

ANTIBIOFILM ACTIVITY OF FIVE NATIVE PLANTS FROM KALIMANTAN ISLAND AGAINST *Staphylococcus aureus*

Joko Priyanto Wibowo^{1✉}, Abdul Rahman Wahid², Dewi Setya Paramitha³, Restu Dara Wewengkang¹ and Medina Nurrahmah¹

¹Faculty of Pharmacy, Universitas Muhammadiyah Banjarmasin, Barito Kuala, Indonesia, 73514.

²Faculty of Health Science, Universitas Muhammadiyah Mataram, Mataram, Indonesia, 83115.

³Faculty of Nursing and Health Science, Universitas Muhammadiyah Banjarmasin, Banjarmasin, 70114.

✉ jp.wibowo@umbjm.ac.id

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ABSTRACT

Infections caused by *Staphylococcus aureus* are an increasing concern in the healthcare sector, particularly due to the rise of antibiotic-resistant strains. Natural products provide a promising alternative source for discovering new antibacterial and antibiofilm agents. This study aimed to evaluate the antibiofilm activity of five plant species natives to Kalimantan Island against *S. aureus*. Plant materials were extracted using the ultrasound-assisted extraction (UAE) method with 70% ethanol at 40 kHz and temperature of 30°C for 30 minutes. Phytochemical screening was performed using standard qualitative tests to detect major secondary metabolite groups, including alkaloids, flavonoids, tannins, saponins, and terpenoids. The antibiofilm assay was carried out using a spectrophotometric microplate method, where biofilm formation was quantified in the presence of plant extracts (500 ppm) compared to negative control (solvent). Data were analyzed statistically using ANOVA ($p < 0.05$) followed by post-hoc tests to determine significant differences among samples. Among the tested extracts, the gelinggang extract exhibited the strongest antibiofilm effect, inhibiting biofilm formation by 84.39 ± 7.21 %, followed by sangkareho (70.63 ± 7.29 %), kratom (66.54 ± 6.11 %) and matoa (58.36 ± 5.91 %). While, limpasu showed the weakest antibiofilm activity (18.22 ± 1.21 %) in this study. The observed activity may be attributed to the presence of phenolic and flavonoid compounds known to disrupt quorum sensing and biofilm matrix synthesis. These findings highlight the potential of Kalimantan's native plants, particularly gelinggang, as promising sources of natural antibiofilm agents for combating antibiotic-resistant *S. aureus* infections.

Keywords: antibiofilm, gelinggang, spectrophotometry, *Staphylococcus aureus*

1. INTRODUCTION

Infectious diseases continue to pose a significant challenge in the healthcare sector, a situation exacerbated by the emergence of various antibiotic-resistant bacterial species (Salam et al., 2023). The consequences of antibiotic resistance are substantial, leading to increased healthcare costs, reduced productivity, prolonged hospital stays, and higher mortality rates (Dadgostar, 2019). This global health concern prompted the World Health Organization (WHO) to release, in 2017 a list of priority pathogens for which new antibiotics are urgently needed (Vogel, Jansen, et al., 2022). A key contributor to antibiotic resistance is the ability of certain bacteria to form biofilms, a structured community of microbial cells enclosed within a self-produced polymeric matrix. Biofilms serve as a defense mechanism, providing resistance against external threats, including antibiotics, and function as virulence factors (Alonso et al., 2021; Zhao et al., 2023). *Staphylococcus aureus* is a Gram-positive bacterium that cause a wide variety of clinical diseases. Infections caused by this pathogen are common both in community-acquired and hospital-acquired settings. In particular, it is notorious for its ability to evade

antibiotic treatment through biofilm formation, which impedes the penetration of antibiotic molecules into bacterial cells (Saud et al., 2023).

Considering the urgent need for alternative antibacterial agents, this study investigates the antibiofilm potential of five native Kalimantan plants, *Pometia pinnata* (matoa), *Callicarpa longifolia* (sangkareho), *Mitragyna speciosa* (kratom), *Cassia alata* (gelinggang), and *Baccaurea macrocarpa* (limpasu), against *S. aureus*. The research aims to evaluate the efficacy of their leaf extracts in inhibiting biofilm formation, thereby contributing to the development of alternative strategies to combat antibiotic resistance. Since these plants have been known in Kalimantan medicine as traditional medicine including for treating infection.

Previous studies have reported the antibacterial properties of these plants: *C. alata* (Fajri et al., 2024), *P. pinnata* (Pakaya et al., 2021), *C. longifolia* (Amalia, 2016), *B. macrocarpa* (Febriana et al., 2023), and *M. speciosa* (Putri et al., 2023). For instance, a study has investigated the potential of the extracts, fractions and compounds of *C. alata* leaves against yeast pathogens, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* as antibiofilm (Karunakaran et al., 2022).

However, to date, no study has specifically investigated their antibiofilm activity against *S. aureus*. The novelty of the present study lies in its exploration of the antibiofilm potential of these five native Kalimantan plants, aiming to identify bioactive secondary metabolites with promising therapeutic applications.

2. METHODS

2.1. Collection and Extraction of Leaves

Fresh leaves of each plant (± 5 kg) were collected from Banjar Regency, South Kalimantan. Prior to the following preparation, the plants has been identified at the Laboratory of Banua Botanical Garden, BRIDA Kalsel (Certificate No. 000/11100/LIT/KRB/2025). The preparation of the crude drugs began with washing to remove dirt and impurities. The leaves were then chopped into small pieces to facilitate drying and dried in a cabinet dryer to prevent fermentation by microbes, degradation of secondary metabolites, and undesirable chemical reactions caused by ultraviolet radiation during sun drying.

The dried crude drugs were ground into powder to increase the surface area and improve extraction yield. The physical quality of the crude drugs powder was tested according to the standards outlined in the *Farmakope Herbal Indonesia* (Indonesian Herbal Pharmacopoeia), 2nd Edition (Direktorat Jenderal Kefarmasian dan Alat Kesehatan, 2017). The powder was then stored in a cool and dry environment in sealed containers until extraction.

Extraction was carried out using the Ultrasound-Assisted Extraction (UAE) method. Approximately 500 grams of crude drugs powder were placed in an erlenmeyer flask, and 70% ethanol (Merck, Germany) (five times the weight of the crude drugs powder) was added until the powder was fully submerged. The extraction was performed using a sonicator (Biobase®, Germany) set at 40 kHz and a temperature of 30 °C for 30 minutes. The extract was filtered using flannel cloth and concentrated using a rotary evaporator (Biobase®, Germany). The concentrated extracts were stored in sealed bottles at a low temperature (below 8 °C) until further analysis (Norhaliza et al., 2022).

2.2. Phytochemical Screening

The presence of secondary metabolites in the leaves was evaluated following the method described by Farnsworth (1966). Briefly, 10 grams of dry leaf powder from each plant were macerated, and the resulting extracts were analyzed for the presence of alkaloids, flavonoids, saponins, steroids, tannins, and triterpenoids. The presence of flavonoids was evaluated by the Shinoda test (magnesium turnings and HCl, observing for orange-red coloration), and alkaloids were detected using alkaloid reagents (Mayer's and Dragendorff's tests, observing for the formation of a precipitate). Saponins were identified by the froth test, where persistent foam indicates saponin content. Tannins were tested with 1% FeCl₃ solution (Merck, Germany), looking for a blue-black or greenish coloration as evidence of tannins. Steroids were assessed using the Liebermann-Burchard reaction (acetic anhydride and concentrated H₂SO₄, yielding a bluish-green

color), and terpenoids were identified by Salkowski's test (conc. H_2SO_4 , forming a reddish-brown interface). A change in color or formation of a precipitate specific to each test indicated the presence of the corresponding phytochemical group.

2.3. Determination of Physicochemical Parameters of *C. alata*

Physicochemical parameters such as moisture content, total ash content, acid insoluble and water-soluble ash contents water and ethanol extractable matter were determined for *C. alata* powder according to the methods described in guidelines (WHO, 2011).

Determination of Moisture Content

The powdered material (1 g) was placed in a moisture dish and dried to a constant weight in an oven at 100 -105 °C. The loss of weight in mg/g of air-dried material was calculated.

Determination of Total Ash Content

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was ignited to a constant weight by gradually increasing the heat to 500 - 600 °C until it was white. The residual ash was allowed to cool in a desiccator. The content of total ash in mg/g of air-dried material was calculated.

Determination of Acid Insoluble Ash Content

Hydrochloride acid (2 N; 25 mL) (Merck, Germany) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water, and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash in mg/g of air-dried material was calculated.

Determination of Water-Soluble Ash Content

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and added to the crucible. The water insoluble matter was collected on an ashless filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to a constant weight. The water-soluble ash content was calculated.

Determination of Ethanol Extractable Matter

Accurately weighed powdered material (4 g) was placed in a glass stoppered conical flask. Ethanol (95%; 100 mL) (Merck, Germany) was added to the flask and it was weighed to obtain the total weight including the flask. Then, the flask was shaken well and kept for 1 h. A reflux condenser was attached to the flask and boiled gently for 1 h, and then it was cooled and weighed. The weight was readjusted to the original total weight by adding required amount of 95% ethanol (Merck, Germany). The contents were filtered rapidly. After that, 25 mL of the filtrate was evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h, cooled in a desiccator, and weighed. The content of extractable matter in mg/g air dried material was calculated. The same procedure was performed to determine water extractable matter.

2.4. Antibiofilm Activity

The antibiofilm activity of the leaf extracts was assessed using a modified version of the protocol by Vogel, Wibowo, et al. (2022):

- a. The extracts were dissolved in 1% DMSO (Merck, Germany) in water and prepared at a final concentration of 250 ppm. A volume of 50 μL of each extract solution was transferred to individual wells of a 96-well plate (VWR, USA).
- b. An overnight culture of *S. aureus* was diluted in nutrient broth (Merck, Germany) to an optical density (OD_{600}) of 1.6. Then, 50 μL of the bacterial suspension was added to each well containing the extract, except for the last column, which served as the control.
- c. The plates were incubated at 37 °C for 18 hours.

- d. After incubation, the contents of each well were discarded, and the wells were washed with sterile distilled water and rinsed three times with 120 μL of phosphate-buffered saline (PBS) (Merck, Germany). The plates were then dried by heating at 60 $^{\circ}\text{C}$ for 1 hour.
- e. Biofilms were stained by adding 120 μL of 0.1% crystal violet (Merck, Germany) solution to each well and incubating at room temperature for 15 minutes. Excess dye was removed with tap water, and the plates were air-dried.
- f. To release the crystal violet–biofilm complex, 150 μL of 30% acetic acid was added to each well.
- g. The released dye solution was diluted 10-fold, and its absorbance was measured at 585 nm using a visible spectrophotometer (Jenway).
- h. The antibiofilm activity was calculated based on the percentage inhibition of biofilm formation. All experiments were conducted in triplicate.

2.5 Statistical Analysis

The results were shown as mean $\pm$ SD (standard deviations) and were analyzed by a one-way ANOVA test. A P-value of ≤ 0.05 was statistically significant. The figures were created using GraphPad Prism software, V.10.3.1.

3. RESULTS AND DISCUSSION

The experiments conducted yielded results as summarized in the following subsections. Fresh leaves of each plant (**Figure 1**) were dried, and their yields were calculated.

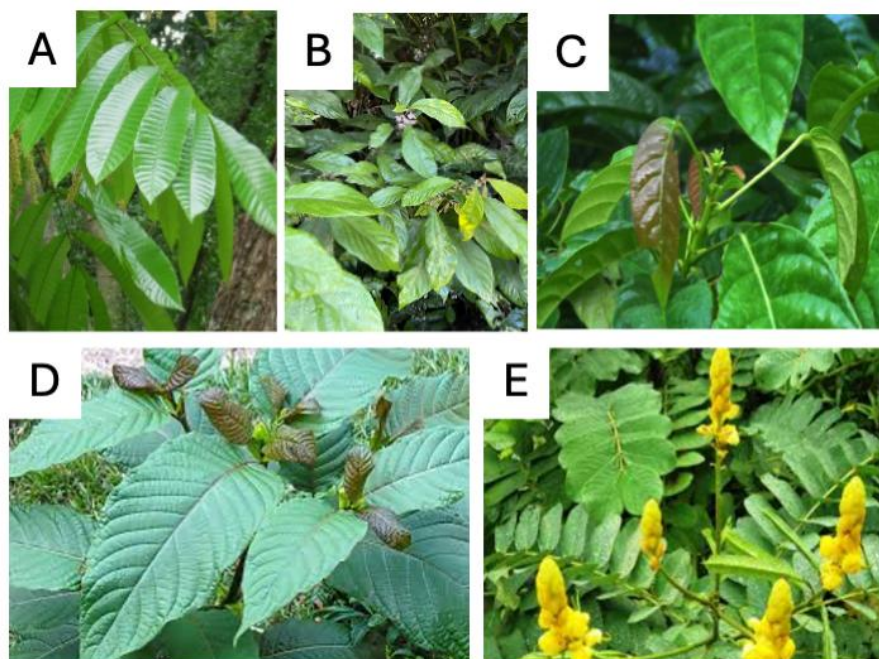


Figure 1. Leaves of native Kalimantan plants: (A) matoa leaves, (B) sangkareho leaves, (C) limpasu leaves, (D) kratom leaves, and (E) gelinggang leaves.

3.1. Yield of Crude Drugs and Extract

In general, the yield of each crude drug was approximately 10–15%. The *crude drugs* that met the quality standard (moisture content $<10\%$) were ground into powder with a 40-mesh size before undergoing extraction using the Ultrasound-Assisted Extraction (UAE) method.

Table 1 presents the yield percentages of crude drugs and extracts obtained from five plant species gelinggang, kratom, limpasu, matoa, and sangkareho—collected from Kalimantan Island. The crude drug yield represents the ratio of dried plant material obtained after initial drying, while the extract yield indicates the percentage of concentrated extract produced after ultrasound-assisted extraction using 70% ethanol.

Table 1. Yield of Crude Drug and Extract

Sample	Yield (%)
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	Gelinggang	Kratom	Limpasu	Matoa	Sangkareho
Crude drugs	19.23	12.77	13.21	15.65	12.20
Extract	13.77	9.16	5.89	14.77	4.50

The yields of both crude drugs and extracts varied among the samples. The highest crude drugs yield (19.23%) was obtained from gelinggang leaves, while the lowest (12.20%) was from sangkareho leaves. This variation can be attributed to differences in the physical characteristics of each leaf. Although the wet weights of the samples were the same, their resulting dry weights differed.

A similar pattern was observed in the extraction process: the weight of the thick extract varied among all samples despite using equal amounts of powdered crude drugs. The matoa extract yielded the highest (14.77%), while sangkareho yielded the lowest (4.50%). These differences may be due to the varying amounts of polar compounds extracted by 96% ethanol in each sample. The high yield in the matoa extract suggests a higher concentration of polar compounds, whereas the low yield in the sangkareho extract indicates fewer polar compounds. This is consistent with 96% ethanol, a polar solvent that primarily dissolves polar substances in the crude drugs.

3.2. Phytochemical Screening

All crude drugs underwent phytochemical screening based on established methods to identify the secondary metabolite content. All samples generally contained the same groups of secondary metabolites, with a few notable differences (**Table 2**). Anthraquinone compounds were detected only in gelinggang leaves, while steroids were absent in limpasu leaves. Terpenoids were present in gelinggang and sangkareho leaves but not in the others.

Table 2. Phytochemical Screening Result

Secondary Metabolite	Gelinggang	Kratom	Limpasu	Matoa	Sangkareho
Alkaloid	+	+	+	+	+
Anthraquinone	+	-	-	-	-
Phenolic	+	+	+	+	+
Flavonoid	+	+	+	+	+
Saponin	+	+	+	+	+
Steroid	+	+	-	+	+
Tannin	+	+	+	+	+
Terpenoid	+	-	-	-	+

Note: (+) = detected

(-) = not detected

The phytochemical screening results align with previous studies, suggesting that the extraction method does not significantly affect the presence of secondary metabolites in these samples.

3.3. Physicochemical Parameters

All crude drugs underwent physicochemical parameter assay based on established methods to obtain the information on the quality of the raw materials. The physicochemical characteristics of the five evaluated medicinal plant samples—Gelinggang, Kratom, Limpasu, Matoa, and Sangkareho—in comparison with established pharmacopoeia standards (**Table 3**). The parameters assessed include moisture content, water- and ethanol-extractable matter, total ash, and acid-insoluble ash. These physicochemical properties serve as critical quality control indicators, reflecting the purity, stability, and potential bioactive content of the plant materials. Variations observed among the species may be attributed to differences in their phytochemical composition, environmental growth conditions, and post-harvest processing. Overall, the results indicate that all samples fall within acceptable limits, supporting their potential use in standardized herbal formulations.

Table 3. Physicochemical parameters

Physicochemical Parameter	Gelinggang	Kratom	Limpasu	Matoa	Sangkareho	Standard Value (%)
Moisture Content	3.46 ± 0.31	5.21 ± 0.41	4.43 ± 0.56	7.46 ± 0.88	4.26 ± 0.63	< 10
Water Extractable Matter	17.83 ± 1.53	15.83 ± 1.22	12.43 ± 1.11	18.11 ± 1.09	13.83 ± 1.24	> 24
Ethanol Extractable Matter	19.52 ± 1.91	18.12 ± 1.81	16.52 ± 1.66	22.52 ± 2.31	15.71 ± 1.98	> 6
Total Ash	16.62 ± 1.62	15.62 ± 1.44	14.22 ± 1.02	16.78 ± 1.92	13.21 ± 1.32	> 10
Acid Insoluble Ash	0.61 ± 0.05	0.51 ± 0.05	0.68 ± 0.06	0.66 ± 0.05	0.55 ± 0.05	< 0.7

The physicochemical evaluation of herbal raw materials is an essential step in ensuring their quality, safety, and efficacy as standardized medicinal sources (WHO, 2011). The present study compared the physicochemical properties of five traditional medicinal plants: Gelinggang (*Cassia alata*), Kratom (*Mitragyna speciosa*), Limpasu (*Baccaurea lanceolata*), Matoa (*Pometia pinnata*), and Sangkareho (*Calicarpa longifolia*).

Moisture content plays a critical role in maintaining the stability of herbal drugs, as higher water content may promote microbial growth and enzymatic degradation (Fadeyi et al., 2013). All five plants exhibited moisture content below the pharmacopoeial limit of 10%, ranging from 3.46 ± 0.31% (Gelinggang) to 7.46 ± 0.88% (Matoa). This indicates acceptable drying and storage conditions, ensuring their stability for pharmaceutical use.

Water and ethanol extractable matter are indicators of the plant's soluble phytoconstituents, including alkaloids, flavonoids, glycosides, and tannins (Angelina et al., 2021). Among the plants tested, Matoa demonstrated the highest water extractable content (18.11 ± 1.09%) and ethanol extractable matter (22.52 ± 2.31%), suggesting a high concentration of bioactive compounds. In contrast, Limpasu showed the lowest water extractable fraction (12.43 ± 1.11%), indicating lower hydrophilic constituents. Importantly, all samples exceeded the minimum ethanol extractable matter standard (>6%), confirming sufficient phytochemical yield for medicinal preparations.

Ash values are useful parameters to evaluate the inorganic composition and detect adulteration or contamination with earthy materials (Nugrahani et al., 2025). The total ash content varied from 13.21 ± 1.32% (Sangkareho) to 16.78 ± 1.92% (Matoa), all above the standard threshold (>10%), indicating substantial mineral content. Furthermore, **acid-insoluble ash**, which reflects siliceous impurities such as sand and soil, remained below the acceptable limit of 0.7% in all samples, with values ranging from 0.51 ± 0.05% (Kratom) to 0.68 ± 0.06% (Limpasu). This confirms the minimal presence of extraneous matter and overall quality compliance.

Overall, the results suggest that the tested medicinal plants meet the minimum pharmacognostic requirements for herbal raw materials. Matoa exhibited the highest extractive values, which may correlate with its ethnomedicinal use as a rich source of bioactive phytochemicals. Meanwhile, Gelinggang showed the lowest moisture content, supporting its stability during storage. These findings provide scientific evidence for the quality parameters of these traditional medicinal plants and can serve as a reference for future standardization and pharmacological investigations.

3.4. Biofilm Activity Test

A biofilm is a community of microorganisms attached to a surface and encased in a self-produced extracellular matrix composed of proteins, DNA, and polysaccharides (Donlan & William Costerton, 2002). Bacteria within biofilms are more resistant to antibiotics than

planktonic forms, complicating the treatment of infections, particularly those caused by *S. aureus*. This pathogen is known to form biofilms on medical devices and tissues, leading to persistent infections such as catheter-related infections, osteomyelitis, and endocarditis (Alonso et al., 2021).

In recent years, research has increasingly explored the potential of plant-derived natural compounds in combating biofilm-associated infections. Five local plant species, gelinggang (*C. alata*), kratom (*M. speciosa*), limpasu (*B. lanceolata*), matoa (*P. pinnata*), and sangkareho (*C. longifolia*), were found to exhibit antibiofilm activity against *S. aureus*. Following our preliminary experiments to determine the dose of the extracts, in this study all extracts showed antibiofilm effects at a concentration of 500 ppm.

In this study, biofilm production was quantitatively measured using a crystal violet staining method. The addition of crystal violet forms a biofilm, crystal violet complex (Figure 2), which is subsequently released from the wells by adding 30% acetic acid. The antibiofilm activity of each leaf extract is reflected in the amount of biofilm formed during incubation (Figure 3). The intensity of the biofilm, crystal violet complex formation correlates with the level of biofilm production, which is influenced by the presence of the leaf extracts added to the *S. aureus* bacterial suspension.

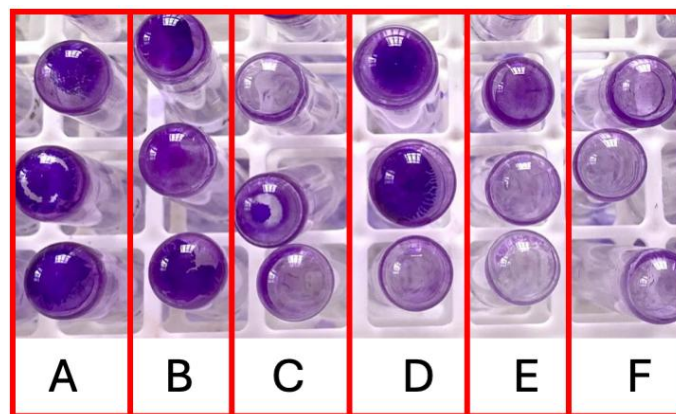


Figure 2. Antibiofilm activity of all samples against *S. aureus* using crystal violet staining: Negative Control (A), Limpasu (B), Kratom (C), Matoa (D), Gelinggang (E), and Sangkareho (F).

The antibiofilm activities of the plant extracts are discussed below based on the experimental findings.

3.1.1. Gelinggang (*C. alata*)

Gelinggang, also known as ketepeng leaf, has long been used in traditional medicine to treat skin infections. While the antibacterial activity of Gelinggang leaf extract has been extensively reported, its antibiofilm activity has only recently attracted scholarly attention. According to the current study, Gelinggang extract exhibited 84.39% antibiofilm activity against *S. aureus*. This efficacy is likely due to flavonoids, tannins, and saponins, compounds known to disrupt biofilm matrices and inhibit bacterial adhesion (Dzoyem et al., 2024).

Previous study has reported that Gelinggang extract demonstrates strong antibacterial activity against *S. aureus*, with an inhibition zone diameter of 16.78 mm (Fajri et al., 2024). This aligns with the present findings, where Gelinggang also showed the highest antibiofilm activity (84.39%). The same bioactive compounds may be responsible for both activities and belong to similar chemical groups.

These active compounds disrupt the integrity of the extracellular matrix, which is critical for biofilm formation and structural stability. The degradation of this matrix renders the bacteria more vulnerable to antibiotics and the host immune response. This study highlights

Gelinggang's promising potential for development as an antibiofilm agent in treating *S. aureus* infections.

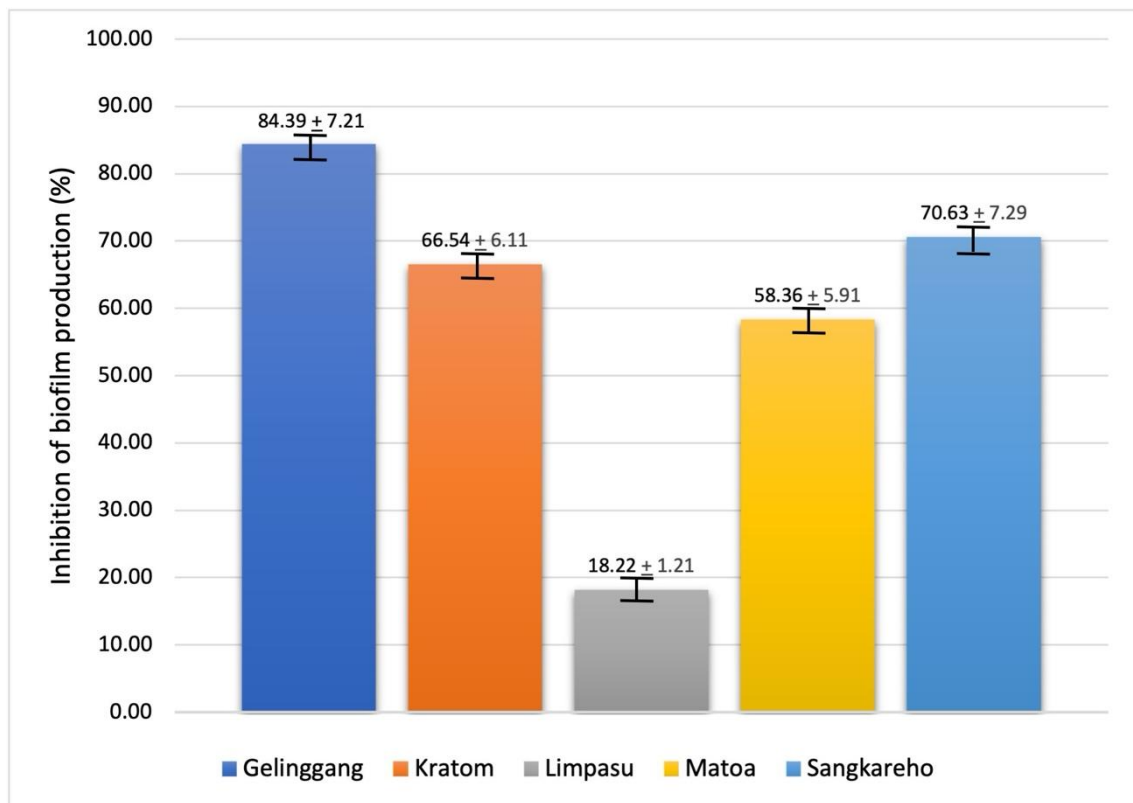


Figure 3. Antibiofilm activity of Gelinggang, Kratom, Limpasu, Matoa, and Sangkareho leaf extracts against *S. aureus*.

3.1.2. Kratom (*M. speciosa*)

Kratom is widely known for its analgesic properties and has traditionally been used to relieve pain. However, its antibacterial and antibiofilm potentials have also become topics of recent research. In this study, Kratom extract exhibited antibiofilm activity of 66.54% against *S. aureus*, an effect attributed to mitragynine and other alkaloids (Karunakaran et al., 2022).

Kratom extract has been reported to possess moderate antibacterial activity (Putri et al., 2023). Similarly, this study demonstrated moderate antibiofilm activity (66.54%). The alkaloids in Kratom are believed to interfere with bacterial communication systems, particularly quorum sensing. Disrupting this signaling mechanism impairs the bacteria's ability to coordinate biofilm formation and maintenance (Hanapi et al., 2021). Therefore, Kratom shows promise as a candidate for developing more effective antibiofilm therapies.

3.1.3. Limpasu (*B. lanceolata*)

Limpasu is an endemic plant that remains relatively under-researched. In this study, its extract showed the lowest antibiofilm activity among the five tested plants, at 18.22%. However, phenolic and terpenoid compounds found in Limpasu have potential roles in disrupting the initial stages of bacterial surface adhesion (Ribić et al., 2025; Salinas et al., 2022). Previous report describes Limpasu extract having a very strong antibacterial activity (Febriana et al., 2023), a finding that contrasts with its weak antibiofilm activity in this study. This suggests that compounds with strong antibacterial properties may not necessarily exhibit similar strength in antibiofilm action. The weak antibiofilm effect may be due to the low concentration of active compounds, the need for synergistic interactions, or slower modes of action. Further research is recommended to enhance the antibiofilm efficacy of Limpasu, including improved extraction techniques or formulation with other antibacterial agents.

3.1.4. Matoa (*P. pinnata*)

Matoa is a tropical plant native to Papua and other Southeast Asian parts. Beyond its edible fruit, its leaves are also a source of bioactive compounds. In this study, matoa leaf extract exhibited antibiofilm activity of 58.36% against *S. aureus*, likely due to its high flavonoid and tannin content (Ribić et al., 2025; Salinas et al., 2022).

Matoa extract has previously shown strong antibacterial activity (Pakaya et al., 2021). The same compounds are believed to reduce bacterial adhesion and inhibit the production of exopolysaccharides, key biofilm matrix components. While Matoa's antibiofilm effect is notable, its efficacy may be improved through dose optimization or co-administration with other agents.

3.1.5. Sangkareho (*C. longifolia*)

Sangkareho leaf extract demonstrated relatively high antibiofilm activity at 70.63%. Traditionally used to treat infections and skin ailments, this plant contains phenolic and terpenoid compounds believed to inhibit biofilm development and promote biofilm matrix degradation (De Rossi et al., 2025; Palomares-Navarro et al., 2022).

Its antibacterial activity has been categorized as moderate (Amalia, 2016; Novaryatiin et al., 2018), but in this study, its antibiofilm effect was considered strong (70.63%). This discrepancy may be due to the presence of compounds that specifically target biofilm-related pathways but have a lesser impact on planktonic bacterial growth.

Given its strong antibiofilm performance, Sangkareho holds significant potential as a candidate for further development as a therapeutic agent against biofilm-associated infections, especially those caused by *S. aureus*.

This study demonstrates that all tested leaf extracts exhibit varying degrees of antibiofilm activity against *S. aureus*. Among the samples, Gelinggang extract showed the highest antibiofilm activity (84.39%), whereas Limpasu exhibited the lowest (18.22%). Interestingly, in comparison to previous reports, the results indicate that antibiofilm and antibacterial activities do not necessarily correlate, suggesting that the secondary metabolites underlying biofilm inhibition differ from those responsible for antibacterial activity.

However, this study has certain limitations. The experiments were conducted under in vitro conditions, which may not fully represent the complex interactions occurring in vivo. Additionally, only a single bacterial strain (*S. aureus*) was tested, limiting the scope of the findings to other clinically relevant pathogens. The study also did not identify or quantify the specific secondary metabolites responsible for the observed activities, which warrants further phytochemical and mechanistic investigations. Future studies incorporating more microbial species, in vivo models, and compound isolation are recommended to elucidate the precise mechanisms and enhance the therapeutic applicability of these plant extracts.

4. CONCLUSION

Among all tested leaf extracts, Gelinggang extract showed the highest antibiofilm activity (84.39 ± 7.21 %) against *S. aureus* (%), while Limpasu displayed the lowest activity (18.22 ± 1.21 %). Compared to previous studies, these results suggest that antibiofilm and antibacterial activities are not always correlated, implying that the secondary metabolites responsible for inhibiting biofilm formation may differ from those contributing to antibacterial effects.

5. ACKNOWLEDGMENT

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6. CONFLICT OF INTEREST

The authors declare no conflict of interest.

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