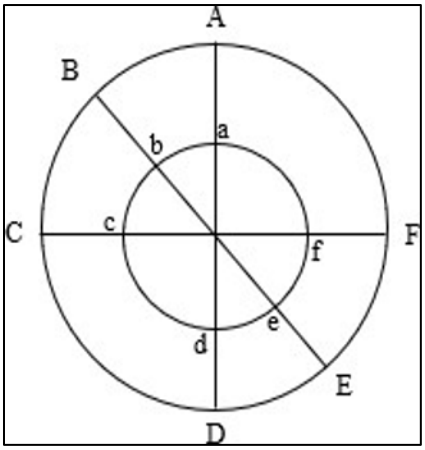
2.7.4. Antifungal test

A 0.1 mL suspension of *Candida albicans* was spread evenly on the surface of Potato Dextrose Agar (PDA) media using a sterile cotton bud. Meanwhile, in the *Aspergillus niger* test, the midpoint method was used on the PDA media. Next, wells were made on the surface of the test media, then each well was filled with 0.1 mL of ethyl acetate extract of muli banana roots with varying concentrations. For *Aspergillus niger*, concentrations of 20%, 25%, 30%, 35%, and 40% were used, while for *Candida albicans*, concentrations of 80%, 85%, 90%, 95%, and 100% were used. Nystatin was used as a positive control, while ethyl acetate was used as a negative control to ensure that the solvent did not provide antifungal activity. Each treatment, including all variations in extract concentration, positive control, and negative control, was carried out 4 times (n = 4) to obtain more accurate data and can be analyzed statistically. The media were then incubated at 28°C for 48 hours for *Candida albicans* and 7 days for *Aspergillus niger*. After the incubation period, antifungal activity was observed by measuring the diameter of the inhibition zone formed using a vernier caliper [11].

2.8. Inhibition Zone Measurement

The diameter of the inhibition zone was measured using a vernier caliper and expressed in millimeters (mm). Measurements were made after incubation in the vertical, horizontal, and diagonal directions, and the average value was calculated using the following formula.

$$\frac{(AD - ad) + (BE - be) + (CF - cf)}{3}$$



Figures 1 Inhibition Zone Measurement Scheme

2.9. Data analysis

In the antibacterial tests, inhibition zone diameter data were analyzed using One-Way ANOVA with SPSS. Prior to the analysis, statistical assumption tests were conducted, including a normality test to determine whether the data were normally distributed and a homogeneity test to determine the similarity of variances between treatment groups. The data met both assumptions ($p > 0.05$), so the analysis was continued with a Post Hoc LSD test to determine differences between treatments.

In the antifungal test, inhibition zone diameter data were analyzed using SPSS after first conducting normality and homogeneity tests. The test results showed that the data were not homogeneous, so the analysis was continued using the nonparametric Kruskal–Wallis test. However, there was a significant difference ($p < 0.05$), so a further Mann–Whitney test was performed to determine the differences between the treatment groups.

3. Results and Discussion

3.1. Secondary Metabolite Compounds of Ethyl Acetate Extract of Muli Banana Roots

Phytochemical tests were conducted to identify the secondary metabolite content in the ethyl acetate extract of muli banana (*Musa acuminata* Colla.) roots. The analysis results showed that the extract contained flavonoids, alkaloids, tannins, terpenoids and triterpenoids, while saponins were not detected. Documentation of the phytochemical test results is presented in Figure 2, while a summary of the test results is presented in Table 1.

Table 1 Phytochemical test results of ethyl acetate extract of muli banana roots

Compound	Results	Information	Method/Reagen
Flavonoids	Positive (+)	A reddish brown color is formed	Mg + HCL
Alkaloids	Positive (+)	A brown precipitate forms	Dragendorff's
Tannins	Positive (+)	A blackish green color is formed	$FeCL_3$
Terpenoids	Positive (+)	A dark brownish red color is formed	Liebermann-Burchard

Triterpenoids	Positive (+)	A purplish red color is formed	Liebermann-Burchard

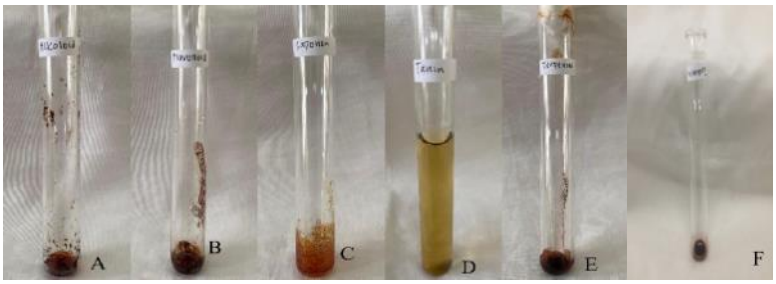
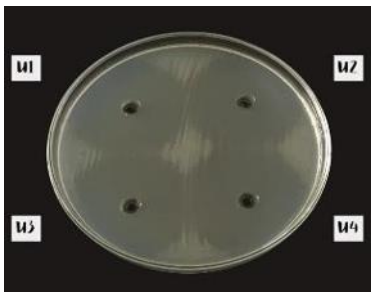


Figure 2 Phytochemical screening results of ethyl acetate extract of *Musa acuminata* Colla.) roots (A) Alkaloids, (B) Flavonoids, (C) Saponins, (D) Tannins, (E) Terpenoids, (F) Tritepenoids

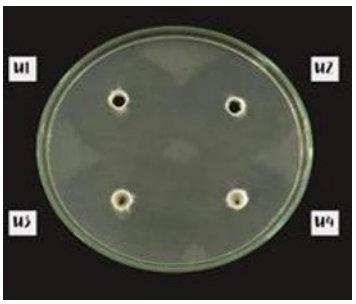
Recent pharmacological evaluations have shown that various parts of the *Musa* genus, particularly the roots and pseudostems, are potential sources of bioactive secondary metabolites. These compounds include phenolic acids, flavonoids, tannins, and terpenoids, which are known to contribute to biological activities, including antimicrobial effects. The profile of extracted metabolites is strongly influenced by the type of solvent used. In this study, ethyl acetate, a semi-polar solvent, was used, making it more selective in extracting compounds with medium to lipophilic polarity [12]. Phytochemical test results showed that the ethyl acetate extract of muli banana roots contained flavonoids, tannins, terpenoids, alkaloids, and triterpenoids, but was negative for saponins. The absence of saponins is thought to be due to saponins being polar compounds and therefore less soluble in ethyl acetate, a semi-polar solvent [13]. The presence of flavonoids, tannins, terpenoids, alkaloids, and triterpenoids provides a scientific basis for the extract's potential antimicrobial activity. These compounds are known to interact with lipid-rich microbial cell membranes, disrupting membrane integrity and permeability. This disruption can cause leakage of intracellular components, leading to growth inhibition and even cell death, which is one of the main mechanisms of antibacterial and antifungal activity [14].

3.2. Antibacterial Activity of Ethyl Acetate Extract of Muli Banana Roots

The biological activity of the extract was tested against *Escherichia coli* and *Staphylococcus aureus* using the well diffusion method. The extract showed inhibitory activity against both test bacteria, indicated by the formation of inhibition zones around the wells at all test concentrations (60%–100%), as shown in Figures 3 and 4. These results are in line with various recent bioprospecting studies that report that various parts of the *Musa acuminata* plant have the potential to be sources of broad-spectrum antibacterial compounds [15].



Figures 3 Inhibition zone of ethyl acetate extract of muli banana roots against *Escherichia coli*



Figures 4 Inhibition zone of ethyl acetate extract of muli banana roots against *Staphylococcus aureus*

Based on the test results, the ethyl acetate extract of muli banana roots showed antibacterial activity against *Escherichia coli*, which was indicated by the formation of inhibition zones at all test concentrations, namely 60%, 70%, 80%, 90%, and 100%. Chloramphenicol was used as a positive control, while ethyl acetate was used as a negative control. The largest average inhibition zone diameter was obtained at a concentration of 100% of 4.42 mm. According to the classification by Davis & Stout, a zone of inhibition of < 5 mm is categorized as weak. This indicates that even though

higher extract concentrations lead to greater inhibitory activity against *Escherichia coli*, the overall potency remains low.

Based on the average inhibition zone diameter data, a statistical analysis was conducted using One-Way ANOVA to determine the effect of extract concentration on antibacterial activity. The results of the analysis showed a significant effect between treatments ($p < 0.05$), which was further evaluated using the Least Significant Difference (LSD) test. The results confirmed that the 100% concentration provided the most statistically optimal antibacterial activity compared to other concentrations ($p < 0.05$). This inhibitory activity is driven by the synergistic action of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids [16].

Ethyl acetate extract of muli banana roots also showed strong antibacterial activity against *Staphylococcus aureus*. In this test, Dettol was used as an antibacterial comparator (positive control) and ethyl acetate as a negative control. Inhibition zones were formed at all concentrations tested, with the largest average diameter obtained at 100% concentration of 12.96 mm. Based on Davis & Stout's criteria [17], the inhibition zones for concentrations of 70% to 100% (ranging from 11.62 mm to 12.96 mm) are classified as strong (10-20 mm), while the 60% concentration (8.20 mm) is classified as moderate (5–10 mm).

These results indicate that increasing the extract concentration is directly proportional to the increase in inhibitory activity against *Staphylococcus aureus*. Statistical analysis using One-Way ANOVA showed a significant effect of extract concentration on the diameter of the resulting inhibition zone. Furthermore, the LSD test showed that the 100% concentration had the highest inhibitory effectiveness compared to other concentrations ($p < 0.05$). The absence of an inhibition zone in the negative control indicates that the antibacterial activity produced comes purely from the active compounds in the ethyl acetate extract of *Musa acuminata* roots, not from the solvent used.

Table 2 Antibacterial activity of ethyl acetate extract of muli banana roots against *E. coli* and *S. aureus*

Treatment	**Concentration (%)**	**Inhibition Zone Against *E. coli* (mm)**	**Inhibition Zone Against *S. aureus* (mm)**
Negative control	Ethyl acetate	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Banana root extract	60	2.71 ± 0.27^{b}	8.20 ± 0.45^{b}
	70	3.08 ± 0.91^{c}	$11{,}62 \pm 0.51^{c}$
	80	3.67 ± 0.97^{c}	$12{,}12 \pm 0.37^{d}$
	90	4.25 ± 0.99^{c}	$12{,}83 \pm 0.26^{d}$
	100	4.42 ± 0.24^{d}	$12{,}96 \pm 0.16^{e}$
Positive control	Chloramphenicol (300 ppm)	35.57 ± 0.05^{e}	-
	Dettol (antibacterial comparator)	-	$37{,}75 \pm 0.17^{f}$

Note: Values are presented as Mean ± SD. Mean values followed by different superscript letters (a-f) within the same column indicate significant differences based on the Least Significant Difference (LSD) post-hoc test ($p < 0.05$).

The inhibitory activity of ethyl acetate extract of *Musa acuminata* roots against *Staphylococcus aureus* shows considerable potential as a natural antibacterial agent. These results align with various recent studies reporting that *Musa acuminata* extract has antibacterial activity against Gram-positive bacteria, including resistant strains. Secondary metabolites such as flavonoids, alkaloids, tannins, and terpenoids are thought to play a role in damaging bacterial cell membranes and inhibiting the growth of microorganisms [5].

Furthermore, antibacterial activity against *Escherichia coli* also indicates that the ethyl acetate extract of *Musa acuminata* roots is able to inhibit the growth of Gram-negative bacteria. This ability indicates that the active compounds in the extract have a fairly broad antibacterial spectrum. The inhibitory activity against *Escherichia coli* is thought to occur due to the interaction of the bioactive compounds with the bacterial cell wall and membrane, thereby disrupting the metabolic processes and growth of bacterial cells [5].

When compared to other parts of the banana plant, the antibacterial potential of *Muli* banana roots exhibits distinct variations. The ethyl acetate extract of *Musa acuminata* peels reported a significantly larger inhibition zone against

Escherichia coli (> 10 mm, classified as strong). Similarly, studies on *Musa acuminata* leaves showed higher efficacy against Gram-negative bacteria due to a higher concentration of mobile tannins and volatile terpenoids on the leaf surface. The relatively weaker activity found in the root extract in this study suggests that while roots accumulate structural defense compounds like triterpenoids, they possess lower yields of low-molecular-weight phenolic compounds compared to the aerial parts (leaves and fruits) of the plant [18].

3.3. Antibacterial Mechanism of Secondary Metabolite Compounds

The antibacterial activity of ethyl acetate extract of *Musa acuminata* roots is thought to be related to its secondary metabolite content, such as alkaloids, flavonoids, tannins, terpenoids, and triterpenoids, which were successfully identified in phytochemical tests. The use of semi-polar ethyl acetate solvent allows for effective extraction of these bioactive compounds, thus providing inhibitory activity against *Escherichia coli* and *Staphylococcus aureus*.

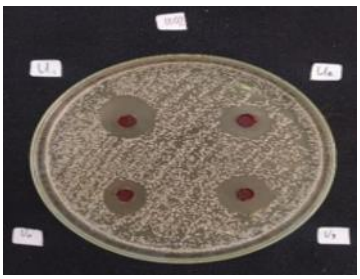
Flavonoid and terpenoid compounds are thought to work by damaging the structure of bacterial cell membranes, thereby increasing membrane permeability and causing leakage of intracellular components. Tannins and alkaloids are known to inhibit enzyme activity and disrupt bacterial cell wall formation, while alkaloids play a role in inhibiting metabolic processes and cellular respiration. The interaction of these various active compounds disrupts bacterial cell function, ultimately inhibiting bacterial growth. Increased inhibitory activity at higher extract concentrations indicates that the greater amount of bioactive compounds provides a more optimal antibacterial effect [19].

In this study, the diameter of the inhibition zone against *Staphylococcus aureus* was larger than that against *Escherichia coli* at a 100% extract concentration, namely 12.96 mm and 4.42 mm, respectively. The difference in inhibitory activity is thought to be influenced by differences in the cell wall structure of the two bacteria. *Staphylococcus aureus* is a Gram-positive bacterium that has a thick peptidoglycan layer but is simpler and does not have an outer membrane, so the bioactive compounds in the extract more easily penetrate and damage the bacterial cell membrane [20].

In contrast, *Escherichia coli*, as a Gram-negative bacterium, has an outer membrane composed of lipopolysaccharide (LPS), which serves as an additional barrier against the entry of antimicrobial compounds. This structure makes it more difficult for the active compounds in the ethyl acetate extract of *Musa acuminata* roots to penetrate Gram-negative bacterial cells, resulting in a smaller inhibition zone than in Gram-positive bacteria. The inhibitory activity against both bacteria is thought to originate from the content of secondary metabolites such as alkaloids, flavonoids, tannins, terpenoids, and triterpenoids, which can disrupt cell membrane permeability, inhibit enzyme activity, and disrupt bacterial metabolic processes. Thus, the ethyl acetate extract of *Musa acuminata* roots shows stronger potency against Gram-positive bacteria than Gram-negative bacteria [5].

3.4. Antifungal Activity of Ethyl Acetate Extract of Banana Roots

The biological activity of the extract was tested against *Aspergillus niger* and *Candida albicans* using the well diffusion method. The test results showed that the ethyl acetate extract of *Musa acuminata* roots has antifungal activity against *Candida albicans*, which is indicated by the formation of an inhibition zone at a concentration of 85%–100%. The largest inhibition zone diameter was obtained at a concentration of 100% with an inhibition zone diameter of 9.6 mm, as shown in Figures 5, indicating that this concentration provided the most optimal inhibitory activity against the growth of *Candida albicans*. Conversely, in the test against *Aspergillus niger*, no inhibition zone was formed at all concentrations tested. These results indicate that the ethyl acetate extract of *Musa acuminata* roots was not yet able to inhibit the growth of *Aspergillus niger* under the conditions and concentrations used in the test.



Figures 5 Inhibition zone of ethyl acetate extract of muli banana roots against *Candida albicans*

Based on the test results, the ethyl acetate extract of muli banana roots did not show antifungal activity against *Aspergillus niger*, which was indicated by the absence of an inhibition zone at all tested concentrations, namely 20%,

25%, 30%, 35%, and 40%. These results indicate that the ethyl acetate extract of muli banana roots was not able to inhibit the growth of *Aspergillus niger* at the concentrations used. The absence of an inhibition zone is thought to be influenced by the characteristics of *Aspergillus niger* as a mold that has a more complex cell wall structure and is composed of chitin and strong polysaccharides, so that the active compounds in the extract have more difficulty penetrating fungal cells. In addition, *Aspergillus niger* has the ability to adapt and grow hyphae quickly, making it more resistant to natural antifungal compounds [21].

In contrast, the ethyl acetate extract of muli banana roots showed antifungal activity against *Candida albicans*, indicated by the formation of inhibition zones at concentrations of 85%, 90%, 95%, and 100%, while no inhibition zone was formed at 80% concentration. Nystatin was used as a positive control and ethyl acetate as a negative control. The largest inhibition zone diameter was obtained at a concentration of 100%, indicating that the higher the extract concentration, the greater the inhibitory activity produced against *Candida albicans*. The difference in inhibitory ability against *Candida albicans* and *Aspergillus niger* is thought to be influenced by the characteristics of the test fungi. *Candida albicans* is a unicellular yeast with a simpler cell structure than mold, so the active compounds in the extract interact more easily with the membrane and cell wall of the fungus [22].

Based on the average inhibition zone diameter data, statistical analysis was performed using the Kruskal–Wallis test followed by the Mann–Whitney test using Bonferroni correction to determine differences between treatments. The results of the analysis showed that the 80% concentration was significantly different from the 85%, 90%, 95%, 100% concentrations, and the positive control of nystatin. The 85% concentration also showed significant differences from the 90%, 95%, 100% concentrations, the positive control, and the negative control of ethyl acetate. In addition, the 90%, 95%, and 100% concentrations were each significantly different from the negative control. All these differences were indicated by an adjusted significance value (Adj. Sig.) <0.05. Based on the results of the statistical analysis, the 100% concentration was declared as the most optimal concentration in inhibiting the growth of *Candida albicans*. This antifungal activity is thought to originate from the content of secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, and triterpenoids which are able to damage the permeability of fungal cell membranes and inhibit cell growth.

Table 3 Antifungal activity of ethyl acetate extract of muli banana roots

Treatment	**Concentration (%)**	**Inhibition Zone Against *Candida albicans* (mm)**
Negative control	Ethyl acetate	0.00 ± 0.00^{a}
Banana root extract	80	0.00 ± 0.00^{a}
	85	$4{,}2 \pm 0.89^{b}$
	90	$6{,}6 \pm 0.46^{c}$
	95	$9{,}1 \pm 0.72^{d}$
	100	$9{,}6 \pm 0.21^{d}$
Positive control	Nystatin	$12{,}4 \pm 0.89^{e}$

Note: Values are presented as Mean ± SD. Mean values followed by different superscript letters (a-e) within the same column indicate significant differences based on Mann-Whitney post-hoc test ($p < 0.05$).

The antifungal activity of ethyl acetate extract of *Musa acuminata* roots against *Candida albicans* shows considerable potential as a natural antifungal. These results align with research reporting that *Musa acuminata* extract has antifungal activity against pathogenic fungi [23]. Secondary metabolites such as flavonoids, alkaloids, tannins, terpenoids, and triterpenoids are thought to play a role in damaging fungal cell membranes, inhibiting ergosterol synthesis, and disrupting cellular metabolism, thereby inhibiting the growth of *Candida albicans*. As a unicellular yeast, *Candida albicans* has a simpler cell structure than mold, allowing the active compounds in the extract to more easily penetrate fungal cell walls and membranes [24].

In contrast, the ethyl acetate extract of *Musa acuminata* roots did not show inhibitory activity against *Aspergillus niger* because no inhibition zone was formed at all tested concentrations. This is thought to be influenced by the characteristics of *Aspergillus niger* as a multicellular mold that has a more complex cell wall structure and is composed of strong chitin, making it more difficult for bioactive compounds to diffuse and penetrate fungal cells. In addition, hyphal growth in molds can also increase resistance to natural antifungal compounds. The difference in response

between *Candida albicans* and *Aspergillus niger* indicates that the ethyl acetate extract of *Musa acuminata* roots has more effective antifungal activity against yeast than mold [25].

3.5. Antifungal Mechanism of Secondary Metabolite Compounds

The antifungal activity of ethyl acetate extract of *Musa acuminata* roots is thought to be related to its secondary metabolite content, such as alkaloids, flavonoids, tannins, terpenoids, and triterpenoids, identified in phytochemical tests. The use of semi-polar ethyl acetate solvent allows for effective extraction of these bioactive compounds, thus providing inhibitory activity against *Candida albicans*. Flavonoids and terpenoids are thought to work by disrupting the permeability of fungal cell membranes, while tannins can inhibit enzyme activity and disrupt cell wall formation [24]. Alkaloids are known to inhibit the metabolic processes of fungal cells, thus inhibiting the growth of microorganisms. The interaction of these various active compounds disrupts cell function, ultimately inhibiting fungal growth [25].

The extract's antifungal activity against *Candida albicans* is thought to occur because the bioactive compounds are able to penetrate the relatively simple yeast cell wall, disrupting the integrity of the cell membrane and the fungal cell's metabolic processes. Membrane damage causes leakage of intracellular components, thus inhibiting *Candida albicans* growth. The higher the extract concentration, the greater the amount of active compounds involved, thus increasing the inhibitory activity [26].

In contrast, the ethyl acetate extract of muli banana roots did not show inhibitory activity against *Aspergillus niger*. This is thought to be due to the characteristics of *Aspergillus niger* as a multicellular mold with a more complex hyphal structure and cell wall, composed of chitin and strong polysaccharides, making it more difficult for bioactive compounds to penetrate fungal cells. This structure causes mold to have a higher resistance to natural antifungal compounds than yeast. Thus, the ethyl acetate extract of muli banana roots showed more effective antifungal activity against yeast than mold [32][27].

4. Conclusion

The ethyl acetate extract of muli banana (*Musa acuminata* Colla.) roots contains alkaloids, flavonoids, tannins, terpenoids, and triterpenoids. The extract significantly inhibited *Escherichia coli* (4.42 mm, weak) and *Staphylococcus aureus* (12.96 mm, strong) at 100% concentration ($p < 0.001$), and inhibited *Candida albicans* at 85%–100% concentration with a maximum inhibition zone of 9.6 mm ($p < 0.001$), while no inhibitory activity was observed against *Aspergillus niger* at any tested concentration. These results indicate that the extract has potential as a natural antibacterial and antifungal agent.

Further research is recommended, including determination of minimum inhibitory concentration (MIC/MBC/MFC), isolation and characterization of active compounds, in vivo efficacy and toxicity testing, and investigation of higher concentrations or alternative solvents to achieve activity against *Aspergillus niger*.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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