

Effectiveness of Ethanol Extract of *Etlingera balikpapanensis* Rhizome as an Antibiofilm Against *Staphylococcus aureus* MRSA In Vitro

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ABSTRACT

The increasing prevalence of antibiotic resistance, particularly in *Methicillin-Resistant Staphylococcus aureus* (MRSA), represents a major challenge in the management of infectious diseases, including skin and soft-tissue infections, pneumonia, bloodstream infections, and device-associated infections, because this pathogen can form biofilms that markedly increase antimicrobial tolerance. Biofilm formation contributes to treatment failure and persistent infection, highlighting the need for alternative strategies, including natural antibiofilm agents. This study aimed to evaluate the antibiofilm activity of the ethanolic extract of *Etlingera balikpapanensis* rhizome against MRSA ATCC 33591 in vitro, assess the effect of different extract concentrations on biofilm formation, and identify the lowest tested concentration producing at least 50% biofilm inhibition. This research employed an in vitro laboratory experimental design. The rhizome was extracted by maceration with 96% ethanol, followed by phytochemical screening and antibiofilm evaluation using a microtiter plate assay with crystal violet staining. The extract was tested at concentrations of 10, 20, and 30 mg/mL after 24, 48, and 72 h of incubation. The ethanolic extract contained alkaloids, flavonoids, saponins, triterpenoids, phenolic compounds, and tannins and exhibited antibiofilm activity at all tested concentrations and incubation times. Biofilm inhibition increased with extract concentration, and 30 mg/mL produced the highest inhibition at each time point. Because the lowest tested concentration (10 mg/mL) already produced $\geq 50\%$ inhibition, the MBIC₅₀ was ≤ 10 mg/mL under the conditions of this assay; the exact MBIC₅₀ could not be determined because concentrations below 10 mg/mL were not tested. In conclusion, the ethanolic extract of *E. balikpapanensis* rhizome shows potential as a natural antibiofilm agent against MRSA ATCC 33591, with the strongest effect observed at 30 mg/mL.

Keywords: *Etlingera balikpapanensis*, MRSA, antibiofilm, in vitro.

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INTRODUCTION

Infectious diseases caused by bacteria remain a challenge in the field of health, particularly due to increasing microbial resistance to antibiotics (Jalalifar *et al.*, 2024; Shaaban *et al.*, 2020). One of the main factors contributing to this resistance is the ability of bacteria to form biofilms, which are complex structures composed of microbial communities protected by an extracellular polymeric substance (EPS) matrix (Samrot *et al.*, 2021). The presence of biofilms causes bacteria to become more tolerant to antibiotics and host immune responses, thereby increasing the risk of treatment failure (Mishra *et al.*, 2020; Mohamed *et al.*, 2020).

Bacteria *Staphylococcus aureus* is a Gram-positive pathogen that plays an important role in various infections, ranging from skin infections to serious systemic infections (Rahman *et al.*, 2018). The development of resistant strains, particularly *Methicillin-resistant Staphylococcus aureus* (MRSA), further complicates clinical problems because these bacteria are resistant to β -lactam antibiotics and capable of forming stable biofilms (Ahmad *et al.*, 2019; Prasetyo & Barliana, 2016). Biofilm formation in MRSA is closely related to the activation of the quorum sensing system, which regulates the expression of virulence genes and EPS production, resulting in persistent and difficult-to-treat infections (Larasati, 2020; Miquel *et al.*, 2016).

The limited effectiveness of conventional antibiotics against biofilms has prompted the search for alternative antibiofilm agents, one of which is derived from natural materials. Plant bioactive compounds are known to inhibit biofilm formation through various mechanisms, including disruption of cell adhesion, disruption of the EPS matrix, and inhibition of the quorum sensing system (Miquel *et al.*, 2016; Mishra *et al.*, 2020). Therefore, the use of traditional medicinal plants is a relevant approach in addressing the problem of antimicrobial resistance.

The *Zingiberaceae* family is widely known to have pharmacological potential, including antimicrobial and antibiofilm activities (Saudah *et al.*, 2022). One of the endemic species of Indonesia from this family is *Etlingera balikpapanensis*, which has been reported to

contain bioactive compounds and have potential as an antimicrobial agent, although scientific studies on it are still limited (Amer & Ibrahim, 2019; Sofia *et al.*, 2016). Based on this background, this study aims to evaluate the antibiofilm activity of ethanol extracts of *Etlingera balikpapanensis* rhizomes against MRSA *Staphylococcus aureus* *in vitro*, analyze the effect of extract concentration variations on biofilm formation, and determine the effective concentration based on the *Minimum Biofilm Inhibitory Concentration* (MBIC₅₀) parameter.

MATERIALS AND METHODS

Research Design

This study is a laboratory experimental study using an *in vitro* method to evaluate the antibiofilm activity of ethanol extract from *Etlingera balikpapanensis* rhizome against MRSA *Staphylococcus aureus*.

Materials and Microorganisms

The main materials used were identified rhizomes of *Etlingera balikpapanensis*, 96% ethanol, distilled water, Brain Heart Infusion (BHI) medium, 1% crystal violet, and tetracycline as a positive control. The test microorganism used was *Staphylococcus aureus* MRSA ATCC 33591, obtained from the culture stock of the Microbiology Laboratory at Muhammadiyah University of East Kalimantan.

Sample Preparation and Extraction

A total of 150 g of *Etlingera balikpapanensis* rhizome powder was macerated continuously in 1500 mL of 96% ethanol for 72 h using the same solvent throughout the extraction period, with periodic stirring. No remaceration or solvent replacement was performed. After maceration, the filtrate was separated by filtration, the solvent was removed using a rotary evaporator, and the extract was further concentrated in a water bath to obtain a thick crude extract for subsequent testing.

Phytochemical Test

The ethanol extract of *Etlingera balikpapanensis* rhizome was subjected to a qualitative phytochemical test to identify the content of alkaloids, flavonoids, saponins, triterpenoids, phenolics, and tannins using specific reagents according to standard procedures (Ilmahdi *et al.*, 2023).

Antibiofilm Activity Test

Antibiofilm activity testing was performed using a 96-well microtiter plate method with crystal violet staining (Henra *et al.*, 2025; Kirmusaoglu, 2019). The extracts were tested at concentrations of 10, 20, and 30 mg/mL with incubation times of 24, 48, and 72 h at 37°C. Tetracycline was used as a positive control, while ethanol was used as a negative control. After incubation, the formed biofilm was stained with 1% crystal violet, rinsed, and then redissolved using 96% ethanol. The optical density (OD) value was measured at a wavelength of 595 nm using a microplate reader.

Data Analysis

The percentage of biofilm formation inhibition was calculated based on the comparison of OD values between the treatment and negative control. The concentration capable of inhibiting $\geq 50\%$ biofilm formation was defined as the Minimum Biofilm Inhibitory Concentration (MBIC₅₀) (Hamzah *et al.*, 2021). Data were presented descriptively to illustrate the effectiveness of the extract's antibiofilm activity at each concentration and incubation time.

RESULTS AND DISCUSSION

Plant Identification Results

The plant material used in this study was Balikpapan ginger rhizome (*Etlingera balikpapanensis*). Plant identification was carried out to ensure the suitability of the species identity with the research object. The identification process was carried out at the Laboratory of Ecology and Tropical Forest Biodiversity Conservation, Faculty of Forestry, Mulawarman University with Number 125/UN17.4.08/LL/2025. The identification results showed that the plant samples used were

identified as *Etlingera balikpapanensis* from the Zingiberaceae family.

Extraction Yield of *Etlingera Balikpapanensi* Rhizome

Extraction was initiated using 150 g of powdered Balikpapan ginger rhizome (Figure 1, Table 1). The rhizome powder was macerated continuously in 1500 mL of 96% ethanol for 72 h using the same solvent throughout the extraction period, with occasional stirring. After completion of maceration, the mixture was filtered using filter paper to obtain the filtrate. The filtrate is then evaporated using a rotary evaporator and concentrated over a water bath to obtain a thick extract from Balikpapan ginger rhizome. This step aims to ensure that the active compounds are optimally extracted and obtained in the form of a concentrated extract that is ready for testing. The main advantage of the maceration method is its ability to extract compounds effectively without using high temperatures or pressure, thereby maintaining the stability and integrity of the active compounds. In addition, this method is able to extract natural components more thoroughly, thereby increasing the efficiency of the extraction process (Rahman *et al.*, 2019).



Figure 1. Crude ethanolic extract of *Etlingera balikpapanensis* rhizome obtained after maceration and solvent evaporation, showing the thick dark-brown extract used to prepare the 10, 20, and 30 mg/mL test solutions.

In the extraction process, 96% ethanol solvent was used because it has good solubility and antimicrobial properties, thereby reducing the risk of contamination (Chairunnisa *et al.*, 2019). Based on the data in the table, the percentage yield of

Balikpapan ginger rhizome extract obtained was 53.18%, and this value meets the extract yield criteria because it is not less than 10% (Ilmahdi *et al.*, 2023) (Senduk, 2020).

Table 1. Extraction yield of the ethanolic extract of *Etlingera balikpapanensis* rhizome obtained by maceration using 96% ethanol.

Sample Weight (g)	Solvent	Extract Weight (g)	Yield (%)	Requirement
150	Etanol 96%	79,77	53,18	>10% (Senduk, 2020)

The working principle of the maceration method is to soak the sample in a suitable organic solvent at room temperature. The solvent will penetrate the cell wall and enter the cell cavity containing the active compound. Several studies have shown that the concentration of the solvent and the duration of maceration are interrelated (Satria *et al.*, 2022). The longer the maceration duration, the greater the amount of extract obtained. The effectiveness of the extraction process greatly affects the yield produced. In addition, factors such as temperature, extraction time, type of solvent, stirring, sample particle size, and the strength of the interaction between the sample and the solvent also determine the extraction results (Febrina *et al.*, 2015). The surface area of the sample also affects the yield because the larger the contact surface, the stronger the interaction between the sample and the solvent (Sineke, 2016).

Phytochemical Composition of the Extract

The purpose of phytochemical testing is to identify the classes of secondary metabolites present in crude drugs and extracts. Secondary metabolites are chemical compounds produced by plants with different types and compositions between species. These compounds are produced as a defense mechanism for plants against disturbances from other organisms and environmental factors (Li Yanqun *et al.*, 2020). The test results are presented in Table 2.

The rhizome extract of *Etlingera balikpapanensis* in this study contains alkaloids,

flavonoids, saponins, triterpenoids, phenolics, and tannins, which have different but complementary antibacterial mechanisms. The presence of alkaloids was confirmed by the formation of white (Mayer), yellow (Wagner), and reddish-brown (Dragendrof) precipitates according to the literature (Ilmahdi *et al.*, 2023), with a mechanism of inhibiting the formation of bacterial cell wall peptidoglycan (Sebastian & Widyarman, 2021). Flavonoids were detected through the formation of a yellow color on the amyl alcohol layer and play a role in inhibiting nucleic acid synthesis, damaging cell membranes, and disrupting bacterial energy metabolism (Nomer *et al.*, 2019). Saponins are characterized by the formation of stable foam and work by increasing cell membrane permeability, causing leakage of cell components (Sapara, 2016). The Liebermann-Burchard test shows a red color change indicating triterpenoids, which can damage the outer membrane porins of bacteria and disrupt the transport of essential substances (Rini & Supriatno, 2017). Phenolics and tannins are indicated by a brownish-green color change after the addition of FeCl₃ (Ilmahdi *et al.*, 2023); phenolics inhibit nucleic acid biosynthesis and energy metabolism (Widyarman *et al.*, 2018), while tannins inhibit cell wall synthesis and inactivate bacterial enzymes (Sapara, 2016).

Overall, the combination of these various secondary metabolites has the potential to provide optimal antibacterial activity.

Table 2. Phytochemical screening results of the ethanolic extract of *Etlingera balikpapanensis* rhizome showing the presence of major secondary metabolites related to antibiofilm activity.

No.	Phytochemical Compound	Reagents	Result	Description	Criteria (Ilmahdi <i>et al.</i> , 2023)
1	Alkaloid	Mayer + HCl 2N	There is a white deposit	(+)	White sediment
		Wagner + HCl 2N	There is yellow sediment	(+)	Brown/yellow deposits
		Dragendrof + HCl 2N	There is a brownish-red deposit	(+)	Orange/brownish red deposits
2	Flavanoid	Concentrated MgHCl powder & amyl alcohol	Yellow color formed on the amyl alcohol layer	(+)	Yellow, orange, or red color forms on the amyl alcohol layer
3	Saponin	Hot water & concentrated HCl	Foam formation	(+)	Foam formation
4	Steroid dan Terpenoid	Lieberman-Burchad	Formed red color	(+)	Forms blue or purple color (Steroid), brownish red color (Triterpenoid)
5	Fenol	Fecl 5%	Brownish green	(+)	Brownish green/blackish
6	Tanin	Fecl 1%	Brownish green	(+)	Brownish green/blackish

Result of MRSA Bacterial Identification

Gram staining is the primary technique for classifying bacteria into two categories: Gram-positive bacteria, which appear purple, and Gram-negative bacteria, which appear red after staining (NauE *et al.*, 2022). This method is most commonly used in bacterial characterization because it facilitates microscopic observation, clarifies cell shape and size, and helps visualize cell structures such as bacterial walls, while also revealing the physicochemical properties of bacteria through the use of dyes (Virgianti,

2017). Microscopic morphological observations show that *Methicillin-Resistant Staphylococcus aureus* is purple in color, cocci in shape, and clustered together, which are characteristics of Gram-positive bacteria (Figure 2). The Gram staining technique can distinguish bacteria based on their cell wall structure. Gram-positive bacteria such as *Methicillin-Resistant Staphylococcus aureus* have a thick cell wall layer that allows them to retain the purple color. This cell wall characteristic is important because it affects the bacteria's response to antibacterial agents and biofilm-inhibiting compounds (Sari *et al.*, 2019).

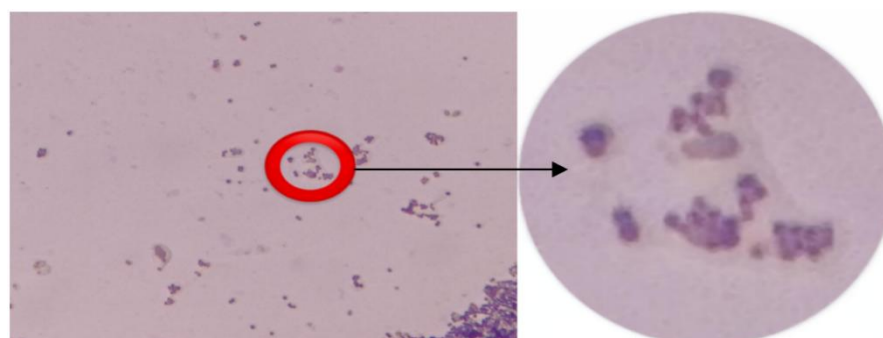


Figure 2. Results of MRSA Bacterial Identification by Gram Staining at 1000× magnification.

Antibiofilm Activity Againsts MRSA (24 Hours)

The antibiofilm activity test of the ethanol extract of Balikpapan ginger rhizome against *Methicillin-Resistant Staphylococcus aureus* at a 24 h incubation period demonstrated its ability to inhibit biofilm formation. The highest inhibitory activity was observed at an extract concentration of 30 mg/mL, with an inhibition percentage of 71.26% w/v. The overall results are presented in Table 3.

The results of the biofilm inhibition assay of Methicillin-Resistant *Staphylococcus aureus* (MRSA) at the mid-phase of biofilm formation (24 h) demonstrated that the ethanol extract of Balikpapan ginger rhizome exhibited significant antibiofilm activity. Based on optical density (OD) measurements using the microtiter plate method with crystal violet staining, all extract concentrations (10, 20, and 30 mg/mL) reduced biofilm formation compared to the negative control containing ethanol only. The reduction in

OD values increased with rising extract concentrations, indicating a concentration-dependent inhibition of MRSA biofilm formation. The inhibition percentages were 63.83% at 10 mg/mL, 68.94% at 20 mg/mL, and the highest at 30 mg/mL concentration (71.26%), which was close to the positive control showing 73.13% inhibition (Table 3). Tetracycline was used as the positive control in this study. This antibiotic is known to inhibit biofilm formation and disrupt quorum sensing during biofilm development (Deryabin & Inchagova, 2018; Dewi *et al.*, 2020). Quorum sensing is a bacterial communication mechanism mediated by autoinducer molecules, which regulate biofilm formation based on cell density. The inhibition results showed that tetracycline (73.13%) exhibited an effectiveness nearly comparable to the Balikpapan ginger extract at 30 mg/mL concentration (71.26%). These findings indicate that the ethanol extract of Balikpapan ginger rhizome has strong potential as an antibiofilm agent against MRSA, consistent with previous findings reported by Dewi *et al.* (2020).

Table 3. Percentage inhibition of MRSA biofilm formation after 24 h of incubation with different concentrations of *E. balikpapanensis* rhizome extract.

Treatment Concentration (mg/mL)	OD Value	Mean OD	OD Percentage (%)
Balikpapan Ginger Extract 10 mg/mL	0,1235	0,1617	63,82%
	0,2065		
	0,1551		
Balikpapan Ginger Extract 20 mg/mL	0,1407	0,1388	68,94%
	0,1543		
	0,1215		
Balikpapan Ginger Extract 30 mg/mL	0,14	0,1284	71,26%
	0,1121		
	0,1332		
Positive control	0,1144	0,1201	73,13%
	0,121		
	0,1249		

According to Asnani *et al.* (2022), antibiofilm activity is considered significant when biofilm inhibition exceeds the MBIC₅₀ cut-off ($\geq 50\%$). In this study, all concentrations of Balikpapan ginger extract surpassed the MBIC₅₀ value, indicating strong antibiofilm activity. At 24 h, MRSA biofilm formation is in the early adhesion phase. Inhibition at this stage occurs mainly

through surface modification by the ethanol extract of Balikpapan ginger rhizome, which creates unfavorable conditions for bacterial attachment. Active compounds such as tannins and flavonoids are known to bind bacterial adhesin proteins, while alkaloids and flavonoids may inhibit cell wall protein synthesis, thereby reducing irreversible bacterial adhesion and

early biofilm formation (Kining *et al.*, 2017). Biofilm formation contributes significantly to MRSA antibiotic resistance by limiting antibiotic penetration. Therefore, early-stage biofilm inhibition, as observed in this study, represents an important alternative strategy for MRSA infection control. Overall, the 24 h

biofilm assay demonstrated that the ethanol extract of Balikpapan ginger rhizome exhibits significant antibiofilm activity against MRSA. The 30 mg/mL concentration was the most effective, producing the highest inhibition and exceeding the MBIC₅₀ threshold, suggesting its potential as a natural antibiofilm agent.

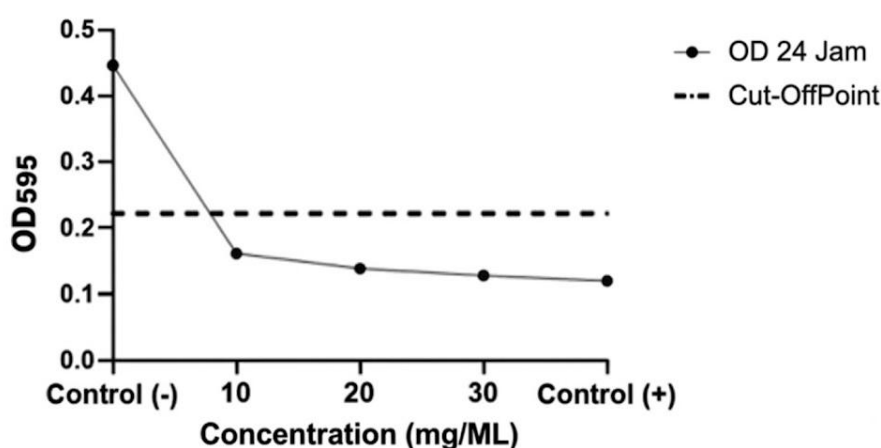


Figure 3. OD595 graph of MRSA biofilm at various treatment concentrations (24 h)

Antibiofilm Activity Against MRSA at 48 Hours

Based on the results of the MRSA (*Methicillin-Resistant Staphylococcus aureus*) biofilm assay at 48 h of incubation, the ethanol extract of Balikpapan ginger rhizome continued

to exhibit biofilm inhibitory activity at all tested concentrations. This was indicated by the treatment showing the highest % OD at a concentration of 30 mg/mL, with a value of 67.39% w/v, reflecting the level of biofilm formation. The overall results are presented in Table 4.

Table 4. Percentage inhibition of MRSA biofilm formation after 48 h of incubation with different concentrations of *Etlingera balikapensis* rhizome extract.

Treatment Concentration (mg/mL)	OD Value	Mean OD	OD Percentage (%)
Balikpapan Ginger Extract 10 mg/mL	0,2344	0,2094	58,30%
	0,2434		
	0,1505		
Balikpapan Ginger Extract 20 mg/mL	0,1848	0,1834	63,48%
	0,2165		
	0,149		
Balikpapan Ginger Extract 30 mg/mL	0,2097	0,1638	67,39%
	0,1299		
	0,1518		
Positive control	0,2078	0,1547	69,20%
	0,1399		
	0,1163		

At a concentration of 10 mg/mL, Balikpapan ginger extract produced a mean OD value of

0.2094 with a biofilm inhibition percentage of 58.31%. The 20 mg/mL concentration showed a

mean OD of 0.1834 with 63.48% inhibition, while the 30 mg/mL concentration resulted in the lowest mean OD value of 0.1638 and the highest inhibition percentage of 67.39%. These values were close to the positive control, which had a mean OD of 0.1547 and an inhibition percentage of 69.21% (Table 4, Figure 4). The decrease in OD₅₉₅ values with increasing extract concentration indicates a dose response relationship, where higher extract concentrations lead to greater suppression of MRSA biofilm formation.

The assessment of antibiofilm activity used a 50% inhibition threshold relative to the negative control. At 48 h, all tested concentrations of Balikpapan ginger extract produced >50% inhibition. Because 10 mg/mL was the lowest concentration tested and already exceeded 50% inhibition, the data indicate an MBIC₅₀ of ≤10 mg/mL rather than establishing 10 mg/mL as the exact MBIC₅₀. The lower OD₅₉₅ values at 10–30 mg/mL compared with the negative control indicate reduced biofilm biomass (Figure 4). The modest reduction in inhibition from 24 to 48 h is

consistent with progressive biofilm maturation, during which the extracellular matrix and physiological heterogeneity can reduce antimicrobial susceptibility (Peng et al., 2023). In *Staphylococcus aureus*, quorum sensing is primarily mediated by the accessory gene regulator (agr) system, in which an autoinducing peptide (AIP) activates the AgrC–AgrA two-component pathway and regulates RNAIII and multiple virulence-associated genes; this differs from the AHL/LasR/RhlR system characteristic of Gram-negative organisms such as *Pseudomonas aeruginosa* (Williams et al., 2023). Therefore, the antibiofilm effect observed in this study should be interpreted cautiously as being consistent with interference in adhesion, matrix accumulation, bacterial growth, and/or regulatory pathways. Direct anti-quorum-sensing activity was not measured in this experiment and requires specific mechanistic assays. Overall, the 48 h results show that the extract continued to suppress MRSA ATCC 33591 biofilm biomass in a concentration-dependent manner.

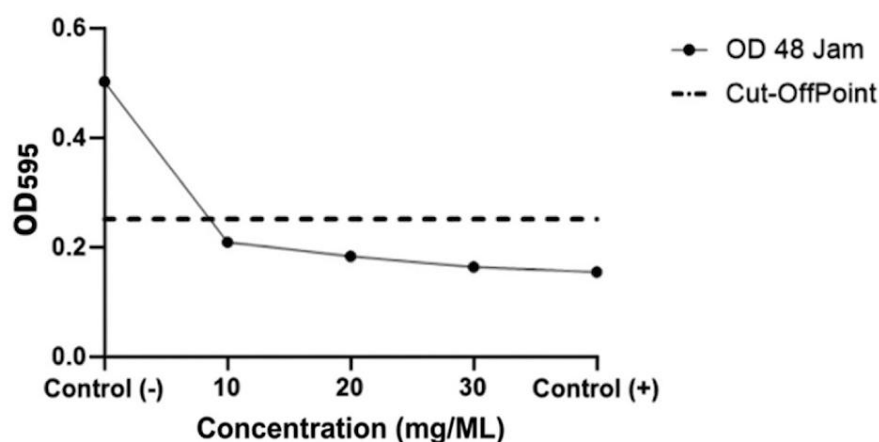


Figure 4. OD₅₉₅ graph of MRSA biofilm at various treatment concentrations (48 h)

Antibiofilm Activity Againsts MRSA (72 Hours)

Based on the results of the 72 h incubation biofilm inhibition assay against *Methicillin-Resistant Staphylococcus aureus* (MRSA), the ethanol extract of Balikpapan ginger rhizome continued to exhibit antibiofilm activity at all tested concentrations. The highest inhibition percentage was observed at a concentration of 30 mg/mL, reaching 63.99% w/v. The overall results are presented in Table 5.

At a concentration of 10 mg/mL, the extract produced an inhibition percentage of 55.28%, a concentration of 20 mg/mL produced 60.02%, and a concentration of 30 mg/mL produced the highest inhibition of 63.99%. This value is close to the positive control, which had an inhibition percentage of 66.32%. This pattern shows a consistent dose-response relationship, where the higher the extract concentration, the greater the ability to inhibit MRSA biofilm formation. The negative control (ethanol) showed the highest OD value and 0% inhibition percentage at all

incubation times, including 72 h, confirming that without active compounds, MRSA is able to form biofilm maximally. The positive control showed the highest inhibition at each time point, but also experienced a decrease from 73.13% (24 h) to 69.21% (48 h) and 66.32% (72 h). This

pattern confirms that the decrease in effectiveness at 72 h did not only occur in ginger extract but also in standard antibiofilm agents, caused by the maturation and increased resistance of the biofilm structure.

Table 5. Percentage inhibition of MRSA biofilm formation after 72 h of incubation with different concentrations of *Etlingera balikpapanensis* rhizome extract.

Treatment Concentration (mg/mL)	OD Value	Mean OD	OD Percentage (%)
Balikpapan Ginger Extract 10 mg/mL	0,3642	0,2277	55,27%
	0,1602		
	0,1587		
Balikpapan Ginger Extract 20 mg/mL	0,3651	0,2036	60,01%
	0,1056		
	0,14		
Balikpapan Ginger Extract 30 mg/mL	0,3205	0,1833	63,99%
	0,1198		
	0,1096		
Positive control	0,1608	0,1715	66,31%
	0,2059		
	0,1478		

Based on the MBIC₅₀ antibiofilm cut-off point according to Asnani *et al.* (2022), an extract is considered to have antibiofilm activity if it is able to inhibit biofilm formation by $\geq 50\%$ compared to the negative control. The results of the study show that all concentrations of Balikpapan ginger extract at 72 h of incubation still exceeded the MBIC₅₀ limit, so it can still be classified as an active antibiofilm agent against MRSA even though the biofilm was in an advanced stage. However, when compared to the 24 h and 48 h incubation times, there was a downward trend in the percentage of inhibition as the incubation time increased, with the highest value at a concentration of 30 mg/mL decreasing from 71.27% (24 h) to 67.39% (48 h) and 63.99% (72 h).

At 72 h of incubation, the biofilm can be considered a later-stage or mature biofilm under many in vitro conditions, although the timing of maturation varies with strain, medium, surface, and experimental model. Mature *S. aureus* biofilms typically exhibit increased structural organization and a more developed extracellular matrix, features that can reduce susceptibility to antimicrobial agents (Peng *et al.*, 2023). In *S. aureus*, quorum sensing is mediated mainly by

the agr system through autoinducing peptides (AIPs) and the AgrC–AgrA signaling pathway, rather than by AHL-dependent LasR/RhlR signaling (Williams *et al.*, 2023). Biofilm inhibition was still observed at 72 h, indicating that the ethanolic extract of *Etlingera balikpapanensis* rhizome remained active against the established biofilm biomass of MRSA ATCC 33591. However, this study did not directly measure agr signaling, AIP production, gene expression, EPS composition, or penetration of individual phytochemicals. Consequently, the observed effect should not be attributed specifically to anti-quorum-sensing activity without additional mechanistic evidence. The lower inhibition at 72 h compared with 24 h is more conservatively interpreted as being associated with the greater complexity and tolerance of older biofilms.

Thus, the decrease in the percentage of biofilm inhibition at 72 h of incubation was not due to a reduction in the potential of the extract, but rather to the increasing complexity of the biofilm structure and the physiological adaptation of bacterial cells over the incubation period (Asnani *et al.*, 2022). The diversity of secondary metabolites in Balikpapan ginger

rhizome extract provides a synergistic antibiofilm effect, which is most effective in the early phase of biofilm formation and still plays a role in the early maturation phase until the biofilm matures. Therefore, Balikpapan ginger

rhizome ethanol extract has the potential as an agent to prevent and inhibit MRSA biofilm, especially when applied in the early stages of biofilm development.

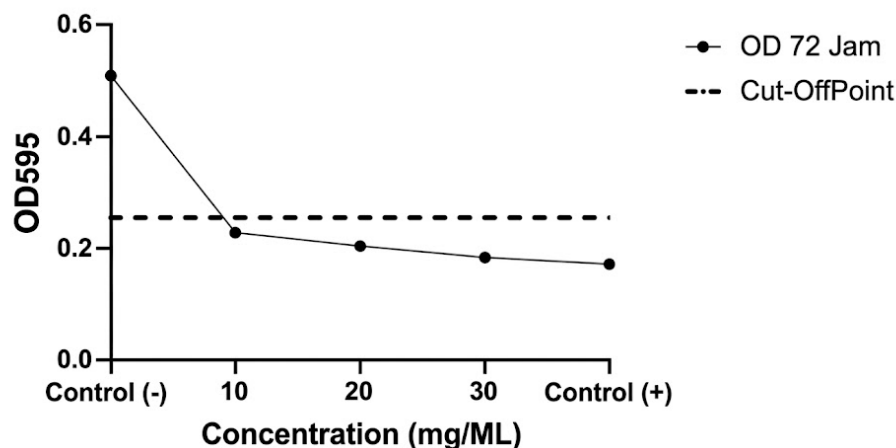


Figure 5. OD595 graph of MRSA biofilm at various treatment concentrations (72 h).

CONCLUSIONS

The ethanol extract of *Etlingera balikapapensis* rhizome showed antibiofilm activity against *Methicillin-Resistant Staphylococcus aureus* ATCC 33591 in vitro. All tested extract concentrations (10, 20, and 30 mg/mL) inhibited biofilm formation by $\geq 50\%$, and 30 mg/mL produced the highest inhibition at 24, 48, and 72 h. Because 10 mg/mL was the lowest concentration tested and already exceeded 50% inhibition, the MBIC₅₀ under these conditions is ≤ 10 mg/mL; additional concentrations below 10 mg/mL are required to determine the exact MBIC₅₀. These findings support the potential of the ethanolic extract as a source of antibiofilm compounds against the tested MRSA strain, but further studies using additional clinical MRSA isolates, cytotoxicity testing, and mechanistic assays are required before broader conclusions or therapeutic applications can be proposed.

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