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TRACING THE ANTIBACTERIAL, ANTIFUNGAL AND ANTI-BIOFILM ACTIVITIES OF ROOT EXTRACT BAJAKAH TAMPALA (SPATHOLOBUS LITTORALIS HASSK)

Endang Setyowati[1], **Elza Fadia Irzani**[1], **Chaerul Fadly Mochtar Luthfi**[2], **Hasyrul Hamzah**[2]

[1]Department of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Kudus, Kudus 59316, Indonesia
[2]Department of Pharmacy, Faculty of Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda 75124 Indonesia

hh241@umkt.ac.id

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ABSTRACT
Biofilm is a common causative factor for urinary tract infections due to catheter usage with a percentage of infection around 70-80%. The Bajakah tampala (Spatholobus littoralis Hassk) is one of native plants of Kalimantan which contains phenolic compounds, flavonoids and tannins which are proven to accelerate wound healing, have antibacterial activity, and have very high and strong antioxidant activity. This study aims to determine the antibacterial and anti-biofilm activity of bajakah root extract (Spatholobus littoralis Hassk) on catheter colonies of Staphylococcus aureus, Escherichia coli and Candida albicans and to determine their mechanism of action in vitro. This research is carried out with an in vitro experimental study design using a microplate reader. Extraction was carried out by maceration method using 96% ethanol solvent. The results showed that the ethanol extract of Bajakah tampala (Spatholobus littoralis Hassk) had antibacterial activity against S. aureus with a concentration of 1% w/v of 88.33% ± 0.01 and anti-biofilm activity of 82.21% ± 0.01. E. Coli bacteria had an antibacterial activity of 84.83% and an anti-biofilm activity of 80.11 at a concentration of 1% w/v. C. albicans had an antifungal activity at a concentration of 1% w/v of 82.31% ± 0.01 and anti-biofilm activity of 77.00% ± 0.01. From these results it can be concluded that the ethanol extract of Bajakah tampala (Spatholobus littoralis Hassk) has antibacterial and antifungal activities and the potential as a new anti-biofilm agent against S.aureus, E. coli and C. albicans

Keywords: Bajakah tampala; Antibiofilm; S.aureus; E.Coli; C.albicans

1. INTRODUCTION

Hospital-acquired infection seems to be difficult to avoid by health workers and patients in medical treatment. One of the nosocomial infections is in the urinary system. The cause of this infection is usually a medical equipment supporting the patient care, namely a catheter. The most common one is catheter associated urinary tract infection (CAUTI). Around 80% of urinary tract infections due to catheters are related to biofilm formation (Nurdin, E., Nurdin, G. M., & Noviyanti, 2020).

Biofilm is a causative factor for health care-related infections with a percentage of around 70-80%. The growth of biofilm on these catheters is also responsible for the death of around 7500 people per year (Nicolle, 2014). Bacteria that often cause the formation of biofilms on catheters and thus become the main cause of nosocomial infections is *Escherichia coli*. However, *Staphylococcus aureus* and *Candida albicans* also cause the formation of biofilms. *Staphylococcus aureus* is also capable of adhering to a medical device surface to form biofilms.

The prevalence of biofilm formation ranged from 65.1-69.8% and 86.7% of biofilm-producing *Staphylococcus aureus*, was multidrug resistant (Hasyrul Hamzah et al., 2023).

Even though a catheter is simple, it can provide significant benefits for patients because it is a type of modern medical device. However, long-term use of a catheter can damage the natural defenses of the urinary tract. Thus, management of patients with catheters is often complicated by infections in which biofilm formation is a major feature (Pelling et al., 2019). explains that the longer the catheter, the greater the possibility of bacteria appearing. Although not responsible for a clinical emergency, a significant increase in biofilm cells often occurs, resulting in the bacteria becoming resistant to antibacterial agents. More than 100 million urethral catheters are sold each year. In addition, according to (Darouiche, 2001), more than 30 million urinary catheters are used annually in the United States.

Antibiotic therapy in general will only kill cells that are planktonic, while the forms of bacteria that are tightly arranged in biofilms will survive. This is because antibiotics cannot penetrate the biofilm layer on the catheter (Mah & O'Toole, 2001). The use of traditional medicine in Indonesia has progressed quite rapidly because it has become an alternative treatment. (Muhlisah, 2007) states that the use of drugs from natural ingredients has a much lower level of danger and long-term risk than the use of synthetic drugs.

One of the famous native plants of Kalimantan in the last two years is Bajakah Tampala (*Spatholobus littoralis* Hassk). Bajakah Tampala contains phenolic compounds, flavonoids, and tannins (Marlina & Samad, 2013). This is reinforced by other studies which state that this plant contains phenolic compounds, flavonoids, and tannins which are proven to accelerate wound healing (Saputera & Ayuchecaria, 2018), with levels of phenolic compounds of 12.33 GAE/mg (Ayuchecaria et al., 2020), have antibacterial activity (Saputera et al., 2019) and have very high antioxidant activity (Ayuchecaria et al., 2020).

The search for anti-biofilm compounds from plants is still limited. Even though biofilm is a health problem worldwide, effective and safe antibiotics have not been found to treat it. Therefore, considering the problems above, this study aims to investigate new anti-biofilm agents from Bajakah root extract effective against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* bacteria, the main causes of biofilm.

2. METHODS

This research is carried out with an in vitro experimental study design using a microplate reader. The maceration method is used to obtain Bajakah Tampala extract (*Spatholobus littoralis* Hassk). This research was conducted from October 2021 to September 2022 at the Microbiology Laboratory of Faculty of Pharmacy at Muhammadiyah University, East Kalimantan, Faculty of Pharmacy, Universitas Muhammadiyah Kudus, Biology Laboratory of Faculty of Pharmacy and LPPT at Gadjah Mada University.

2.1. Plant Determination Results

Plant determination was carried out at the Mulawarman Herbarium, Laboratory of Ecology and Conservation of Tropical Forest Biodiversity at the Faculty of Forestry at Mulawarman University. The results showed that the plant was the Bajakah tampala with the species *Spatholobus littoralis* Hassk. from the Fabaceae family.

2.2. Sample Extraction

Extraction was carried out by maceration method using 96% ethanol solvent. The maceration method was chosen because it is the simplest method (Novriyanti et al., 2022) and 96% ethanol was chosen as a solvent because 96% ethanol is universal which can extract less polar, semi-polar to polar compounds. In addition, 96% ethanol is also able to extract flavonoids, alkaloids, saponins, antraquinones, and glycosides (Sinung et al., 2019). After the extraction

process, a viscous extract of the stem of the Bajakah Tampala (*Spatholobus littoralis* Hassk) with a weight of 49.38 g was obtained.

2.3. Secondary Metabolite Testing

2.3.1. Alkaloid Test

Extracts and fractions (2 mL) were put into test tubes. Then added with HCl 2N as much as 1 ml and then dripped with 3 drops of Dragendorff reagent, a positive test is indicated by the formation of an orange precipitate (Novriyanti et al., 2022).

2.3.2. Phenolic test

Extracts and fractions (1 mL) are put into a test tube, then 3 drops of 1% FeCl3 reagent are added, A positive test is indicated by the formation of a black color (Novriyanti et al., 2022).

2.3.3. Flavonoid Test

Extracts and fractions (2 mL). Put into a test tube, then added 0.05 mg of Mg powder and 1 mL of concentrated HCl and shaken, Positive test is indicated by the formation of red, yellow or orange color (Novriyanti et al., 2022).

2.3.4. Saponin Test

Extracts and fractions (2-3 mL) are put into a test tube, then added with 10 mL of warm water, and then shaken vigorously for 10 seconds, A positive test is indicated by the formation of a stable froth as high as 1-10 cm for 10 minutes (Novriyanti et al., 2022).

2.3.5. Steroid and Terpenoid test

Extracts and fractions (2 mL) are put into a test tube, then 1-3 drops of Lieberman Burchard reagent are added and the solution is shaken gently, A positive test for steroids is indicated by the formation of a blue or green color, while terpenoids give a brownish red color (Novriyanti et al., 2022).

2.3.6. Tannin Test

Extracts and fractions (1 ml), put into a test tube, then added 1-3 drops of 10% FeCl3 solution, Positive test is indicated by the formation of greenish-black color (Novriyanti et al., 2022)

2.3.7. Anthocyanin Test

Extracts and fractions (2 ml) were put into a test tube, then add NaOH 2N drop by drop. If the red color turns blue-green and fades slowly, it indicates the presence of anthocyanins (Trinovani et al., 2022).

2.4. Preparation of Bacterial Subcultures

Standard biofilm-forming *Staphylococcus aureus* was cultured in Brain-heart infusion broth (BHI) medium and incubated at 37ºC for 72 h. The optical densities (OD_{600}) of microbial cultures were adjusted to 0.1 (equal of the 0.5 Mc Farland standard ~1.5 x 10^8 CFU/ml), and subsequently diluted in fresh medium to OD_{600} 0.01 for each microbial species (Hasyrul Hamzah et al., 2023).

2.5. Antibacterial and Antifungal Testing

An antibacterial test was carried out using the microdilution method. The test was carried out on a microtiter plate flat-bottom polystyrene 96 wells with a series of test compound concentrations of 1%, 0.5%, 0.25%, 0.125% w/v. The control used was drug control using ciproloxacin and fluconazole. Growth control in the form of microbial suspension and solvent control was adjusted with the solvent of the test compound into each wells microplate, BHI media and bacterial suspension, and RPMI media were added for fungal suspension and then incubated at 37 °C for 24 hours for bacteria and 72 hours for fungi (Hamzah et al., 2018).

2.6. Mono-species Biofilm Test on Catheter

96% ethanol, allowed to dry, and the catheter inserted into the well. A total of 100 L of media containing bacterial suspension, normal human urine, and the test compound was added to each microtiter well plate containing the catheter, then incubated at ± 37°C for 24 hours for the middle phase. After the incubation period, the plates were washed using 150 L of sterile distilled water. The catheter was then scraped and transferred to a new plate, and 125 L of 1% crystal violet solution was added. Then the biofilm was washed with running water, and 200 L of 96% ethanol was added. The results of biofilm inhibition were read using an Optical Density (OD) 595 nm microplate reader. The test was carried out with three repetitions (Hamzah et al., 2018).

3. RESULTS AND DISCUSSION

3.1. Phytochemical Screening

As shown in **Table 1**, that the results of the qualitative phytochemical screening test showed that the ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) positively contained alkaloids, flavonoids, saponins, tannins and phenols. Based on the results of the alkaloid screening, the Bajakah Tampala peel extract positively contained alkaloid compounds. According to research conducted by (Saputera & Marpaung, 2019), the factors that influence differences in compound content are differences in plant growing areas.

Table 1. Phytochemical screening results

Compound	Phytochemical Screening		
	Test Method	**Indicator**	**Results**
Alkaloids	Meyer	White precipitate	-
	Wagner	Brown precipitate	+
	Dragendroff	Orange precipitate	+
Flavonoids	NaOH	Changes color when compared to the control solution	+
	H_2SO_4 Concentrated		+
	Mg-HCL Concentrated		+
Saponins	Warm aquadest	Stable foam	+
tannins	$FeCl_3$ 1% 3-5 drops	Blackish green or blackish blue	+
Phenol	$FeCl_3$ 5% 2-5 drops	Greenish blue or green	+

Explanation: += Positive; −: Negative

3.2. Antimicrobial Test

3.2.1. Antifungals Against *C. albicans*

Antifungals are antibiotics which is able to hinder up to kills fungal growth. Antifungal has two meanings namely fungicidal and fungistatic. Fungicide is defined as a compound that can kill fungi, while fungistatic can inhibits the growth of fungi without turn it off. As seen in **Figure 1**, the antifungal test using microdilution showed that at a concentration of 1% w/v the Bajakah Tampala ethanol extract was able to provide inhibitory activity against *C. albicans* as much as 82.31% ± 0.01, while Fluconazole as a positive control provided inhibition growth of 88.10% ± 0.01. These results show that the ethanol extract of Bajakah Tampala has antifungal activity against *C. albicans*.

The ethanol extract of Bajakah Tampala has antifungal activity against *C.albicans* of 85.77% at a concentration of 1% w/v, with the control drug fluconazole providing activity of 74.20%. ± 0.01 on the fungus Candida albicans. The process of inhibition of the biosynthesis of fungal nucleic acids is caused by alkaloid compounds, which result in the growth of the fungus not developing and the fungus becoming dead and other compounds, namely flavonoids, which have pharmacological effects as antifungals (Hamzah et al., 2020). Bioactive compounds that function as antifungals are triterpenoids which are classified as terpenoids. Fungal growth will be inhibited even though it passes through the cytoplasmic membrane and the increase in fungal

spores caused by the presence of terpenoids (Mochtar, C. F., Saleh, L. O., Hamzah, H., & Ilyas, 2022).

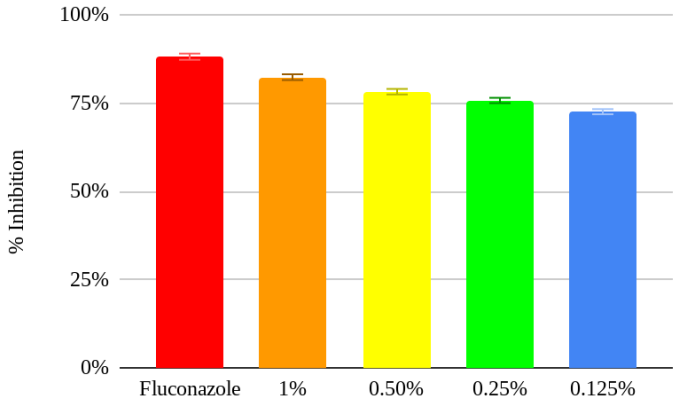

Figure 1. Antifungal activity of the ethanol extract of Bajakah Tampala

3.2.2. Antibacterial Against *S. aureus*

As seen in **Figure 2**, the results of the antibacterial test against *S. aureus* using microdilution showed that at a concentration of 1% w/v the ethanol extract of Bajakah Tampala was able to provide inhibitory activity against S. aureus of 88.33% ± 0.01, while chloramphenicol as a positive control gave growth inhibition of 89.01% ± 0.01. These results provide evidence that the ethanol extract of Bajakah Tampala has antibacterial activity against S. aureus. These results are in line with research conducted by (Mochtar, C. F., Saleh, L. O., Hamzah, H., & Ilyas, 2022). That the 1% concentration of the Bajakah Tampala ethanol extract has an antibacterial activity of S. aureus of 82.30% and (Kurniawan, 2019) reported that at a concentration of 100% it had an inhibition diameter of 12.25 ± 0.5 mm and the Bajakah Tampala Ethanol Fraction (*Spatholobus Littoralis* Hassk.) at a concentration of 80% with an inhibition diameter of 8.25 ± 0.5mm (Agustin et al., 2018).

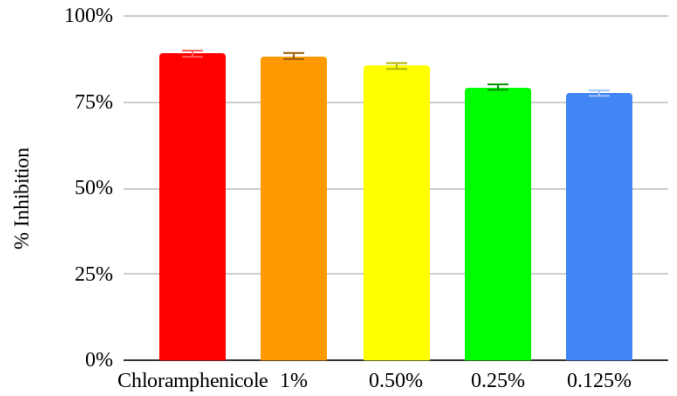

Figure 2. Antibacterial activity of the ethanol extract of Bajakah Tampala against *S. aureus*

Compounds that have antibacterial activity are alkaloids by slowing cell respiration and have a function when intercalating DNA (Hamzah et al., 2020). In addition, saponins also have antibacterial activity by interfering with the surface tension of the cell wall, so when the surface tension is disturbed, the antibacterial substance can easily enter the cell and interfere with metabolism, causing bacterial death to occur (Agustin et al., 2018).

3.2.3. Antibacterial Against *E. coli*

As seen in **Figure 3**, the ethanol extract of Bajakah Tampala (*Spatholobus littoralis* Hassk) provided an antibacterial activity of 84.83% at a concentration of 1% w/v and this activity was almost the same as the control drug chloramphenicol of 87.17%. These results indicate that the ethanol extract of Bajakah Tampala (*Spatholobus littoralis Hassk*) is able to inhibit the growth of *E. Coli* above 50%. The 50% concentration of the Bajakah Tampala ethanol extract has antibacterial activity against *E.Coli* with an average diameter of the inhibition zone of 20.32 mm (Saputera et al., 2019).

Compounds that are known to play a role in inhibiting bacterial growth include flavonoids, saponins, and tannins, where these substances function as antibacterials with different mechanisms (Febrianti et al., 2018). In tannin compounds, bacterial growth is inhibited by interfering with protein transport, inactivating cell adhesins and inactivating enzymes in bacterial cells (Agustin et al., 2018).

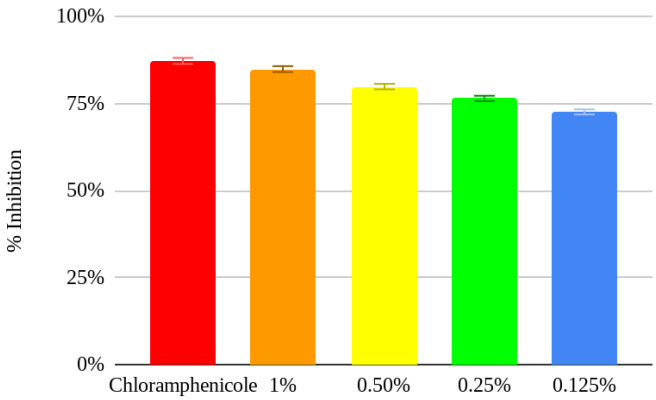

Figure 3. Antibacterial activity of the ethanol extract of Bajakah Tampala against *E. coli*

3.3. Antibiofilm Test

3.3.1. Antibiofilm Activity Against *C. albicans* (Anti-biofilm Activity of the Ethanol Extract of Bajakah Tampala in the Mid-24-hour Phase)

As seen in **Figure 4**, Bajakah tampala ethanol extract at a concentration of 1% w/v gave inhibitory activity against *C. albicans* biofilms of 77.00% ± 0.01, while Fluconazole showed inhibition of *C. albicans* biofilm formation of 80.67% ± 0.01, From the results above, it has been shown that the same extract concentration tested (1% w/v) showed weaker activity against inhibition of biofilm formation (77.00% ± 0.01) compared to planktonic (82.31 % ± 0.01).

The results showed that microbes in the form of biofilms were more difficult to inhibit than in planktonic ones. This may be because the microbes in planktonic are single cells, while the microbes in biofilms tend to live together (many colonies), attach and grow on the surface, and form multi-layered structures encased by an EPS matrix, which makes biofilms more resistant to antibiotics and antimicrobials (Hamzah, Rasdianah, et al., 2021).

3.3.2. Antibiofilm Activity Against *S. aureus* (Anti-biofilm Activity of the Ethanol Extract of Bajakah Tampala in the Mid-24-hour Phase)

In **Figure 5**, the results of the study show that the ethanol extract of the Bajakah tampala plant 1% w/v gave the highest activity of all extract concentrations as an anti-biofilm against *S. areus* in the mid phase of 80.23% ± 0.01. Meanwhile, the control drug in the form of chloramphenicol with a concentration of 1% w/v gave an activity of 82.21% ± 0.01. In this study, there was a decrease in inhibitory activity compared to inhibitory activity in planktonic. This is because biofilms produce EPS structures that function as microbial protection from drug compounds. Microbes in biofilms differ from planktonic cells in various ways of growing. One

consequence of these differences is that the microbes in biofilms have been shown to be more resistant to antibiotics and antimicrobials (Hamzah, Siregar, et al., 2021). However, the results of this study provide evidence that the ethanol extract of Bajakah Tampala has potential as a new anti-biofilm agent.

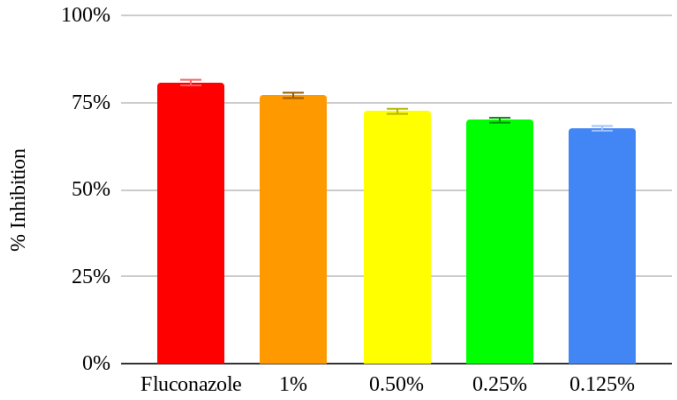

Figure 4. Middle phase anti-biofilm activity of Bajakah Tampala ethanol extract against *C.albicans* biofilm monospecies in catheters

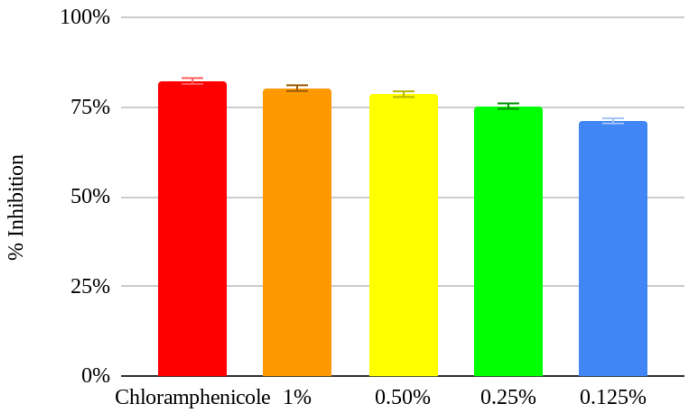

Figure 5. Middle phase anti-biofilm activity of Bajakah Tampala ethanol extract against *S. aureus* biofilm monospecies in catheters

3.3.3. Antibiofilm Activity Against E. coli (Anti-biofilm Activity of the Ethanol Extract of Bajakah Tampala in the Mid-24-hour Phase)

In **Figure 6**, it can be seen that the ethanol extract of Bajakah Tampala can inhibit the formation of E. coli biofilms in the intermediate phase from 1% to 0.125% where at 1% b/v level it provides activity of 80.11%, while the control drug chloramphenicol is 82.65%. However, there is a decrease in the inhibition of ethanol extract of bajakah tampala and chloramphenicol when biofilm formation occurs, this occurs because biofilms provide protection against E. coli from antibiotic treatment and the immune system. These bacteria can be up to 1000-fold more resistant to antibiotics than planktonic bacteria. Tolerance to antibiotics is mainly due to the following mechanisms: low antimicrobial penetration, reduced growth rate and stress response, cell survival, efflux pumps, and HGT (Ballen, Cepas., et al 2022).

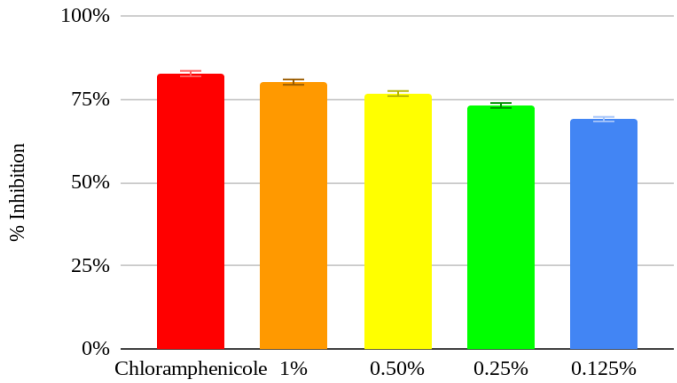

Figure 6. Mid-phase anti-biofilm activity of Bajakah Tampala ethanol extract against Middle phase anti-biofilm activity of Bajakah Tampala ethanol extract against *E. Coli* biofilm monospecies in catheters

4. CONCLUSION

From the results of the qualitative phytochemical screening, the ethanol extract of Bajakah tampala (*Spatholobus littoralis* Hassk) positively contained alkaloids, flavonoids, saponins, tannins and phenols. The ethanol extract of Bajakah tampala (*Spatholobus littoralis* Hassk) has antibacterial activity against *S. aureus* and *E. coli* bacteria. It also has antifungal activity against *C. albicans* where the higher the concentration of the extract, the higher the antibacterial and antifungal activities are produced. Microbial activity in biofilms has been shown to be more resistant to antibiotics and antimicrobials, but based on the results of the anti-biofilm activity testing, the ethanol extract of Bajakah Tampala has potential as a new anti-biofilm agent against *S. aureus, E. coli and C. albicans.*

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6. CONFLICT OF INTEREST

All authors declare no conflict of interest.

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