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Activity Test Of White Frangipani Plant Stem Extract (*Pulmeria Rubra L*) Against Gram Positive Bacteria *Staphylococcus Aureus* ATCC 25923

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Abstract

This study aims to test the antibacterial potential of ethanol extract of white frangipani stems (Plumeria rubra L.) against Staphylococcus aureus ATCC 25923 as a Gram-negative bacterium.-positive for causing skin infections. The study used a true experimental laboratory design with a quantitative approach. The population was white frangipani plants on Jl. Palem, Cemani, Grogol, Sukoharjo, with samples in the form of fresh stems. The extract was made by maceration with 96% ethanol using a rotary evaporator, then standardized and phytochemically screened for alkaloids, flavonoids, saponins, and tannins. Antibacterial activity was tested using the well diffusion and agar disc diffusion methods, and the inhibition zone data were analyzed descriptively (mean ± SD) based on the strength classification criteria of <5 mm, 5–10 mm, 10–20 mm, and >20 mm. The results showed that the white frangipani stem extract contained alkaloids, flavonoids, saponins, and tannins, and produced a strong inhibition zone (10–20 mm) at concentrations of 20%, 40%, and 60% against Staphylococcus aureus ATCC 25923, with the highest inhibition zone of the positive control. In conclusion, the ethanol extract of white frangipani stems has the potential as a natural antibacterial agent against Gram-negative bacteria.-positive, although further studies regarding MIC/MBC and toxicity are needed.

Keywords: Antibacterial Activity, GramPositive Bacteria, Natural Extract, Staphylococcus Aureus, White Frangipani.

INTRODUCTION

Infection with pathogenic microorganisms, particularly Staphylococcus aureus bacteria, continues to be a global public health problem that demands new treatment strategies, particularly in the context of skin diseases. Nearly 30–50% of skin infection cases worldwide are associated with Staphylococcus aureus, encompassing various superficial conditions such as boils, impetigo, folliculitis, to deeper infections such as cellulitis and even staphylococcal scalded skin syndrome (SSSS), which require intensive care in healthcare facilities (Lestari, 2022; Halodoc, 2023). In Indonesia, the prevalence of skin infections is reported to range from 4.60–12.95%, ranking third among the ten most common diseases, with a significant percentage being due to the involvement of microorganisms, including Staphylococcus spp. (Lestari, 2022; UKRIDA Ejournal, 2023). This phenomenon indicates that bacterial-based skin infections remain a significant burden in terms of both morbidity and healthcare costs, making the search for safe, effective, and affordable therapeutic alternatives a highly relevant scientific and practical issue.

Globally, increasing microbial resistance to antibiotics is a major challenge in managing bacterial infections, including Staphylococcus aureus. The use of systemic antibiotics to treat skin infections and other bacterial infections has resulted in an increase in the proportion of resistant Staphylococcus aureus isolates, including methicillin-resistant S. aureus (MRSA), complicating empirical therapy and increasing mortality (Mehraj et al., 2014; IML Research, 2026). In Indonesia, national monitoring shows the widespread prevalence of Staphylococcus spp., with the proportion of cases resulting from exposure to these bacteria reaching 25–65%, with the national average being around 38% (Ministry of Health, 2018). These data confirm that research into alternative antibacterial agents, including those from natural sources, is not only academically valuable but also practically urgent in reducing the pressure on conventional antibiotic use and reducing the risk of resistant infections.

In a specific context, this research focuses on the development of natural antibacterial agents from Indonesian herbal plants, specifically the white frangipani tree (Plumeria rubra L.), to treat skin infections triggered by Staphylococcus aureus. Indonesia is known as one of the countries with an extraordinary wealth of medicinal plants, most of which have been traditionally used in various therapeutic formulations, both for external and internal treatment (Laksanawati, 2022; Anggraini, 2021). The frangipani tree (Plumeria spp.) is an ornamental plant commonly found in home gardens, public cemeteries, and park areas. The dominant variant in Indonesia is Plumeria rubra, which has a large stem structure but is almost not optimally utilized, making it a source of biomass with little economic value (Laksanawati, 2022). On the other hand, various phytochemical studies show that parts of the frangipani plant contain alkaloids, tannins, saponins, and flavonoids which have been shown to have antimicrobial activity in vitro, so the pharmacological potential of this species is worth exploring further (Laksanawati, 2022; Rahman & Yusuf, 2023).

Previous research has shown that ethanol extract of white frangipani leaves (Plumeria acuminata) is able to inhibit the growth of Streptococcus mutans with a minimum concentration of 25% which is still effective in the inhibition test (Hudatama, 2016). A subsequent study by Mardaningrat et al. (2023) confirmed that ethanol extract of white frangipani flowers (Plumeria rubra L.) also has antibacterial activity against Streptococcus pyogenes, with an average inhibition zone of 7.2–9.6 mm at concentrations of 25–100%, indicating a medium inhibition category at the highest concentration (Mardaningrat et al., 2023). This finding is consistent with several other studies that reported the antibacterial effects of various Plumeria spp. extracts against Gram-negative microorganisms.-Positive results are supported by the presence of flavonoids and tannins, which act as proton donors and inhibit bacterial cell wall and membrane synthesis (Rahman & Yusuf, 2023; Sudhakar et al., 2022). However, some of these studies are still limited to the leaves and flowers, focusing on Streptococcus spp., thus not providing a complete picture of the antibacterial potential of other plant components or the optimal concentration of the extract against other pathogenic species relevant to skin infections.

From a critical perspective, several methodological and contextual shortcomings in previous research can be identified. First, most studies only evaluated the antibacterial activity of leaf or flower extracts, while the proportionally larger and largely underutilized plumeria stems remain underexplored as a source of bioactive compounds (Laksanawati, 2022; Sudhakar et al., 2022). Second, the bacterial target is still dominated by Streptococcus spp., so there is no strong evidence regarding the effectiveness of white plumeria extract, especially from the stems, against Staphylococcus aureus, a major cause of skin infections in Indonesia (Lestari, 2022; Anggraini, 2021). Third, several previous studies did not systematically determine the optimal extract concentration for inhibiting bacterial growth, so the concentration-effect response curve has not been characterized in detail. This limitation opens up a research gap that still requires a more comprehensive study to identify the active compound profile of white frangipani stem extract (Plumeria rubra L.) and test its antibacterial effectiveness against Staphylococcus aureus ATCC 25923, including determining its optimal concentration.

Based on this gap, this study was designed to answer the main problems: (1) what is the profile of active compounds contained in the white frangipani stem extract (Plumeria rubra L.), (2) how effective is the white frangipani stem extract in inhibiting the growth of Staphylococcus aureus ATCC 25923 bacteria, and (3) what is the most optimal extract concentration in inhibiting the growth of these bacteria. These three problems were formulated sharply to ensure that the study not only confirmed the presence of antibacterial activity, but also tested the effective concentration and related the findings to the composition of active compounds expressed quantitatively and qualitatively. Thus, this study not only contributes to the development of natural antibacterial agents for skin infections; but also enriches the literature base regarding the utilization of plant parts that have been underutilized, in the context of increasing microbial resistance.

The main objectives of this study are (1) to identify the active compounds in the white frangipani stem extract (Plumeria rubra L.), (2) to prove the effectiveness of the white frangipani stem extract in inhibiting the growth of Staphylococcus aureus ATCC 25923, and (3) to determine the optimal concentration of the extract to inhibit the growth of the bacteria. This activity is important to be carried out in a national and global context, considering the increasing concern about resistant Staphylococcus aureus infections and the limited discovery of new antibiotics in the last decade (IML Research, 2026; Ministry of Health of the Republic of Indonesia, 2018). The novelty of this study lies in the combination of phytochemical approaches, antibacterial tests, and determination of optimal concentrations in the white frangipani stem, which have not been widely reported previously. Theoretically, the results of this study will complement the understanding of the relationship between the structure of active compounds in Plumeria rubra L. and antibacterial activity against Staphylococcus aureus, while practically, these findings can provide a scientific basis for the development of natural topical formulations for safer and more sustainable skin infection therapy amidst the global antibiotic crisis.

RESEARCH METHODS

This study was designed as a laboratory experimental study with a true experimental design, which aims to test the cause-and-effect relationship between the administration of white frangipani stem extract (Plumeria rubra L.) concentrations on the reduction of Staphylococcus aureus ATCC 25923 bacterial growth. In a methodological framework, experimental research is defined as a study involving the manipulation of independent variables, control of external variables, and systematic observation of changes in the dependent variable (Sugiyono, 2021; Sudaryono, 2022). This study adopted a quantitative experimental approach because the data obtained were in the form of numerical measurements (inhibition zone diameter, percentage of compound content, etc.) which were then analyzed statistically to test the hypothesis of the extract's effectiveness. This quantitative experimental approach is suitable for testing optimal concentrations and comparing treatment effects because it allows strict control of environmental conditions and the use of a valid control design (Sugiyono, 2021; Sudaryono, 2022).

Data collection was carried out using various standardized physical and chemical instruments in the microbiology laboratory, including rotary evaporators, ovens, moisture balances, Laminar Air Flow (LAF), incubators, microscopes, calipers, analytical balances, and a series of sterile glassware and microbial culture equipment (volumetric pipettes, test tubes, petri dishes, disc paper, etc.) according to the agar diffusion test protocol (Pipit, 2020; Anggraini, 2021). Antibacterial activity data were obtained from measuring the diameter of the inhibition zone (in mm) using the disc diffusion method and agar well diffusion using a caliper with an accuracy of 0.01 mm, then classified into weak, medium, strong, and very strong categories based on the ranges of 0–5, 5–10, 10–20, and >20 mm (Pipit, 2020). Phytochemical screening and standardization data for simplicia/extracts are presented in qualitative and semi-quantitative forms (e.g., positive/negative, percentage of water, ash, heavy metals, and yield). The data analysis techniques applied include descriptive statistical descriptions (mean ± SD) for the inhibition zone at each extract concentration, as well as comparison of values between treatments to illustrate the concentration-effect response pattern against Staphylococcus aureus ATCC 25923, according to the experimental quantitative analysis paradigm (Sugiyono, 2021; Sudaryono, 2022).

The population in this study was all white frangipani plants (Plumeria rubra L.) growing in the area of Jl. Palem, Cemani, Grogol, Sukoharjo Regency, Central Java Province. The research sample used was fresh white frangipani plant stems taken from the location, with the inclusion criteria being mature plants, not attacked by obvious pests and diseases, and harvested uniformly at the same stem height to ensure phytochemical uniformity (Laksanawati, 2022; Oktaviana, 2017). Sampling was carried out using a purposive sampling technique, namely selecting frangipani stems that visually meet the eligibility criteria for medicinal raw materials, according to the standard guidelines for processing herbal simplicia (Ministry of Health of the Republic of Indonesia, 2000; Silverman, 2023). The bacterial sample used was Staphylococcus aureus ATCC 25923 as a certified reference strain and is often used in testing antibacterial activity according to international standards (ATCC, 2023; Microbiologics, 2025).

The research procedure was carried out systematically through several main stages. The first stage was the identification and determination of the plant (Plumeria rubra L.) which was carried out at the Hortus Medicus Functional Implementation Unit (UPF) of Dr. Sardjito Tawangmangu General Hospital, Karanganyar, to ensure the authenticity of the species according to botanical literature (Laksanawati, 2022). The second stage was the processing of the simplicia, namely cleaning the frangipani stems with running water, cutting them into small pieces, drying them using an oven at 60 °C to obtain a minimum water content, grinding them using a blender, and sieving with a 40 mesh to obtain a uniform simplicia powder, with the criteria for simplicia yield <10% according to pharmacopoeial standards (Oktaviana, 2017; Silverman, 2023; Ministry of Health of the Republic of Indonesia, 2000). The simplicia is then standardized through drying shrinkage tests, water content (with moisture balance), and ash content (heating in a 600 °C furnace for 7 hours), according to the simplicia standardization guidelines (Ministry of Health of the Republic of Indonesia, 2000; Adolph, 2016; Chanida, 2024).

The third stage is ethanol extraction. 500 g of dried white frangipani stems were macerated using 96% ethanol at a ratio of 1:15 (1 part material : 15 parts solvent) for 3 days in a closed container, stirring occasionally, and filtered after 24 hours, followed by remaceration with fresh solvent (2.5 L) to maximize extraction. The combined filtrate was evaporated using a rotary evaporator at 60 °C, then concentrated in a water bath until a thick extract was obtained and the yield was calculated using the formula: yield (%) = (extract weight / simplicia weight) × 100 (Hasibuan, 2020). The extract was then standardized with an ethanol-free test (checking for the absence of ester aroma after heating with H_2SO_4 and acetic acid), water content (with moisture balance), and examination of heavy metal contamination of lead (Pb) and cadmium (Cd) using K_2CrO_4 and NaOH reagents (Priamsari MR, 2019; Utami, 2017; Ningsih, 2024). The next stage was phytochemical screening of alkaloids, flavonoids, saponins, and tannins using classical methods using various reagents (Mayer, Dragendorff, Wagner, Mg/HCl, $FeCl_3$) to confirm the presence of the main bioactive compounds in the white frangipani stem extract (Hasibuan, 2020; Marsila, 2025).

The fourth stage is the antibacterial activity test against Staphylococcus aureus ATCC 25923. The tools and media used were sterilized in an autoclave at 121 °C for 15–20 minutes, while metal equipment was sterilized by direct annihilation (Anggraini, 2021). The bacteria were rejuvenated on Nutrient Agar (NA) slants, made into a suspension with a density equivalent to 0.5 McFarland, and inoculated on NA petri media using agar disc and well diffusion techniques. White frangipani stem extract was tested at concentrations of 20%, 40%, and 60%, with negative controls (solvent) and positive controls (standard antibiotics), according to the agar diffusion inhibition zone test method commonly applied in testing the antibacterial activity of herbal extracts (Pipit, 2020; Marsila, 2025). The inhibition zone was measured after 24 hours of incubation at 37 °C, and the inhibition zone diameter data were analyzed descriptively to determine the most effective concentration (Pipit, 2020). The entire research procedure was systematically designed from material processing, standardization, phytochemical screening, to antibacterial testing, to meet the principles of controlled experiments and be able to provide valid evidence of the effectiveness of white frangipani stem extract against Staphylococcus aureus ATCC 25923 (Sugiyono, 2021; Sudaryono, 2022).

RESULTS AND DISCUSSION

Plant Determination

The botanical identification of the white frangipani stem (Plumeria rubra L.) was completed at the Hortus Medicus Functional Implementation Unit (UPF) of Dr. Sardjito Tawangmangu General Hospital, Karanganyar, Central Java, which was aimed at validating the originality and suitability of the white frangipani stem (Plumeria rubra L.) which would be used as a research specimen.

Preparation of Simple Ingredients

The specimens used in this study were 5 kg of whole, wet stems of white frangipani (Plumeria rubra L.) harvested from Jl. Palem 1 C5, Cemani RT 05/RW 09, Grogol, Sukoharjo. The stems were cleaned with running water, cut to the required size, and then dried in an oven at 60 °C to reduce the water content without damaging the active ingredients. Once dry, the crude drug was ground using a blender and sieved with a 40-mesh sieve to obtain a uniform powder. From 500 g of powdered crude drug of white frangipani stems, a yield of 10% was obtained, indicating optimal yield because it meets standard criteria and indicates a moderate water content (Wijaya, 2022).

Table 1. Results of the yield of simple drugs

Sample	Wet weight of simple substance (g)	Dry weight of simple substance (g)	Research result	Requirements (Ministry of Health of the Republic of Indonesia, 2017)
White Plumeria Stem (Pulmeria Rubra L)	5000 g	500 g	10%	≥10%

Standardization of Simple Drugs

Drying shrinkage test

Table 2. Table of results of drying shrinkage test of simple drugs

Replication sample	I	II	III	Average
White Plumeria Stem (Pulmeria Rubra L)	6%	6.5%	5.5%	6%

Referring to the table above, it was found that the drying shrinkage of the white frangipani (Plumeria rubra L.) stem powder in specimen I reached 6%, specimen II reached 6.5%, specimen III reached 5.5%, with an average of 6%. Through the finding of the average drying shrinkage of the white frangipani (Plumeria rubra L.) stem of 6%, it can be judged to comply with the criteria, because the drying shrinkage benchmark is ≤ 10% (FHI, 2023).

Table 2. Results of water content test of simple drugs

Replication sample	I	II	III	Average
White Plumeria Stem (Pulmeria Rubra L)	7.49%	8.20%	8.69%	8.12%

Referring to the table above, the results of the white frangipani stem (Plumeria rubra L.) stem liquid test results were 7.49% in specimen I, 8.20% in specimen II, and 8.69% in specimen III, with an average of 8.12%. The average findings on the white frangipani stem (Plumeria rubra L.) stem can be concluded to have a fairly ideal water content, because the optimal water content criteria are ≤ 10%.(Barrow, 2019).

Ash content test

Table 4. Results of the ash content test of the simple drug

Replication sample	I	II	III	Average
White Plumeria Stem (Pulmeria Rubra L)	7%	8.5%	6.5%	7.33%

Referring to the table above, the ash percentage output for specimen I reached 7%, specimen II reached 8.5%, and specimen III reached 6.5%, with an average of 7.33%. The ash percentage inspection findings obtained can be deemed to have met the criteria, as the optimal total ash content is ≤ 8% (Evifania, 2020).

Extract Preparation

Table 3. Extract yield results

Sample	Powder Weight (g)	Extract weight (g)	Yield
White Plumeria Stem (Pulmeria Rubra L)	500 g	44.38 g	8.87%

Referring to the previous table, the yield of white frangipani (Plumeria rubra L.) stem extract reached 8.87%. This confirms that the yield of white frangipani (Plumeria rubra L.) stem extract meets the criteria set by the Indonesian Herbal Pharmacopoeia, as its volume did not exceed the 13.1% limit.

Extract Standardization

The indicator for ethanol-free testing is the detection of specific residues present in the extract. The ethanol-free test aims to verify that the extract is sterile from ethanol, ensuring its safety during research. The first step in the ethanol-free test is to pour 1 ml of concentrated extract into a test tube. Next, add 2 drops of H2SO4 and 2 drops of acetic acid, then heat. Based on the test results, the extract is considered ethanol-free when no ester odor is detected.(Priamsari MR, 2019)**.**

Table 6. Ethanol-free test results

Sample	Ethanol Free Test	Results
White Frangipani Stem	2 ml thick extract + 2 drops of concentrated acetic acid (HCL) + drops of acetic acid (H2SO4)	There is no characteristic ethanol ester odor.

Table 4. Results of the extract water content test

Replication sample	I	II	III	Average
White Plumeria Stem (Pulmeria Rubra L)	8.70%	8.69%	8.63%	8.63%

Referring to the table above, the evaluation results for the percentage of water in the white frangipani (Plumeria rubra L.) stem extract show that specimen I reached 8.70%, specimen II reached 8.69%, and specimen III reached 8.63%, with an average of 8.63%. This fact validates that the white frangipani (Plumeria rubra L.) stem extract maintains an ideal moisture content, as the benchmark for a good moisture content is ≤ 10%.(Barrow, 2019).

Metal contamination evaluation is aimed at detecting the presence of metal contamination in white frangipani stem extract.(Ningsih, 2024). Referring to the results of the heavy metal contamination inspection, it was found that white frangipani stem extract contained no Pb or Cd contamination. The white frangipani stem extract was proven to be undetectable or below the approved peak threshold, thus being classified as safe and meeting the qualifications as an herbal product.

Table 5. Test for free contamination of Pb and Cd metals

Sample	Compound	Test	Library	Results
White Frangipani Stem Extract	Lead Metal (Pb)	Extract 2 ml + K2CrO4 (Chromic Acid)	Yellow precipitate forms(Faizal, 2025)	No precipitate formed (-)
	Cadmium Metal (Cd)	1 mg extract + NaOH	White precipitate forms(Faizal, 2025)	No white precipitate (-) is formed.

Phytochemical Screening

Table 6. Phytochemical Screening

Phytochemical Test	Reagent	Research result	Information
Alkaloid	*Mayer Drangendorf Wagner*	Yellow sediment, Orange sediment Brown sediment	+
Flavonoid	Mg + concentrated HCL	Red sediment	+
Saponin	Extract + Aquades	Foam deposits	+
Tannin	Ethanol + FeCl3	Greenish black sediment	+

Phytochemical testing of white frangipani stem extract (Plumeria rubra L.) showed the presence of alkaloids, flavonoids, saponins, and tannins. In the alkaloid test, 2 g of extract was dissolved in 2N HCl and distilled water, heated, and then Mayer, Dragendorff, and Wagner reagents were added, producing yellow, orange, and brown precipitates as a positive indication of the presence of alkaloids. The flavonoid test began by mixing 1 ml of extract with 70% ethanol, heating, and adding Mg and concentrated HCl, producing a red color indicating the presence of flavonoids. The saponin test used 1 g of extract with distilled water, heated, shaken, and allowed to stand, producing a stable foam indicating the presence of saponins, compounds with surfactant properties. Finally, the tannin test used 2 ml of extract in ethanol added with 1% $FeCl_3$, producing a greenish-black color, indicating the presence of tannin compounds that react with the phenol group.

Antibacterial Activity Test

Evaluation of the antibacterial effectiveness of white frangipani (Plumeria rubra L.) stem extract was conducted using the agar diffusion method in a microbiology laboratory. Prior to testing, all glassware, media, and culture equipment (pipettes, petri dishes, test tubes, etc.) were sterilized using an autoclave at 121 °C for 15–20 minutes at 15 psi, ensuring that almost all microorganisms, including spores, were inactivated by high-pressure steam (Anggraini, 2021; autoclaving is considered an effective pressure steam sterilization method for laboratory equipment). Loop needles and tweezers were sterilized by burning directly over a burner flame, ready for use for microbial transfer.

The test was carried out using the disc diffusion method using sterile disc paper with a diameter of 6 mm, which was dipped into each concentration of white frangipani stem extract, then placed on the surface of the agar media in a petri dish (Anggraini, 2021; this technique is commonly used to assess bacterial sensitivity to antibacterial agents through the diameter of the inhibition zone). Nutrient Agar (NA) media was made by dissolving 0.46 g of NA in 20 ml of distilled water (equivalent to 23 g/1000 ml), then divided into three test tubes, wrapped in aluminum foil, and sterilized in an autoclave at 121 °C for 15 minutes so that the media became solid and ready to be used for rejuvenation and antibacterial testing (Anggraini, 2021). Staphylococcus aureus ATCC 25923 microbes were rejuvenated by streaking one culture loop on a slanted NA medium using a sterile loop, then incubated at 37 °C for 24 hours to obtain a pure culture ready to be inoculated on the test medium.

Identification of Staphylococcus aureus bacteria is done by Gram staining, namely by applying one loop of bacteria to a glass object, adding distilled water, spreading it thinly, fixing it with a spirit flame, then dripping it with crystal violet, Lugol, 96% alcohol for 1 minute, and finally safranin, which produces purple bacteria (Gram-positive) if the staining is successful (Anggraini, 2021). After obtaining a ready culture, a bacterial suspension is made by taking 1–2 loop needles of Staphylococcus aureus and adding them to 5 ml of 0.9% NaCl, then vortexing until the turbidity is equivalent to the McFarland 0.5 standard (equivalent to approximately $1–1.5 \times 10^8$ CFU/mL), which is the reference standard bacterial concentration in microbial sensitivity testing. The final suspension for testing is made by taking 1 loop from the culture that has been incubated for 24 hours, mixing it with 1 ml of sterile NaCl, homogenizing by vortexing, incubating at 37 °C for 24 hours, and then comparing the turbidity with the McFarland 0.5 standard so that the bacterial concentration is suitable for testing antibacterial activity consistently and measurably.

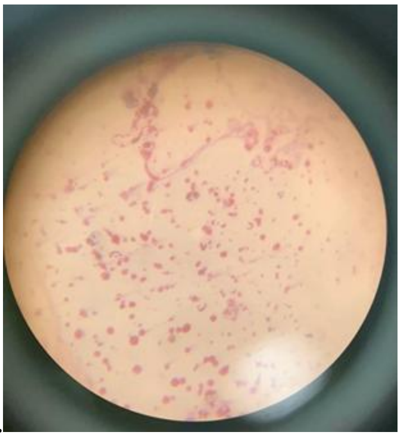

Figure 1. Gram staining

Antibacterial activity testing was performed using the agar well diffusion method, where incubation was limited to no more than 24 hours to prevent bacterial growth in the inhibition zone. A 0.1 ml suspension of Staphylococcus aureus was inoculated into 15 ml of NA media, then wells were made where white frangipani stem extract was added at concentrations of 20%, 40%, and 60%. The petri dish was incubated at 37 °C for 24 hours, then the diameter of the inhibition zone was measured with a caliper with an accuracy of 0.01 mm from the outermost clear area around the well (Pipit, 2020). Based on this diameter, antibacterial effectiveness was classified: <5 mm (weak), 5–10 mm (medium), 10–20 mm (strong), and >20 mm (very strong).

Table 7. Inhibition zone diameter results

Treatment	Inhibition Zone Diameter (mm)			Average (mm)	Category
	R1	R2	R3		
F1 (Positive control)	18	20	23	20.33	Very strong
F2 (20%)	12.5	13.5	14	13.33	Strong
F3 (40%)	12	11.5	14.5	12.67	Strong
F4 (60%)	12.5	12.5	12.5	12.5	Strong

Referring to the calculation results of the diameter of the inhibition zone of white frangipani stem extract on Staphylococcus aureus microbes, it is composed of several variations of the highest positive control dose, followed by doses of 20% and 40%, along with a dose of 60%.

The inhibition zone output of the positive control against Staphylococcus aureus bacteria held an average inhibition zone diameter of 20.33 mm. The output of each of the 3 replications of the inhibition zone of the positive control against Staphylococcus aureus bacteria reached 18 mm, 20 mm, and 23 mm. The average output of the 3 replications of the inhibition zone of the 20% extract dose against Staphylococcus aureus microbes was 13.33 mm. The output of each of the 3 replications of the inhibition zone reached 12.5 mm, 13.5 mm, and 14 mm. The average output of the 3 replications of the inhibition zone of the 40% extract dose against Staphylococcus aureus bacteria was 12.67 mm. The output of each of the 3 replications of the inhibition zone reached 12 mm, 11.5 mm, and 14.5 mm. The average inhibition zone of three replicates of the 60% extract dose against Staphylococcus aureus bacteria was 12.5 mm. The inhibition zone of each replicate reached 12.5 mm, 12.5 mm, and 12.5

mm, respectively. The difference in inhibition zone output in the positive control extract of white frangipani stems showed a very large inhibition zone against Staphylococcus aureus bacteria, compared to the 20%, 40%, and 60% extract doses.

The positive control dose (ethanol solvent) in white frangipani stems has the potential to hold the most ideal inhibition zone, as it represents a very strong classification. The 20% extract dose, 40% extract dose, and 60% extract dose represent a strong classification. This fact makes the positive control dose in this study capable of inhibiting the proliferation of Staphylococcus aureus microbes. The positive control dose was determined as the ideal dose to produce more potent antibacterial efficacy against Staphylococcus aureus bacteria, as the results of the inhibition zone diameter calculation scored the highest.

CONCLUSION

This study shows that the ethanol extract of white frangipani (Plumeria rubra L.) stem has significant antibacterial potential against Staphylococcus aureus ATCC 25923. The results of phytochemical screening confirmed the presence of alkaloids, flavonoids, saponins, and tannins that act as bioactive compounds, while the standardization test showed that the yield of simplex and extract met the standards, reasonable water and ash content, and no heavy metal contamination was revealed. In the antibacterial activity test using the well diffusion method, white frangipani stem extract with concentrations of 20%, 40%, and 60% produced a strong inhibition zone (10–20 mm), although the positive control showed the highest activity, thus confirming the effectiveness of certain extract concentrations in inhibiting the growth of Gram-negative bacteria.-These findings support the use of white frangipani stems as a source of natural antibacterial agents that can be further developed into topical formulations for the treatment of skin infections.

However, this study has several limitations. The design used only reached a qualitative inhibition zone test without determining the minimal inhibitory concentration (MIC) or minimal bactericidal concentration (MBC), so the concentration-effect mechanism and toxicity of the extract to mammalian cells have not been comprehensively characterized. Furthermore, the test was only conducted on a single standard strain of S. aureus ATCC 25923, so it cannot yet describe the response to resistant clinical isolates or other bacterial species relevant to skin infections. Further research is recommended to evaluate the extract's activity against resistant S. aureus isolates, determine the MIC/MBC, and assess in vitro and in vivo toxicity before developing topical formulations. Practically, the results of this study provide a scientific basis for utilizing the underutilized white frangipani stem as a raw material for antibacterial extracts, thereby contributing to the development of more sustainable skin infection therapies amid the global antibiotic resistance crisis.

REFERENCES

Adolph, R. (2016). Testing of water content and specific parameters of simple drugs. Cerebellum Journal, 6(1), 17–20.

Anggraini, PH (2021). Inhibitory power test of extracts and fractions n-hexane, ethyl acetate fraction, water fraction of cherry leaves (Muntingia calabura L.) against Staphylococcus aureus ATCC 25923 bacteria. Duta Pharma Journal, 1(2), 8–19.

Anisa, S. (2023). Phytochemical screening and antibacterial test of karamunting fruit extract (Melastoma malabathricum) against Staphylococcus aureus bacteria. Indonesian Scientific Journal of Pharmacy, 5(1), 1–10.

Astridwiyanti, AA (2019). Effectiveness test of ethanol extract of red dragon fruit (Hylocereus polyrhizus) peel against Staphylococcus aureus ATCC 25923 in vitro. Medical Science Digest, 10(3), 482–486.

Barrow, S. D. (2019). Sustainability and herbal drug standardization. Switzerland: Springer.

Candrawati, TH (2025). Profile of Staphylococcus aureus originating from nasal cavity swabs of food handlers at the University of Mataram canteen. Journal of Tropical Biology, 25(2), 1611–1622.
Chanida, ZT (2024). Characterization of gold nanoparticle serum (Au) bioreductant from sunflower seed extract (Helianthus annuus L.). Journal of Food Agroindustry, 4(3), 158–171.
Dzakwan, MLF (2025). Biosynthesis of silver nanoparticles (AgNPs) using frangipani flowers (Plumeria sp.) and their potential as antibacterials against Staphylococcus aureus. Journal of Pharmaceutical Sciences, Jember Pharmacy Academy, 8(2), 76–91.
Evifania, RD (2020). Testing of specific and nonspecific parameters of senggani leaf (Melastoma malabathricum L.) simplicia. Cerebellum Journal, 6(1), 17–20.
Faizal, RF (2025). Analysis of heavy metal contamination of cadmium (Cd) and lead (Pb) in the food safety laboratory (UPTDBPSMP) of the West Sumatra Provincial Food Service. Journal of Food Agroindustry, 4(3), 158–171.
Halodoc. (2023). Staphylococcus aureus and the diseases it causes. Retrieved April 10, 2026, from https://www.halodoc.com/artikel/mengenal-staphylococcus-aureus-dan-penyakit-yang-ditimbulkannya
Hasibuan, AS (2020). Phytochemical screening of ethanol extract of shallot bulbs (Allium cepa L.). Jurnal Farmasimed (JFM), 2(2), 45–49.
Hudatama, A. (2016). Antibacterial activity test of ethanol extract of white frangipani leaves (Plumeria acuminata) against the growth of Streptococcus mutans. Indonesian Journal of Pharmacy, 5(1), 1–8.
ILM Research. (2026). Antibiotic resistance and mortality due to resistant infections. Global Antimicrobial Resistance Watch Report.
Jayani, NI (2021). Standardization of simplex of tempuyung leaves (Sonchi folium) cultivated at the Ubaya Training Center, Trawas, Mojokerto. Journal of Pharmacy Science and Technology, 1(1), 68–79.
Julianto, TS (2019). Phytochemistry: Review of secondary metabolites and phytochemical screening (53rd Edition). Jakarta: EGC Medical Book Publisher.
Juliawaty, LD (2024). Phytochemistry and bioactivity of the Indonesian plant Cryptocarya (medang). Journal of Medicinal Plants Indonesia, 10(2), 45–53.
Khairunisa, N., & Yulianti, L. (2023). Effectiveness of basil leaf extract & red betel leaf extract as antimicrobials for Staphylococcus aureus that causes furuncles. Fakumi Medical Journal, 3(2), 106–111.
Krismayadi, HE (2024). Standardization of the quality of simple drugs and ethanol extracts of basil leaves. Pharmacy Genius, 3(2), 67–81.
Kurniawan, HM (2023). Antibiotic sensitivity test against Escherichia coli and Staphylococcus aureus bacteria in vitro. Proceedings of the National Seminar on Computer Technology and Science, 1(1), 712–718.
Lady, DY (2020). The effect of maceration (soaking) time on the viscosity of betel leaf extract (Piper betle). Journal of Tinctura Pharmacy, 2(1), 34–41.
Laksanawati, TA (2022). Purification and activity testing of sulfhydryl protease from plumeria (Plumeria obtusa) stems. Agribios, 20(2), 235–240.
Maisarah, MC (2023). Characteristics and functions of alkaloid compounds as antifungals in plants. Jurnal Serambi Biologi, 8(2), 231–236.
Marsela, S. (2022). Antibacterial activity test of white frangipani stem sap (Plumeria acuminata WT Ait.) against the growth of Pseudomonas aeruginosa bacteria. Indonesian Pharmaceutical Journal, 6(1), 1–6.
Marsila, RF (2025). Extraction and identification of bioactive compounds from basil (Ocimum basilicum L.) leaf extract. Jurnal Lentera Ilmiah Kesehatan, 3(8), 1–12.
Maryam, FT (2020). Measurement of specific and non-specific parameters of ethanol extract of matoa leaves (Pometia pinnata JR & G. Forst.). Jurnal Mandala Pharmacon Indonesia, 6(1), 1–12.

Mehraj, V., et al. (2014). Global burden and epidemiology of methicillin-resistant Staphylococcus aureus infections. Journal of Infection and Public Health, 7(6), 409–418.

Ministry of Health of the Republic of Indonesia. (2018). National monitoring of the distribution of Staphylococcus spp. bacteria in Indonesia. Ministry of Health of the Republic of Indonesia.

Ministry of Health of the Republic of Indonesia. (2000). Indonesian Pharmacopoeia (VIth edition). Ministry of Health of the Republic of Indonesia.

Moh, E. (2021). Antibacterial activity test of water extract of shallot skin (Allium cepa L.) against Staphylococcus aureus bacteria. Pharmacognosy Magazine, 75(17), 399–405.

Naga, JA (2022). Antibacterial activity test of ethanol extract of starfruit leaves (Averrhoa bilimbi L.) against Staphylococcus aureus bacteria. Indonesian Journal of Pharmacy, 4(1), 1–8.

Najib, AM (2017). Standardization of water extract of guava leaves and green tea. Indonesian Phytopharmaceutical Journal, 4(2), 241–245.

Ningsih, WS (2024). The use of sweet mango seed starch (Mangifera indica L.) as a disintegrant on the physical properties of paracetamol tablets using the wet granulation method. Journal of Pharmaceutical and Health Sciences Students, 2(1), 160–169.

Oktaviana, BR (2017). Antifungal activity of methanol extract of white frangipani flowers (Plumeria acuminata) against Aspergillus clavatus. Jurnal Labora Medika, 1(2), 26–31.

Pipit, ME (2020). Formulation of deodorant preparation from ethanol extract of awarawar leaves (Ficus septica Burm.) as an antibacterial against Staphylococcus aureus. Journal GEEJ, 7(2), 1–10.

Priamsari, MR (2019). Phytochemical screening and burn healing activity of ethanolic extract of Morinda citrifolia L. on rabbit skin (Oryctolagus cuniculus). Journal of Pharmacy, 8(1), 22–28.

Priamsari, MR (2021). Antibacterial activity test of ethanolic extract of noni leaves (Morinda citrifolia L.) against Staphylococcus pyogenes bacteria in vitro. Journal of Pharmacy, 9(2), 15–20.

Puspitawati, DP (2025). Single and combined extracts of basil leaves (Ocimum sanctum L.) and cosmos caudatus leaves (Kunth) against Escherichia coli bacteria. Journal of Pharmacy Indonesia, 9(3), 2919–2926.

Rachma, SK (2024). Viability of methicillin-resistant Staphylococcus aureus (MRSA) bacteria preserved at different temperatures and times. Quangga: Journal of Education and Biology, 16(2), 144–152.

Rahman, MA, & Yusuf, A. (2023). Evaluation of antimicrobial activity and phytochemical screening of Plumeria rubra L. extracts. Journal of Medicinal Plants, 11(3), 1–10.

Rahmasiahi, HS (2023). Phytochemical screening of methanol extract of pandan leaves (Pandanus amarilifolius Roxb.). Journal of Pharmaceutical Science and Herbal Technology, 1(1), 32–39.

Restyana, AI (2019). Formulation and topical antibacterial test of papaya (Carica papaya L.) seed extract microemulsion on Staphylococcus aureus bacteria. Jurnal Wiyata, 1(0), 73–79.

Rianti, ED (2022). AC electric field strength in inhibiting the growth of Staphylococcus aureus and Escherichia coli colonies. Biome: Scientific Journal of Biology, 11(1), 79–88.

Rosmini, LS (2020). Guidance on medicinal plant cultivation techniques for the provision of herbal medicinal simplicia for the community. Dinamisia: Journal of Community Service, 5(2), 294–299.

Setiawati, A. (2015). Increasing the resistance of Staphylococcus aureus bacterial cultures to amoxicillin using the gradual adaptive method. Indonesian Journal of Pharmacy, 7(3), 190–194.

Setyani, IK (2021). Standardization of simple and extract of cubeb fruit (Piper cubeba Lf) as raw materials for herbal capsule preparations for shortness of breath. JPSCR: Journal of Pharmaceutical Science and Clinical Research, 6(3), 238–245.

Silverman, ML (2023). Indonesian Herbal Pharmacopoeia (2nd edition). Jakarta: EGC Medical Book Publisher.

Sudaryono. (2022). Educational research methods. Yogyakarta: Sanata Dharma University Press.

Sugiyono. (2021). Quantitative, qualitative, and R&D research methods. Bandung: Alfabeta.

Sulistyarsi, A., & Andriani, S. (2018). Comparative test of white frangipani leaf extract (Plumeria acuminata WT Ait.) as an inhibitor of the growth of Salmonella typhi and Staphylococcus aureus bacteria (in vitro). Proceedings of the National Seminar on SIMBIOSIS, 16–22.

Usman, H., & Darmawan, A. (2012). Introduction to statistics (2nd edition). Jakarta: PT Bumi Aksara.

Utami, YP (2017). Standardization of simple drugs and ethanol extract of leilem leaves (Clerodendrum sp.). Journal of Pharmaceutical and Medical Sciences, 2(1), 32–39.

Wahyuni, I., & Febrina, KS (2020). Antibacterial activity test of ethanol extract of gardenia leaves (Gardenia jasminoides Ellis) against Streptococcus mutans bacteria. Journal of Science and Health, 2(1), 399–404.

Wijaya, A., & Andriyanto, B. (2022). Determination of water content of basil leaf (Ocimum sp.) simplex. Indonesian Journal of Pharmaceutical Research, 4(2), 185–195.

Zhang, W. X. (2024). The effect of procrastination on physical exercise among college students: The chain effect of exercise commitment and action control. International Journal of Mental Health Promotion, 26(8), 611–622.