TRACING ANTIBIOFILM ACTIVITY AND BIOFILM ERADICATION OF BAJAKAH TAMPALA (SPATHOLOBUS LITTORALIS HASSK) ETHANOL EXTRACT AGAINST PSEUDOMONAS AERUGINOSA BIOFILM

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Abstract: The Bajakah tampala plant (*Spatholobus littoralis* Hassk) is one of the plants that grows in Indonesia, precisely on the island of Borneo, which has antibacterial activity but its antibiofilm activity against *Pseudomonas aeruginosa* ATCC 27853 has never been reported. The search for new antibiofilms against *P. aeruginosa* biofilms is important in preventing biofilm-associated infections. This study aimed to determine the effectiveness of Bajakah tampala (*Spatholobus littoralis* Hassk) ethanol extract in inhibiting the formation of *P. aeruginosa* biofilm. The planktonic and biofilm inhibition tests were carried out using the *microtiter broth* method. Antibiofilm activity of Bajakah tampala (*Spatholobus littoralis* Hassk) ethanol extract against *P. aeruginosa* was analyzed by calculating the *minimum biofilm inhibitory concentration* ($MBIC_{50}$) and biofilm eradication activity were analyzed by calculating the *minimum biofilm eradiaction concentration* ($MBEC_{50}$). The ethanol extract of Bajakah tampala (*Spatholobus littoralis* Hassk) 1% gave *P. aeruginosa* antibacterial activity of 83.20% ± 0.01 and mid-phase antibiofilm activity of 79.30% ± 0.01, maturation phase 76.30 % ± 0.01 and eradication activity of 70.77 % ± 0.01. *Scanning electron microscopy* results also showed that the ethanol extract of Bajakah tampala (*Spatholobus littoralis* Hassk) could damage the matrix of *extracellular polymeric substances* biofilm *P. aeruginosa*. Therefore, the ethanol extract of Bajakah tampala (*Spatholobus littoralis* Hassk) has the potential to be developed as a candidate for new antibiofilm drugs against *P. aeruginosa* biofilms.

Keywords: Bajakah Tampala, Biofilms, *Pseudomonas aeruginosa, Spatholobus littoralis* Hassk

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative bacterium that is commonly found in nosocomial infections [1]. This bacterium is commonly found in patients in the *Intensive Care Unit* (ICU) which is the cause of infection in patients who have decreased immunity. *Pseudomonas aeruginosa* is a bacterium that can adapt in an environment that is low in oxygen and nutrients, even this bacteria can grow in temperatures of 4-42°C [2]. Dharmayanti & Sukrama (2019) stated that the use of antibiotics in the treatment of *Pseudomonas aeruginosa* is also difficult due to the high level of antibiotic resistance against *Pseudomonas aeruginosa* [2]. It was recorded that as many as 51,000 *Pseudomonas aeruginosa* infections each year, more than 13% of cases of infection are *multi-drug resistant* (MDR) so that *Pseudomonas aeruginosa* has a lot of antiiotic resistance which makes this bacteria difficult to treat.

Indonesia is one of the megabiodiversity countries because it has the second largest tropical forest in the world and has more than 20,000 types of medicinal plants, but only 1,000 species have been recorded and have been used for traditional medicine, but only about 300 species [3]. People in Indonesia have a habit of using traditional medicine as another alternative to treat various diseases. Traditional medicine uses natural ingredients based on plants, these plants contain chemical compounds called secondary metabolites. Bajakah tampala plant (*Spatholobus littoralis* Hassk) is one of the plants that can be used by the people of the interior of Kalimantan as traditional medicine.

The Dayak community has always used the root of bajakah as a medicine to restore stamina when they are active in the forest, the community also uses it to treat several kinds of diseases.

Based on a qualitative preliminary test conducted by Lysa et al (2022) Bajakah tampala contains saponins, flavonoids, phenolic compounds and tannins. The content of these secondary metabolites can treat several degenerative diseases, such as diabetes, cancer, tumors and others. This can be

strengthened by using another study conducted [5] which states that the root of bajakah tampala can treat cancer. Ayuchecaria et al., (2020) stated that the extract of Bajakah tampala stems contained phenolic compounds as much as 12.33 mg GAE/g [6]. Bajakah tampala has also been shown to increase the speed of the wound healing process [7]. Hamzah's research (2022) also reported that the tampala rhizome had antibacterial and antibiofilm activity against C. albicans [8].

Until now, testing the activity of Bajakah Tampala (*Spatholobus littoralis* Hassk) against *P. aeruginosa* ATCC 27853 biofilm has not been reported. Therefore, this study aimed to test the activity of Bajakah Tampala (*Spatholobus littoralis* Hassk) against *P. aeruginosa* biofilms.

MATERIAL AND METHODS

Materials The material used in this study was Bajakah Tampala (*Spatholobus littoralis* Hassk) (Figure 1 collected from the Forests in East Kalimantan). The plant was determined at the Faculty of Forestry, Universitas Mulawarman. Other materials were standard biofilm-forming P. *aeruginosa* isolate (ATCC 27853), chloramphenicol, 1% DMSO, NaCl, McFarland standard 0.5, sterile distilled water, Brain Heart Infusion (BHI) media, phosphate buffer saline (PBS) solution, and crystals violet 1%. The instrument used in this study were Laminar Air Flow, incubator (IF2B) (Sakura, Japan), micropipette Pipetman (Gilson, France), multichannel micropipette (Socorex, Switzerland), *microplate flat-bottom polystyrene 96 well* (Iwaki, Japan), *microtiter plate reader* (Optic Ivymen System 2100-C, Spain), spectrophotometer UV Genesys 10 UV Scanning, 335903 (Thermo Scientific Spectronic, US), autoclave (Sakura, Japan), and analytical balance (AB204-5, Switzerland).

Figure 1. Bajakah Tampala (Spatholobus littoralis Hassk)

Antibacterial test

An antibacterial test was carried out using the microdilution method. The test was carried out on microtiter plate flat-bottom polystyrene 96 wells with a series of levels of test compounds: 1, 0.5, 0.25, and 0.125% w/v. The control used was chloramphenicol 1% w/v. Growth control in the form of a microbial suspension and solvent control adjusted to the solvent of the test compound. The microplate wells were inserted BHI media and bacterial suspension, then incubated at 37°C for 24 hours. Microplate absorbance reading process using a microplate reader at a wavelength of 595 nm [9].

Test of inhibition of biofilm formation mid-phase and maturation-phase using the microbroth dilution method

A *96-well flat-bottom polystyrene microtiter plate* was used to assess the effect of the test isolates on the formation of mono-species *P. aeruginosa* biofilms [10]. About 100 µL of media containing ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) with a series of concentrations was added to each well. A medium without microbial growth was used as a control medium, and a microbial suspension was used as a negative control. A microbial suspension was used as a positive control, given 1% chloramphenicol w/v. The plates were then incubated at 37°C for 24 hours to form the mid-phase biofilm and 48 hours to form the maturation phase biofilm. Next, the plate was washed using distilled water three times and dried at room temperature for five minutes to remove the remaining water. A total of 125 µL of 1% crystal violet solution was added to each well to color the formed biofilm (both dead cells and live cells, which were also components of the biofilm), then incubated at room temperature. After incubation, the microplate was washed with running water three times to remove the remaining crystal violet, and 200 µL of 96% ethanol was added to each well to dissolve the formed biofilm. The OD readings were carried out with a *microplate reader* at a wavelength of 595 nm. The OD value was then used to calculate the percent inhibition in Formula 1. The sample level that could inhibit at least 50% biofilm formation was considered *Minimal Biofilm Inhibition Concentration* ($MBIC_{50}$).

$$\frac{(OD_{negative\ control\ mean} - OD_{test\ sample\ mean})}{OD\ negative\ control\ mean} X\ 100$$

The level of the sample that can inhibit at least 50% of biofilm formation is considered as Minimal Biofilm Inhibition Concentration $MBIC_{50}$ [10][11].

***Pseudomonas aeruginosa* biofilm eradication activity from Bajakah Tampala (*Spatholobus littoralis* Hassk) Tests for biofilm eradication**

were almost similar to biofilm inhibition, but the processing time differed. The biofilm eradication test takes five days, while the biofilm inhibition takes about 1-2 days, depending on the inhibition desired. The biofilm was inoculated with a *microtiter plate*. After incubation at 37°C for 48 hours, the plates were washed with 150 µL of sterile distilled water three times to remove nonadherent cells. A total of 100 µL of media containing ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) with a series concentration was added to each well that had been washed, then reincubated at 37°C for 48 hours. Chloramphenicol at a concentration of 1% w/v were used as positive controls. After incubation, the plates were washed three times with 200 mL of sterile PBS to remove adhering cells. Biofilm eradication was quantified with 125 µL 1% crystal violet solution into each well, then incubated at room temperature for 15 minutes. After incubation, the microplate was washed with PBS, and 200 µL of 96% ethanol was added to each well to dissolve the formed [9].

***Scanning electron microscopy* (SEM) test**

Scanning electron microscopy (SEM) was performed on *P. aeruginosa* biofilm. Cells were standardized on media grown directly on cover slips and incubated at 37^0C for 24 hours for the intermediate phase. After the middle of the biofilm, carefully cover the slip with as much PBS as twice, followed by

washing with 2% paraformaldehyde, 2% glutaraldehyde, 0.15 M sodium cacodylate and prepared for SEM. The specimen is a sputter coated with a gold layer and observed below *Scanning electron microscopy* JEOL JSM-6400. Image processed using photoshop software [12].

RESULTS AND DISCUSSION

Antibacterial of Bajakah tampala against P. aeruginosa

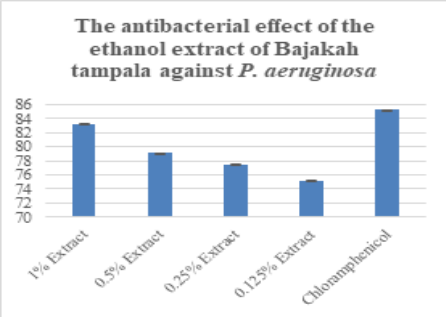

Figure 2. The antibacterial effect of the ethanol extract of Bajakah tampala against P. aeruginosa.

In these results it was reported that the ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) gave antibacterial activity of 83.20% at a concentration of 1% w/v and this activity was almost the same as the control drug chloramphenicol of 85.23% (Figure 2). These results indicated that the ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) was able to inhibit the growth of P. *aeruginosa* above 50%. The results of this study indicate that increasing the concentration can significantly increase the inhibition of bacterial growth. The results of previous studies also provide evidence that Bajakah Tampala has antibacterial activity against bacteria [13].

The activity of Bajakah tampala ethanol extract on P. aeruginosa biofilms in the mid-phase (24 hours), maturation-phase (48 hours) and eradication activity.

The results of the study in Figure 3. show that the 1% ethanol extract of Bajakah Tampala gave P. *aeruginosa* antibiofilm activity in the middle phase of 79.30%. While the control drug Chloramphenicol 1% was 82.30%. In the mid-phase of the biofilm, the activity of Bajakah Tampala ethanol extract decreased compared to its activity against antibacterial. This is because the process of inhibition as an antibiofilm and antibacterial is very different. Biofilm is a collection of microbial cells irreversibly attached to a surface and encased in a matrix of *Extracellular Polymeric Substances* (EPS) that it produces itself and shows phenotypic changes such as changes in growth rates and changes in gene transcription from planktonic cells or their free cells [14].

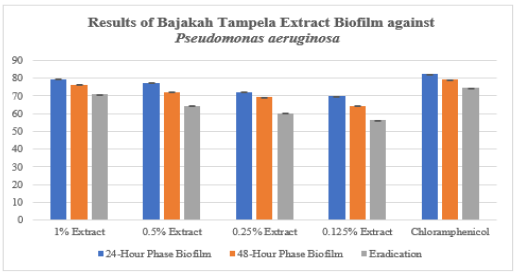

Figure 3. The antibiofilm extract of Bajakah tampala against P. aeruginosa

The results of the study in Figure 2 also show that the 1% ethanol extract of Bajakah Tampala provides antibiofilm activity of *P. aeruginosa* in the maturation phase of 76.32%. These results show evidence that the ethanol extract of Bajakah Tampala has decreased activity compared to the planktonic phase and 24-hour biofilm phase. This is because in this phase the *P. aeruginosa* biofilm EPS matrix produced is more and the biofilm structure formed is denser and more complex, this can be seen from the *P. aeruginosa* biofilm slime layer attached to the wells ring. This causes the Bajakah Tampala ethanol extract to have difficulty penetrating target cells. The growth of the 48 hour phase of biofilm has a longer time compared to the 24 hour phase, therefore the biofilm community formed in this phase is more and more organized with each other, thus forming a kind of 3-dimensional group that will communicate with each other when there are foreign objects that will come into contact with each other. join their community [15].

In the eradication phase, 1% of bajakah tampala extract was able to eradication *P. aeruginosa* bacteria by 70.77% while the control drug chloramphenicol was 74.52%. In eradication there was a decrease in the inhibitory activity of the biofilm because the EPS material produced by bacteria wrapped in a biofilm was very complex and structured and the biofilm also played its role using communication between cells (Quarum sensing). However, the results of this study provide the latest information because Bajakah Tampala has inhibitory activity and biofilm eradication.

Test results of P. aerugiosa using Scanning Electron Microscopy

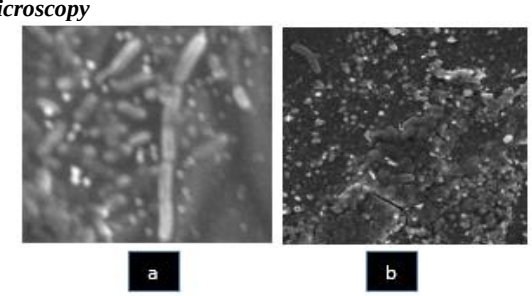

Figure 4. Scanning Electron Microscopy (SEM) Results of Bajakah Tampala Extract against P. aeruginosa Bacteria (a. Before administration of test extract) (b. After administration of test extract)

The results of *Scanning Electron Microscopy* showed that untreated P. *aeruginosa* biofilms showed thicker cells, intact morphology and neatly structured wrapped by an EPS matrix

(Figure 4a). This is because biofilm bacteria can synergistically form groups or communities with one species with another species and physically and physiologically the biofilm structure is thicker and stronger [15].

The administration of Bajakah tampala extract to P. *aeruginosa* biofilm (Figure 4b) showed that there were changes in morphology and structure of the broken cells. In addition, the *P. aeruginosa* biofilm EPS matrix also becomes damaged so that the *P. aeruginosa* biofilm cells lysis.

CONCLUSION

Bajakah Tampala Extract (*Spatholobus littoralis* Hassk) has activity as a strong antibacterial and antibiofilm, besides that Bajakah Tampala Extract can also eradicate P. *aeruginosa* biofilm and damage the EPS matrix of P. *aeruginosa* biofilm, therefore Bajakah Tampala extract is very potential to be used as as a candidate for new antibiofilm drugs.

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Ethical Approval: This study did not use experimental animals so it does not require approval from the ethics committee.

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Conflict Of Interest: The authors have no conflicts of interest to declare that are relevant to the content of this article.

Informed Consent: The research focused on the biofilm of *P.aeruginosa*, one of the microbes that causes wound infection with diabetic ulcers on the foot, using a plant from the Indonesian Borneo Island, Bajakah Tampala.

Authorship

H. Hamzah : main idea of research, biofilm testing, final approval, data analysis
S.U.T Pratiwi : main idea of research, biofilm testing
A. Jabbar : Plant determination and extract manufacture
W. Rahmah : research assistant
A. S. Hafifah : research assistant
C. Fadly : sampling

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