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Effects of Red Galangal Rhizome Extract (*Alpinia purpurata*) as an Inhibitor of *Staphylococcus aureus* Bacterial Biofilm

Sri Winarsih[1], Ardian Rizal[2], Estyka Ratu[3*]

[1]Lecturer, Department of Microbiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[2]Lecturer, Department of Cardiology, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia
[3]Student, Medical Education Study Program, Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia

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***Corresponding author:**
Estyka Ratu

E-mail address:
estyka.ratu@gmail.com

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A B S T R A C T

Staphylococcus aureus is a type of bacteria that causes infection through the virulence mechanism of biofilm formation, namely forming a layer by removing the matrix as a form of defense from the immune system and from antibacterial agents. This bacterium makes it very easy to form biofilms, thereby reducing the effectiveness of therapy with antibiotics. Red galangal is a spice plant that grows a lot in Indonesia. It is known to contain flavonoids, tannins, and terpenoids, which are thought to have activity in inhibiting biofilm formation. This study aimed to determine the effect of red galangal rhizome extract in inhibiting the formation of biofilms of *Staphylococcus aureus* bacteria. The method used is the test tube method using crystal violet dye. The results of the tube test method were photographed and quantified into the mean gray value (MGV) found in the Adobe Photoshop CS6 application. The thicker the biofilm is indicated by the lower the MGV value. Red galangal extract was obtained by maceration using 96% ethanol solvent, while the extract concentrations for treatment were 50%, 25%, 12.5%, 6.25%, 3.125%, 1.56%, and 0%. The results of statistical analysis showed that the higher the concentration of the extract, the thinner the biofilm ring formed (Pearson correlation, r = -0.980, p = 0.000). In conclusion, the ethanol extract of red galangal rhizome has the potential to inhibit *Staphylococcus aureus* biofilm formation with minimal biofilm inhibition at a concentration of 12.5%.

1. Introduction

Staphylococcus aureus is a bacterium that is pathogenic to the skin and mucous membranes of the human body. *Staphylococcus aureus* is a gram-positive bacterium of the *Staphylococcaceae* family that has the ability to multiply and spread widely in body tissues and produce several extracellular substances that cause several diseases such as bacteremia, pneumonia, endocarditis, osteoarticular, hematogenous acute osteomyelitis, infections of the skin and soft tissue, meningitis, lung infections, and infections related to equipment medical. *Staphylococcus aureus* has been resistant to several antibiotics, namely Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-intermediate *Staphylococcus aureus* (VISA), and Vancomycin-resistant *Staphylococcus aureus* (VRSA).[1-3]

The existence of this resistance is caused by the ability of the bacteria *Staphylococcus aureus to* easily form biofilms. In research by Abolella et al. (2009), urine and blood isolates have different strengths *S. aureus* in forming biofilms, in the 18th blood isolate from 32 samples (56.2%), *S. aureus* produced biofilms, whereas in the 15th urine isolate out of 18 samples (83.3%) *S. aureus* produce biofilms. Biofilm is a collection of microorganisms attached to each other on a surface and covered with an exopolysaccharides matrix (EPS) produced by the bacteria themselves. Biofilm formation begins with the attachment of

bacteria to a solid surface with protein adhesin, followed by maturation and dispersion or release phases. In the maturation phase, bacteria begin to form an extra polysaccharide matrix (EPS). Biofilms can spread to other body parts or surfaces in the dispersion phase. Furthermore, biofilm formation is mediated by quorum sensing, which is the role of quorum sensing as a mechanism of communication between cells and bacteria. In addition, the formation of biofilms plays a role in the occurrence of microbial infections in the human body. This is a major problem and is difficult to treat effectively by the host's immune system, and is resistant to treatment with antimicrobials. This protective mechanism is considered to inhibit the absorption and penetration of antibiotics through the biofilm matrix.[4-7]

One of the herbal plants that can inhibit bacterial biofilms, *Staphylococcus aureus,* is red galangal (*Alpinia purpurata),* which is a member of the genus *Alpine*. Genus *Alpine* contains active compounds in the rhizome, buds, leaves, flowers, fruits, and seeds. This red galangal rhizome contains a class of compounds, namely flavonoids, terpenoids, and soil. The group of compounds in red galangal rhizome are flavonoids, terpenoids, and soil, and there is ethanol extraction in it. Flavonoids play a role in anti-biofilm by inhibiting quorum sensing (QS), called the cell communication system between bacteria in biofilm formation. Terpenoids also have the role of changing cell permeability as penetration into the lipid bilayer membrane, thereby disrupting the fluidity of the biofilm surface and reducing the ability to form adherence and adhesion of bacteria.[8-10] This study aimed to determine the effect of red galangal rhizome extract (*Alpinia purpurata)* as an inhibitor of *Staphylococcus aureus* bacterial biofilm.

2. Methods

The research design is true experimental, using the tube method to determine the effect of the concentration of red galangal rhizome extract on the inhibition of biofilm formation of *Staphylococcus aureus*. The method used is the tube test method. The sample of this research is a sample of red galangal rhizome extract (*Alpinia purpurata*), and the bacteria used were *Staphylococcus aureus* obtained from the laboratory of microbiology, Faculty of Medicine, Universitas Brawijaya. This study used 8 concentrations, namely 0% (control), 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50% and 50% + medium. The dependent variable in this study was the ring thickness of the biofilm formation *Staphylococcus aureus* on the tube wall as measured from the value mean gray value using Adobe Photoshop CS6.

The research procedure was started by preparing the ethanol extract of red galangal rhizome (*Alpinia purpurata)* by using the maceration and evaporation methods carried out at the pharmacology laboratory, Faculty of Medicine, Universitas Brawijaya. Then do the bacterial preparation of *Staphylococcus aureus* by carrying out a bacterial identification test using gram staining, catalase test, coagulase test, bacterial culture with NAP (nutrient agar plate), and MSA culture (Mannitol salt agar). Followed by the manufacture of a bacterial liquid seed of 10^8 CFU/mL. This study used concentrations of ethanol extract of red galangal rhizome of 0% (control), 1.56%, 3.125%, 6.25%, 12.5%, 25%, 50%, and 50% + medium, then repeated four repetitions. time. The biofilm ringed tube, which is the result of the tube method, is then photographed using a digital camera and quantified into a value mean gray value contained in the application Adobe Photoshop CS6. Then, the results were analyzed using the SPSS 20.0 statistical analysis application for Windows.

3. Results and Discussion

Red galangal rhizome extract (*Alpinia purpurata)* has a thick consistency, yellowish brown color, and sour smell.

Figure 1. The final result of red galangal rhizome extract (*Alpinia purpurata*) on the bottle.

The results of the identification test obtained bacteria with gram-positive cocci with clustered colonies like purple grapes, with NAP culture results (nutrient agar plate) in the form of golden yellow bacterial colony growth, then MSA culture results (Mannitol salt agar) showed the formation of a bright yellow area (halo) caused by the ability of bacteria *Staphylococcus aureus* in fermenting mannitol which is characterized by the formation of an acidic area (yellow), and the catalase test shows the formation of positive bubbles and the coagulase test shows the occurrence of clumping within 10 seconds, which proves that this bacterium is a *Staphylococcus aureus*.

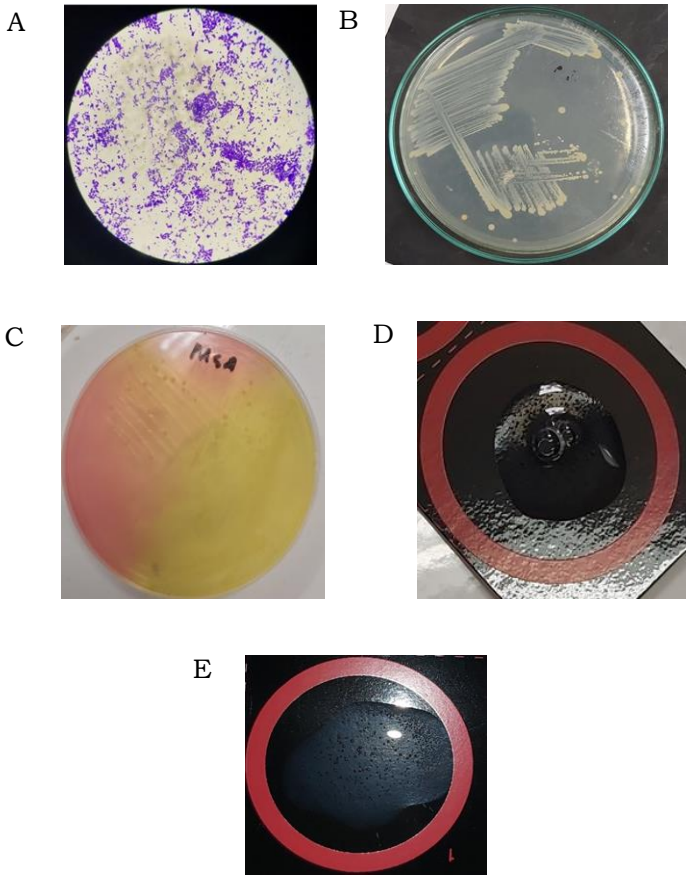

Figure 2. Bacterial identification test results: (A) Gram staining showed gram-positive cocci, (B) NAP culture (nutrient agar plate) obtained growth of golden yellow bacterial colonies, (C) MSA culture (Mannitol salt agar) indicates the formation of a bright yellow area (halo) caused by the ability of bacteria *Staphylococcus aureus* in fermenting mannitol which is characterized by the formation of an acid area (yellow), (D) the catalase test shows the formation of positive bubbles, (E) the coagulase test the occurrence of clumping within 10 seconds, which proves that this bacterium is a *Staphylococcus aureus*.

The results of the tube method are quantified with the mean gray value contained in the application Adobe Photoshop CS6. Then, the data is processed into a graph showing that each increase in extract concentration results in an increase mean gray value. This means that with each increase in the concentration of the extract, the thickness of the biofilm ring that is formed gets thinner.

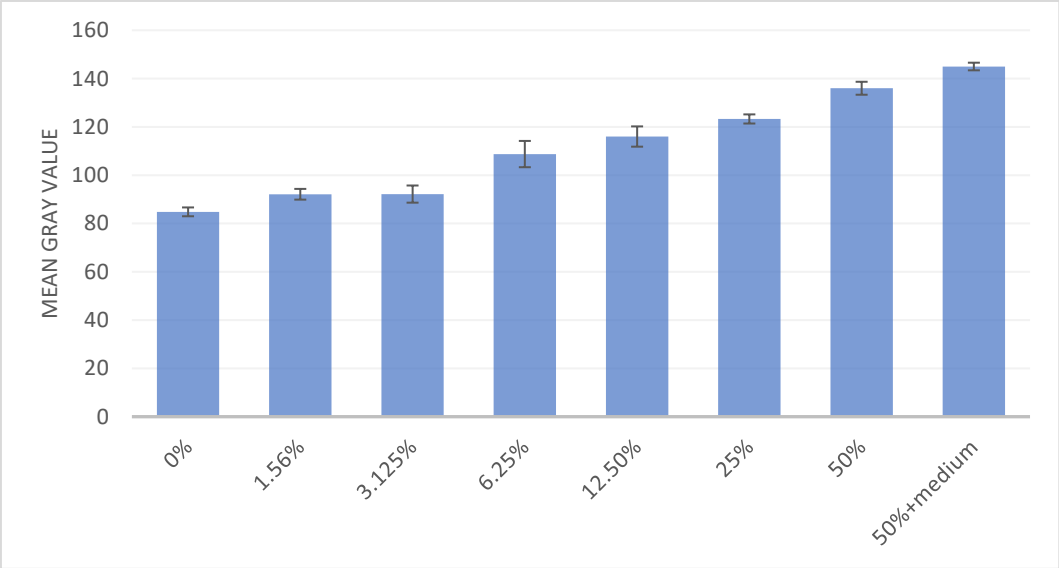

Figure 3. Comparison graph between the concentrations of red galangal rhizome ethanol extract and mean gray value obtained.

In this study, it was obtained that the minimum biofilm inhibitory content (MIC) was present at a concentration of 12.5%, which was determined by finding the value mean gray value in the treatment group that is not smaller than 10% of the value Mean Gray Value empty tube. In this study, it was found that 10% of the mean gray value empty tube at a concentration of 12.5% is worth 79.38. So that the minimum biofilm inhibition level in this study was obtained at a concentration of 12.5%. Observational data at concentrations of 0% to 50% show an average increase in mean gray value, which is directly proportional to the increase in the concentration of red galangal rhizome extract. So, the higher the concentration of red galangal rhizome extract used, the higher the mean gray value, which indicates the lower the color intensity, the stronger the inhibition of biofilm formation that occurs. This shows that the red galangal rhizome extract (*Alpinia purpurata*), which contains flavonoids, tannins, and terpenoids, can inhibit the formation of biofilms from bacteria *Staphylococcus aureus*. The ability of red galangal rhizome extract (*Alpinia purpurata*) allegedly due to the presence of compounds it has. Tannins, which are known to be contained in red galangal rhizomes, play a role in inhibiting bacterial adhesion and adherence. This condition will inhibit the formation of the exopolysaccharide matrix. In line with tannins, terpenoid compounds in the form of 1.8-cineole, chavicol, β-caryophyllene, and α-cellular on red galangal rhizome can interfere with bacterial adherence, which is important in maintaining the existence of biofilms. The active substances of flavonoids play a role in inhibiting the formation of biofilms through inhibition quorum sensing, a form of communication between bacterial cells in facilitating the formation of biofilms.[11-14]

From the measurement results, the mean gray value is then statistical data processing using IBM SPSS version 20 for Windows. The statistical test used

is a parametric test because the data is normally distributed, and the variance of the data is the same (homogeneous), namely the Oneway ANOVA test and Pearson correlation. Obtained a significance value of p = 0.000 (p <0.005). This explains that there is a significant difference in effect between each concentration of red galangal rhizome extract. Next, for the results of the Pearson correlation test, the value of r = -0.980 was obtained, which proved that there was a strong correlation between the increase in the concentration of red galangal rhizome extract and the value mean gray value. These results indicate a negative correlation or opposite and are very strong. Other studies have shown that red galangal rhizome extract has a very strong inhibitory effect on the growth of *Staphylococcus aureus* with concentrations of 20%, 30%, and 40%. It is possible that red galangal rhizome extract has the effect of inhibiting biofilms on gram-positive bacteria. In another study, red galangal rhizome extract had moderate inhibition at concentrations of 20% and 40% and strong inhibition at 80% concentration of bacterial growth. *Pseudomonas aeruginosa*. It is possible that red galangal rhizome extract is also able to exert a biofilm inhibitory effect on other gram-negative bacteria. Other research shows that the extract *Alpinia galanga* is capable of inhibiting biofilm formation of *Staphylococcus aureus* at a minimum concentration of 15%. Meanwhile, in a similar study on biofilm inhibition, *Staphylococcus aureus* used ethanol extract of basil leaves. The results showed that the higher the concentration of basil leaf extract, the greater the inhibition of biofilm formation in *Staphylococcus aureus* in vitro, with minimal biofilm inhibition at a concentration of 30%. Minimum concentration differences are possible because there are differences in compound content and the extraction process of the materials used.[15-20]

4. Conclusion

There is a linear relationship between the concentration of red galangal rhizome extract (*Alpinia purpurata*) on biofilm formation S*taphylococcus aureus*, where the higher the concentration given, the higher the value mean gray value. Red galangal rhizome extract (*Alpinia purpurata*) has a biofilm-inhibiting effect on *Staphylococcus aureus* with minimum biofilm inhibitory concentration (MBIC), namely at a concentration of 12.5%.

5. References

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