

Antibacterial Activity Test of Red Gedi Leaf Ethanol Extract (*Abelmoschus manihot* L. Medik) against *Staphylococcus aureus* Bacteria

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Abstract

A critical threat to global public health is posed by antimicrobial resistance, with particular urgency highlighted by Methicillin-Resistant *Staphylococcus aureus* (MRSA), thereby necessitating the rapid discovery of alternative botanical antibacterial agents. Although traditional therapeutic applications in North Sulawesi have long utilized red gedi leaves (*Abelmoschus manihot* L. Medik), the corresponding antibacterial efficacy against *S. aureus* remains largely uncharacterized. In this investigation, the antibacterial capacity of an ethanolic extract derived from red gedi leaves was evaluated against *S. aureus*, and its minimum effective threshold was determined. Extraction was achieved via maceration in 96% ethanol, with subsequent disk diffusion assays conducted at concentrations of 3%, 5%, and 10%; meanwhile, tetracycline and 10% dimethyl sulfoxide were employed as positive and negative controls, respectively. The resulting zones of inhibition were subjected to statistical evaluation via a one-way analysis of variance (One-Way ANOVA). Moderate antibacterial suppression was elicited by all tested extract concentrations, with recorded zones of inhibition spanning 7.28 ± 1.00 mm, 7.82 ± 0.86 mm, and 8.59 ± 1.48 mm, respectively. Conversely, robust antibacterial susceptibility was demonstrated by tetracycline, yielding an inhibition zone of 18.61 ± 0.59 mm, whereas no inhibitory effects were registered by the negative control. No statistically significant variations in the zones of inhibition were exposed across the various extract concentrations by the One-Way ANOVA ($F = 0.991$, $p = 0.425$), suggesting that an escalation in crude extract concentration did not yield a commensurate increase in antibacterial potency. Ultimately, moderate antibacterial efficacy against *S. aureus* is exhibited by the ethanolic extract of red gedi leaves, confirming its viability as a natural therapeutic candidate. Nonetheless, the identification of an optimal dosage remains to be established through broader concentration gradients and subsequent verification via Minimum Inhibitory Concentration (MIC) assays.

Introduction

The most critical jeopardy to public health on a global scale is currently represented by antimicrobial resistance (AMR) (Ferri et al., 2017; Majumder et al., 2020; Hossen & Mascellino, 2026; Cameron et al., 2022). According to dataset insights compiled by the Global Burden of Disease, an estimated 4.71 million fatalities are linked to AMR, among which 1.14 million casualties are explicitly precipitated by this phenomenon. The primary driver of global mortality within this domain is identified as Methicillin-Resistant *Staphylococcus aureus* (MRSA), with documented fatalities associated with this pathogen having escalated to more than twofold the figures recorded in 1990 (Naghavi et al., 2024). In line with the WHO's List of Priority Pathogens, MRSA remains categorized as a high-priority threat (Organization, 2024). In Indonesia, surveillance data for 2024 shows that the prevalence of MRSA is in the range of 37–44% of the total *S. aureus* isolates tested (Artanti et al., 2026). This phenomenon confirms that antibiotic-resistant *S. aureus* remains a serious health challenge that requires a sustained solution.

A diverse spectrum of pathologies spanning from superficial cutaneous lesions to life-threatening conditions including bacteremia, endocarditis, osteomyelitis, and biomaterial-associated infections is induced by the Gram-positive pathogen *Staphylococcus aureus* (Keerthi et al., 2026; Paharik & Horswill, 2016; Nawan et al., 2025; Kaushik et al., 2026; Tong et al., 2015). In Indonesia, the prevalence of MRSA in clinical isolates varies between 0.3% to 52%, with the highest rates reported in large hospitals such as in Jakarta (Syahniar et al., 2020). This high prevalence has implications for therapy failure, increased duration of hospitalization, treatment costs, and patient morbidity and mortality, especially due to resistance to first-line antibiotics such as beta-lactam (Ventola, 2015; Cerceo et al., 2016; Mirha et al., 2024). This situation reinforces the urgency of developing alternative antibacterial agents, including from natural materials, to reduce dependence on conventional antibiotics and slow the rate of resistance (Abdallah et al., 2023; Ekwueme et al., 2025; AL-Azzawi et al., 2024).

One of Indonesia's natural resources that has the potential to be a natural antibacterial is red gedi leaves (*Abelmoschus manihot* L. Medik). Red gedi leaves are empirically used as a medicine in North Sulawesi and eastern Indonesia (Anggraini et al., 2020; Indrawati & Setijorini, 2024). This leaf ethanol extract is rich in flavonoids, saponins, tannins, quinones, and steroids (Tandi et al., 2026). These compounds act as antibacterials through the destruction of cell membranes, either by forming protein complexes (flavonoids and tannins) or by lowering the surface tension of the cell wall (saponins). Ojong et al. (2026) and Wiradana et al. (2025) said that, this phytochemical profile makes red gedi leaves a potential antibacterial candidate, especially against Gram-positive bacteria such as *S. aureus* which are susceptible to phenolic compound disruptions.

Research on the antibacterial activity of ethanol extract of red gedi leaves against *S. aureus* is still limited. Most of the recent research on gedi plants has focused more on different species of gedi and different test bacteria, for example the inhibition test of ethanol extract of green gedi leaves against *Streptococcus mutans* (Paerah et al., 2022) or more highlight the other pharmacological activities of gedi merah such as the antioxidant activity and quality safety parameters of the extract, rather than its antibacterial activity (Tandi et al., 2026). This gap underlies the need for research that specifically tests the antibacterial activity of ethanol extract of red gedi leaves against *S. aureus* through concentration variations, so that a more comprehensive picture can be obtained of its potential and inhibitory strength.

Based on this urgency, this study was carried out to evaluate the antibacterial effectiveness of ethanol extract of red gedi leaves (*Abelmoschus manihot* L. Medik) against *Staphylococcus aureus*, which included the analysis of the amount of inhibition and determination of effective concentration. Therefore, this study is focused on testing antibacterial activity in vitro to analyze the potential inhibition of bacterial growth by the extract.

Method

Tools and Materials

The tools used include: autoclave (Nuair®), hot plate (Cole-Parmer®), incubator (Mettler®), digital caliper (WP-150B®), micro pipette (Eppendorf®), oven (Falc®), rotary evaporator (THOMAS), ® analytical scale (HWH).® The materials used include: pure culture of *Staphylococcus aureus* bacteria, tetracycline discs, red gedi leaves (*Abelmoschus manihot* L. Medik), 96% ethanol, DMSO (Dimethyl Sulfoxide) 10%, nutrient agar, Mueller Hinton Agar.

Sample Setup

Sample Processing

The red gedi leaves (*Abelmoschus manihot* L. Medik) used were taken from North Minahasa Regency, North Sulawesi. Samples of red gedi leaves are washed with running water until clean, then sorted wet, then sliced, then dried by aerating, then dried dry, then pollinated until a lump is obtained, then extracted by the maceration method.

Extract Manufacturing

A total of 200 g of simplicia powder was extracted using the maceration method with 1.5 L of 96% ethanol for 5 days at room temperature. The process is carried out in a light-insulated container with periodic stirring. The maserrate is separated, then the residue is rinsed with the same solvent until a final volume of 2 L is obtained.

Antibacterial Activity Test

Sterilization Tools

All equipment used in this antibacterial activity research goes through a sterilization procedure first. The scaled glassware is sterilized in an autoclave at 121°C for 15 minutes, the ose needle and tweezers are sterilized by direct spraying on the flame, and the non-scaled glassware is sterilized in the oven at 170°C for 2 hours (Amiliah et al., 2021; Kusumaning et al., 2022)

Turbidity Standard Creation (Mc. Farland Solution)

A standard turbidity solution is prepared by mixing 99.5 mL of 0.36 N H₂SO₄ solution with 0.5 mL of BaCl₂ solution · 2H₂O 1.175%. The mixture is shaken until it forms a cloudy solution that is used as a standard turbidity reference for bacterial suspensions (Rossalinda et al., 2021).

Medium Manufacturing

A total of 5.75 g of Agar Nutrients is dissolved in 250 mL of aquadest and heated on a hot plate until a clear solution is obtained, with the pH of the solution set to 7. The sterilization process is carried out using an autoclave at a temperature of 121°C for 15 minutes. For the manufacture of an inclined agar medium intended for bacterial inoculation, as much as 5 mL of the media is put into the test tube, then let it sit until it solidifies at an inclination position of 30° (Maimunah et al., 2020).

Test Bacteria Rejuvenation

Rejuvenation is carried out by transferring one to four microbial oses from each pure stock into the nutrient medium so that they are new in the test tube in an oblique form. Scraped on a medium nutrient agar and then incubated at 370C for 1 x 24 hours (Ningsih et al., 2014).

Manufacture of Bacterial Suspension

A total of 2 mL of 0.9% NaCl solution is inserted into the test tube. Next, the test bacteria from the media to be taken using sterile ose wire and suspended into the NaCl solution until a turbidity level equivalent to the Mc. Farland standard is obtained (Rossalinda et al., 2021).

Activity Test of Red Gedi Leaf Ethanol Extract

Before conducting the test, a sample of red gedi leaf extract with a concentration of 3%, 5% and 10% was suspended using 10% DMSO. Then the paper disk is soaked for approximately 15 minutes on the prepared sample. Furthermore, the medium nutrient agar as much as 15 mL is put into each chocolate bottle then 20 µL of *S. aureus* suspension is added then homogenized,

then poured into a petri dish and allowed to solidify. After compacting, a paper disk containing ethanol extract of red gedi leaves with a concentration of 3%, 5%, 10%, tetracycline as a positive control and DMSO 10% as a negative control is inserted into a petri dish containing the media. It was then incubated at 37°C for 1 x 24 hours and measured in the inhibition zone.

Data Analysis

Evaluation of the variances across the diverse treatment groups was executed through the application of a One-Way ANOVA statistical analysis on the collected inhibition zone diameter measurements. In instances where meaningful variances were detected, post-hoc comparisons were subsequently performed utilizing Tukey's test, operating under a 95% confidence interval where statistical significance was established at $p < 0.05$. The execution of all computational and statistical procedures was facilitated using the SPSS software package (version 25).

Result and Discussion

Extraction of 200 g of red gedi leaf *simplicia* (*Abelmoschus manihot* L. Medik) by maceration method using 96% ethanol produced a thick green extract of 24.3258 g. The selection of 96% maceration and ethanol solvents is relevant for the isolation of plant bioactive compounds, as studies on the leaf extracts of other plants also show that more polar or semi-polar solvents often provide high yields as well as richer phytochemical profiles, especially flavonoids, phenolics, tannins, and saponins (Backiam et al., 2023; Wasihun et al., 2023).

Testing using the disc diffusion method showed that the entire concentration of the extract was able to form an inhibitory zone against *S. aureus*, while the negative control (DMSO 10%) showed no inhibitory activity (Table 1, Figure 1), which confirmed that the antibacterial activity came from the active compounds of the extract and not the solvent. The measured mean inhibition zone diameters were 7.28 ± 1.00 mm, 7.82 ± 0.86 mm, and 8.59 ± 1.48 mm, while tetracycline-positive control resulted in an inhibition zone of 18.61 ± 0.59 mm. Referring to the classification of Davis and Stout (1971), all variations in the concentration of extracts are categorized as having moderate activity, while tetracycline is classified as strong. Statistical analysis showed that the data were normally distributed with homogeneous variance, but the One-Way ANOVA test revealed no significant difference between concentration groups ($F = 0.991$; $p = 0.425$), so further post-hoc testing of Tukey was not performed.

Table 1. Measurement results of the diameter of the inhibition zone of ethanol extract of red gedi leaves

Test Solution	Replication	Diameter of Red Gedi Leaf Inhibition Zone (mm)	Interpretation
Positive Control	I	17,93	Strong
	II	18,93	
	III	18,98	
Average		18,61	
Concentration 3%	I	8,24	Medium
	II	7,36	
	III	6,25	
Average		7,28	
Concentration 5%	I	6,84	Medium
	II	8,15	
	III	8,47	

Average		7,82	
Concentration 10%	I	6,95	Medium
	II	9,81	
	III	9,01	
Average		8,59	
Negative Control	I	0	No activity
	II	0	
	III	0	
Average		0	

Description:

Positive (+) control : Tetracycline

Concentration 3% : Red gedi leaf ethanol extract 3%

Concentration 5% : Red gedi leaf ethanol extract 5%

Concentration 10% : Red gedi leaf ethanol extract 10%

Negative control (-) : DMSO (Dimethyl Sulfoxide)

The formation of an inhibition zone in the entire concentration of this extract is supported by the content of flavonoids, saponins, tannins, quinones, and steroids in the ethanol extract of red gedi leaves (Tandi et al., 2026), which works through membrane protein binding and cell wall surface tension decrease (Cowan, 1999). Recent evidence of the mechanism of action suggests that exposure to flavonoids in *S. aureus* increases intracellular protein and nucleic acid leakage due to cell membrane damage (Zhou et al., 2023), with a multitarget mechanism that is cell wall disruption, protein synthesis inhibition, and biofilm inhibition (Zhang et al., 2025).

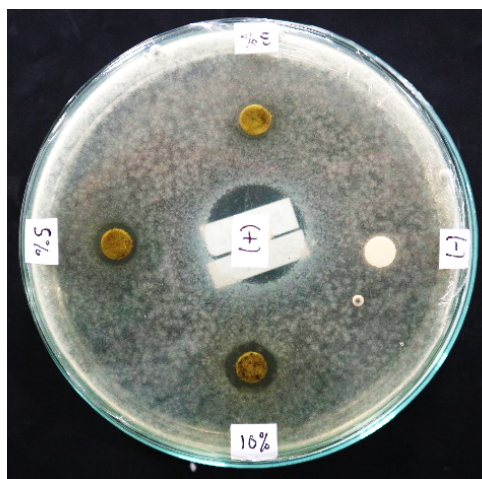


Figure 1. Ethanol Extract Inhibition Zone of Red Gedi Leaves

However, the increase in inhibition zones with the increase in concentrations was not statistically significant, possibly because the concentration range tested (3–10%) was relatively narrow (only a threefold difference), coupled with the characteristics of the disc diffusion method that were influenced by the solubility and polarity of the compounds, so that the increase in the concentration of the crude extract was not always directly proportional to the amount of active compounds reaching the medium. This is consistent with the findings on papaya leaf extract against *S. aureus*, where a concentration range of 10–80% (eight times) remains resulting in a medium category inhibition zone (3.92–8.24 mm), suggesting that the

relationship between concentration and response to plant extract assays using disc diffusion methods is often non-linear (Zahra et al., 2025).

This non-linearity pattern is consistent with previous studies on the relationship of total phenolic and flavonoid content to the antibacterial activity of *Abelmoschus manihot*, where total phenolic levels contributed to 71% variation in the antibacterial activity of gedi extract against *E. coli*, as well as a similar correlation between total flavonoid levels and the antibacterial activity of the extract and the fraction of green gedi leaves (Kambey et al., 2019). Thus, the antibacterial potential of the extract was influenced more by the specific active compound profile than simply the percentage of crude extract concentration, which theoretically answers why the increased concentration in this study did not result in a statistically significant increase in the inhibition zone.

The results of this study show that ethanol extract of red gedi leaves has moderate antibacterial activity against *Staphylococcus aureus* and is evidence that red gedi leaves can be a natural antibacterial candidate that deserves further research through the standardization of extracts, Minimum Inhibitory Levels (KHM) tests, identification of active compounds, and testing on clinically resistant isolates.

Conclusion

Ethanol extract of red gedi leaf (*Abelmoschus manihot* L. Medik) was shown to have antibacterial activity against *Staphylococcus aureus*, characterized by the formation of an inhibition zone at all test concentrations while negative control of DMSO 10% showed no inhibiting activity, with the amount of inhibition force of 7.28 ± 1.00 mm, 7.82 ± 0.86 mm, and 8.59 ± 1.48 mm at concentrations of 3%, 5%, and 10%, all of which were classified as moderate according to the classification of Davis and Stout (1971), respectively. In contrast to the positive control of tetracycline which is classified as strong (18.61 ± 0.59 mm); however, the results of the One-Way ANOVA test showed that there was no significant difference in inhibition zones between concentrations ($F = 0.991$; $p = 0.425$), so although the 10% concentration showed the highest average numerically, it could not be determined to be the most statistically effective concentration in the 3–10% range tested.

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