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Antimicrobial Activity of Ethanol Extract of *Abrus precatorius* L. Roots against Planktonic Cells and Biofilm of Urine and Blood Methicillin Sensitive *Staphylococcus aureus* (MSSA) Isolate

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Abstract. The purpose of this study was to compare the antibiofilm activity of ethanol extract of *Abrus precatorius* L. roots at various concentrations of the Indonesian isolate of *Staphylococcus aureus* biofilm. The method used included inhibition test of planktonic bacteria and inhibition test of bacterial biofilm. The variable measured is the optical density (OD) of biofilm formation tested by ELISA reader. Inhibition test of biofilm formation is carried out using the microtiter plate method. The results showed that ethanol extract of *A. precatorius* L. roots had a significant differences in the inhibitory effect on biofilm formation between treatment group and positive control. The linear regression test showed that antibiofilm activity of ethanol extract of *A. precatorius* L. roots was slightly stronger on blood MSSA isolate. Therefore, the ethanol extract of *A. precatorius* L. roots have antibiofilm activity for MSSA isolate.

Keywords: Antimicrobial, *A. precatorius* ., MSSA

1. Introduction

Staphylococcus aureus is a cause of opportunistic and nosocomial infections, especially in patients with implanted medical devices [1]. *S. aureus* has been recognized as a major pathogen causing infection for more than 100 years. Infection caused by *S. aureus* often recurs, because of its ability to form biofilms. The physiological structure of *S. aureus* biofilm which has a strong bacterial cell attachment to implanted medical devices, forms endotoxin to fight the immune response of the host, does not have the immune system to eliminate bacteria that develop in biofilms, and the exchange of plasmids in biofilms that carry resistant genes This particular antimicrobial that causes this infection is difficult to cure [2].

Biofilms of *S. aureus* are often found in medical devices placed on the patient's body, such as urinary catheters and Central Venous Catheters (CVC), which often cause urinary tract infections and sepsis, with an incidence of 10–50% and 3-5%. In urine and blood isolates there were differences in the strength of *S. aureus* in forming biofilms, in the 18th blood isolates from 32 samples (56.2%) of *S. aureus* produced biofilms [3], whereas in the 15th urine isolates from 18 samples (83.3%) of *S. aureus* produces biofilms [4].

Abrus precatorius L. is a shrub and propagates endemic to Indonesia which has activity as an antibiofilm. Based on the secondary metabolites it contained, the roots of *A. precatorius* L. contained isoflavonoids and quinones-abruquinones A, B, C, D, E, F [5], and 7,5-dihydroxy-6,49-



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dimethoxyisoflavone 7-ObD- galactopyranoside [6]. Research has never been conducted on the extent of antibiofilm activity on biofilm cell growth and inhibition of *S. aureus* urine and blood isolates. The aim of this study was to determine the antibiofilm activity of the root extract of *A. precatorius* L. as an antibiofilm of *S. aureus*, and to compare the effectiveness of the root extract of *A. precatorius* L. as an antibiofilm of *S. aureus* blood and urine isolates. It is hoped that this research can provide new information on alternative ingredients for handling *S. aureus* biofilms.

2. Experimental Method

S. aureus (MSSA) bacteria was obtained from the Department of Microbiology, Faculty of Medicine, Airlangga University. The sample used in the study was *A. precatorius* L. leaves obtained from Sumenep, East Java, Indonesia. The bacterial isolates were observed for colony morphology which included shape, elevation, edge, diameter and color. Cell morphology tests were carried out using Gram staining and bacterial physiology testing using Microbact identification kits.

The root powder of *A. precatorius* L. 20 gr was extracted by maceration method for 24 hours which was carried out three times using ethanol solvent. The extract obtained was evaporated with rotary vacuum evaporator to obtain thick ethanol extract.

The implementation of *S. aureus* biofilm inhibition of urine and blood isolates was determined based on Yamanaka (2008) method [7], and OD measurements using ELISA reader. The bacterial suspension was filled in a 96 wells 150 μL flat-bottom microtiter plate and incubated at 37 $^{\circ}\text{C}$ for 24 hours to form a biofilm. 50 μL of various concentrations of *A. precatorius* L. extract were added, ie 800 mg mL^{-1} , 400 mg mL^{-1} , 200 mg mL^{-1} , 100 mg mL^{-1} , 50 mg mL^{-1} , 25 mg mL^{-1} and 0 mg mL^{-1} into each wells and incubated at 37 $^{\circ}\text{C}$ for 24 hours. All liquids in each wells are removed using a pipette so that the biofilm is attached to the wall in wells. Each biofilm-containing wells was washed 4 times with PBS pH 7 200 μL . Added cristal violet dye 0.5% 200 μL and waited for 15 minutes, then washed with 200 μL of distilled water. Wells which has been colored aerated at room temperature in a sterile state. Wells added 95% ethanol 200 μL . The 125 μL suspension was inserted in the new wells and read with an ELISA reader with $\lambda 595$ nm and obtained the OD power values for each treatment of *S. aureus* biofilm isolate urine and blood.

3. Results and Discussion

In the re-identification stage, there were rounded, yellow colonized specimens, 5-7 mm of colony diameter, coccus-shaped cells, convex elevation, slippery edges, gram-positive and clustered cells. Physiologically identified characterizations containing nitrate, glucose, mannitol, ONPG, Urease, sucrose and catalase. *S. aureus* biofilm inhibition isolates urine and blood by extracts of *A. precatorius* L. can be seen in **Figures 1** and **2**.

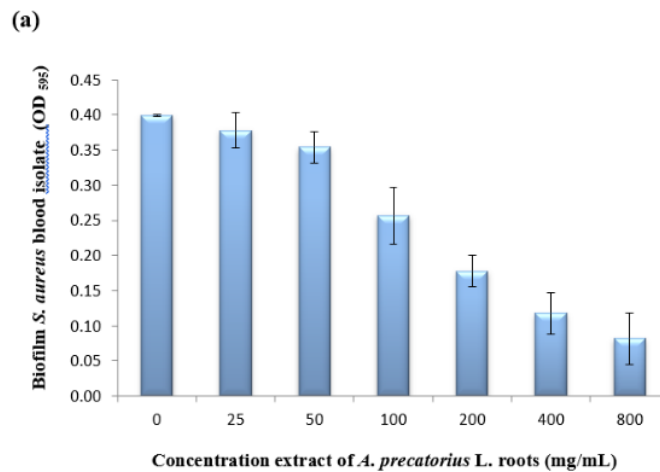


Figure 1. Biofilm inhibition of *S. aureus* blood isolates at various concentrations of *A. precatorius* L. extract.

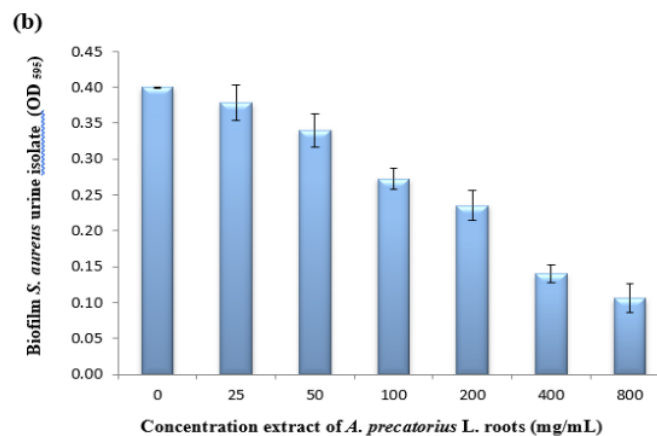


Figure 2. Biofilm inhibition of *S. aureus* urine isolates at various concentrations of *A. precatorius* L. extract.

The results of statistical analysis on both *S. aureus* urine and blood bacterial isolates obtained OD within normal and homogeneous limits for each isolate. In blood and urine isolates, biofilm formation has decreased significantly in the treatment with the lowest concentration of 25 mg/mL. In **Figure 1** and **2**, it can be seen that in a range of concentrations of *A. precatorius* L. extracts induces biofilm formation before finally inhibiting its formation. However, there was no significant change in the value of the OD and from the one way ANOVA test, the optimal concentration for *A. precatorius* L. extract to inhibit biofilm formation was obtained at a concentration of 25 mg/mL for both isolates because of independent testing. t-test to determine the effect of 25 mg/mL of *A. precatorius* L. extract on *S. aureus* biofilm formation compared with positive control of each isolate.

The results of simple linear regression test, the determination coefficient value is -957 for urine isolates and -969 for blood isolates. From these figures it can be concluded that 96% decrease in *S. aureus* isolate urine biofilm formation and 97% decrease in *S. aureus* isolates blood was influenced by the presence of 25 mg/mL of *A. precatorius* L. extract. This shows that the administration of *A. precatorius* L. extract of 25 mg/mL has a strong activity against inhibition of *S. aureus* biofilm formation both urine isolates and blood isolates, and there were no significant differences in inhibitory activity of biofilm formation from the two isolates. The slight difference in the value of simple linear regression tests in both isolates can occur due to differences in strength in forming biofilms between the two isolates. This is caused by differences in strains from *S. aureus* bacteria. The use of different strains, it is probable that these two strains have phenotypic differences related to the strength of biofilm formation. So, there is no known difference in the strength of each strain in forming biofilms. The difference in the value of a simple linear regression test can also be caused by the presence of genotypic instability. According to Nuryastuti (2010) [8], genotypic instability in *ica*-locus can cause reversible changes in phenotype, *ica*-positive phenotype can change to *ica*-negative and this switching ability is also different in different strains. This can be influenced by the culture environment and also the freshness of the culture. Extract characteristics also affect the results obtained. The *A. precatorius* L. extract used in this study has the characteristics of coarse and coarse crude extracts. The properties of this extract can affect the OD value of biofilms because the reading of OD values uses the principle of light bending by bacteria in colored biofilms by crystal violet. If there are residual extracts that bind to violet crystals that are still left on the microtiter when read in the ELISA reader, it will read as false positive.

4. Conclusions

A. precatorius L. extract has antibiofilm activity in *S. aureus* (MSSA) blood and urine isolates. There was no significant difference in the activity of *A. precatorius* L. extract as an antibiofilm for *S. aureus* urine and blood isolates. Minimal concentration of *A. precatorius* L. extract to inhibit biofilm (MBIC) of *S. aureus* urine and blood isolates was 25 mg/mL.

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