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Research Article
Tracking of the Antibiofilm Activities of Lakum Leaf Extract (*Causonis trifolia* Linn.) Against *Staphylococcus aureus*

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Abstract

Background and Objective: Biofilms as a bacterial defense are relatively more difficult to eradicate with antibiotics, thus pathogenic bacteria in their biofilm form can cause serious problems for human health. Lakum *(Causonis trifolia* L.) is an herbaceous plant with many biological activities, one of which is an antimicrobial compound containing flavonoids, squalene, nimbidin, saponins, anthocyanins, tannins, myricetin, others. This study aimed to determine the antibiofilm activity of Lakum leaf extract against *Staphylococcus aureus* bacteria and the active compounds that play a role in inhibiting monomicrobial biofilms. **Materials and Methods:** This research method was carried out with an *in vitro* experimental study design using observations of phytochemical screening test results and the effectiveness of Lakum leaf antibiofilm on *Staphylococcus aureus* through microplate reader readings that measure optical density values. **Results:** This study showed that Lakum leaves contain alkaloids, flavonoids, phenolics, polyphenols, tannins and saponins. In addition, Lakum leaves gave biofilm inhibitory activity in the middle and maturation phase with the highest concentration in 1% extract of 76.95±0.0007 and 72.85± 0.0003%, respectively. Meanwhile, the lowest concentration was 0.125% extract of 65.65±0.0001% in the middle phase and 59.71±0.0003% in the maturation phase. **Conclusion:** That Lakum leaves have biofilm inhibitory activity on *Staphylococcus aureus* with flavonoid compounds, tannins and polyphenols that work as active substances in inhibiting the biofilm formation.

Key words: Antibiofilm, biofilm, *Causonis trifolia*, CAUTI, Lakum leaf, resistant

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In the world of health, precisely in medical care in hospitals, one thing that cannot be avoided by health workers and patients is infection. No matter how much the government and medical personnel minimize the occurrence of nosocomial infections inpatients, patient families and hospital workers, there is still a possibility that someone will be infected[1].

The nosocomial infection itself is an infection that someone gets from the hospital environment because the hospital itself is a place where all diseases gather. Although nosocomial infection is not a direct cause of death, in general, nosocomial infection is a very important concern because it is associated with morbidity and mortality[2].

One of the nosocomial infections is in the urinary system. The cause of this infection is usually medical equipment supporting patient care, namely a catheter commonly referred to as a Catheter-Associated Urinary Tract Infection (CAUTI). Catheters in hospitals are used to assist patients in draining urine out of the body. Patients who are usually given a catheter are unconscious patients, patients with urinary problems, surgery patients, injured patients and so on[3]. Because of this, the catheter is the most commonly used medical device worldwide. It is known that approximately 80% of urinary tract infections due to catheters are associated with biofilm formation[4].

In addition, according to Nicolle[4], biofilm is a factor in the occurrence of infections related to health care with a percentage of around 70-80%. The growth of biofilm on these catheters is also a contributing factor in the death of around 7500 people per year[5]. 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 on the catheter[6].

Even though it is a simple tool, this tool can provide considerable benefits for many people, especially patients in the hospital because it is a type of modern medicine and long-term use of a catheter can damage the natural defenses of the urinary tract. Thus the management of catheterized patients is often complicated by infection in which biofilm formation is a key feature[7]. The longer the catheter, the greater the possibility that bacteriuria will appear. Although not responsible for clinical emergencies, significant increases often occur in biofilm cells, resulting in bacteria becoming resistant to antibacterial agents[8]. According to Saint *et al.*[9], more than 100 million urethral catheters are sold annually. In addition, according to Tiwari and Mishra[10], more than 30 million urinary catheters are used annually in the United States.

As is generally known, Indonesia is a country rich in plants that can be used as traditional medicine. Even though modern medicine or synthetic medicine is still circulating in the market, the use of traditional medicine in Indonesia is progressing quite rapidly because it is an alternative treatment[11] because it has many advantages such as being easier to obtain, cheaper, easier to make yourself at home[12], derived from plants and natural ingredients also have side effects, but the level of danger and risk of long-term use is much lower than synthetic drugs[13].

Lakum plant (*Causonis trifolia* L.) is an herbaceous plant in the Vitaceae family that grows wild in many places such as yards, riverbanks, plantations, bushes, forests and so on, so this plant is very low to be endangered in the wild[14,15].

Lakum plants are plants that grow creeping or climbing with other plants as support[14]. It is known that all parts of the Lakum plant have biological activity as antidiabetic[16], fracture healing[17], antidiabetic[18], hyperlipidemia[19], antitumor[20], anticancer, hypoglycemic, antiviral, antiprotozoal, diuretic[21], anemia, anti-inflammatory, antimicrobial[22], antibacterial[23] and so on.

Lakum leaves (*Causonis trifolia* L.) contain flavonoids (cyanidin)[16], terpenoids, steroids, squalene, nimbidin and saponins[19], anthocyanins, vitamin C[22], tannins, stilbenes (piceid, resveratrol, viniferin, ampelopsin), hydrocyanic acid, delphinidin, kaempferol, myricetin, quercetin, triterpenes, epifriedelanol[16] and so on.

According to Hamzah *et al.*[24], biofilms can form on monomicrobial or polymicrobial bacteria. According to research conducted by Nurdin *et al.*[3], *Staphylococcus aureus* bacteria is one of the bacteria that causes biofilm formation based on the results of catheter urine culture as much as 45% of other bacteria. Based on research conducted by Opeña and Sotto[21], Lakum leaves have antimicrobial activity against *Staphylococcus aureus* bacteria. The study showed that the growth of *Staphylococcus aureus* bacteria was completely inhibited by Lakum leaf extract.

Until now, studies on the search for anti-biofilm compounds in catheters from plants are still limited, even though biofilm is a health problem throughout the world because effective and safe antibiotics have not been found to treat it. Therefore, based on the problems above, in this study, a search for new anti-biofilm agents from Lakum leaf extract was carried out against *Staphylococcus aureus* bacteria, which is one of the biggest causes of biofilms on catheters.

MATERIALS AND METHODS

Study area: This research was carried out in the Laboratory of the Universitas Muhammadiyah Kalimantan Timur, Faculty of Pharmacy and Department of Pharmacy, Islamic University of Indonesia from October, 2021 to May, 2022.

This research was carried out using an *in vitro* experimental study design using observations of the results of phytochemical screening tests to determine the content of secondary metabolites in Lakum leaf extract and to determine the effectiveness of Lakum leaf anti-biofilm (*Causonis trifolia* L.) on *Staphylococcus aureus* through microplate reader reading which measures the absorbance value of the bacteria. Plants based on their optical density values to measure the percent inhibition of biofilms on Lakum leaves[25].

Research tools and materials: In this study, the research equipment used was an oven (Bioevopeak), glass jar (Kedaung), wooden stirrer, measuring cup (Iwaki), filter paper, Erlenmeyer (Iwaki), funnel, porcelain cup, rotary evaporator (Buchi), water bath (Thermo), aluminum foil, plastic wrap, stainless basin, stir bar, microtube, brown paper, autoclave (All American, test tube (Iwaki), test tube rack, Petri dish (Iwaki), micropipette (Dragon Lab), micropipette tip, plate reader 96 wells (Iwaki), analytical balance (Fujitsu FS-AR210, tools glasses, incubators (Labnet), ose needles, Bunsen, micro readers (HiPo-Biosan and Lenovo), microscopes (Olympus), laminar air flow (LAF) (Bioevopeak).

While the materials used in this study were Lakum leaf extract (*Causonis trifolia*) *S. aureus* bacterial culture (ATCC 25923), sodium broth media (NB), sodium agar media (NA), sterile distilled water and crystal violet (1% in distilled water), 96% ethanol, Clindamycin, Mayer's reagent, Bouchardat's reagent, Dragendorff's reagent, Magnesium (Mg), concentrated Hydrochloric Acid (concentrated HCl), amyl alcohol, Liebermann's reagent and others.

Methods

Sampling: Lakum leaf samples (*Causonis trifolia*) were obtained from Tenggarong City, Kutai Kartanegara Regency, East Kalimantan Province, Indonesia as much as 3 kg with a total viscous extract obtained of 37.3 g and a yield of 9.18%.

Plant determination: Lakum plants, to be precise, Lakum leaves to be used in this study were determined based on the morphological characteristics of the plant. The plant determination process will be carried out at the Forestry Laboratory of Mulawarman University, Samarinda.

Making simplicia powder: The simplicia of the Lakum leaves that have been taken will be wet sorted to separate dirt or foreign materials and other unwanted plant parts from the simplicia. After carrying out wet sorting, proceed with washing, chopping and drying the simplicia. The simplicia drying process is carried out using the sun until the simplicia is completely dry (obtained moisture content $\leq$10%)[21,26,27].

Then after the simplicia drying process is complete, is continued by carrying out dry sorting to separate foreign materials and simplicia that have not dried properly so that simplicia is guaranteed to be completely free of foreign materials. After that, the simplicia pollination process was carried out using a blender[21,26,27].

Preparation of Lakum leaf extract (*Causonis trifolia*): Lakum leaves were extracted using the maceration method in which 200 g of powdered Lakum leaves were weighed and 2250 mL of 96% ethanol was added. Extraction was carried out for 3×24 hrs or for 3 days. After extraction, the maceration results were filtered to separate the soluble extract and filtrate. Then the extract dissolves in the rotary evaporator until the extract thickens slightly or the solvent and extract have separated sufficiently. After that, the results of the rotary evaporator extract were in a water bath until the extract thickened.

Phytochemical analysis testing

Alkaloid compounds:

- **Test filtrate:** Take sufficient extract and put it in a beaker glass or Erlenmeyer then adds 1 mL of 2N HCl and 9 mL of distilled water. After that, homogenize the solution while heating over a water bath for 2 min then cool and filter
- **Mayer's test:** Put ±2 mL of the test filtrate into the test tube. Then add 1-2 drops of Mayer's reagent into the tube. If a cloudy white or yellow precipitate forms in the solution, then the positive extract contains alkaloids
- **Bouchardat's test:** Put ±2 mL of the test filtrate into the test tube. Then a few drops of Bouchardat's reagent were added to the tube. If the solution produces a blackish-brown color, then the positive extract contains alkaloids
- **Dragendorff's test:** Put ±2 mL of the test filtrate into the test tube. Then added a few drops of Dragendorff's reagent. If an orange or brick-red precipitate forms in the solution, then the positive extract contains alkaloids
- **Wagner's test:** Put ±2 mL of the test filtrate into the test tube. Then a few drops of Wagner's reagent were added through the test tube wall. If the solution produces a reddish-brown color then the positive extract contains alkaloids

Flavonoid compounds:

- **Test with amyl alcohol:** Take a thick extract to taste and put it in a beaker glass or Erlenmeyer. Then added 10 mL of hot water and boiled for 5 min. Then after 5 min immediately filtered

After that, 5 mL of the test filtrate was added and 50 mg, 1 mL concentrated HCl and 2 mL of amyl alcohol were added to the test tube. After that, it was shaken and allowed to separate. If the solution produces a red or orange color in the amyl alcohol layer, then the positive extract contains flavonoids.

- **Test with 10% NaOH:** Add 10% NaOH reagent into the isolate that has been put in a test tube. If the solution has a specific color change, then the positive extract contains flavonoids

Phenolic compounds: As much as 50 mg of Lakum leaf extract was dissolved in 5 mL of distilled water. Then add a few drops of 5% neutral ferric chloride. If the solution produces a dark green color, then the positive extract contains phenolic.

Polyphenol compounds: Add 10% FeCl solution in distilled water. Then put it in the isolate that has been put in a test tube. If the solution produces a strong green, red, purple, blue, or black color, then the positive extract contains polyphenols.

Terpenoid compounds, sterols and steroids: Evaporate as much as 2 mL of the extract then the residue obtained from the evaporation process is dissolved again in 0.5 mL of chloroform, then added with 0.5 mL of anhydrous acetic acid. Furthermore, the solution was dripped with concentrated sulfuric acid as much as 2 mL through the wall of the test tube. If the solution produces a bluish-green color, then the positive extract contains terpenoids, sterols and steroids.

Another method for identifying these three compounds is by placing 1 mL of the extract solution in a test tube, then adding a few drops of Liebermann-Burchard's reagent. If the solution produces a green or blue color, then the positive extract contains steroids. If the solution produces a purple or red color, then the positive extract contains terpenoids.

Tannin compounds: Take enough thick extract and add 10 mL of distilled water. Then the solution was heated for 3 min and cooled. After cooling, the filtrate was filtered and diluted again. After filtering, put it in a test tube and add 1-2 drops of $FeCl_3$ reagent. If the solution produces a black-green color, then the positive extract contains tannins.

Another method for identifying these tannin compounds is by inserting $\pm$2 mL of the filtrate into a test tube. Then 2-3 drops of 1% FeCl solution were added. If the solution produces a green or bluish-black color, then the positive extract contains tannins.

Saponin compounds: Take enough thick extract and add 10 mL of hot distilled water (hot water). Then the filtrate was cooled and shaken vigorously. If the solution produces foam after shaking and does not disappear for $\geq$5 min, then the positive extract contains saponins.

Preparation of bacteria for assay: *Staphylococcus aureus* bacteria were cultured in sodium broth (SB) medium and incubated for 24 hrs at 37°C. The optical density of 600 of the bacterial culture is adjusted to the McFarland 0.1 standard, namely 0.5-1.5$\times 10^8$ CFU mL^{-1} by looking at the level of bacterial density using the help of a spectrophotometer. If the density of *Staphylococcus aureus* bacteria cells is not in accordance with the McFarland standard, then the bacterial media needs to be diluted using new growth media until the level of bacterial density meets the McFarland standard, which was OD_{600} 0.01. If the cell density was in accordance with the McFarland standard, then the biofilm test bacteria can be used for testing[28-31].

Assay of biofilm inhibitory activity: A total of 5 µL of microbial suspension (10^7 CFU mL^{-1}) and 75 µL of media containing test extracts with concentration series (0.125, 0.25, 0.5 and 1% w/v) were added to each well of the microtiter plate. In addition, 20 µL of aquadest control, 75 µL of media control, ethanol control and drug control in other wells were given as a comparison of the test results. Once ready, the plates were incubated for 24 and 48 hrs[28-31].

After completion of incubation for a predetermined time, the plate is washed using sterile aquadest three times to remove cells that are not attached. Then add 100 µL of 1% crystal violet to each well filled with the test sample and let it stand for 15 min. After that, the crystal violet solution was removed from the plate and then washed again using sterile aquadest three times. After washing, 100 µL of 96% ethanol was given to each well filled with the test sample and then tested the sample in a microreader[28-31].

As a control media, media without microbial growth was used and microbial suspensions were used as growth controls. As a drug control, a microbial suspension was used which was given Clindamycin at a rate of 1% w/v. The plates were then incubated at 37°C for 24 and 48 hrs. After that, the plate was washed using aquadest three times. To remove excess water after washing, the plates were dried for 5 min at room temperature[28-31].

Then, 125 µL of 1% crystal violet was added to each well to give color to the biofilm that formed. In this case, the building blocks of biofilms are dead cells and living cells. After that, the plate was incubated for 15 min at room temperature. After incubation, the microplates were washed again with running water to clean the remaining crystal violet and to dissolve the biofilm that formed, 200 µL of 96% ethanol was added to each well[28-31].

Optical density (OD) readings were carried out with a microplate reader at a wavelength of 620 nm. The test was carried out with three replications. The data obtained from the analysis of biofilm inhibition was in the form of OD values for each concentration of the test compound and the control without the test compound (growth control) obtained from readings with a microplate reader[28-31].

To determine the percentage of bacterial inhibition, the calculation used the OD value from the research analysis using the following equation[28-31]:

$$\text{Inhibition (\%)} = \frac{\text{Negative control mean OD-Test sample mean OD}}{\text{Negative control mean OD}} \times 100$$

The concentration of the sample which can inhibit at least 50% of biofilm formation is considered $MBIC_{50}$ (Minimal Biofilm Inhibition Concentration)[28-31].

Statistical analysis: The research data obtained were subjected to statistical testing in the form of the One Way ANOVA test. Before carrying out the test, a normality test will be carried out with a value of $p \geq 0.05$ to ensure that the data is normally distributed and a variance test is carried out, so that the data must be homogeneous.

RESULTS AND DISCUSSION

Phytochemical screening of Lakum (*Causonis trifolia*) leaves: Phytochemical screening of Lakum leaf extract was carried out to find out which groups of compounds it contains. The compounds tested were alkaloids, flavonoids, phenolics, polyphenols, triterpenoids, sterols, steroids, tannins and saponins. The results of the phytochemical screening of Lakum leaves can be seen in Table 1.

Based on the results of the research conducted, Lakum leaves (*Causonis trifolia*) taken from Tenggarong District, Loa Ipuh Village, Tenggarong City, which grows wild in the plantations and settlements of the Tenggarong people, tested positive for containing alkaloids, flavonoids, phenolic compounds, polyphenols, tannins and saponins. This was in accordance with the statements of some researchers[21,32-34].

However, the leaves of Lakum (*Causonis trifolia*) are negative for triterpenoids, sterols, steroids and steroids. In this case, there are several factors that affect the absence of these compounds even though in some areas these compounds are contained in the same plant.

Factors that affect this are the age of the plants taken, the living environment of the plants[27] the location or altitude[35,36] and the nutrient content of the soil where the plants grow[35], the method of drying or the extraction process[37], the type of solvent used for the extraction process[37] and so on.

Table 1: Results of Lakum leaf phytochemical screening test (*Causonis trifolia* L.)

Compound	Reagents/reactants	Test results	Information
Alkaloid	Mayer	+	Produces a white precipitate
	Bouchardat	+	Produces a dark brown precipitate
	Dragendorff	+	Produces a brick red precipitate
	Wagner	-	Does not produce a white precipitate
Flavonoid	Concentrated (mg) amyl alcohol (HCl)	+	An orange layer forms on the amyl alcohol
	10% NaOH	+	Produces a greenish yellow color
Phenolic	Ferric chloride	+	Produces a dark green color
Polyphenol	10% FeCl	+	Produces a deep green color
Triterpenoid	Liebermann-burchard	-	Did not experience any discoloration
	Chloroform, anhydrous, concentrated H_2SO_4	-	No greenish/brownish ring is formed
Sterols	Liebermann-Burchard	-	Did not experience any discoloration
	Chloroform, anhydrous, concentrated H_2SO_4	-	No greenish blue ring is formed
Steroids	Liebermann-Burchard	-	Did not experience any discoloration
	Chloroform, anhydrous, concentrated H_2SO_4	-	No greenish/brownish ring is formed
Tannin	$FeCl_3$	+	Produces a dark green or blackish green color
	1% FeCl	+	Produces a deep green color
Saponin	HCl 2N	+	Produces foam after shaking and does not dissipate for $\geq$5 min

Table 2: Antibiofilm results of Lakum leaf extract (*Causonis trifolia* L.) against *Staphylococcus aureus* bacteria in the middle phase (24 hrs) and maturation phase (48 hrs)

Sample	Middle phase (24 hrs)	Maturation phase (48 hrs)
Negative control (pure bacteria)	0.00%	0.00%
Positive control (clindamycin 1%)	80.90%	77.30%
Aquadest control	4.98%	2.78%
Ethanol control	4.23%	3.39%
Media control	3.05%	2.15%
Extract 1%	76.95%	72.85%
Extract 0.5%	73.60%	68.89%
Extract 0.25%	68.50%	63.18%
Extract 0.125%	65.65%	59.71%

Results of antibiofilm activity against *Staphylococcus aureus*: In this study, the anti-biofilm potential of *Causonis trifolia* L. was tested in the inhibition phase of the biofilm against *Staphylococcus aureus* bacteria. The results obtained were that the Lakum leaf extract had an inhibitory effect on the growth of biofilms in the 48 hrs or maturation phase. The results of Lakum leaf anti-biofilm against *Staphylococcus aureus* bacteria can be seen in Table 2 and Fig. 1.

The anti-biofilm potency test of Lakum leaves (*Causonis trifolia* L.) was carried out during the maturation phase, namely the monomicrobial biofilm inhibition test against *S. aureus* bacteria. Lakum leaf extract exhibited biofilm inhibitory activity in the mid-phase (24 hrs) and the maturation phase (48 hrs) at all extract concentrations ranging from 1-0.125% w/v.

The highest inhibitory activity of Lakum leaf extract (*Causonis trifolia* L.) was in the mid and maturation phase in this study at 1% extract concentration, namely 76.95±0.0007 and 72.85±0.0003%. Meanwhile, the lowest biofilm inhibition activity was at a concentration of 0. Extract 0.125%, namely 65.65±0.0001% in the mid-phase and 59.71±0.0003% in the maturation phase.

In the control drug, Clindamycin 1% alone gave biofilm inhibitory activity in the mid-phase of 80.9±0.0004% and the maturation phase was 77.3±0.0004%. Based on the results of this study, the $MBIC_{50}$ value of the ethanol extract of Lakum leaves (*Causonis trifolia* L.) was at a concentration of 0.125% w/v in the maturation phase, which was 59.71±0.0003%. Meanwhile, the MIC value of this extract was found at the lowest concentration, namely 0.125% with the inhibition in the mid-phase (24 hrs) and maturation (48 hrs) of 65.65±0.0001 and 59.71±0.0003%.

According to Gosal *et al.*[38] and Elisabeth[39], there is greater inhibition in the middle phase compared to the maturation phase. This was because the biofilm EPS (extracellular polymeric substances) matrix produced in the middle phase is not as complex and structured as in the maturation phase so the ethanol extract of Lakum leaves inhibits the growth of biofilms more than in the maturation phase.

According to Elisabeth[39], during the maturation phase (48 hrs) the formation of biofilm cells has produced EPS which adheres to the surface of the bacterial cells and attaches to a collection of *Staphylococcus aureus* bacteria to form microcolonies. According to Purbowati[40], Kannappan *et al.*[41] and Hamzah *et al.*[42], during the maturation phase (48 hrs) biofilms will decrease anti-biofilm activity compared to the mid-phase (24 hrs). This was because the growth time of biofilms in the maturation phase is longer and the biofilm defense system will form a stronger defense system and biofilm cells will continue to grow for several hours[43,44].

Biofilm growth during the maturation phase results in a thicker and more complex cell structure when compared to the mid-phase. The mucus layer produced in the maturation phase is a difference in cell structure that can be seen because in this phase it can be seen that the mucus layer is more numerous and thick and is attached to the wells. This is what makes it difficult for some antibiotics to penetrate the EPS biofilm layer. In addition, according to Achinas *et al.*[44] and Muhammad *et al.*[45], the increase in biofilm EPS production occurs due to slow bacterial growth. This was due to the hydration of biofilm EPS which prevents dryness in some natural biofilms. This EPS also contributes to the antimicrobial resistance of the biofilm which causes obstructs the mass transport of antibiotics through the biofilm[46,47].

Even so, the inhibitory activity of the ethanol extract of Lakum leaves (*Causonis trifolia* L.) gave a good biofilm inhibitory activity in the mid-phase of 76.95±0.0007% and this result was almost equivalent to the control drug Clindamycin 1%, which was 80.9+0.0004%. In addition, in the maturation phase, Lakum leaf extract still provided good biofilm inhibition activity, although not as large as in the middle phase, which was 72.85±0.0003% and these results were close to the control of clindamycin 1% which was 77.3±0.0004%. Thus it was stated that the ethanol extract of the leaves of Lakum (*Causonis trifolia* L.) had biofilm inhibitor activity until the maturation phase.

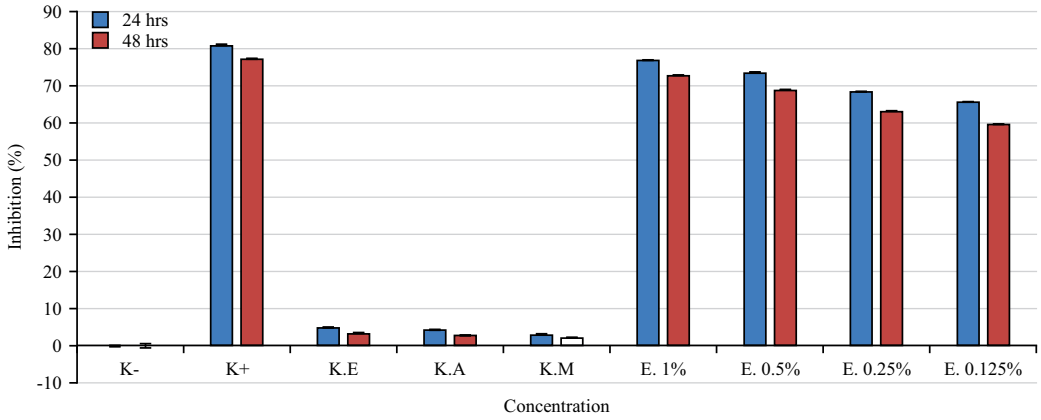

Fig. 1: Antibiofilm activity of Lakum leaf extract (*Causonis trifolia* L.) on *Staphylococcus aureus* bacteria
Blue: Middle phase (24 hrs), Red: Maturation phase (48 hrs), K-: Negative control, K+: Positive control, K.E: Ethanol control, K.A: Aquadest control, K.M: Media control, E. 1%: Extract 1%, E. 0.5%: Extract 0.5%, E. 0.25%: Extract 0.25% and E. 0.125%: Extract 0.125%

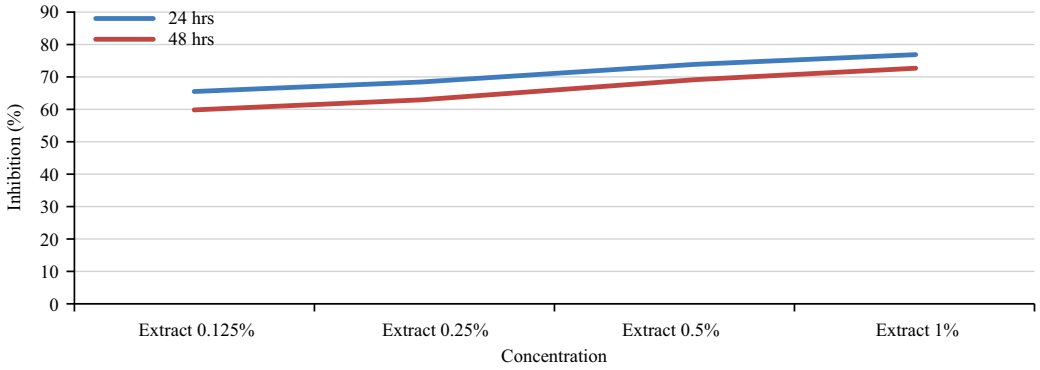

Fig. 2: Graph of antibiofilm activity results of Lakum leaves (*Causonis trifolia* L.) against *Staphylococcus aureus* bacteria
Blue: Middle phase (24 hrs) and Red: Maturation phase (48 hrs)

According to Sari *et al.*[33], the ethanol extract of Lakum leaves has a very strong inhibitory effect on the growth of *Staphylococcus aureus* bacteria. At a concentration of 25%, Lakum leaf extract had an inhibition of 25.65 mm and at the lowest concentration of 10% it was 21.08±1.50 mm. Meanwhile, according to Opeña and Sotto[21], Lakum leaf extract has *Staphylococcus aureus* antibacterial activity with an inhibition zone of 12 mm. In addition, according to Princeton *et al.*[47] Lakum leaf extract has antibacterial activity against *Staphylococcus aureus* with the highest inhibition zone of 25.33 mm and the lowest inhibition zone produced is 18.33 mm.

However, in this study, previous research as research literature regarding the biofilm inhibition test of Lakum leaf extract did not yet exist and this research is the first study regarding testing the anti-biofilm activity of Lakum leaf extract against *Staphylococcus aureus* bacteria.

However, in this study, there were several secondary metabolites that played the most active role in inhibiting monomicrobial biofilms on *Staphylococcus aureus* bacteria. In general, the mechanism for inhibiting biofilm growth is by means of active compounds interfering with communication signals (Quorum sensing) between bacteria that work to form biofilms by penetrating the bacterial cell wall resulting in the inactivation of the genes in the bacteria that form EPS synthesis so that the EPS layer cannot be formed[48,49].

Whereas specifically, according to Ma *et al.*[49] flavonoids and tannins play an important role in inhibiting biofilm formation. In the formation of *Staphylococcus aureus* biofilm, regulation occurs through ica-dependent biofilm production. In this regulation, flavonoid and tannin compounds directly inhibit the expression of the icaA and icaD genes[49,50] where these genes regulate the formation of polysaccharide intercellular adhesin (PIA) by activating sigma factor

σ^B which can activate staphylococcal accessory regulator A (sarA). If sarA is activated, then the ica promoter will also be activated[51,52]. Apart from being able to inhibit the formation of biofilms, flavonoids and tannins also play an active role as antibacterials in which these two compounds work by inhibiting bacterial cell adhesion which is the main factor in the formation of biofilms in the process of attaching bacteria to substrate surfaces and between bacteria[50].

In addition, Jagani *et al.*[52] and Yamanaka-Okada *et al.*[53] stated that flavonoids and tannins are a class of polyphenolic compounds that play an active role in the formation of biofilms where these compounds work directly on an important factor, namely the process of bacterial cell adhesion to the substrate where this compound will reduce the hydrophobic nature of the bacteria so that the formation of biofilms becomes inhibited.

During the research, there were some research limitations occurred. The first limitation of this research is the lack of sources of previous research regarding the anti-biofilm activity of Lakum leaf extract against *Staphylococcus aureus* bacteria, so there is no source for comparison of test results. Apart from that, due to the COVID-19 pandemic, the duration of the laboratory's working hours was cut so that the research process could not run long and freely in one day. In addition, due to the limited tools and materials used in the laboratory. So that when purchasing the necessary tools and materials, you have to wait some time until the research can be continued with the appropriate tools and materials.

CONCLUSION

Lakum leaves (*Causonis trifolia* L.) originating from Tenggarong Sub-District, East Kalimantan is known to contain positive alkaloids, flavonoids, phenolics, polyphenols, tannins and saponins. However, the Lakum leaves were declared to have no triterpenoid, sterol or steroid compounds using 2 phytochemical tests. Lakum leaves (*Causonis trifolia* L.) originating from the Tenggarong Sub-District, East Kalimantan was stated to be able to provide biofilm inhibition activity in the mid-phase with a large percentage of inhibition produced at 0.125-1% w/v of 65.65±0.0001, 68.5±0.0009, 73.6±0.0004 and 76.95±0.0007%. While in the maturation phase with the same concentration, it was 59.71±0.0003, 63.18±0.0006, 68.89±0.0003 and 72.85±0.0003%. The percentage of inhibition produced by the Lakum leaf extract was almost equivalent to the control drug clindamycin 1% which provided percentage of inhibition of the biofilm against *Staphylococcus aureus* bacteria by 80.9±0.0004% in the middle phase and by 77.3±0.0004% in the maturation phase. The active compounds that work as biofilm inhibitors are flavonoids, tannins and polyphenols.

SIGNIFICANCE STATEMENT

This research was conducted to obtain the results of the anti-biofilm activity in Lakum leaf extract against *Staphylococcus aureus* bacteria and active compounds that play an important role in inhibiting monomicrobial biofilms. Lakum leaves have biofilm inhibitory activity against *Staphylococcus aureus* with flavonoids, tannins and polyphenols which work as active substances in inhibiting the formation of biofilms where biofilms become one of the nosocomial infections in the urinary tract system called Catheter-Associated Urinary Tract Infection (CAUTI). It is known that approximately 80% of urinary tract infections due to catheters are related to biofilm formation.

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