







Exploring the Bioactivity of Bajakah Tampala Plant (*Spatholobus littoralis* Hassk) as Polymicrobial Antibiofilm on Catheters

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Abstract: Catheter-associated urinary tract infections (CAUTIs or CAUTI) are among the most common healthcare-associated infections (HAIs) found in hospitals. Urinary catheters are a significant factor in CAUTIs; 70 to 80% of the incidence of these infections is caused by urinary catheters. The National Healthcare Safety Network reported an incidence of CAUTI of approximately 3.1-7.5 infections per 1,000 catheters per day. This study aims to determine the effectiveness of Bajakah Tampala ethanol extract against polymicrobial biofilms on catheters. The microtiter broth method determined antibiotic and antifungal tests, biofilm inhibition, and eradication activity. The effectiveness of the Bajakah Tampala antibiofilm on polymicrobial biofilms was analyzed by calculating the minimum biofilm inhibitor concentration (MBIC50) and the minimum biofilm eradication concentration (MBEC50). The ethanol extract of Bajakah Tampala also has antibiofilm activity against monospecific *S. aureus*, *E. coli*, and *C. albicans* in the middle phase. It provides activity against polymicrobial biofilms on the catheter in the middle and maturation phase. The results also prove that Bajakah Tampala's ethanol extract can eradicate polymicrobial biofilms on catheters. Therefore, Bajakah Tampala has the potential to be developed as a candidate for new antibiofilm agents against polymicrobial biofilms on catheters.

Keywords: Bajakah Tampala; biofilm; antibiofilm; polymicrobial; catheter.

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1. Introduction

Indonesia is an archipelagic country with a rich diversity of plants [1]. This wealth is a national asset that must be developed to increase the nation's resilience and sovereignty. One of Indonesia's islands with a diversity of plants is Kalimantan. Kalimantan has long been known to have many diverse medicinal plants and is one of Indonesia's most significant sources of flora diversity. One of the famous Kalimantan plants in the last two years is *Spatholobus littoralis* Hassk (local name Bajakah Tampala) [2]. Bajakah Tampala has been proven to accelerate wound healing [2]. In addition, other studies have also shown that Bajakah Tampala

has antibacterial activity [3]. Bajakah, which in the Dayak language means the root is not a specific plant species. Recently, Bajakah Tampala has become the center of public attention because it is believed to cure breast cancer [4,5]. Catheter-associated urinary tract infections (CAUTIs or CAUTI) are among the most common healthcare-associated infections (HAIs) found in hospitals. Urinary catheters are a significant factor in CAUTI, with 70 to 80% of current infections caused by biofilms [6–9]. Biofilm growth on catheters is associated with urinary tract nosocomial infections and causes about 7500 deaths annually [6,10,11]. Until now, studies on the search for antibiofilm compounds in catheters from plants are still very few, even though biofilm is a health problem worldwide because no effective and safe antibiotics have been found to treat it [12–14]. Therefore, based on the issues above, this research will search for new antibiofilm agents from the ethanol extract of Bajakah Tampala against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans* [15–17].

2. Materials and Methods

2.1. Equipment.

The equipment used is glassware, Laminar Air Flow, incubator (IF-2B) (Sakura, Japan), micropipette pipetman (Gilson, France), microplate flat-bottom polystyrene 96 well (Iwaki, Japan), microtiter plate reader (Optic Ivymen). System 2100-C, Spain), 24 well flat-bottom polystyrene microplate (Iwaki, Japan), spectrophotometry (Genesys 10 UV Scanning, 335903) (Thermo Scientific Spectronic, USA), autoclave (Sakura, Japan), coverslip (2001212mM) (SPL), white tip, yellow tip, blue tip, silica gel F254 10 x 10 cm (Merck, Germany), analytical balance (AB204-5, Switzerland), Scanning electron microscopy (JEOL JSM-6400, Japan).

2.2. Materials.

Bajakah Tampala ethanol extract; culture of *C. albicans* ATCC 10231; *S. aureus* ATCC 25923; *E. coli* ATCC 25922; Media Roswell Park Memorial Institute 1640 (RPMI 1640) (Sigma, USA); Distilled Water (Aquadest) Sterile; Crystal Violet (Merck, Germany) (1% in Aquadest); N-Hexan (Merck, Germany); Ethyl Acetate (Merck, Germany); Brain Heart Infusion (BHI); Ethanol 95% (Merck, Germany); Glutaraldehyde Solution (Sigma, USA).

2.3. Preparation of test bacteria and fungi.

Staphylococcus aureus and *Escherichia coli* bacteria were all grown within 24 hours at 37°C in BHI (Brain Heart Infusion) media. *Candida albicans* was grown for 72 hours at 37°C in Sabouraud Dextrose Broth (SDB). The optical density 600 of the microbial culture was adjusted to 0.1 (equivalent to the McFarland standard $0.5 - 1.5 \times 10^8$ CFU/mL) and then diluted in a new growth medium to OD600 0.01 for bacteria and OD520 0.38 for *C. albicans*.

2.4. Thin-layer chromatography analysis.

Merck's silica gel 60 F254 preparative plate was prepared with a length of 20 cm and a width of 20 cm. The thick extract that has been diluted with ethanol is smeared along the bottom edge of the plate and aerated for a while. The plate is put into a chamber containing the eluent, namely n-hexane: ethyl acetate, in a ratio of 3:1. The plate is allowed to elute until the eluent reaches the upper boundary of the plate, then it is removed and dried in air. Detection was carried out using UV light at 254 nm, 366 nm, and spray reagents, anisaldehyde, sulfuric acid,

Liberman-Bourchart, and Dragendroff. The aim is to see the compounds contained in the ethanol extract of Bajakah Tampala.

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, a flat-bottom polystyrene 96-well plate, with a series of test compound concentrations of 1%, 0.5%, 0.25%, and 0.125% w/v. The control used was drug control using ciprofloxacin and fluconazole. Growth control in the form of a microbial suspension and solvent control was adjusted with the solvent of the test compound in each well of the microplate. BHI media, 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.

2.6. Inhibition of mono-species biofilm formation.

A total of 100 µL of microbial suspension (10^7 CFU/mL) was added to each well of the microtiter plate. Specifically, *Candida albicans* were incubated at a temperature of $\pm 37^\circ\text{C}$ for 90 minutes for the biofilm attachment phase. After that, the plate was washed three times using 100 µL of sterile distilled water to remove nonadherent cells. A total of 100 µL of media containing the test extract with concentration series (1%, 0.5%, 0.25%, 0.125% w/v) was added to each well that had been washed. A medium without microbial growth was used as a control medium, and a microbial suspension was used as a growth control. A microbial suspension was used as a controlled drug, which was given antifungal fluconazole and antibacterial ciprofloxacin at a concentration of 1% w/v. The plate was then incubated at 37°C for 24 hours.

Optical density (OD) readings were carried out with a microplate reader at a wavelength of 595 nm. The test was carried out with three replications. Data obtained from the analysis of biofilm inhibition in the form of OD values of each concentration of the test compound and control without the test compound (growth control) were obtained from reading with a microplate reader. The OD value is then used to calculate the percent inhibition in the following equation:

$$\%Inhibitor = \frac{OD\ negative\ control\ mean - OD\ test\ sample\ mean}{OD\ negative\ control\ mean} \times 100 \quad (1)$$

The sample level inhibiting at least 50% of biofilm formation, iMBIC₅₀ (minimal biofilm inhibition concentration).

2.7. Polymicrobial biofilm test on catheter.

In the polymicrobial biofilm inhibition test, the catheter was cut 1 cm long, then sterilized in 96% ethanol, allowed to dry, and inserted into the wells [18,19]. A total of 100 µL of media containing bacterial suspension, normal human urine, and test compounds was added to each well of the microtiter plate that already included a catheter, then incubated at a temperature of $\pm 37^\circ\text{C}$ for 24 hours for the middle phase and 48 hours for the ripening phase. Meanwhile, in the biofilm eradication test, each well that had been catheterized was put in media containing a suspension of bacteria and normal human urine for 48 hours at 37°C. After the incubation period, the plates were washed using 150 µL of sterile distilled water. A total of 100 µL of media containing ethanol extract of Bajakah Tampala with a concentration series (1% - 0.125% w/v) was added to each well that had been washed and incubated for 48 hours

at 37°C. The catheter was then scraped and transferred to a new plate, and 125 µL of 1% crystal violet solution was added. Then, the biofilm was washed with running water, and 200 µL of 96% ethanol was added.

The biofilm eradication results were read using a microplate reader with an optical density (OD) at 595 nm. The test was carried out with three replications. The test was carried out in three repetitions. The inhibition percentage for each extract concentration was calculated using the formula below:

$$\%Inhibitor = \frac{OD\ negative\ control\ mean - OD\ test\ sample\ mean}{OD\ negative\ control\ mean} \times 100 \quad (2)$$

The sample level that can inhibit at least 50% of biofilm formation is considered MBIC₅₀ (minimum biofilm inhibition concentration), and the sample level that can degrade at least 50% of biofilm formation is regarded as MBEC₅₀ (minimal biofilm elimination concentration) [20,21].

2.8. Scanning electron microscopy (SEM) observation.

Observation of biofilms by scanning electron microscopy (SEM) was carried out in the laboratory. The coverslip was put into a 24-well round-bottom polystyrene microtiter plate containing the test suspension, treated in the same way as the biofilm formation inhibition test. After being incubated at 37°C for 24 hours, the coverslip was carefully washed three times with sterile distilled water, then fixed with 2.5% (v/v) glutaraldehyde in cacodylate buffer for ± 24 hours to kill the cells without changing cell structure to be studied. Furthermore, dehydration using ethanol was carried out for 30 minutes to reduce the water content and not interfere with the observation process. The sample was then observed under Scanning Electron Microscopy (SEM) with a voltage of 10 kV [22–24].

3. Results and Discussion

3.1. Bajakah Tampala sample setup.

The initial step of this study involved collecting samples, as illustrated in Figure 1 (Bark of Bajakah Tampala). The samples were gathered from a forested area in Berau Regency, East Kalimantan Province.



Figure 1. The sample of Bajakah Tampala.

3.2. Plant determination results.

The determination results show that the Bajakah Tampala used in the study can be ascertained to be of the type *Spatholobus littoralis* Hassk and the Leguminosae tribe.

3.3. Extract manufacture.

In this phase, the dried Bajakah Tampala bark enters the extraction process using the maceration method on the stem and bark, as is presented in Figure 2. The Batang and Bajakah Tampala bark powder was weighed and then macerated with methanol until the simplicia was utterly submerged in the solvent. Maceration was carried out for 3 x 24 hours and was repeated until a clear filtrate was obtained. The maceration results were then separated from the simplicia dregs using Whatman filter paper and then evaporated using a rotary vacuum evaporator at a temperature of 40-45°C at a speed of 65-90 rpm to obtain a thick extract.



Figure 2. Bajakah Tampala condensed extract.

3.4. TLC results.

This study aims to determine the class of compounds contained in the ethanol extract of Bajakah Tampala using the TLC (thin layer chromatography) method. Starting with Bajakah Tampala dissolved in ethanol solvent using hexane: ethyl acetate (3:1) mobile phase by observing using 254 nm and 366 nm UV lamps and silica gel GF254 plates. Each extract was tested by thin-layer chromatography (TLC) to determine the profile of the target compound. Chromatographic profiles obtained from the extract provide information on the target compound. The target compound was monitored from the spots on the chromatogram profile using specific reagents and general reagents. Specific reagents function to see certain groups of compounds and initial screening in preliminary tests to determine the activity of a compound, while general reagents function to know the complexity of compounds in the extract [25–28].

Figure 3 shows the chromatogram profile of the flavonoid compounds contained in the ethanol extract of Bajakah Tampala. The content of this flavonoid compound was indicated by the formation of blue-yellow spots under UV 366 (Figure 3.1b) and the formation of yellow spots after being sprayed with sitroborate (Figure 3.1c). This is by the statement [29] that flavonoids will have yellow, green, or blue fluorescence when observed under UV 366 light after being sprayed with sitroborate, and showing yellow spots under visible light after being sprayed with sitroborate. The value of the Retardation factor (Rf) of the Bajakah Tampala ethanol extract was 0.137 and 0.312, respectively. Furthermore, the ethanol extract in Bajakah

Tampala contains alkaloids (Figure 3.2). This is evidenced by detecting visible light (Figure 3.2a), which looks orange after being sprayed using Dragendroff. This follows the statement [29,30] that compounds containing alkaloids will appear orange to brown after being sprayed with Dragendroff reagent. The comparison used in detecting this alkaloid is quinine [7,16,31].

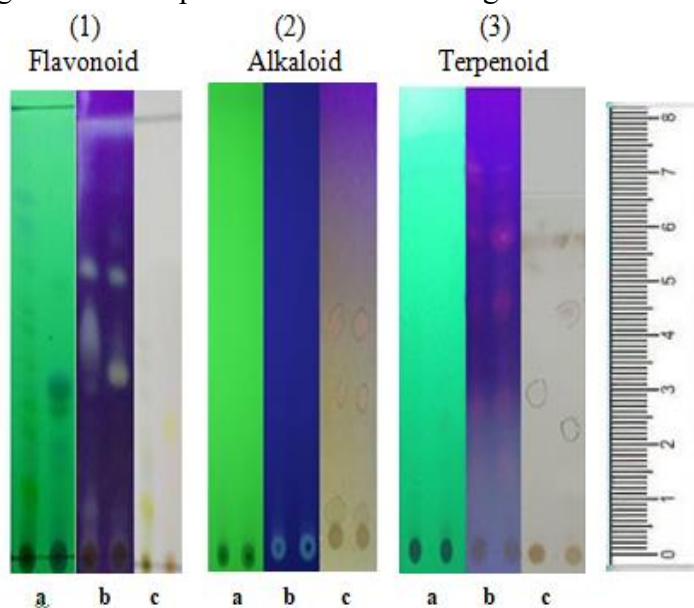


Figure 3. Chromatogram profiles of (1) flavonoids; (2) Alkaloids; (3) Terpenoids with (a) UV light at 254 nm; (b) UV light at 366 nm; (c) after spraying using specific reagents.

In the TLC test, the ethanol extract of Bajakah Tampala contains terpenoid compounds (Figure 3.3); this is indicated by the visible light observation showing a purplish red color after spraying anisaldehyde sulfuric acid (Figure 3.3a,b,c). At UV 254, observations (Figure 3a,b,c) showed blue fluorescence spots, while UV 366 (Figure 3.3a) showed red fluorescence spots. This follows the statement. That ulphate or anisaldehyde sulfuric acid will give a blue to blue-violet color, sometimes red, yellow, dark blue, purple, green, or yellow-brown in visible light. In this test, the detection of terpenoid compounds uses a comparison of thymol compounds. The value of the Retardation factor (R_f) of bajakah tampala extract was 0.375 and 0.55 [24,32,33].

3.5. Test of antibacterial activity of *Staphylococcus aureus*, *Escherichia coli*, and antifungal *Candida albicans* on ethanol extract of Bajakah Tampala.

Figure 4 shows that Bajakah Tampala ethanol extract can provide antibacterial activity against *S. aureus*, *E. coli*, and *C. albicans* at concentrations of 1% to 0.125%.

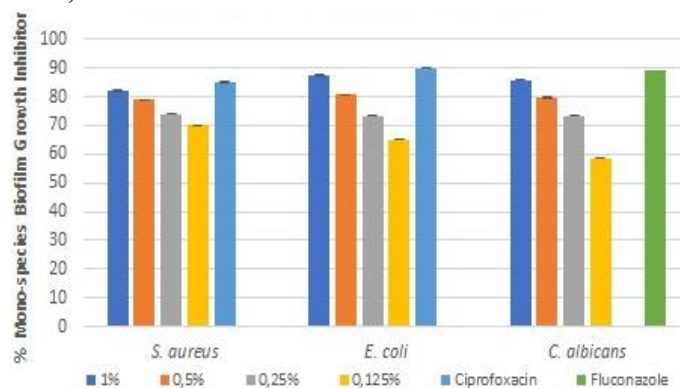


Figure 4. Antibacterial activity of *S. aureus*, *E. coli*, and antifungal *C. albicans* on ethanol extract of Bajakah Tampala.

The antibacterial activity of the best Bajakah Tampala ethanol extract was shown in the antibacterial activity of *E. coli* of 87.46%, *S. aureus* of 82.30%, and antifungal *C.albicans* of 85.77% at a concentration of 1% w/v.

3.6. The activity of Bajakah Tampala ethanol extract as a polymicrobial antibiofilm on catheters.

From Figure 5, can be observe in the middle phase (24 hours), Bajakah Tampala ethanol extract at a concentration of 1% – 0.125% w/v gave an inhibitory activity of polymicrobial antibiofilm on the catheter above 50%, where the most significant activity was at a concentration of 1% w/v of $73.46\% \pm 0, 01$ and higher than the control drug fluconazole by 55.70 ± 0.01 but lower inactivity than the control drug ciprofloxacin i.e., $74.72\% \pm 0.01$. In the maturation phase (48 hours), the 1% - 0.5% w/v pirated ethanol extract gave an inhibitory activity of polymicrobial antibiofilm on the catheter above 50%. A concentration of 1% gave the best activity of $70.24\% \pm 0.01$, and control fluconazole 1% w/v was $50.98\% \pm 0.01$, and control ciprofloxacin had an activity almost equivalent to $69.82\% \pm 0.01$.

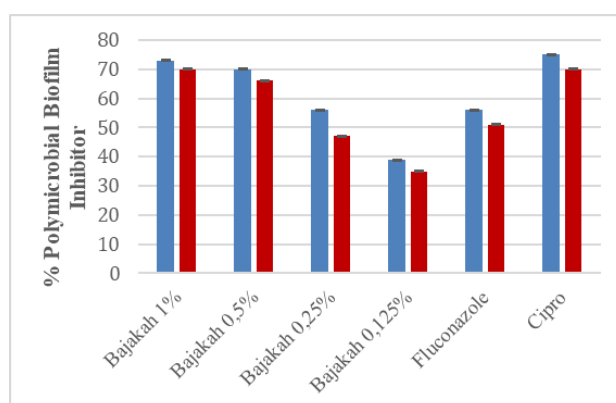


Figure 5. The activity of Bajakah Tampala ethanol extract as a polymicrobial antibiofilm on catheters. Blue = mid (24 Hours), Red = ripening (48 Hours).

These results show that during the ripening phase (48 hours), the Bajakah Tampala ethanol extract experienced a decrease in antibiofilm activity compared to the antibacterial phase, mono-species biofilm, and the intermediate phase (24 hours). In this phase, the biofilm-forming microbes were already attached to the substrate, so the Bajakah Tampala extract was challenging to kill the biofilm compared to the intermediate phase (24 hours). In the maturation phase, microbes form a strong biofilm defense system and build a cell communication mechanism called quorum sensing [17,34,35]. This follows Andersson's statement that the EPS matrix in biofilms can help cells survive longer by providing a protective layer against antibiotics [18].

3.7. Eradication activity of polymicrobial biofilm on a catheter from Bajakah Tampala ethanol extract.

Our results, represented in Figure 6, prove that Bajakah Tampala 1% w/v ethanol extract can eradicate 50% of polymicrobial biofilms on catheters composed of *S. aureus*, *E. coli*, and *C.albicans* by 55.13 ± 0.01 . The EPS matrix in biofilms can help microorganism cells survive longer than in planktonic conditions because they are organized/connected to form a kind of three-dimensional so-called cell communication [36,37]. The highest biofilm eradication activity on the catheter was seen at a concentration of 1% (w/v), with an eradication

activity of 55.13%, and the lowest biofilm eradication activity was at a concentration of 0.125% (w/v), with an eradication activity of 31.08%. Meanwhile, the control drug, fluconazole, was 48.25%, and ciprofloxacin was 68.10%.

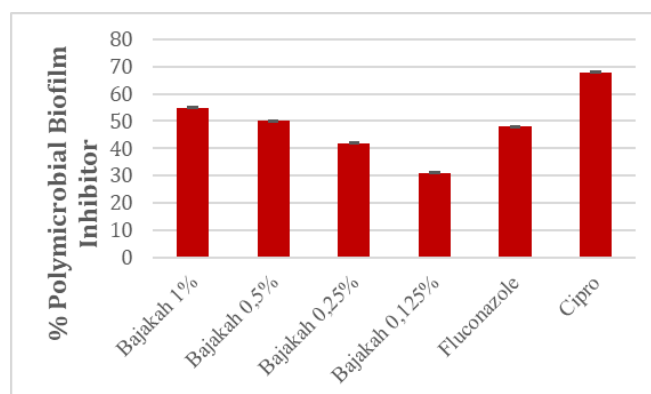


Figure 6. Polymicrobial eradication activity in Bajakah Tampala.

This study showed that the ethanol extract of Bajakah Tampala experienced a decrease in biofilm inhibitory activity compared to the intermediate (24 hours) and maturation phase (48 hours) of the catheter biofilm. This is because the biofilm growth time in this phase is much longer than the 24 and 48-hour phases [31,38,39]. So that the resulting matrix is more and more, which can be seen in the catheter ring, which is covered with biofilm characterized as a mucus layer; in this phase, the biofilm is formed in a complex and structured manner, so that the test compound finds it rather difficult to penetrate the biofilm. In addition, in this phase, the biofilm formation on the catheter is neatly arranged, and the nutrients produced are sufficient for life [40–42]. Cell communication between bacteria and fungi makes it difficult for the test compound to penetrate the biofilm defenses on the catheter protected by the EPS matrix. These results follow the statement of Hamzah that the biofilm formed on the catheter provides a complex community group and synergizes with each other, where the EPS matrix produced is so large that the drug is difficult to penetrate the target cells [11,43,44]. This study provides information that the ethanol extract of Bajakah Tampala provides evidence that it is capable of eradicating/degrading polymicrobial biofilms formed by *S. aureus*, *E. coli*, and *C. albicans* [45,46].

3.8. Scanning electron microscopy (SEM) test results.

In Figure 7, it is a polymicrobial biofilm grown on a catheter (without treatment).

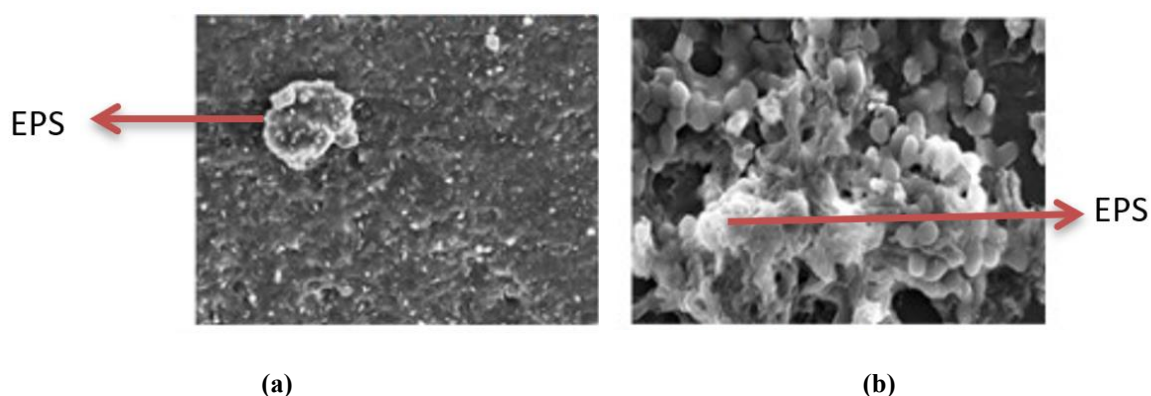


Figure 7. (a) Result of scanning electron microscopy biofilm with no treatment; (b) result of scanning electron microscopy biofilm with administration of Bajakah tampala extract.

Prior to the administration of 0.5% w/v Bajakah Tampala ethanol extract, bacterial cells were seen with thick, strong, smooth walls, and the formation of a biofilm matrix characterized by the presence of accumulating cells.

In Figure 8, after administering the ethanol extract with the patch 0.5% w/v against the polymicrobial on the catheter, it shows that cell damage occurs, so that the ethanol extract with the patch on the drug can enter or penetrate the cells and cause cell leakage, so that the biofilm cells become lysed or die.

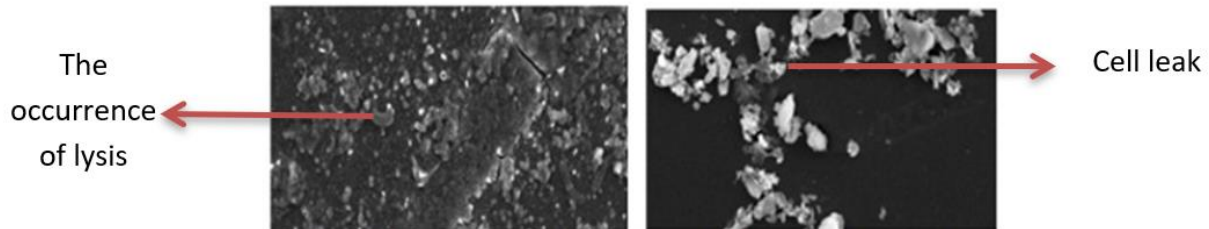


Figure 8. After giving Bajakah Tampala 0.5 w/v ethanol extract to the polymicrobial biofilm on the catheter.

4. Conclusions

The results of this study showed that the ethanol extract of Bajakah Tampala had antibacterial and antibiofilm inhibitory activity on the catheter in vitro on microorganisms *S. aureus*, *E. coli*, and *C. albicans* with the MBIC₅₀ value in the middle phase at a concentration of 0.25% w/v the gemstone phase was 0.5% w/v and the MBEC₅₀ value for eradication activity was at a concentration level of 1% w/v. The mechanism of action of the polymicrobial antibiofilm activity of Bajakah Tampala ethanol extract on the catheter is damaging the EPS matrix of the polymicrobial biofilm, causing cell leakage and lysis.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Institutional Review Board Statement

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Informed Consent Statement

Not applicable.

Data Availability Statement

Data supporting the findings of this study are available upon reasonable request from the corresponding author.

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Conflicts of Interest

The authors declare no conflict of interest.

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