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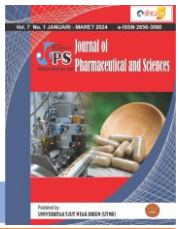

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Antibacterial efficacy of 70% ethanolic extract of kepok banana leaves (*Musa Paradisiaca* Linn) against *Staphylococcus aureus*: implications for natural antimicrobial development

Efektivitas antibakteri ekstrak etanol 70% daun pisang kepok (*Musa paradisiaca* Linn.) terhadap *Staphylococcus aureus*: implikasi untuk pengembangan antimikroba alami

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Abstract

Bacterial infections remain a health problem, especially in tropical countries such as Indonesia. The increasing occurrence of antibiotic resistance has resulted in the need for the development of alternative antibacterial agents based on natural resources. Kepok banana leaves (*Musa paradisiaca* Linn.) contain secondary metabolites that may possess antibacterial activity. This study aimed to determine the secondary-metabolite groups and antibacterial activity of a 70% ethanolic extract of kepok banana leaves (*Musa paradisiaca* Linn.) against *Staphylococcus aureus*. The study included plant identification, phytochemical screening, heavy metal analysis for Pb and Cd, extraction by maceration using 70% ethanol, and antibacterial activity testing using the disk diffusion method. The antibacterial test was performed using extract concentrations of 5%, 10%, 15%, 20%, and 25%, with three replications for each treatment. Phytochemical screening revealed the presence of flavonoids, alkaloids, saponins, terpenoids, and tannins in the ethanolic extract of kepok banana leaves. Pb and Cd were not detected in the extract under the conditions of the analysis. The extract inhibited the growth of *Staphylococcus aureus* at all tested concentrations, with inhibition zones categorized as strong, and the inhibition zone increased with increasing extract concentration. These findings indicate that the 70% ethanolic extract of kepok banana leaves has potential as a source of natural antibacterial compounds against *Staphylococcus aureus*. The observed activity may be associated with the secondary metabolites detected in the extract; however, their individual contributions and possible interactions were not directly investigated.

***Keywords*: antibacterial activity, Musa paradisiaca Linn., phytochemical screening, Staphylococcus aureus.**

Abstrak

Infeksi bakteri masih menjadi masalah kesehatan, terutama di negara-negara tropis seperti Indonesia. Meningkatnya kejadian resistensi antibiotik telah mendorong kebutuhan untuk mengembangkan agen antibakteri alternatif berbasis sumber daya alam. Daun pisang kepok (*Musa paradisiaca* Linn.) mengandung metabolit sekunder yang berpotensi memiliki aktivitas antibakteri. Penelitian ini bertujuan untuk mengetahui kelompok metabolit sekunder dan aktivitas antibakteri ekstrak etanol 70% daun pisang kepok (*Musa paradisiaca* Linn.) terhadap *Staphylococcus aureus*. Penelitian meliputi identifikasi tanaman, skrining fitokimia, analisis logam berat Pb dan Cd, ekstraksi dengan metode maserasi menggunakan etanol 70%, dan pengujian aktivitas antibakteri menggunakan metode difusi cakram. Uji antibakteri dilakukan menggunakan konsentrasi ekstrak 5%, 10%, 15%, 20%, dan 25%, dengan tiga replikasi untuk setiap perlakuan. Hasil skrining fitokimia menunjukkan adanya flavonoid, alkaloid, saponin, terpenoid, dan tanin dalam ekstrak etanol daun pisang kepok. Pb dan Cd tidak terdeteksi dalam ekstrak berdasarkan kondisi analisis yang digunakan. Ekstrak mampu menghambat pertumbuhan *Staphylococcus aureus* pada seluruh konsentrasi yang diuji dengan kategori zona hambat kuat, dan diameter zona hambat meningkat seiring dengan peningkatan konsentrasi ekstrak. Hasil ini menunjukkan bahwa ekstrak etanol 70% daun pisang kepok berpotensi menjadi sumber senyawa antibakteri alami terhadap *Staphylococcus aureus*. Aktivitas tersebut diduga berkaitan dengan metabolit sekunder yang terdeteksi dalam ekstrak, meskipun kontribusi dan kemungkinan interaksi masing-masing senyawa belum dianalisis secara langsung.

***Keywords*: aktivitas antibakteri, Musa paradisiaca Linn., skrining fitokimia, Staphylococcus aureus**

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Introduction

Bacteria are a type of harmful germs that enter the body and multiply to cause infectious diseases. The microorganisms that infect people commonly are some of the Escherichia coli, Salmonella typhi, Streptococcus mutans, and *Staphylococcus aureus*. *Staphylococcus aureus*, a Gram-positive coccus-shaped bacterium, grows in grape-like clusters [1]. It is normally present as a flora on human skin and mucous membranes. However, S. aureus can be an opportunistic pathogen that causes different infectious diseases when the immune system is weak or tissue is damaged (Abdallah, 2016). Can cause skin infections such as cellulitis, acne, boils, impetigo, and abscesses. This bacterium also causes more serious diseases such as pneumonia, osteomyelitis, meningitis, and endocarditis [2]. Therefore, controlling the proliferation of S. aureus is one of the important strategies in the treatment of infectious diseases.

Antibacterial drugs are often used to treat infections caused by bacteria. Antibacterial compounds are the substances that can prevent the multiplication of bacteria that cause disease or kill the bacteria [3]. However, the irrational and overuse of antibiotics has resulted in more cases of antibiotic resistance in some countries. Antibiotic resistance is when bacteria become resistant to antibiotics that used to work to kill or stop them [4]. The disease is caused by a number of factors, including inappropriate use of antibiotics, poor environmental cleanliness, increased population mobility, and an increasing number of people with compromised immune system [5]. The development of antibiotic resistance fuels the need to discover new, safer, and more potent sources of antibacterial activity.

One of the widely developed strategies is the use of natural materials as sources of alternative antibacterial chemicals [6]. The utilisation of plants as traditional medicinal material has been transmitted from generation to generation and is still evolving. Medicinal plants are known to possess diverse secondary metabolites such as flavonoids, alkaloids, tannins, and saponins that may possess antibacterial properties [5]. Indonesia is a tropical country with a very high level of biodiversity including various plants that can be used as natural medicine [7]. Musa paradisiaca L. is one of the plants used by the community with high frequency because almost all parts of the banana can be used for various purposes [3]. Banana kepok (Musa paradisiaca L.) is one of banana types usually found in Indonesia. The fruit and leaves of this banana are mostly used. Kepok banana leaves have antibacterial and antioxidant properties that can be utilised in the medical field in addition to food wrappers [8]. In order to create a source of natural antibacterial agents which can be used in pharmaceutical and medical industries, studies on the efficacy of ethanol extract from kepok banana leaves against the development of *Staphylococcus aureus* need to be performed.

Methods

Apparatus

The apparatus used in this study included porcelain dishes (Iwaki®), test tubes (Iwaki®), Petri dishes (Iwaki®), digital analytical scales (Ohaus®), an electric stove (Maspion®), Erlenmeyer flasks (Iwaki®), an autoclave (All American®), inoculating needles (OneMed®), a vortex mixer (Gemmy®), dropper pipettes (Iwaki®), an incubator (Memmert®, Germany), micropipettes (Socorex®), a sterile spreader (Normax®), glass stirring rods (Iwaki®), graduated cylinders (Iwaki®), beakers (Iwaki®), a Bunsen burner (Pyrex®), matches (Tokai®), syringes (Terumo®), aluminum foil (Klin Pak®), a hot plate (Thermo Scientific®), tweezers (RRC®), a ruler (Butterfly®), a test-tube rack (Normax®), a water bath (Memmert®, Germany), and a stainless-steel spatula (RRC®).

Materials

The materials used in this study included powdered kepok banana leaves (*Musa paradisiaca* Linn.), distilled water (Brataco®), 70% ethanol (Brataco®), methanol (Brataco®), petroleum ether (Merck®), magnesium powder (Merck®), Mayer's reagent (Merck®), 2 N hydrochloric acid (Merck®), Dragendorff's reagent (Merck®), diethyl ether (Merck®), acetic anhydride (Merck®), concentrated sulfuric acid (Merck®), hot water, dimethyl sulfoxide (DMSO) (Merck®), 1% ferric chloride ($FeCl_3$) solution (Merck®), Mueller–Hinton Agar (MHA) medium (Merck®), 0.9% sodium chloride solution (Otsuka®), chloramphenicol (Kimia Farma®), sterile paper discs (Oxoid®), and Mueller–Hinton Broth (MHB) medium (Merck®).

Collection, Identification, and Preparation of Musa paradisiaca Linn. Leaf Simplicia

Kepok banana leaves (*Musa paradisiaca* Linn.) were collected from Panji District, Situbondo Regency. The leaves selected for the study were fresh, dark green, free from visible damage, and not wilted. Plant identification was conducted at the Biology Laboratory, Ahmad Dahlan University, Yogyakarta, to confirm the botanical identity of the samples. The preparation of the simplicia began with the selection of leaves according to the predetermined criteria. Wet sorting was then performed to remove the petioles, damaged parts, and other unwanted materials. The leaves were washed under running water to remove adhering dirt and subsequently dried in an oven at 40°C for 24 h. After drying, the leaves were subjected to a second sorting process to remove possible contaminants. The dried leaves were then ground using a blender and sieved through a No. 60 mesh sieve. The moisture content of the simplicia was maintained below 10% [9,10].

Extraction of Musa paradisiaca Linn. Leaves

The dried simplicia powder was extracted by maceration using 70% ethanol at a ratio of 1:10. Briefly, 100 g of simplicia powder was soaked in 1 L of 70% ethanol. The maceration process was conducted for three consecutive 24-h periods with periodic stirring and protection from direct sunlight. After three days, the mixture was filtered, and the residue was discarded. The filtrate was concentrated using a rotary evaporator at 78°C to remove the solvent and was subsequently concentrated in a water bath [11].

Phytochemical Screening

Phytochemical screening was conducted as a preliminary qualitative analysis to identify groups of secondary metabolites present in the extract [12]. The tested compounds included flavonoids, alkaloids, saponins, terpenoids, and tannins. For the flavonoid test, the extract was treated with methanol and petroleum ether. The methanol layer was evaporated, and the residue was dissolved in 70% ethanol and treated with magnesium powder and 2 N hydrochloric acid. The development of an orange to purple-red color indicated a positive reaction. For the alkaloid test, the extract was boiled with 2 N hydrochloric acid and distilled water, cooled, and filtered.

The filtrate was separately tested using Mayer's and Dragendorff's reagents. A positive reaction with Mayer's reagent was indicated by the formation of a white precipitate, whereas a positive reaction with Dragendorff's reagent was indicated by the development of an orange-brown color. For the saponin test, hot water was added to the extract, and the mixture was shaken vigorously. The formation of stable foam that remained after the addition of 2 N hydrochloric acid indicated a positive reaction. For the tannin test, a 1% $FeCl_3$ solution was added to the filtrate obtained after boiling the extract. The development of a violet-green color indicated the presence of tannins. For the terpenoid test, the extract filtrate was treated with diethyl ether, acetic anhydride, and concentrated sulfuric acid. The development of a blue, green, or red color indicated a positive reaction for terpenoids [13].

The phytochemical screening results were interpreted qualitatively. A positive result (+) indicated the formation of the characteristic color, precipitate, or foam associated with the tested metabolite, whereas a negative result (−) indicated the absence of a visible positive reaction under the test conditions.

Heavy Metal Analysis

Heavy metal analysis was conducted to determine the potential contamination of the kepok banana leaf extract by lead (Pb) and cadmium (Cd). The determination of Pb and Cd content was performed using Atomic Absorption Spectrophotometry (AAS) [14].

Sterilization of Equipment

All equipment and materials used in microbiological testing are first sterilized. Glassware such as test tubes and petri dishes are sterilized using an oven at 180°C for 2 hours [15]. Equipment and materials that

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cannot withstand prolonged dry heating are sterilized using an autoclave at 121°C for 15 minutes. Meanwhile, glass spreaders, inoculating loops, and tweezers are sterilized by incineration using a Bunsen burner until they turn red [16].

Preparation of Mueller–Hinton Agar Medium

Mueller–Hinton Agar (MHA) medium was prepared according to the manufacturer's instructions and the EUCAST recommendations for antimicrobial susceptibility testing [17]. The required amount of MHA powder was dissolved in distilled water and heated on a hot plate until completely homogenized. The medium was then sterilized in an autoclave at 121°C. After sterilization, the medium was aseptically poured into sterile Petri dishes while still warm and allowed to solidify at room temperature before use.

Preparation of McFarland Turbidity Standard

The bacterial suspension was adjusted to a turbidity equivalent to a 0.5 McFarland standard before being used in the antibacterial assay. The standard and bacterial suspension were mixed thoroughly and visually compared to ensure similar turbidity prior to inoculation. [18].

Preparation of Bacterial Culture and Test Bacterial Suspension

The test bacterium, *Staphylococcus aureus*, was cultured at 37°C for 3–4 h. Subsequently, 1 mL of the bacterial culture was transferred into a tube containing 9 mL of sterile 0.9% sodium chloride solution. The suspension was homogenized and adjusted to a turbidity equivalent to 0.5 McFarland before use in the antibacterial assay [16].

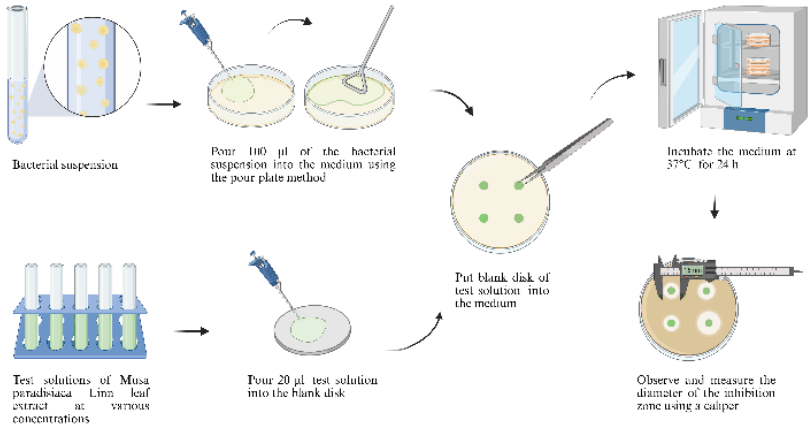

Figure 1. Experimental Stages of the Antibacterial Activity Assay

Preparation of Test Solutions

The 70% ethanolic extract of kepok banana leaves was prepared at concentrations of 5%, 10%, 15%, 20%, and 25%. The 5% solution was prepared by dissolving 0.05 g of extract in 1 mL of DMSO, whereas the 10%, 15%, 20%, and 25% solutions were prepared by dissolving 0.10 g, 0.15 g, 0.20 g, and 0.25 g of extract, respectively, in 1 mL of DMSO. Each solution was stirred until the extract was adequately dispersed and homogenized.

Antibacterial Activity Assay Using the Disc Diffusion Method

The antibacterial activity was evaluated using the disc diffusion method against *Staphylococcus aureus*. Sterile paper discs with a diameter of 6 mm were used. A 100 µL aliquot of the bacterial suspension adjusted to 0.5 McFarland was aseptically spread evenly over the surface of the solidified Mueller–Hinton Agar medium using a sterile spreader. The inoculated plates were allowed to stand for 15–30 min under aseptic conditions to facilitate absorption of the bacterial suspension into the agar surface. Subsequently, 20 µL of each test solution was applied to separate sterile paper discs. The discs were allowed to stand briefly under aseptic conditions to reduce excess solvent and were then placed on the surface of the inoculated agar. Chloramphenicol was used as the positive control, while DMSO was used as the negative control. The plates were incubated in an inverted position at 37°C for 24 hours [19]. Antibacterial activity was observed based on

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the measurement of the diameter of the inhibition zone or the clear zone formed around the disk paper, measured using a caliper[20].

Data Analysis

The antibacterial activity data were obtained from the measurement of inhibition zone diameters and expressed as mean ± standard deviation (SD) based on three independent replications. The data were analyzed using a one-way analysis of variance (ANOVA) at a 95% confidence level ($\alpha = 0.05$) to determine differences among the extract concentrations, positive control, and negative control. When a significant difference was observed ($p < 0.05$), a post hoc multiple-comparison test was performed to identify differences between specific treatment groups. Before conducting ANOVA, the normality and homogeneity of variance of the data were evaluated. The normality and homogeneity test results should be reported together with their statistical values and p-values. The statistical analysis was used to evaluate differences in inhibition zone diameters among the treatment groups.

Results and Discussion

Plant Authentication and Characterization of Musa paradisiaca Linn. Leaf Simplicia

As each plant species has a unique secondary metabolite profile, precise identification is crucial before further testing is performed [21]. To confirm the botanical identity of the kepok banana leaves used in this study, plant identification was conducted at the Biology Laboratory, Ahmad Dahlan University, Yogyakarta, based on the morphological characteristics of the plant. The results of the determination confirmed that the samples were kepok banana leaves (*Musa paradisiaca* Linn.). To complement the macroscopic determination, microscopic examination was conducted to identify the diagnostic anatomical features of the leaf simplicia.

The results presented in Figure 2 revealed the presence of parenchyma tissue, stomata, parenchyma containing stomata, scalariform vascular bundles, covering trichomes, and fibers at 40× magnification. These microscopic characteristics are consistent with the anatomical profile of *Musa paradisiaca* Linn. and are commonly used as reference parameters in the authentication and standardization of medicinal plant materials. The findings further verify the identity and quality of the kepok banana leaf samples employed in this study.

Parenchyma (40×)	Parenchyma with Stomata (40×)	Scalariform Vascular Bundle (40×)	Covering Trichome (40×)	Fiber (40×)	Stomata (40×)

Figure 2. Microscopic Identification of Musa paradisiaca Linn. Leaf Simplicia

Results of Heavy Metal Analysis of Musa paradisiaca Linn. Leaf Extract

Heavy metal analysis conducted at the LPTP Laboratory, Ahmad Dahlan University, Yogyakarta, showed that Pb and Cd were not detected in the *Musa paradisiaca* Linn. leaf extract. These findings indicate that Pb and Cd contamination was not detected in the extract under the conditions of the analysis [22]). However, this result applies only to Pb and Cd, as other heavy metals were not analyzed or reported in this study.

Phytochemical Profile of 70% Ethanolic Extract of Musa paradisiaca Linn. Leaves

The identification of chemical constituents was carried out through phytochemical screening to determine the presence of secondary metabolite groups with potential biological activities, particularly antibacterial effects. The compounds evaluated included flavonoids, alkaloids, saponins, terpenoids, and tannins. The phytochemical screening results of the 70% ethanolic extract of kepok banana leaves (*Musa paradisiaca* Linn.) are presented in Table 1.

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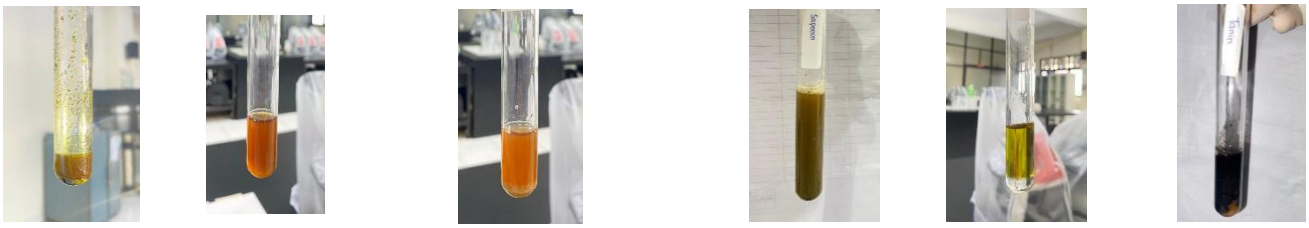
Table 1. Phytochemical Screening Results of the 70% Ethanolic Extract of Musa paradisiaca Linn. Leaves

Compound	Reagent	Screening result	Observation
Flavonoid	HCl	Formation of an orange-red color	+
Alkaloid	Mayer's reagent	No white precipitate formed	-
	Dragendorff's reagent	Formation of an orange-brown color	+
Saponin	2 N HCl	Formation of stable foam	+
Terpenoid	H_2SO_4	Formation of a green color	+
Tanin	1% $FeCl_3$	Formation of a violet-green color	+

Table 2. Phytochemical Screening Results of Musa paradisiaca Linn. Leaf Extract



Flavonoid	Alkoloid (Mayer)	Alkoloid (Dragendrof)	Saponin	Terpenoid	Tanin

Notes:
(+) Indicates a positive qualitative reaction based on the formation of a characteristic color, precipitate, or foam
(−) Indicates the absence of a visible positive reaction under the test conditions.

The phytochemical screening results indicated that the Kepok banana leaf extract contained flavonoids, alkaloids, saponins, terpenoids, and tannins. The presence of flavonoids was confirmed by the development of an orange-red color following the addition of magnesium powder and hydrochloric acid, indicating the reduction reaction of the flavonoid nucleus. Flavonoids are known to exhibit antibacterial activity through the inhibition of nucleic acid synthesis, disruption of bacterial cell membrane function, and inhibition of bacterial enzymatic activity [23]. Alkaloids were positively detected using Dragendorff's reagent, as indicated by the appearance of an orange-brown color, although no white precipitate was observed in Mayer's test. The different results obtained from the two reagents may be related to their different reaction principles and sensitivities. Therefore, the result indicates a positive qualitative reaction for alkaloid-related compounds detected by Dragendorff's reagent, whereas Mayer's test produced a negative reaction under the test conditions. Alkaloids have been reported to exert antibacterial effects by inhibiting peptidoglycan synthesis in the bacterial cell wall, leading to cell lysis [24].

The saponin test yielded positive results, as evidenced by the formation of stable foam even after the addition of 2 N HCl. Saponins are known to reduce the surface tension of bacterial cell membranes, thereby increasing membrane permeability and causing leakage of intracellular components [25]. Likewise, the positive terpenoid reaction, indicated by the formation of a green color, suggested the presence of terpenoid-related compounds in the extract. These compounds may contribute to antibacterial activity through disruption of bacterial membrane integrity. Tannins were also detected, as demonstrated by the formation of a violet-green color following the addition of $FeCl_3$. Tannins exhibit antibacterial activity by precipitating bacterial cell proteins and inhibiting essential enzymes, thereby interfering with bacterial metabolism [26,27]. The presence of these secondary metabolites may contribute to the antibacterial activity of the Kepok banana leaf extract. Overall, the phytochemical screening confirmed the presence of flavonoids, Dragendorff-positive alkaloid-related compounds, saponins, terpenoids, and tannins in the extract. However, because the screening was qualitative, the findings do not establish the concentration or definitive chemical identity of each compound. These findings support the potential of *Musa paradisiaca* Linn. leaf extract as a natural antibacterial agent.

Antibacterial Activity of 70% Ethanolic Extract of *Musa paradisiaca* Linn. Leaves against *Staphylococcus aureus*

The antibacterial activity test in this study was carried out using the disk diffusion method. In this method, the test solution was applied onto sterile paper disks with a diameter of 6 mm, which were then placed on the surface of Mueller–Hinton Agar (MHA) medium previously inoculated with the test bacteria.

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For the assay against *Staphylococcus aureus*, chloramphenicol was used as the positive control, while dimethyl sulfoxide (DMSO) served as the negative control. The inhibition zone was categorized as weak when the diameter was ≤5 mm, moderate when it ranged from >5 to <10 mm, strong when it ranged from 10 to <20 mm, and very strong when the diameter was ≥20 mm [27].

Table 3. Inhibition Zone Diameter and Standard Deviation of *Musa paradisiaca* Linn. Leaf Extract Against the Growth of *Staphylococcus aureus*

No	Experimental Group	Replication (mm)			Mean ± SD	Category
		I	II	III		
1	5% Extract	13,23	13,37	13,63	13,41±0,20	Strong
2	10% Extract	14,12	14,64	14,64	14,46±0,30	Strong
3	15% Extract	15,04	15,18	15,28	15,16±0,12	Strong
4	20% Extract	15,96	16,14	16,34	16,14±0,19	Strong
5	25% Extract	17,41	17,82	18,47	17,90±0,53	Strong
6	PositiveControl (Chloramphenicol)	20,61	21,05	21,75	21,13±0,57	Very Strong
7	Negative Control (DMSO)	0	0	0	0	No Inhibition

Based on the research results, the 70% ethanolic extract of kepok banana leaves was proven to possess antibacterial activity against *Staphylococcus aureus*, as indicated by the formation of an inhibition zone around the paper discs after 24 h of incubation. The formation of the inhibition zone indicates the inhibitory effect of bioactive compounds in the extract on the growth of the test bacteria. The strength of the antibacterial activity can subsequently be interpreted based on the diameter of the inhibition zone formed. According to the classification of Davis and Stout (1971), an inhibition zone with a diameter of ≤5 mm is categorized as weak, >5 to <10 mm as moderate, 10 to <20 mm as strong, and ≥20 mm as very strong

Table 3 shows that the concentration of 5% w/v had an inhibition zone diameter of 13.41 ± 0.20 mm, which was categorized as strong; the concentration of 10% w/v had an inhibition zone diameter of 14.46 ± 0.30 mm, which was categorized as strong; the concentration of 15% w/v had an inhibition zone diameter of 15.16 ± 0.12 mm, which was categorized as strong; the concentration of 20% w/v had an inhibition zone diameter of 16.14 ± 0.19 mm, which was categorized as strong; and the highest concentration, 25% w/v, had an inhibition zone diameter of 17.90 ± 0.53 mm, which was categorized as strong. The positive control, chloramphenicol, had an inhibition zone diameter of 21.13 ± 0.57 mm and was categorized as very strong. The negative control (DMSO) showed no inhibition zone, indicating that the solvent did not exhibit antibacterial activity under the test conditions. The higher concentration of the ethanolic extract of kepok banana leaves produced a larger inhibition zone, which may be related to the greater amount of antibacterial compounds present in the extract [28].

Based on the antibacterial activity test of kepok banana leaf extract against *Staphylococcus aureus* using the disk diffusion method, the extract showed inhibitory activity against the growth of *S. aureus*. This activity was indicated by the appearance of a clear zone around the paper discs. Clear zones were observed around the discs containing the positive control (chloramphenicol) and the kepok banana leaf extract, whereas no clear zone was observed around the discs containing the negative control (DMSO).

Statistical Analysis of Antibacterial Activity

Statistical analysis was conducted to determine whether the differences in inhibition zone diameters among the 70% ethanolic extract concentrations, positive control, and negative control were statistically significant. The normality and homogeneity of variance of the data were evaluated before the ANOVA was performed. The data were then analyzed using a one-way analysis of variance (ANOVA), followed by a post hoc multiple-comparison test when a significant overall difference was observed. One-way ANOVA is used to compare the mean values of more than two groups. A p-value of < 0.05 was considered statistically significant. The post hoc test was subsequently used to identify the specific pairs of groups that differed significantly [29].

The post hoc results showed that the p-values ranged from $p = 0.002$ to $p < 0.001$, with one comparison showing $p = 0.024$. According to Di Leo [30] , a p-value below 0.05 indicates that the observed difference is statistically significant under the tested conditions. Therefore, the differences in inhibition zone diameters among the tested groups were statistically significant, indicating that extract concentration and control

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treatment were associated with differences in the antibacterial response. However, the statistical results do not by themselves establish the specific chemical mechanism responsible for the observed activity.

Table 4. P-Values of Post Hoc Comparisons of Inhibition Zone Diameters Among Treatment Groups Against *Staphylococcus aureus*

	K1	K2	K3	K4	K5	K+	K-
K1		0,002(**)	0,000(**)	0,000(**)	0,000(**)	0,000(**)	0,000(**)
K2	0,002(**)		0,024(*)	0,000(**)	0,000(**)	0,000(**)	0,000(**)
K3	0,000(**)	0,024(*)		0,003(**)	0,000(**)	0,000(**)	0,000(**)
K4	0,000(**)	0,000(**)	0,003(**)		0,000(**)	0,000(**)	0,000(**)
K5	0,000(**)	0,000(**)	0,000(**)	0,000(**)		0,000(**)	0,000(**)
K+	0,000(**)	0,000(**)	0,000(**)	0,000(**)	0,000(**)		0,000(**)
K-	0,000(**)	0,000(**)	0,000(**)	0,000(**)	0,000(**)	0,000(**)	

Notes:
* (Significant): Occurs when the value is within the range of $0.01 < p \leq 0.05$.
** (Highly Significant): Occurs when the value is very small, namely $p \leq 0.01$.
K1 = 5% concentration of kepok banana leaf ethanol extract
K2 = 10% concentration of kepok banana leaf ethanol extract
K3 = 15% concentration of kepok banana leaf ethanol extract
K4 = 20% concentration of kepok banana leaf ethanol extract
K5 = 25% concentration of kepok banana leaf ethanol extract
K+ = Positive control (Chloramphenicol)
K− = Negative control (DMSO)

Differences in antibacterial activity among the groups are thought to be associated with the secondary metabolite compounds present in kepok banana leaves, such as flavonoids, tannins, saponins, and alkaloids. Flavonoids are known to damage bacterial cell membranes and inhibit nucleic acid synthesis. Tannins can disrupt bacterial cell wall permeability through protein precipitation, while saponins act by increasing membrane permeability, thereby causing leakage of cellular contents. Alkaloids play a role in inhibiting peptidoglycan formation in the bacterial cell wall, thereby disrupting bacterial growth [31]. These mechanisms are considered possible explanations for the observed antibacterial activity, although they were not directly examined in this study.

The statistical test results also showed significant differences between the extract treatment groups and the negative control (DMSO). This finding indicates that the inhibition zones observed in the extract groups were unlikely to be attributable to the solvent under the tested conditions. Meanwhile, the significant differences between the extract treatment groups and the positive control (chloramphenicol) indicate that the antibacterial activity of the extract differed significantly from that of the standard antibiotic. Although the extract showed antibacterial activity, its inhibition zone at the highest concentration remained lower than that of chloramphenicol.

Overall, the statistical analysis indicated that the 70% ethanolic extract of kepok banana leaves showed antibacterial activity against *Staphylococcus aureus* and that the treatment groups differed significantly in terms of inhibition zone diameter. These findings support the potential of kepok banana leaves as a source of natural antibacterial compounds. Nevertheless, the results are limited to the disk diffusion assay and should not be interpreted as evidence of clinical efficacy or definitive therapeutic effectiveness.

Study Limitations and Future Research

This study has several limitations. The antibacterial activity of the 70% ethanolic extract of kepok banana leaves was evaluated only using the disk diffusion method. Therefore, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extract could not be determined. These tests were not conducted because of limitations in laboratory facilities and resources. In addition, the phytochemical analysis performed in this study was qualitative and only indicated the presence or absence of visible reactions. The concentration and definitive chemical identity of the detected compounds were not determined. Further studies should determine the MIC and MBC values using a broth microdilution method. Chromatographic analysis, toxicity testing, formulation stability testing, and topical safety evaluation are also recommended before the extract is considered for therapeutic application.

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Conclusions

Based on the results of the study, it can be concluded that the 70% ethanolic extract of kepok banana leaves (*Musa paradisiaca* Linn.) contained flavonoids, alkaloids, saponins, terpenoids, and tannins, which may contribute to its antibacterial activity. The extract produced strong in vitro inhibition against *Staphylococcus aureus* at all tested concentrations (5–25%) using the disk diffusion method. The inhibition zone increased with increasing extract concentration, although the inhibition zone produced by the 25% extract remained lower than that of chloramphenicol. Pb and Cd were not detected in the extract under the conditions of the analysis. However, these findings only describe the results of Pb and Cd analysis and do not establish the overall safety of the extract. Therefore, the 70% ethanolic extract of kepok banana leaves shows potential as a source of natural antibacterial compounds, but further studies are required to determine its MIC and MBC values and to evaluate its chemical profile, toxicity, formulation stability, and topical safety.

Conflict of Interest

This study was conducted independently and objectively following standard scientific methodologies. All data were obtained and analyzed empirically without external influence or conflicts of interest. Scientific integrity was maintained through complete documentation and transparent analytical procedures. The findings are solely based on the evidence generated in this research.

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