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Bioactivity Tracing of the Ethanol Extract of Bajakah Tampala (*Spatholobus littoralis* Hassk.) Typical Plant of Kalimantan Island as Antibiofilm of *Staphylococcus aureus*

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Abstract

BACKGROUND: Free radicals (oxidants) can cause skin irritation/damage which can be a manifestation of minor skin infections. Not only that, one of the complications of the disease that can arise is diabetes mellitus (DM) with diabetic foot ulcers (LKD). LKD is very susceptible to exposure to microorganisms and develops into diabetic foot infection (DFI). DFI is associated with the presence of biofilms in wounds especially those caused by Staphylococcus aureus infection. Bajakah tampala (Spatholobus littoralis hassk) is one of the native plants of Indonesia which has been known to have antibacterial activity, while its antibiofilm activity has not been studied. Evaluation of antibiofilms from the tampala bajakah plant can be of good novelty value, as well as support success in the treatment of infectious.

AIM: This study aims to determine the effectiveness of the ethanol extract of the Bajakah tampala plant from East Kalimantan in inhibiting and eradicating the formation of S. aureus biofilms.

MATERIALS AND METHODS: The planktonic and biofilm inhibition tests were carried out using the microtiter broth method. Antibiofilm activity of Bajakah tampala ethanol extract against S. aureus was analyzed by calculating the minimum biofilm inhibitor concentration (MBIC50) and the biofilm eradication activity calculating the minimum biofilm eradication concentration (MBEC50). In addition, we also carried out additional verification tests using the DPPH method by calculating the inhibitory concentration (IC50) parameter value.

RESULTS: The results showed that the 1% bajakah tampala extract gave mid-phase antibiofilm activity of 80.23% w/v ± 0.01, maturation phase of 77.23% w/v ± 0.01 and eradication with a large inhibition of 75.56% w/v ± 0.01. In the DPPH test, the IC50 value was 5.9 ppm with a very strong category.

CONCLUSION: Thus, it can be concluded that the ethanolic extract of the Bajakah tampala plant has a high potential to be developed as a candidate for new antibiofilm drugs against S. aureus biofilms.

Introduction

Infective diseases are still a major problem in the health sector. One of the most common microorganisms that attack humans is *Staphylococcus aureus* (54.5%). *S. aureus* is a Gram-positive bacterium that causes infectious diseases of the skin [1]. These bacteria can cause various types of infections ranging from minor skin infections, food poisoning, to systemic infections [2]. Infection is one of the complications that are often found in the care of patients with DM. One in two patients with LKD develops diabetic foot infection (DFI) [3]. The prognosis of LKD patients who experience infection is quite poor, such as amputation and death [4]. It was reported every 30 s lower limb amputation as a result of diabetes [5]. LKD is reported to be very susceptible to exposure to microorganisms [6]. Microorganisms found in LKD are producers of biofilms that can inhibit the healing process.

One way to treat diseases caused by *S. aureus* infection is by giving antibiotics. However, in some cases, antibiotics against *S. aureus* are less effective because the bacteria are already resistant to several antibiotics [7]. In addition, one of the virulence factors for *S. aureus* to be resistant to antibiotics and immune cells in the body is the ability to form biofilms [8].

A biofilm is defined as a collection of microorganisms and their associated extracellular products on their surface and generally adheres to biological and non-biological substrates [9]. In some cases, the biofilm is inhabited by a single species

while in other cases, it is inhabited by diverse microbes. Many surfaces which when viewed from the combination of moisture and nutrients present in them are susceptible to biofilm formation if microorganisms are present. Some of these surfaces include living tissue, medical devices, piped drinking water treatment systems, and industrial and natural aquatic systems that support the formation of biofilms [9]. Biofilms are currently considered to be the main mediators of infection, with an estimated 80% of infections associated with biofilm formation [10]. This is because the formation of biofilms on microorganisms can increase tolerance to antimicrobials and disinfectants. Thus, biofilms play a major role in the development of resistance and chronic disease. Antibiotic therapy in general will only kill planktonic cells, while the tightly packed form of bacteria in the biofilm will survive. This is because antibiotics cannot penetrate the biofilm layer [11].

Currently, the use of traditional medicinal plants in Indonesia is progressing quite rapidly. Medicines derived from traditional medicinal plants are now starting to be reused by the community as an alternative treatment [12]. One of the medicinal plants that are widely used for treatment is the Bajakah Tampala plant [13]. Research by Saputera & Ayuchecaria (2018) shows that the rods of Bajakah contain phenolic compounds, flavonoids, tannins, and saponins and are known to have antibacterial activity [14]. In addition, based on research by Luis *et al.* [15], it shows that *Spatholobus* extract has antibacterial activity. The genus Spatholobus has many species including *Spatholobus suberectus* and *Spatholobus littoralis* Hassk., the content of secondary metabolites contained in pirates can treat various degenerative diseases, such as diabetes, cancer, tumors, and others [14].

Until now, research on the Bajakah Tampala plant against *S. aureus* biofilms has never been reported. Therefore, this study will explore the bioactivity of the ethanol extract of the Bajakah Tampala plant as an antibiofilm for *S. aureus* bacteria.

Materials and Methods

Instruments: Laminar Discuss Stream, hatchery (IF-2B) (Sakura, Japan), micropipette pipetman (Gilson, France), multichannel micropipette (Socorex, Swiss), microplate flat-bottom polystyrene 96 well (Iwaki, Japan), microtiter plate peruser (Optic Ivymen Framework 2100-C, Spain), spectrophotometer (Genesys 10 UV Filtering, 335903) (Thermo Logical Spectronic, USA), autoclave (Sakura, Japan), and expository scale (AB204 -5, Switzerland).

The material used in this study was the ethanol extract of the Tampala Bajakah collected from the Forest in East Kalimantan, isolates of *S. aureus* which form biofilm standards (*S. aureus* ATCC 25923) from the collection of the Microbiology Laboratory of the Faculty of the Faculty of Pharmacy UGM, Chloramphenicol 1% w/v, DMSO 1% w/v, NaCl, McFarland widespread 0.5, sterile distilled water, Sabouraud Dextrose Broth (SDB) Media, RPMI Media, PBS (phosphate buffer saline), 1% w/v Quartz Violet Solution, gloves disposable, and mask.

Preparation of extract

Simplicia powder making

The manufacture of simplicia Bajakah Tampala stems begins by doing a wet sorting of Bajakah Tampala rods. Then wash the Tampala Bajakah rod with clean water. The stems are then drained to reduce the amount of rinse water so that the remaining impurities in the washing rinse water are also removed. The rods were weighed to obtain the wet weight. The stems are chopped for easy drying and grinding. The slices are then dried in direct sunlight for 3 days. Chopped using a blender into powder and then weighed as the weight of simplicia powder.

Making of Tampala Bajakah Bark extract

The ethanol extract of Bajakah Tampala stem was made by maceration method. A total of 250 g of simplicia powder were put into a maceration vessel. Add 2.5 L of 96% ethanol (1:10 ratio) or until fully submerged. Store in a place protected from sunlight for 3 × 24 h, stirring occasionally. Maserat is separated by filtering. Remaceration was carried out by adding 2 L of 96% ethanol. Remaceration was carried out 3 × 24 h. The filtrate was then evaporated using a rotary evaporator at a temperature of 50°C to accelerate the separation of the solvent with the efficacious extract. The half evaporated extract was re-evaporated using a water bath at 50°C to ensure that there was still residual solvent in the extract so that it became a thick extract.

Bacterial strains

S. aureus was grown within 24 h at 37°C in SDB. The optical density (OD) 600 of the microbial culture was adjusted to 0.1 (equivalent to the McFarland standard 0.5–1.5 × 108 CFU/mL) and then diluted in a new growth medium to 0.01 OD600.

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 h. Microplate absorbance reading process using a *microplate reader* at a wavelength of 595 nm.

Test of inhibition of biofilm formation mid-phase (24 h) and maturation phase (48 h) 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 *S. aureus* biofilms [15]. About 100 μL of media containing ethanol extract of Bajakah Tampala 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 h to form the mid-phase biofilm and 48 h to form the maturation phase biofilm. Next, the plate was washed using distilled water 3 times and dried at room temperature for 5 min to remove the remaining water. A total of 125 μL of 1% crystal violet solution were 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 3 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. The sample level that could inhibit at least 50% biofilm formation was considered minimal biofilm inhibition concentration ($MBIC_{50}$).

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

The result of inhibition that can inhibit the formation of biofilms of at least 50% is considered to be the minimum concentration of MBIC50 biofilm inhibition [15], [16].

S. aureus biofilm eradication activity from Bajakah Tampala

Tests for biofilm eradication (degradation) were almost similar to biofilm inhibition, but the processing time differed. The biofilm degradation test takes 5 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 h, the plates were washed with 150 μL of sterile distilled water 3 times to remove non-adherent cells. A total of 100 L of media containing ethanol extract of Bajakah Tampala with a series concentration were added to each well that had been washed, then reincubated at 37°C for 48 h. Chloramphenicol at a concentration of 1% w/v was used as positive controls. After incubation, the plates were washed 3 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 min. After incubation, the microplate was washed with PBS, 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 [12], [17].

Bajakah Tampala plant extract antioxidant test

Antioxidant activity was tested using the DPPH radical scavenging method [18]. A series of sample solutions were made from the four concentrated extracts with various concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, using DMSO as solvent. To determine the antioxidant activity, 0.2 ml of the sample solution was pipetted with a micropipette into the vial, then 3.8 ml of 50 M DPPH solution was added. The solution mixture was homogenized and left for 30 min in the dark. Absorption was measured with a UV–Vis spectrophotometer at a wavelength of 517 nm.

Scanning electron microscopy (SEM) test

For SEM observations, *Candida albicans* biofilms were formed on the sterile polyvinyl chloride coverslips (with 0.13–17 mm thickness and 22 mm diameter) inside a 12-well microtiter plates (Corning1 Costar1, Sigma-Aldrich, Missouri, USA) in the presence of 0.25% w/v of ethanolic extract of Bajakah Tampala for 72 h at 35°C, as described in the previous section. A biofilm grown in the absence of the test drug served as a control. Briefly, the coverslips were removed, washed twice with sterile PBS (0.1 M and pH = 7.2), and placed in a primary fixative solution (glutaraldehyde 0.15 M 2.5% [vol/vol] in PBS) at 48°C for 60 min. The coverslips were subsequently rinsed 2 times with PBS for 5 min, then treated with the secondary fixative (osmium tetroxide OsO4 1% w/v) for1 h. The samples were subsequently washed with distilled water, dehydrated in an ethanol series (70% for 10 min, 95% for 10 min, and 100% for 20 min), and air-dried overnight in a desiccator. The coverslip was coated twice with platinum vanadium using a sputter ion (Bal-Tec SCD 005) followed by bonding to carbon double side tape for examination by SEM.

Results and Discussion

Antibiofilm activity of ethanol extract of Bajakah Tampala against S. aureus biofilm phase (24 h)

In this study, we evaluated the antibiofilm potency of the Bajakah Tampala plant against the mono-species biofilm inhibition of *S. aureus*. The results showed that the ethanol extract of the Bajakah Tampala plant was able to inhibit more than 50% of *S. aureus* biofilm formation. The Bajakah Tampala plant itself is known to contain flavonoid compounds, saponins, tannins, and phenolics, so it has potential as an antimicrobial [19]. The results of the study in Figure 1 show that the ethanol extract of the Bajakah Tampala plant 1% w/v gave the highest activity compared to variations in the concentration of other extracts as *S. aureus* antibiofilm in the middle phase, which was 80.23% ± 0.01, while the results with the control drug in the form of chloramphenicol with a concentration of 1% w/v, in an activity of 82.21% ± 0.01. This result is in line with research by Hamzah, 2021, where it is known that Bajakah Tampala plant compounds provide activity faster than biofilm growth so that biofilm growth cannot form a complex structure and produces more extracellular polymeric substances (EPS) matrix which will provide a strong defense against *S. aureus* [20]. Another theory by Hamzah *et al.* [21] also states that the mechanism of inhibition of biofilm growth can be by penetrating the bacterial cell wall so that it can interfere with communication signals (quorum sensing) between bacteria that play a role in biofilm formation or inactivate genes in bacteria that trigger EPS synthesis [10], so that the test results show a fairly high inhibitory activity.

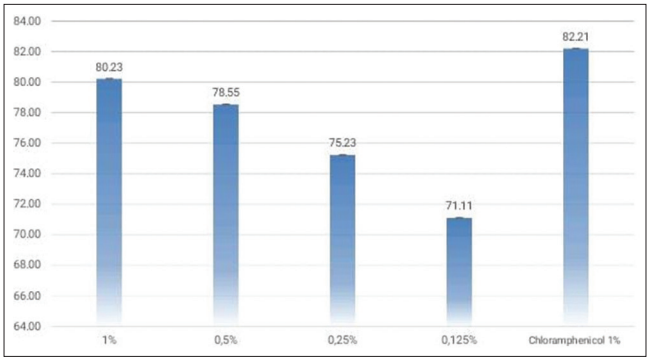

Figure 1: Antibiofilm activity of Staphylococcus aureus ethanol extract of Bajakah Tampala

In addition, in the mid-phase of the biofilm, the ethanol extract activity of Bajakah Tampala was different for each concentration. This can be seen from the inhibitory activity due to antibacterial activity. The size of the inhibition is influenced by the concentration of the given extract, increasing the concentration of the extract causes an increase in the content of the active ingredient that functions as an antibacterial so that its ability to inhibit the growth of a bacterium is also greater [22].

Antibiofilm activity of Bajakah Tampala ethanol extract against S. aureus biofilm phase (48 h)

The research carried out in Figure 2 shows that the Bajakah Tampala plant extract was able to inhibit biofilms at the 48 h maturation phase with the highest activity based on the concentration of the extract, which was shown at a concentration of 1% w/v, the Bajakah Tampala plant extract produced an inhibition of 77.23% ± 0.01, and control drug using chloramphenicol with a concentration of 1% w/v gave an inhibitory activity of 79.97% ± 0.01. In this phase, the effect of the ethanol extract of the Bajakah Tampala plant decreased in activity compared to the middle phase. This is because in this phase, *S. aureus* biofilm is fully formed so that *S. aureus* gets strong enough protection.

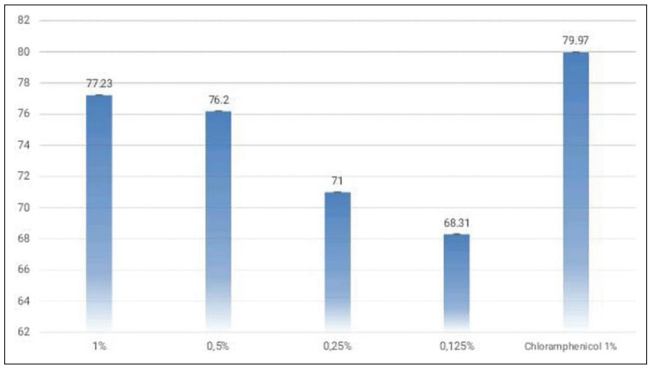

Figure 2: Effect of ethanol extract of Bajakah Tampala on mono-species Staphylococcus aureus in the maturation phase (48 h)

Several studies have also shown that the formation of biofilms at the 48 h phase is stimulated by continued hyphal growth and the production of EPS, which consist of cell walls of polysaccharides and proteins. Mature biofilms consist of yeast with hyphal substances that form a complex network that is covered with EPS and moves away from the surface [23]. In addition, research on the growth of the 48 h phase of biofilm is known to have a longer time compared to the 24 h phase, therefore, the biofilm communities formed in this phase are more numerous and organized with each other, thus forming a kind of stronger three-dimensional group that will communicate with each other so as to make the defense more difficult to penetrate [24]. This decrease in activity was also caused by the different amount of EPS between the middle phase and the maturation phase where the amount of EPS continued to increase according to the age of the biofilm [26].

In addition, the difference in concentration in this phase also plays a major role in providing inhibitory activity, according to Sonya's research [26]. In one study of the effectiveness of the extract on the biofilm formation of *S. aureus* bacteria, it was explained that a decrease in the average value of biofilm formation started at concentration and increased with increasing concentration [27].

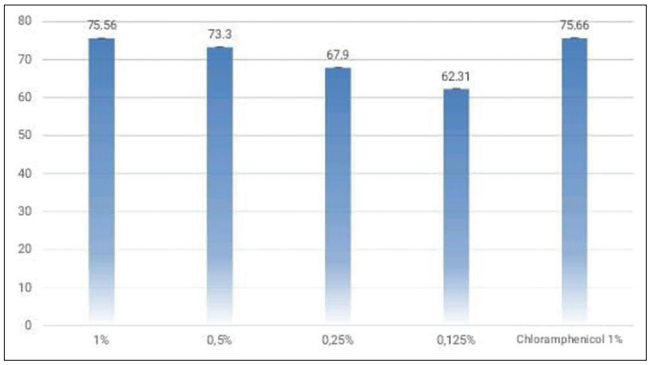

Figure 3: Activity of ethanol extract of Bajakah Tampala in eradicating Staphylococcus aureus biofilm

Biofilm Eradication activity of ethanol extract of Bajakah Tampala against S. aureus biofilm

After applying crystal violet stain, it was found that at a concentration of 1% w/v, the ethanol extract of Bajakah Tampala could inhibit *S. aureus* biofilm by 75.56% ± 0.01, while chloramphenicol reduced as much as 75.66% ± 0.01 biofilm *S. aureus*, as shown in Figure 3.

At the same concentration, the activity of the tested extracts in degrading *S. aureus* biofilm formation was weaker (75.56% ± 0.01) than the activity in inhibiting *S. aureus* biofilm formation (24 h) 80.23% ± 0.01 and phase (48 h) 77.23% ± 0.01. The decrease in the activity of the ethanol extract of Bajakah Tampala in eradicating *S. aureus* biofilms was thought to be because the degradation phase was more difficult in terms of biofilm damage, compared to microbes that formed biofilms in the intermediate and inhibitory phases. In the degradation phase, it is known that the longer the growth time of the biofilm, this also contributes to the resistance of the biofilm to antibiotics, making it more difficult to penetrate [28]. Biofilms are also known to contain microgradients of metabolites. This chemical gradient affects changes in antibiotic potency, the ability of biofilms to self-oxygenate, modulates the action of aminoglycosides so that bacteria are more resistant to antibiotics [29]. This is also known to make it more difficult for biofilm defenses to be penetrated.

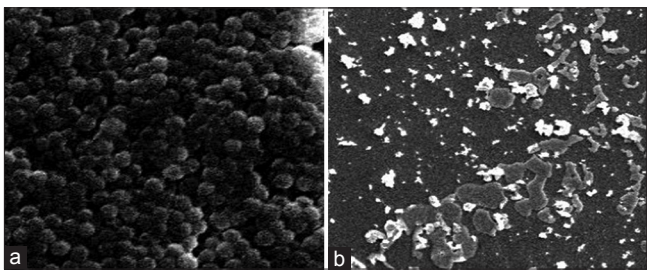

Figure 4: Scanning electron microscopy, (a) before being given Bajakah Tampala ethanol extract, (b) after being given Bajakah Tampala ethanol extract

SEM result of S. aureus administrated ethanol extract of Bajakah Tampala

The activity of Bajakah Tampala ethanol extract in inhibiting and destroying *S. aureus* biofilm was confirmed by SEM analysis. The results confirmed that untreated *S. aureus* had high cellular density within the EPS matrix protecting *S. aureus* (Figure 4) [22]. This indicates that a biofilm has formed. Biofilm matrix in Gram-positive bacteria has the characteristics of a thick biofilm matrix, between cells where the attachment is very strong and accumulates, there are very clearly visible tunnels [30]. These characteristics are shown in Figure 4a. Figure 4b shows that there was damage to the biofilm cells due to the application of the Bajakah Tampala plant extract on the bacterial biofilm.

The biofilm matrix serves as a link between adhesive and cohesive interactions, which provides mechanical stability to the biofilm, controls cell dispersion of the biofilm, and can also act as a nutrient source provider for cell communication [15]. *S. aureus* biofilm matrix also acts as a protective cellular biofilm and a major barrier to cell biofilm defense during attacks from the immune system and antibacterial drug treatment.

Antioxidant Testing of Tampala Bajakah Extract

Table 1 shows result used for look for concentration effective extract for dampen radical free of DPPH or IC_{50} value.

Table 1: IC_{50} values of samples extract plant Tampala _

Data	Equation of line	y value	Value x or IC_{50}
Replication I	y=6.5286x+10.301	50	5.980218389
Replication II	y=6.3846x+11.688	50	6.001253133
Replication III	y=6.5286x+10.301	50	6.081341912
Comparator (Vitamin C)	y=10.092x+20.242	50	2.948672216

The high or low antioxidant activity of the sample using the DPPH radical scavenging method is known from the percentage of inhibition. The greater the percentage inhibition of the sample, the higher the antioxidant activity. Meanwhile, the IC_{50} value is the effective concentration of the extract needed to reduce 50% of the total DPPH, in other words, it has an inhibition percentage of 50%.

We performed three replications to calculate the IC_{50} value of the Tampala Bajakah extract. Based on the data in Table 1, the IC_{50} value of all test samples for variations in the concentration of the extract shows an IC_{50} value of less than 50. The results showed that the average IC_{50} value of the Bajakah Tampala extract was 6.020973811. From the test results, the strongest antioxidant was shown by replication I where the results obtained were 5.980218389 ppm with a very strong category. In accordance with the IC_{50} value parameter in Table 2, this indicates that the Bajakah Tampala plant has a very strong antioxidant activity (IC_{50} value <50).

Table 2: Antioxidant properties based on IC_{50} value (Tristantini *et al*., 2016)

IC_{50} value antioxidant properties	
<50 ppm	Very strong
50–100 ppm	Strong
100–150 ppm	Currently
150–200 ppm	Weak

It is also known from all the literature articles that the Bajakah plant has antioxidant compounds that are classified as very strong because it has an IC_{50} value below 50 ppm [31]. Based on research conducted by Saputera & Ayuchecaria [14] on Bajakah stems from Central Kalimantan, it was found that Bajakah stems contain phenolic compounds, flavonoids, tannins, and saponins and have very effective bioactivity as a wound healer tested on rats. White male and pirated plants have bioactivity as antioxidants. Based on the results of this study, it can also be said that it is true that the Bajakah Tampala plant has a fairly high antioxidant activity and has the potential to be used as a medicine for various diseases.

Conclusion

The results showed that the ethanol extract of Bajakah Tampala had antibiofilm and antioxidant activity. The ethanol extract of the Bajakah Tampala plant was able to inhibit *S. aureus* biofilm at the mid-24 h phase and the 48 h maturation phase. Moreover, it was found that the antioxidant activity was included in the strong category. Thus, the ethanol extract of Bajakah Tampala has the potential to be developed as a candidate for *S. aureus* antibiofilm, as well as an anti-free radical/antioxidant compound.

References

1. Situmeang B, Ilham I, Ibrahim AM, Amin F, Mahardika M, Bialangi N, *et al*. ntioxidant and antibacterial activity of methanol extract fraction of kesambi stem bark (*Shleichera oleosa*). Jurnal Kimia J Chem. 2022;16(1):53-9. https://doi.org/10.24843/JCHEM.2022.v16.i01.p07
2. Tobi CH, Saptarini O, Rahmawat I. Anti-biofilm activity of areca seed (*Areca catechu* L.) terhadap *Staphylococcus aureus* ATCC 25923. J Pharm Sci Clin Res. 2022;7(1):56. https://doi.org/10.20961/jpscr.v7i1.43698
3. Hurlow JJ, Humphreys GJ, Bowling FL, McBain AJ. Diabetic foot infection: A critical complication. Int Wound J. 2018;15(5):814-21. https://doi.org/10.1111/iwj.12932
   PMid:29808598
4. Ndosi M, Wright-Hughes A, Brown S, Backhouse M, Lipsky BA, Bhogal M, *et al*. Prognosis of the infected diabetic foot ulcer: A 12-month prospective observational study. Diabet Med 2018;35(1):78-88 https://doi.org/10.1111/dme.13537
   PMid:29083500
5. IDF. International Diabetes Federation Diabetes Atlas. 8thed.,2017
6. Sinaga MS, Tarigan R. The use of materials in wound care at RSUD Dr. Djasamen Saragih Pematang Siantar. J Keperawatan Klinis. 2012;2(1):1-5.
7. Diyantika D, Mufida DC, Misnawi M. Perubahan morfologi *Staphylococcus aureus* akibat paparan ekstrak etanol biji kakao (*Theobroma cacao*) secara *in vitro*. Pustaka Kesehatan. 2014;2(2):337-45.
8. Cakranegara R, Rachmawaty FJ. Potential of Herbal Medicine in Formation Inhibition of Biofilm Staphylococcus aureus ATCC25923. 2020:1-36. Available from: https://dspace.uii.ac.id/123456789/28100.
9. Purbowati R. The relationship of biofilms to infection: Implications for public health and strategies to control it. J Ilmiah Kedokteran Wijaya Kusuma. 2018;5(1):1-14. https://doi.org/10.30742/jikw.v5i1.1
10. Archer NK, Mazaitis MJ, Costerton JW, Leid JG, Powers ME, Shirtliff ME. *Staphylococcus aureus* biofilms: Properties, regulation, and roles in human disease. Virulence. 2011;2(5):445-59. https://doi.org/10.4161/viru.2.5.17724
    PMid:21921685
11. Lestari DR, Soegianto L, Hermanu LS. Antibacterial and anti-biofilm potential of Bintaro flower ethanol extract (*Cerbera odollam*) terhadap *Staphylococcus aureus* atcc 6538. J Pharm Sci. 2017;4(1):30-5.
12. Hamzah H, Rasdianah N, Nurwijayanto A, Nandini E. Activity of calincing leaf ethanol extract against Candida albicans biofilm. J Farmasetis. 2021;10(1):21-8. https://doi.org/10.32583/farmasetis.v10i1.1319
13. Abdulrahman, Utami SR, Widia, Roanisca O. Study of secondary metabolites of bajakah stems (*Spatholobus littoralis Hassk*.) In development as anticancer herbal medicines and antioxidants. Seminar Nasional Penelitian Dan Pengabdian Pada Masyarakat. 2021;5:46-9.
14. Saputera MM, Ayuchecaria N. Testing the effectiveness of bajakah tampala (*Spatholobus littoralis Hassk*) ethanolic extract on wound healing time. J Ilmiah Ibnu Sina. 2018;3(2):318-27. https://doi.org/10.36387/jiis.v3i2.185
15. Pierce CG, Vila T, Romo JA, Montelongo-Jauregui D, Wall G, Ramasubramanian A, *et al*. The *Candida albicans* biofilm matrix: Composition, structure and function. J Fungi (Basel). 2017;3(1):14. https://doi.org/10.3390/jof3010014
    PMid:28516088
16. Nuryastuti T, Setiawati S, Ngatidjan N, Mustofa M, Jumina J, Fitriastuti D, Mardjan MID. Antibiofilm activity of (1)-N-2-methoxybenzyl-1, 10-phenanthrolinium bromide against Candida albicans. J Mycol Méd. 2018;28(2):367-373. https://doi.org/10.1016/j.mycmed.2017.12.010
17. Pratiwi SU, Lagendijk EL, Hertiani T, De Weert SA, Van Den Hondel CA. Antimicrobial effects of Indonesian medicinal plants extracts on planktonic and biofilm growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. J Hortic. 2015;7(4):183-91. https://doi.org.10.4172/2376-0354.1000119
18. Selvi AT, Joseph GS, Jayaprakasha GK. Inhibition of growth and aflatoxin production in *Aspergillus flavus* by *Garcinia indica* extract and its antioxidant activity. Food Microbiology. 2003;20(4):455-460. https://doi.org/10.1016/S0740-0020(02)00142-9
19. Rachman T. Potential bioactive compound Spatholobus litoralis Hassk is bioactive compounds as antimicrobial and anticancer MCF-7 by in vitro and in silico methods. Angew Chem Int Ed. 2018;6(11):951-2.
20. Hamzah H, Hertiani T, Sylvia UT, Nuryastuti T. Effect of saponins on planktonic and mono-species inhibition of *Candida albicans* atcc 10231 biofilms in the intermediate, maturation and degradation phases. Majalah Farmaseutik. 2021;17(2):198-205.

https://doi.org/10.22146/farmaseutik.v17i2.54444

21. Hamzah H, Siregar KAAK, Suffiana Y, Yudhawan I, Nurwijayanto A. Antibacterial and antibiofilm activity of Begonia multangula Blume. leaf extract against *Candida albicans*. Food Research. 2022;6(1):268-268. https://doi.org/10.26656/fr.2017.6(1).560
22. Alfiah RR, Khotimah S, Turnip M. Effectiveness of methanol extract of sembung creeper leaves (*Mikania micrantha Kunth*) on the growth of *Candida albicans* fungus. J Protobiont. 2015;4(1):52-7.
23. Harriott MM, Noverr MC. Ability of *Candida albicans* mutants to induce *Staphylococcus aureus* vancomycin resistance during polymicrobial biofilm formation. Antimicrob Agents Chemother. 2010;54(9):3746-55. https://doi.org/10.1128/AAC.00573-10

    PMid:20566760
24. Hamzah H, Hertiani T, Pratiwi SUT, Nuryastuti T. The inhibition activity of tannin on the formation of mono-species and polymicrobial biofilm *Escherichia coli*, Staphylococcus aureus, Pseudomonas aeruginosa, and *Candida albicans*. Trad Med J. 2019;24(2):110-118.
25. Homenta H. Bacterial biofilm infection. J E-Biomedik. 2016;4(1):1-11. https://doi.org/10.35790/ebm.4.1.2016.11736
26. Federika AS, Rukmo M, Setyabudi S. Antibiofilm activity of flavonoid mangosteen pericarp extract against porphyromonas gingivalis bacteria. Conservative Dentistry J. 2020;10(1):27–30. https://doi.org/10.20473/cdj.v10i1.2020.27-30
27. Winarsih S, Khasanah U, Alfatah AH. Anti-biofilm activity of the ethyl acetate fraction of Putri malu (*Mimosa Pudica*) leaf extract on Methicilin-resistant *Staphylococcus Aureus* (Mrsa) bacteria *in vitro*. Majalah Kesehatan. 2019;6(2):76-85. https://doi.org/10.21776/ub.majalahkesehatan.006.02.1
28. Hamzah H, Pratiwi SUT, Hertiani T. Efficacy of Thymol and Eugenol Against Polymicrobial Biofilm. Indones J Pharm. 2018;29(4):214-221. https://doi.org/10.14499/indonesianjpharm29iss4pp221
29. Yolazenia Y, Budiman BJ, Irfandy D. Bacterial biofilmin patients with chronic rhinosinusitis. J Kesehatan Melayu. 2018;1(2):106. https://doi.org/10.26891/jkm.v1i2.2018.106-113
30. Marlina D, Kurniati M, Hamid F, Larasathi F, Irnawita F. Visualization of Eschericia coli biofilm matrix with bacteriological peptone, sucrose and ethanol media. Jurnal Kesehatan. 2018;9(1):26. https://doi.org/10.26630/jk.v9i1.730
31. Amiani W, Fahrizal MR, Aprelea RN. The content of secondary metabolites and the activity of pirated plants as antioxidant agents. J Health Sains. 2022;3(4):2003-5.