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Antimicrobial activity of ethanol extracts from petioles and leaf midribs of Muli banana (*Musa acuminata* Colla) against pathogenic bacteria and fungi

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Abstract

Antimicrobial resistance and the rising incidence of infections caused by pathogenic bacteria and fungi remain major public health challenges, increasing the need for safer, naturally derived antimicrobial agents. The muli banana (*Musa acuminata* Colla), particularly its petioles and leaf midribs, is known to contain bioactive compounds with antimicrobial potential. This study aimed to identify the secondary metabolite content and evaluate the in vitro antimicrobial activity of ethanol extracts from the petioles and leaf midribs of muli banana against *Staphylococcus aureus*, *Escherichia coli*, *Candida albicans*, and *Aspergillus niger*. A Completely Randomized Design was used; secondary metabolites were identified through phytochemical screening, and antimicrobial activity was evaluated using the agar well diffusion method, with inhibition zone diameter as the measured parameter. Phytochemical screening revealed the presence of flavonoids, tannins, saponins, and alkaloids. The extract exhibited moderate inhibitory activity against *S. aureus*, with the largest zone of 13.8 mm at 90% concentration, and moderate, concentration dependent activity against *C. albicans* that increased significantly with concentration (Kruskal-Wallis, $p<0.05$), reaching 10.32 mm at 70%. Activity against *E. coli* was weak and detected only at 90% concentration (2.9 mm), with a significant difference compared to other concentrations ($p<0.05$), whereas no inhibitory activity was observed against *A. niger* at any concentration tested ($p>0.05$). These findings indicate that ethanol extract of muli banana petioles and leaf midribs has potential as a natural antimicrobial agent, with efficacy varying according to microorganism type and extract concentration.

Keywords: Muli Banana; Ethanol Extract; Inhibition Zone; Antimicrobial Activity; Secondary Metabolites

1. Introduction

Antimicrobial resistance is one of the growing global health challenges. Antibiotic resistance is the ability of bacteria to survive and multiply despite exposure to antibiotics that would normally inhibit the growth or kill disease causing bacteria [1]. Antibiotic resistance develops through various mechanisms, including genetic mutations, the production of enzymes that degrade antibiotics, efflux pumps, horizontal gene transfer, and adaptive physiological changes [2]. Inappropriate antibiotic use is known to be one of the main factors driving the emergence of bacterial resistance to antibiotics [3]. In 2014, the Review on Antimicrobial Resistance projected that deaths due to antimicrobial resistance could reach 10 million cases per year by 2050. This situation has led to a decline in the effectiveness of antimicrobial therapy and underscores the need to identify new antimicrobial agents from natural sources [4].

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Bacteria and fungi are groups of microorganisms frequently involved in human infections. *Staphylococcus aureus* is a Gram-positive, pathogenic, and highly adaptable bacterium capable of developing resistance to various antibiotics [5], most notably as *methicillin-resistant S. aureus* (MRSA), a multidrug-resistant (MDR) pathogen [6]. Meanwhile, *Escherichia coli* is a Gram-negative bacterium frequently reported to exhibit antibiotic resistance and can cause various infections in both humans and animals [7].

In addition to bacterial infections, fungal infections remain a global health problem with high prevalence and mortality, particularly among individuals with weakened immune systems, such as those with HIV/AIDS, cancer, transplant recipients, and users of immunosuppressive drugs [8]. Pathogenic fungi can cause disease in humans, animals, and plants [9], and *C. albicans* and *A. niger* are among those most frequently associated with opportunistic infections in humans. Invasive candidiasis, in particular, has a high mortality rate and remains one of the most common fungal infections in humans [8], with *C. albicans* and *C. tropicalis* most frequently responsible for both superficial and systemic disease [10].

The continuous use of antibiotics and synthetic antifungals can lead to the emergence of resistant microorganisms, and synthetic antimicrobial agents are also known to cause side effects, including disruption of the body's normal flora. In fungal infections specifically, limited efficacy, side effects, poor tissue penetration, and increasing resistance to antifungal drugs highlight the need for safer and more effective treatment alternatives [10]. Plant derived extracts have recently been shown to be effective as natural disinfectants, offering a promising alternative to synthetic chemical disinfectants [11]. Therefore, the exploration of bioactive compounds from plants has become a widely developed approach in the search for new antimicrobial agents.

The muli banana (*Musa acuminata* Colla) is a plant with potential as a source of natural antimicrobial compounds. Research by Kanedi et al. [12] showed that muli banana contains various bioactive compounds, including lycopene, phenols, flavonoids, saponins, alkaloids, and tannins, with antimicrobial potential. The petioles and leaf midribs of banana contain alkaloids, flavonoids, saponins, tannins, phenolics, glycosides, steroids, and terpenoids that contribute to antimicrobial activity. Flavonoids and phenolic compounds are known to damage fungal cell membranes and inhibit intracellular enzyme activity, while saponins increase the permeability of pathogen cell membranes, and antifungal compounds in general exert their effects through enzyme inhibition, disruption of protein and nucleic acid synthesis, and damage to fungal cell membranes [10]. In muli banana specifically, ethanol extract of the root has recently been reported to possess antibiotic activity against *Escherichia coli* and antiseptic activity against *S. aureus* [13], while comparable plant derived secondary metabolites have also demonstrated antibacterial and antioxidant activity in other species [14], and ethanol extracts from woody plant tissues have similarly shown inhibitory activity against *S. aureus* and *C. albicans* [15].

Previous studies have also demonstrated the antimicrobial potential of the genus Musa more broadly. Sivasamugham et al. [16] showed that ethanol extract of *Musa acuminata* leaves contains alkaloids, flavonoids, glycosides, terpenoids, tannins, and saponins, and is capable of inhibiting MRSA growth at certain concentrations. Jouneghani et al. [17] reported that ethanol extract of the pseudostem of *Musa* sp. exhibited antibacterial activity against *E. coli* and *S. aureus*, while Shaukat et al. [18] showed that ethanol extract of muli banana peel inhibited the growth of multidrug-resistant bacteria, including *E. coli*. Afifah et al. [19] further reported that bio-hand soap containing ethanol extract of Ambon banana leaf stalks was able to inhibit *E. coli* growth. The concentration ranges used in the present study were determined based on these precedents, together with findings from Loyaga et al. [20] and Al-Hayali et al. [21] on antifungal testing of banana-derived extracts, and from Helda et al. [22] on antibacterial testing of other plant extracts, which together indicate that concentrations ≥60% are commonly effective for ethanol-based plant extracts (detailed concentration variations for each organism are provided in the Materials and Methods section).

Although various studies have reported the antimicrobial activity of several parts of the banana plant including the root of muli banana, which exhibits antibiotic activity against *E. coli* and antiseptic activity against *S. aureus* [13] research on the antimicrobial activity of ethanol extracts from the petioles and leaf midribs of muli banana, particularly against pathogenic fungi, remains limited. Therefore, this study aims to identify the phytochemical composition and determine the in vitro antimicrobial activity of ethanol extracts from the petioles and leaf midribs of *Musa acuminata* Colla against *E. coli*, *S. aureus*, *C. albicans*, and *A. niger*

2. Material and methods

2.1. Materials and Equipment

The main samples used were the petioles and leaf midribs of muli banana (*Musa acuminata* Colla). Solvents and reagents included 96% ethanol, Dragendorff's reagent, hydrochloric acid (HCl), concentrated sulfuric acid (H_2SO_4), glacial acetic acid (CH_3COOH), magnesium metal powder, and ferric chloride ($FeCl_3$), together with Potato Dextrose Agar (PDA) medium, 0.9% NaCl solution, Nystatin, Chloramphenicol, Dettol antiseptic solution, 70% alcohol, and sterile distilled water for microbiological testing. Test microorganisms comprised the fungi *Candida albicans* and *Aspergillus niger*, and the bacteria *Staphylococcus aureus* and *Escherichia coli*. Equipment used included an analytical balance, rotary evaporator, laminar air flow (LAF) cabinet, autoclave, oven, hot plate, fungal and bacterial incubators, magnetic stirrer, vortex mixer, pH meter, Bunsen burner, and vernier caliper, together with standard laboratory glassware and consumables (Petri dishes, test tubes, Erlenmeyer flasks, micropipettes, sterile cotton, sterile cotton swabs, microtips, and inoculation needles).

2.2. Sample Preparation

The samples used in this study were the petioles and midribs of muli banana leaves. The samples were cleaned and coarsely chopped, then dried by sun-drying in indirect sunlight and assisted by an oven. The dried samples were ground into powder using a blender, and the resulting powder was sieved to obtain a fine, uniform powder. The result was placed in a sealed glass container [23]

2.3. Preparation of Muli Banana Petiole and Leaf Midrib Extract

Extraction was performed using the maceration method with 96% ethanol as the solvent. A 400 g sample was dissolved in 4 L of solvent. Soaking was carried out for 3 days with occasional stirring. After 3 days, the sample was filtered using filter paper to produce a filtrate. The filtrate obtained was then evaporated using a rotary evaporator to obtain an ethanol extract [23].

2.4. Phytochemical Screening

Crude ethanol extracts of the petioles and leaf midribs of muli banana were qualitatively screened for secondary metabolites alkaloids, flavonoids, triterpenoids, steroids, saponins, and tannins using standard colorimetric and precipitation assays [24].

For the alkaloid test, 0.5 g of extract was dissolved in 2 N hydrochloric acid and distilled water, heated, filtered, and treated with Dragendorff's reagent; turbidity or precipitate formation indicated a positive result. For the tannin test, 1 g of extract was boiled in distilled water, filtered, and treated with 1% iron (III) chloride; a blackish-green or blackish-blue color indicated tannins. The saponin test used the foam method, in which extract dissolved in ethanol and treated with HCl was vigorously shaken; stable foam formation indicated saponins. For the flavonoid test, extract boiled in ethanol was treated with magnesium powder and concentrated HCl; a pink, red, or orange color indicated flavonoids. For the triterpenoid and steroid test, extract was treated with glacial acetic acid and concentrated sulfuric acid; a green or blue color indicated steroids, while a red or purple color indicated triterpenoids.

2.4.1. Determination of Antimicrobial Activity

Antifungal activity was tested using the agar well diffusion method on Potato Dextrose Agar (PDA) against *A. niger* and *C. albicans*. Antibacterial and antiseptic activity were evaluated using the same well diffusion method on Nutrient Agar (NA) against *E. coli* (isolated from the Microbiology Laboratory collection, Faculty of Mathematics and Natural Sciences, University of Lampung) and *S. aureus* (ATCC 25923). This approach is consistent with previous assessments of antimicrobial and antioxidant activity in plant- and fungus-derived secondary metabolites, [25], and complements an earlier study on the same plant species that evaluated the root extract of muli banana against *E. coli* and *S. aureus* using the Kirby-Bauer disc diffusion method [13].

2.4.2. Preparation of culture media

PDA medium (1.95 g in 50 mL distilled water) was prepared by heating until clear, sterilized by autoclaving (121°C, 15 min), supplemented with chloramphenicol (50 ppm), and poured into Petri dishes (±25 mL) under aseptic conditions [10]. NA medium (28 g in 1000 mL distilled water) was homogenized, sterilized by autoclaving (121°C, 15 min), and dispensed into Petri dishes (20–25 mL) and slanted test tubes for bacterial subculturing [26].

2.4.3. Preparation of fungal and bacterial inoculum

C. albicans was subcultured on PDA by streaking and incubated at 30°C for 3 days; a loopful of the culture was suspended in 9 mL of 0.9% saline and adjusted to the 0.5 McFarland standard [27]. *A. niger* was subcultured using the spot method and incubated at 25–28°C for 5–7 days until sporulation occurred [28]. *S. aureus* (ATCC 25923) and *E. coli* were subcultured on slanted NA and incubated at 37°C for 24 hours; a loopful of each culture was suspended in sterile 0.9% NaCl and adjusted to the 0.5 McFarland standard [29].

2.4.4. Extract concentration treatments

Antifungal activity was tested at five extract concentrations with positive (nystatin) and negative (sterile distilled water) controls: 15, 25, 35, 45, and 55% for *A. niger*, and 30, 40, 50, 60, and 70% for *C. albicans*. Antibacterial and antiseptic activity were tested at concentrations of 60, 70, 80, 90, and 100% for both *E. coli* and *S. aureus*, with chloramphenicol and Dettol, respectively, as positive controls and sterile distilled water as the negative control.

2.4.5. Antimicrobial activity testing procedure

For *C. albicans*, 0.1 mL of fungal inoculum was swabbed evenly onto PDA medium in 9-cm Petri dishes; four wells (6 mm diameter) were punched per dish across two replicate dishes and filled with 100 µL of extract or control, with four replicates in total, and inhibition zones were measured after incubating at 37°C for 72 hours [10]. For *A. niger*, the extract and controls were applied to wells punched in chloramphenicol-supplemented PDA, after which spores were spot-inoculated at the center of each plate and incubated at 25–28°C for 7 days; inhibition was indicated by a growth-free zone surrounding the wells [28]. For *S. aureus* and *E. coli*, 0.1 mL of bacterial suspension was spread evenly onto NA medium, four wells were punched per dish, and 0.1 mL of extract or the corresponding control (Dettol for the antiseptic test, chloramphenicol for the antibacterial test) was added to each well; plates were incubated at 37°C for 24 hours, and inhibition zone diameters were measured with a Vernier caliper.

2.4.6. Inhibition Zone Measurement

Antimicrobial activity can be observed by the formation of inhibition zones on the agar. The diameter is measured using a Vernier caliper and expressed in millimeters (mm). Measurements are taken after the incubation period has ended, in both the vertical and horizontal directions. The diameter of the inhibition zone can be calculated using the following formula:

$$\frac{(Dv-Ds)+(Dh-Ds)}{2} \qquad (30)$$

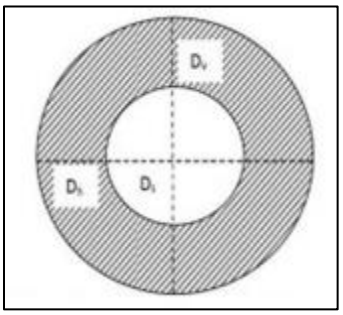

Figure 1 Measurement setup for the resistance zone. Dv, vertical diameter; Dh, horizontal diameter; Ds, well or disc diameter

The results of the diffusion test were assessed visually by measuring the diameter of the inhibition zone. The strength of inhibition was classified as very strong (>20 mm), strong (16–20 mm), moderate (10–15 mm), and weak (<10 mm) [31]

2.5. Statistical Analysis

The data analyzed were derived from observations of the diameter of the inhibition zone. The data were first tested for normality and homogeneity. A normality test was conducted, where a p-value > 0.05 was considered indicative of normally distributed data. Statistical significance was set at a p-value < 0.05. If the data were not normally distributed, a non-parametric Kruskal-Wallis test was performed to determine the antimicrobial efficacy of the ethanol extract

concentrations of the petioles and midribs of Muli banana leaves. This was followed by a paired test using the *Pairwise Comparison* or *Mann–Whitney* post-hoc test to demonstrate that there were significant differences between treatments. All statistical analyses were performed using IBM SPSS statistics 20 software [32]

3. Results and discussion

3.1. Phytochemical Screening

Phytochemical analysis of ethanol extracts from the petioles and leaf midribs of the muli banana revealed the presence of secondary metabolites such as tannins, flavonoids, saponins, and alkaloids, while no steroid or triterpenoid compounds were detected. This is consistent with the results of phytochemical tests using ethanol as a solvent, which identified lycopene, phenols, flavonoids, saponins, alkaloids, and tannins compounds known for their antimicrobial properties, including antifungal activity [12]. The results of the phytochemical analysis of the ethanol extracts of the petioles and leaf midribs of the Muli banana are presented in Table 1

Table 1 The results of phytochemical screening of ethanol extracts from the petioles and leaf midribs of Muli banana for antifungal activity

Phytochemical Analysis	Reagent	Results	Description
Alkaloids	Dragendorff	+	Turbidity and sediment
Flavonoids	Mg + HCl thick	+	A red or orange color appears
Saponins	Hot distilled water and concentrated HCl	+	A stable foam emulsion is formed
Tannins	Hot distilled water and 3% FeCl3	+	It has a blackish-green color
Steroid and triterpenoid	$CH_3COOH + H_2SO_4$ thick	-	Dark brown

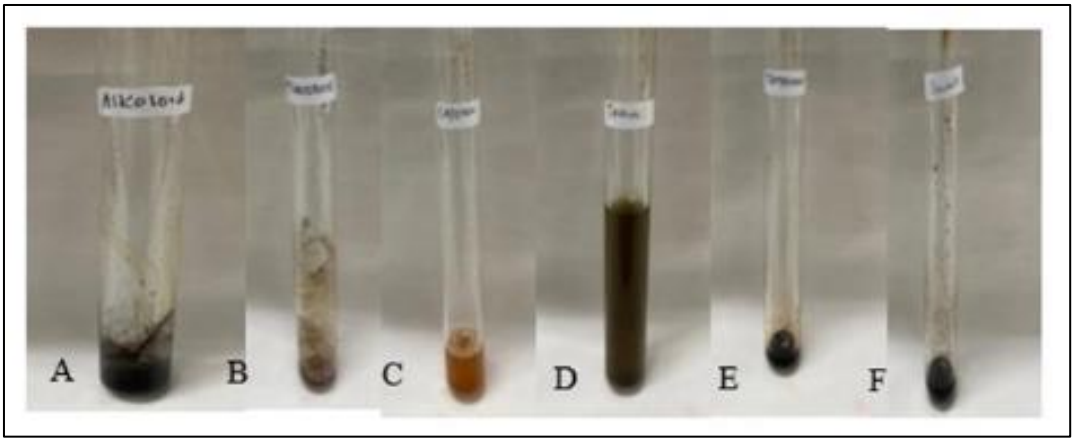

Figure 2 Results of phytochemical screening of ethanol extracts from the petioles and leaf midribs of (*Musa acuminata* Colla.) (A) Alkaloids, (B) Flavonoids, (C) Saponins, (D) Tannins, (E) Steroids, (F) Triterpenoids

Based on the results of the study, the petioles and midribs of muli banana leaves produced secondary metabolites in the form of flavonoids, tannins, alkaloids, and saponins after extraction using 96% ethanol, whereas triterpenoids and steroids were not detected in the phytochemical analysis. 96% ethanol is a highly polar solvent. This is due to the chemical structure of ethanol, which contains a polar hydroxyl group (-OH) and a non-polar ethyl chain, enabling it to interact with various compounds such as alkaloids, flavonoids, tannins, and saponins that have potential as antifungal agents [33].

3.2. Antifungal Inhibition Zone

Ethanol extracts of the petioles and leaf midribs of the muli banana at concentrations of 50%, 60%, and 70% exhibited sufficient antifungal activity to inhibit the growth of *C. albicans*, with average inhibition zone diameters of 7.1 mm, 8.37 mm, and 10.32 mm, respectively. Furthermore, in the positive control using 100,000 IU of Nystatin, a large inhibition

zone formed on *C. albicans* (25.51 mm). Meanwhile, ethanol extracts of the petioles and midribs of Muli banana leaves at concentrations of 30% and 40% did not produce any inhibition zones. The results of the antifungal inhibition zone test can be seen in Figure 3

Figure 3 Antifungal test results of ethanol extracts from the petioles and leaf midribs of Muli banana against Candida albicans

The inhibition zone data were analyzed using the Kruskal-Wallis test because the data were not normally distributed and were not homogeneous. The results of the Kruskal-Wallis test showed a significance value of 0.000 ($p < 0.05$), indicating that the application of various concentrations had a significant effect on the formation of inhibition zones in the fungi. Next, a paired comparison test was conducted between each treatment using the *Pairwise Comparison* post-hoc test, as shown in Table 2.

Table 2 Results of the analysis of data on the inhibition zone diameters of ethanol extracts of the petioles and midribs of Muli banana leaves against *Candida albicans*

Treatment	Concentration (%)	Mean diameter of the inhibition zone (Mean ± SD)
Negative control	Sterile distilled water	0.00 ± 0.00^{a}
Ethanol extract of the petioles and leaf midribs of the muli banana	30	0.00 ± 0.00^{a}
	40	0.00 ± 0.00^{a}
	50	7.1 ± 0.27^{ab}
	60	8.37 ± 0.35^{ab}
	70	10.32 ± 0.32^{ab}
Positive control	Nystatin	25.51 ± 0.19^{b}

Note: Values are presented as mean ± SD. Means with different superscript letters (a, b, and ab) in the same column indicate significant differences based on the Pairwise Comparison test ($p < 0.05$).

The results of the *Pairwise Comparison* test indicate that there are significant differences between the positive control and the negative control, as well as between the 30% and 40% concentrations (Adj. Sig < 0.05), where the significance level has been adjusted using the Bonferroni correction, which is a very strict statistical adjustment. Statistically, the increases in measurements at the 50%, 60%, and 70% concentrations (7.1 mm, 8.37 mm, and 10.32 mm) are not considered extreme enough to be deemed mathematically consistent differences when compared to the increase observed in the positive control (25.51 mm).

The results of antifungal activity testing against *A. niger* showed that treatment with the extract at various concentrations, namely 15%, 25%, 35%, 45%, and 55% did not produce any inhibition zones in the wells. This indicates that at these concentrations, the extract was unable to inhibit the growth of *A. niger* in vitro. In contrast, the positive control showed the formation of a clear inhibition zone in all replicates with an average of 12.18 mm.

The inhibition zone data were then analyzed using the Kruskal-Wallis test to determine the effectiveness of the extract concentrations as antifungals. The results of the Kruskal-Wallis test showed a significance value of 0.000 ($p < 0.05$), indicating that the application of various concentrations had a significant effect on the formation of inhibition zones in the fungi. Next a paired comparison test was conducted between each treatment using the *Pairwise Comparison* post-hoc test, as shown in Table 3.

Table 3 Results of the analysis of measurements of the inhibition zone diameter of ethanol extracts from the petioles and midribs of Muli banana leaves against *Aspergillus niger*

Treatment	Concentration (%)	Mean diameter of the inhibition zone (Mean ± SD)
Negative control	Sterile distilled water	0.00±0.00[a]
Ethanol extract of the petioles and leaf midribs of the muli banana	15	0.00±0.00[a]
	25	0.00±0.00[a]
	35	0.00±0.00[a]
	45	0.00±0.00[a]
	55	0.00±0.00[a]
Positive control	Nystatin	12.18±1.90[b]

Note: Values are presented as mean ± SD. Means with different superscript letters (a, and b) in the same column indicate significant differences based on the Pairwise Comparison test ($p < 0.05$).

The results of the Pairwise Comparison post-hoc test confirmed that significant differences ($p < 0.05$, Bonferroni-adjusted) were observed only between the positive control and all extract concentration groups (30%, 40%, 50%, 60%, and 70%). This significant difference stems from the contrast in inhibition zone values between the positive control and all treatment groups, although the extract concentration groups themselves did not differ from the negative control.

Meanwhile, a comparison of the extract concentrations revealed no significant differences ($p > 0.05$), with an average inhibition zone of 0 mm. This indicates that, within the tested concentration range, the extract was unable to inhibit the growth of *A. niger*, and thus its effectiveness did not match that of the standard control.

3.3. Inhibition Zones in Antibacterial and Antiseptic Tests

Ethanol extracts of the petioles and midribs of Muli banana leaves at concentrations of 60%, 70%, 80%, 90%, and 100% exhibited antiseptic activity against *S. aureus*, with average inhibition zone diameters of 10.7 mm, 12.6 mm, 13.6 mm, 13.8 mm, and 13.4 mm. The largest inhibition zone diameter was obtained at a concentration of 90% with an average of 13.8 mm. Furthermore, in the positive control using Dettol, a larger inhibition zone was formed compared to all extract concentrations. The results of the antiseptic inhibition zones at a concentration of 90% can be seen in Figure 4.

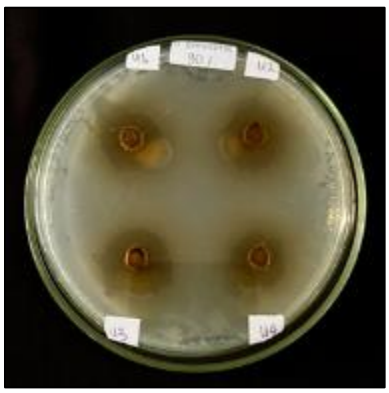

Figure 4 Antiseptic test results of a 90% ethanol extract of the petioles and leaf midribs of Muli banana leaves against *S. aureus.*

The inhibition zone data were analyzed using the Kruskal-Wallis test because the data were not normally distributed and were not homogeneous. The results of the Kruskal-Wallis test showed a p-value of 0.490 ($p > 0.05$), indicating no

significant effect of the various extract concentrations on inhibition zone formation against *S. aureus*. A paired comparison test was nonetheless conducted between each treatment using the *Mann-Whitney* post-hoc test, as shown in Table 4.

Table 4 Results of the analysis of data on the inhibition zone diameters of ethanol extracts from the petioles and midribs of Muli banana leaves against *S. aureus* and *E. coli*

Antimicrobial Tests	Concentration (%)	Mean diameter of the inhibition zone (Mean ± SD)
Antibacterial Test (*E. coli*)	Negative control	0 ± 0^{a}
	Positive control	31.5 ± 1.23^{c}
	60	0 ± 0^{a}
	70	0 ± 0^{a}
	80	0 ± 0^{a}
	90	2.9 ± 3.3^{b}
	100	0 ± 0^{a}
Antiseptic Test (*S. aureus*)	Negative control	0 ± 0^{a}
	Positive control	35.1 ± 1.81^{b}
	60	10.7 ± 0.82^{a}
	70	12.6 ± 0.97^{a}
	80	13.6 ± 1.29^{a}
	90	13.8 ± 1.19^{a}
	100	13.4 ± 1.7^{a}

Note: Values are presented as mean ± SD. Means with different superscript letters (a, and b) in the same column indicate significant differences based on the Mann-Whitney test ($p < 0.05$).

Further *Mann-Whitney* tests showed that all comparisons between groups had p-values greater than 0.05; therefore, it can be concluded that there were no significant differences between any pairs of extract concentrations tested.

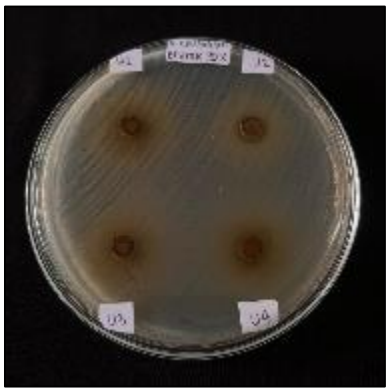

Figure 5 Results of antibacterial testing of a 90% ethanol extract of the petioles and leaf midribs of Muli banana leaves against *Escherichia coli*

Ethanol extracts of the petioles and leaf midribs of the muli banana exhibited limited antibacterial activity against *E. coli*. Inhibition zones formed only at a 90% concentration, with an average diameter of 2.9 mm. Meanwhile, no inhibition zones formed at concentrations of 60%, 70%, 80%, and 100%. Furthermore, the positive control using chloramphenicol produced a larger inhibition zone compared to the extracts. The results of the antibacterial inhibition zones at a 90% concentration can be seen in Figure 5

The inhibition zone data were analyzed using the Kruskal-Wallis test because the data were not normally distributed and were not homogeneous. The results of the Kruskal-Wallis test showed a significance level of <0.001 ($p < 0.05$), indicating that the application of various concentrations had a significant effect on the formation of inhibition zones in *E. coli*. Next, a paired comparison test was conducted between each treatment using the *Mann-Whitney* post-hoc test, as shown in Table 4

The results of the *Mann-Whitney* post-hoc test for antibacterial activity with Bonferroni correction in Table 4 show that a significant difference was found when comparing the 90% concentration with all other concentrations, namely, 60%, 70%, 80%, and 100%, with a p-value of 0.022 ($p < 0.05$).

3.3.1. The Effect of 96% Ethanol Solvent on the Extraction of Secondary Metabolites from Extracts of the Petioles and Midribs of Muli Banana Leaves and Their Mechanism of Action as Antifungal Agents

The use of 96% ethanol as a solvent plays a crucial role in determining the types of secondary metabolites that are successfully extracted. Based on the results of the study, the petioles and midribs of the muli banana leaf produced secondary metabolites in the form of flavonoids, tannins, alkaloids, and saponins after extraction using 96% ethanol, whereas triterpenoids and steroids were not detected in the phytochemical analysis. Triterpenoids and steroids are generally non-polar to semi-polar in nature, making them less optimally extracted using a predominantly polar solvent like 96% ethanol, especially if no further fractionation is performed with non-polar solvents such as n-hexane or chloroform [34]. The use of 96% ethanol as a polar solvent tends to be more selective in extracting polar compounds such as flavonoids, tannins, saponins, and alkaloids, but is less effective in dissolving non-polar compounds such as triterpenoids and steroids. This is consistent with the research by Aristina et al. [35], which showed negative results for triterpenoids in the ethanol extract of muli banana peel.

Secondary metabolites inhibit fungal growth through four mechanisms: inhibition of chitin and β-glucan cell wall synthesis, inhibition and disruption of cell membrane permeability, inhibition of structural and functional protein synthesis, and DNA damage [36]. Flavonoids are widely reported to exhibit antimicrobial, antifungal, antiviral, anticancer, and antitumor properties [25]. Research indicates that flavonoids may exhibit dual mechanisms of action, such as binding to ergosterol, inhibiting biofilm formation, and inducing oxidative stress, which collectively suppress fungal proliferation [37]. Flavonoids form complexes with soluble proteins, leading to impaired permeability of fungal cell membranes [15]. Flavonoids are lipophilic and can bind to ergosterol in the cell membrane, causing increased membrane permeability and leakage of intracellular components such as ions and essential metabolites. Flavonoids also play a role in inhibiting the formation of fungal cell structures, including cell walls, thereby weakening the cells and making them more susceptible to damage [20].

Alkaloids exhibit diverse antifungal mechanisms of action, targeting different active sites in various fungal species. Alkaloids are known for their low toxicity, making them strong candidates for the development of new antifungal agents. Due to the increasing resistance of pathogenic fungi such as *C. albicans* and *A. niger*, alkaloids have shown potential as inhibitors and fungicidal agents [38]. Alkaloids and flavonoids possess the strongest antifungal activity because they are capable of targeting various critical structures within fungal cells. These compounds cause damage to the cell membrane and then strongly bind to ergosterol, forming pores that lead to membrane leakage. This results in irreversible damage to the cell and fungal cell death [39].

Tannins are able to inhibit chitin synthesis for fungal cell wall formation and to damage cell membranes, thereby inhibiting fungal growth [24]. Tannins can also bind to metal ions required by fungi for growth and enzymatic activity, thereby inhibiting cellular metabolism. The combination of structural damage caused by alkaloids and protein inactivation by tannins makes these two compounds effective as antifungal agents with a multi-target mechanism of action that has the potential to reduce the risk of antifungal resistance [40].

The mechanism of fungistatic activity by saponin compounds occurs through a reduction in cell membrane surface tension due to the disruption of sterol structures, which play a crucial role in fungal cell wall synthesis [41]. Saponins are able to bind to membrane sterols, thereby increasing permeability and causing a loss of cell membrane integrity, as indicated by the leakage of proteins and nucleic acids. In *C. albicans* fungi, saponins are similarly reported to disrupt cell integrity [14]. Additionally, saponins inhibit ergosterol biosynthesis by suppressing the expression of key genes in the sterol synthesis pathway, thereby weakening the stability and function of fungal cell membranes [42].

3.3.2. Secondary Metabolites in Ethanol Extracts of Muli Banana Petioles and Leaf midribs, and Their Inhibitory Mechanisms as Antibacterial and Antiseptic Agents

Flavonoids and alkaloids are known to exhibit antibacterial activity through various mechanisms that target the structure and function of bacterial cells. The mechanisms of action of antibacterial compounds generally include cell membrane damage, inhibition of cell wall synthesis, disruption of nucleic acid and protein synthesis, and inhibition of metabolic pathways involved in bacterial growth [43].

Flavonoids are a type of secondary metabolite widely found in various plant species and have been reported to possess broad-spectrum antibacterial activity [44]. The antibacterial activity of flavonoids occurs through several mechanisms, including damaging bacterial cell membranes, inhibiting enzymes involved in nucleic acid synthesis, disrupting the respiratory chain, and inhibiting the formation of bacterial cell walls [44]. Flavonoids can also interact with DNA, thereby inhibiting the synthesis of DNA and RNA required for bacterial cell growth and division [45]. Additionally, flavonoids can form complexes with membrane proteins, leading to cell membrane damage, increased leakage of intracellular components, and inhibition of bacterial energy metabolism. The lipophilic nature of flavonoids also facilitates their interaction with bacterial cell membranes, particularly in Gram-positive bacteria, thereby contributing to their antibacterial activity [44].

Alkaloids are a class of secondary metabolites that possess a broad spectrum of antibacterial activity, including against bacteria that have developed resistance to antibiotics [43]. These compounds act through various mechanisms, such as inhibiting nucleic acid and protein synthesis, disrupting cell membrane function, and acting as DNA intercalators that inhibit replication and transcription. Additionally, alkaloids can inhibit the activity of efflux pumps involved in bacterial resistance mechanisms, thereby increasing the accumulation of antibacterial compounds within the cell. Alkaloids can also inhibit the activity of various essential enzymes involved in bacterial metabolic pathways, thereby inhibiting bacterial growth and proliferation [43].

The presence of flavonoids and alkaloids in the ethanol extract of the petioles and midribs of Muli banana leaves is believed to contribute to the formation of inhibition zones in tests against *S. aureus* and *E. coli.* However, the resulting inhibitory effectiveness differs between the two bacteria. This difference is thought to be related to the structural characteristics of the cell walls of Gram-positive and Gram-negative bacteria, which affect the ability of active compounds to diffuse and reach intracellular targets. Therefore, the antibacterial and antiseptic activities of the extract are influenced not only by the types of secondary metabolites it contains but also by the characteristics of the target microorganisms.

The antibacterial mechanism of saponins is related to their ability to interact with bacterial cell membranes, thereby increasing membrane permeability and causing damage to cell integrity. This damage results in the leakage of intracellular components and disruption of bacterial metabolic processes [46]. Meanwhile, tannins act through a more complex mechanism, including inhibition of cell wall synthesis, disruption of cytoplasmic membrane function, chelation of metal ions required for metabolism, and inhibition of enzyme activity and fatty acid biosynthesis. Consequently, bacterial cell growth and division are inhibited, thereby reducing bacterial viability [47].

3.3.3. Differences in the Responses of Candida albicans and Aspergillus niger to the Antifungal Activity of Ethanol Extracts from the Petioles and Leaf midribs of Muli Banana Leaves

Filamentous fungi such as *A. niger* have biological characteristics that differ significantly from those of yeasts such as Candida albicans, particularly in terms of cell wall structure, hyphal formation, and response mechanisms to antifungal agents. The growth of *Aspergillus* in the form of branched mycelium makes the penetration of antifungal compounds more difficult, as its cell wall is composed of chitin, β-glucan, and complex polysaccharides that are thicker and more organized than those of unicellular yeasts [48]. These structural differences are evident in the antifungal activity test results of this study. Ethanol extracts of the petioles and leaf midribs of muli banana were able to form inhibition zones against *C. albicans* but did not show inhibition zones against *A. niger*. For *C. albicans*, an inhibition zone began to form at a 50% concentration (7.1 mm), increased at a 60% concentration (8.37 mm), and reached its largest diameter at a 70% concentration (10.32 mm). Conversely, all concentration variations of the extract against *A. niger* (15%, 25%, 35%, 45%, and 55%) showed negative results with an inhibition zone diameter of 0 mm.

The results of the study showed that the extract did not form an inhibition zone against *A. niger*, but was able to inhibit the growth of *C. albicans*, with the diameter of the inhibition zone increasing as the extract concentration increased [39]. This difference indicates that the extract is more effective against yeast than against molds, which is thought to be related to the ability of secondary metabolites such as flavonoids, tannins, and alkaloids to disrupt the permeability of yeast cell membranes, which are simpler compared to the more complex hyphal structure of molds [48]. In *C. albicans*,

secondary metabolites can more easily diffuse and disrupt cell membrane integrity, leading to leakage of intracellular components and disruption of cellular metabolism. This results in the formation of an increasingly larger inhibition zone at higher extract concentrations. Conversely, the more complex structure of *A. niger's* mycelium and cell wall makes it difficult for active compounds to reach cellular targets optimally, preventing the formation of an inhibition zone around the well [19].

A. niger possesses physiological adaptation capabilities that enable cells to survive when exposed to bioactive plant compounds. Exposure to antifungal compounds can trigger a cellular stress response characterized by increased synthesis of cell wall components such as chitin and β-glucans to strengthen the cell wall structure and maintain cell membrane integrity [49]. This is consistent with the results of research by Castano-Duque et al. [49], which showed that cagaita leaf extract containing phenols, tannins, flavonoids, and saponins was only able to reduce the growth of *A. niger* mycelium by a few percent at concentrations up to 10,000 mg/L; where the fungus still grew (only inhibited or fungicidal). *A. niger* can also activate cellular defense systems, such as increasing the activity of antioxidant enzymes to reduce oxidative stress and activating membrane transporter proteins that function to remove toxic compounds from the cell. This adaptation mechanism helps reduce the accumulation of antifungal compounds within the cell so that metabolic processes continue. Through this physiological response, *A. niger* is still able to maintain survival even though its growth can be inhibited by exposure to secondary metabolite compounds.

C. albicans is a unicellular yeast with a relatively simpler cell wall and is more susceptible to external disturbances. Physiologically, *C. albicans* has a faster metabolism and is highly dependent on cell membrane integrity, making it more susceptible to membrane damaging antifungal compounds. *C. albicans* has a more limited defense mechanism, although it is still able to adapt through biofilm formation, morphological changes (yeast to pseudohyphae), and increased membrane protein expression to reduce the accumulation of toxic compounds [50]. Therefore, *C. albicans* shows a higher sensitivity to secondary metabolite compounds in ethanol extracts of the petioles and leaf midribs of muli banana, as seen from the formation of inhibition zones at concentrations of 50%, 60%, and 70%. This is supported by research by Djenontin et al. [51], which shows that filamentous fungi such as *A. niger* have a more complex defense system than yeast, including cell wall regulation and oxidative stress response, while *Candida* is more susceptible to membrane disruption and shows a biofilm response as the main defense mechanism.

3.3.4. Differences in Cell Wall Structure and Sensitivity of Gram-Positive (Staphylococcus aureus) and Gram-Negative (Escherichia coli) Bacteria to Antibacterial and Antiseptic Compounds

The difference in antibacterial and antiseptic activity of ethanol extracts of the petioles and leaf midribs of muli banana against *S. aureus* and *E. coli* is thought to be influenced by differences in the cell wall structure of the two bacteria. *S. aureus* is a Gram-positive bacterium that has a thick peptidoglycan layer with a relatively simpler cell wall structure. In contrast, *E. coli* is a Gram-negative bacterium that has a thinner peptidoglycan layer, but is equipped with an outer membrane containing lipopolysaccharides that acts as a barrier to the entry of various antimicrobial compounds.

The results showed that the ethanol extract of the petioles and leaf midribs of muli banana was able to form an inhibition zone at all test concentrations against *S. aureus*, while in *E. coli* the inhibition zone was only formed at a concentration of 90%. These results indicate that *S. aureus* has a higher sensitivity to the extract than *E. coli*. This difference in sensitivity is thought to be caused by the cell wall structure of Gram-positive bacteria which is more easily penetrated by secondary metabolite compounds compared to Gram-negative bacteria which have an outer membrane as a barrier to the diffusion of antibacterial compounds [52].

In Gram-positive bacteria, the absence of a lipopolysaccharide layer allows antimicrobial compounds, both hydrophilic and hydrophobic, to diffuse more easily into the peptidoglycan layer. Active compounds can then interact with cell wall components, causing damage to the cell structure, ultimately leading to lysis due to osmotic pressure from within the cell. Conversely, in Gram-negative bacteria, the outer membrane, composed of lipopolysaccharides, only allows certain molecules to pass through the porin protein, thus limiting the penetration of antibacterial compounds. In addition to functioning as a physical barrier, Gram-negative bacteria also have a peptidoglycan recycling mechanism that plays a role in maintaining cell wall stability. This pathway allows the results of peptidoglycan degradation to be reused for the formation of new cell walls, thus helping bacteria maintain cell integrity when exposed to antimicrobial compounds. The presence of this mechanism is thought to contribute to the lower inhibitory activity of the extract against *E. coli* compared to *S. aureus*. A comparatively weaker antibacterial effect against *E. coli* relative to *S. aureus* has similarly been reported for the root extract of muli banana [13], supporting the consistency of this Gram-dependent pattern across different parts of the same plant

4. Conclusion

Ethanol extract of the petioles and leaf midribs of muli banana (*Musa acuminata* Colla) contains flavonoids, saponins, tannins, and alkaloids that have potential as antimicrobial agents. The extract exhibited its highest mean inhibition zone against *Staphylococcus aureus* at a concentration of 90% (13.8 mm) and *Candida albicans* at a concentration of 70% (10.32 mm), but its activity was low against *Escherichia coli* and did not show inhibition against *Aspergillus niger*. This study shows the potential of utilizing waste petioles and leaf midribs of muli banana as a source of natural antimicrobials that can be further developed through identification of active compounds, toxicity testing, and product formulation to support the provision of safe, affordable, and beneficial antimicrobial alternatives for the community.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

This study did not involve human participants or animals; therefore, ethical approval was not required.

Statement of informed consent

This study did not involve human subjects; therefore, informed consent was not required.

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