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Effectiveness of Etanol Extract Bajakah Wood (*Spatholobus sp.*) on the Growth of *Staphylococcus* aureus ATCC 25923

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ABSTRACT

Background: Infectious diseases are still an important issue for Indonesia. One of the opportunistic bacteria that causes various infectious diseases is *Staphylococcus aureus*. There is a need for new alternative treatments that have the same high potential as antibiotics. Bajakah wood (*Spatholobus sp*.) is a plant that grows abundantly in the tropical forests of Central Kalimantan. This plant has the potential antibacterial potential because it contains secondary metabolite compounds, namely alkaloids, tannins and glycosides.
Objective: This study aims to determine the effectiveness of Bajakah wood (*Spatholobus sp*.) on the growth of *S. aureus* ATCC 25923 bacteria through antimicrobe sensitivity test.
Methods: In this study, three different methods were used, the disc method, the well method and the *Whatman* paper method using a concentration of 5%; 15%; 25%; 50% and 100% extract.
Results: The results showed that the ethanol extract of Bajakah Wood (*Spatholobus sp*.) had antibacterial activity against *S. aureus* ATCC 25923 at the lowest concentration, namely 5%. The disk method obtained an average inhibition zone of 11.1 mm, the well method obtained an average inhibition zone of 16.8 mm and the *Whatman* paper method obtained an average inhibition zone of 10.8 mm.
Conclusion: From this study, the ethanol extract of Bajakah wood (*Spatholobus sp*.) has antibacterial potential against *S. aureus* bacteria with its secondary metabolite compounds.

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1. Introduction

Staphylococcus aureus remains one of the pathogenic bacteria commonly involved in infectious diseases worldwide[1]. *S. aureus* is a type of bacteria that has gram-positive properties, has a round shape, and has a size of about 0.5-1.5 µm. These bacteria appear bluish purple with Gram stain, do not form spores, and are non-motile or immobile[2]. *S. aureus* is also opportunistic which is one of the causes of various infectious diseases[3]. Transmission of this bacterial infection can be through direct contact with sufferers, using shared personal items, or food contamination[2].

Cases of infection caused by *S. aureus* bacteria continue to increase along with the problem of antibiotic resistance for the treatment of these bacteria. The most effective antibiotic and often used to treat *S. aureus* bacterial infections is amoxicillin because of its excellent peroral absorption. However, after 1942, cases of *S. aureus* resistance began to be documented, after which these cases increased. The resistance of *S. aureus* to methicillin (penicillin group) is called *Methicillin-Resistence Stpahylocuccus aureus* (MRSA). More than 86% of cases of S. aureus resistance to the penicillin group[3].

New alternative treatments are needed that have the same high potential as antibiotics. One of them is by utilizing medicinal plants as herbal medicines. Bajakah wood (*Spatholobus sp*.) is one of the plants that grow in the tropical forests of Central Kalimantan. Bajakah (*Spatholobus sp*.) is a Liana class plant, namely vines on other plants that can be found growing naturally in Central Kalimantan. Lianas have distinctive characteristics in tropical rainforest ecosystems, where these plants form a crown layer in the middle of the forest. Bajakah roots grow through various types of trees until they reach the crown layer and get maximum sunlight[4]. Bajakah has a single brown tree morphology, growing up to the top of the tree it is climbing to a height of 50 meters. The trunk is porous, large, strong and sturdy. There are 29 genus *Spatholabus* growing spread in the tropical forests of Indonesia[5]. Bajakah has various types, including those that have been studied are bajakah tampala, bajakah lamei, and bajakah kalawit[6].

Many of the local people use Bajakah wood as a medicine to restore energy when doing activities in the forest[7]. The chemical compounds contained in Bajakah

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Wood can have the effect of increasing the body's immunity, so it has the potential to be developed as an herbal medicine[8]. The results of phytochemicals carried out on bajakah wood and root extracts are that this plant contains secondary metabolites in the form of active compounds including alkaloids, flavonoids, and terponoids[9-10]. Flavonoids function as antioxidants against bacteria and pathogens[11].

2. Methods

This study used an experimental post-test only control group design, conducted by comparing the experimental group with the control group. Measurement of the experimental group was carried out after the group was treated, namely with five treatment concentrations of 5%, 15%, 25% 50%, and 100%. Then compared with the control group which was given amoxicillin disk 25 µg. The method used to extract Bajakah Wood (*Spatholobus sp*.) is by maceration method using 70% ethanol solvent. The method used to test antibacterial activity is the disc method, the well method and the *Whatman* paper method.

The tools used in this study are Laminar Air Flow, digital scales, magnetic stirrer, petri dish, jar bottle, ose, tweezers, autoclave, incubator, erlenmeyer, sterile cotton swab, Mc Farland densitometer, marker, sterile gloves, and digital caliper. The materials used were distilled water, paper discs, amoxicillin discs, aluminum foil, Mc Farland solution, Mueller Hinton Agar (MHA) media, Bajakah Wood (*Spatholobus sp*.), *S. aureus* ATCC 25923 bacterial culture, and 70% ethanol solvent. The sample used in this study was Bajakah wood (*Spatholobus* sp.) found in Longkang, Jaar Village, East Dusun District, East Barito Regency, Central Kalimantan.

A. Preparation of Bajakah Wood Simplisia

The process of making simplisia is carried out by separating the skin on Bajakah Wood then washed using clean water and drained. Next, the wood is cut into small pieces and dried in the sun. The dried Bajakah wood was then crushed into powder using a blender.

B. Phytochemical Screening of Bajakah Wood

In this study, a phytochemical testing process was carried out to determine the content of secondary metabolite compounds contained in Bajakah Wood (*Spatholobus sp*.). Testing alkaloid compounds by taking 1 ml of ethanol extract of Bajakah Wood and adding 1 ml of HCl into a test tube. Homogenize the solution. Add a few drops of Dragendroff reagent to the solution. Observe the changes that occur, if an orange-brown precipitate forms, it shows a positive result.

Alkaloid testing is done by taking 0.2 ml of Bajakah Wood ethanol extract and placing it in a test tube. Add 1 ml of Folin-Ciocalteulalu reagent to the test tube. Then add 15.8 ml of distilled water. Homogenize the solution. Let the solution stand for 8 minutes. Add 3 ml of 10% Na_2CO_3 solution to the mixture. The 10% Na_2CO_3 solution is obtained by dissolving 10 grams of Na_2CO_3 in 100 ml of distilled water. Let the solution stand at room temperature for one hour. Then use a UV-Vis spectrophotometer to measure the maximum wavelength of the solution. The wavelength that is generally used to measure phenol content is 725 nm. Repeat 3 times to obtain the average value and obtain the desired phenol content. Observe the color change that occurs after the reaction. If the resulting color is blue, red, purple, green, blackish green, or blackish blue, it shows a positive result[12].

Testing phenolic compounds by taking 1 ml of ethanol extract of Bajakah Wood and adding 10 ml of distilled water into a test tube. Cool the solution. Shake the solution vigorously for 10 seconds to produce foam. Let the solution stand for about 10 minutes, a stable foam will form. Add 1 drop of 2 N HCl to the test tube. Observe the changes that occur, if the foam does not disappear or remains stable after adding HCl, it shows a positive result[13].

Testing for tannin compounds by taking 6 ml of ethanol extract of Bajakah wood and heating the solution using a heater. Divide the solution into three different test tubes, with each tube having the same volume. Add 1% NaCl solution into the first tube. Add 1% NaCl solution and 5% gelatin into the second tube. Add 1% FeCl3 solution into the third tube. Observe the changes that occur after the addition of these substances. The result shows positive if in the second tube a white precipitate forms and in the third tube a color change to purple or blue-black[13].

C. Preparation of Bajakah Wood Ethanol Extract

The extraction method used in this study is the maceration method with 70% ethanol solvent. Bajakah wood simplisia powder was put into a maceration vessel and 70% ethanol solvent was added to the vessel in a ratio of 1:10 (simplisia powder: ethanol) or until fully submerged. Then homogenize for 2-3 hours and covered with aluminium foil, left for 3x24 hours and stored in a place that is not exposed to direct sunlight. After the soaking process is complete, the mixture of simplisia and ethanol is then filtered to separate the filtrate from the insoluble simplisia powder. Using a rotary evaporator evaporate the filtrate at 50°C. The result is a thick brown extract[14].

D. Preparation of Bajakah Wood Ethanol Extract Suspension

The extract suspension was diluted using distilled water and divided into 5 concentrations, namely 5%, 15%, 25%, 50%, and 100%.

E. Preparation of *S. aureus* ATCC 25923 Bacterial Suspension

S. aureus ATCC 25923 bacteria were inoculated from pure culture using a sterile ose into a tube containing physiological NaCl solution. Then stirred gently to suspend the bacteria with physiological NaCl solution until the turbidity is similar to Mc Farland solution which is the turbidity standard of the bacterial suspension to be used.

F. Preparation of MHA (*Mueller Hinton Agar*) Media

The agar powder was dissolved with distilled water and stirred until there were no lumps or sediment. The container is covered with aluminium foil and sterilized using an

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autoclave at 121°C within 3 hours. Next, the media was allowed to warm up (around 50°C), then poured into Petri dishes and allowed to solidify at room temperature.

G. Antibacterial Activity Test

The disc method was performed by soaking the disc paper in each concentration for 15 minutes. The disc paper and amoxicillin disk as control were then placed on the surface of MHA media that had been inoculated with *S. aureus* ATCC 25923 bacteria.

The wells method was performed by making holes in the MHA media that had previously been inoculated with *S. aureus* ATCC 25923 bacteria using a sterile plastic pipette where the tip had been cut first. Then the holes were injected with ethanol extract of Bajakah Wood (*Spatholobus sp*.) at each concentration of 5%; 15%; 25%; 50% and 100%.

The *Whatman* filter paper method is done by folding the filter paper three times and then perforating it using a hole punch to form a disk with three layers. The disk was then soaked for 15 minutes in the ethanol extract of Bajakah Wood (*Spatholobus sp.*) at each concentration. Next, the disk was placed on the surface of MHA media that had been inoculated with *S. aureus* ATCC 25923 bacteria.

All Petri dishes were closed and incubated at 37°C for 24 hours. After incubation, the inhibition zone formed around the hole was measured using a digital caliper in millimetres.

3. Result

This study used an experimental post-test only control group design. This study was conducted after obtaining ethical approval from the Ethics Commission of the Faculty of Medicine, University of North Sumatra with letter number 839/KEPK/USU/2023. This study was conducted from August to October 2023 in a span of three months. The preparation of ethanol extract of Bajakah Wood (*Spatholobus sp*.) was carried out at the Organic Chemistry Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, and testing of ethanol extract of Bajakah Wood (*Spatholobus sp*.) against *S. aureus* ATCC 25923 bacteria was carried out at the Microbiology Laboratory, Faculty of Medicine, Universitas Sumatera Utara.

Before the extract preparation process, phytochemical screening of Bajakah Wood was carried out to determine the content of secondary metabolite compounds contained. The table below shows the results of phytochemical screening that Bajakah Wood samples contain alkaloids, tannins, and glycosides.

Table 1. Bajakah Wood Phytochemical Screening Results

No	Secondary Metabolite Compounds	Reactor	Results
1.	Alkaloid	Bouchardart	+
		Maeyer	+
		Dragendroff	+
		Wagner	+
2.	Steroida and Triterpenoid	Salkowsky	-
		Lieberman-Burchad	-
3.	Saponin	Akuadest+Alkohol 96%	-
4.	Flavonoida	$FeCl_3$ 5%	-
		$Mg_{(s)} + HCl_{(p)}$	-
		NaOH 10%	-
		$H_2SO_{4(p)}$	-
5.	Tanin	$FeCl_3$ 1%	+
6.	Glikosida	Mollish	+

(-) No Secondary Metabolite Compound Detected
(+) Secondary Metabolite Compound Detected

The extraction method used in this study is the maceration method with 70% ethanol solvent. The symplisia used was 2 kg of Bajakah wood powder (*Spatholobus sp*.) and a total of 20 L of 70% ethanol solvent. The yield value obtained can be seen in the table below.

Table 2. Weight of Bajakah Wood Extract

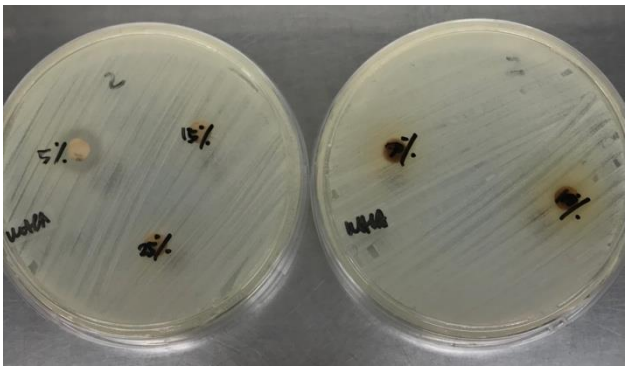
Sample	Type of Solvent	Weight of Dry Sample	Weight of Extract	Yield
Bajakah wood	Etanol 70%	2 kg	386 g	19,3%

Making extracts with maceration techniques is done by adding 70% ethanol in a ratio of 1: 10 (simplisia powder: ethanol) to the simplisia. The mixture of simplisia and ethanol is then filtered using filter paper to separate the filtrate from the simplisia powder. The filtrate was then evaporated using a rotary evaporator and water bath to accelerate the separation of the solvent from the extract.

In the study, the measurement of antibacterial activity used the diffusion method, namely the disc method, the well method and the *Whatman* paper method by calculating the diameter of the inhibition zone formed on the surface of the media. The results obtained are classified based on the classification of inhibition response; 21-30 mm diameter has a strong growth inhibition response, 11-20 mm diameter has a moderate growth inhibition response, and 1-10 mm diameter has a weak growth inhibition response[15].

A. Test of antibacterial effectiveness of bajakah wood (*Spatholobus sp*.) against the growth of bacteria *S. aureus* ATCC 25923 with disc method

In testing the antibacterial activity of ethanol extract of Bajakah Wood (*Spatholobus sp*.) against bacteria *S. aureus* ATCC 25923 by disc method at concentrations of 5%; 15%; 25%; 50% and 100% the inhibition zone arises only at the lowest concentration of 5% with an average diameter of 11.1 mm (in table 3). At other higher concentrations, the inhibition zone did not appear at all. When viewed in the inhibition zone response, the 5% concentration with the disc method has a moderate response in inhibiting the growth of *S. aureus* ATCC 25923 bacteria[15].



(a)

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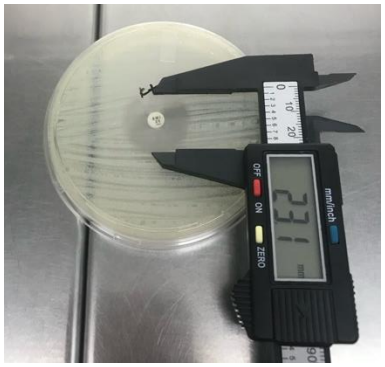

(b)

Figure 1. (a) The results of the diameter of the inhibition zone of the ethanol extract of Bajakah Wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 in the second repetition using the disc method. Left media 5% (12.1 mm); 15% and 25% concentration and right media 50% and 100% concentration and (b) Inhibition zone diameter result control amoxicillin disk 25µg (23 mm)

Table 3. Measurement of inhibition zone of ethanol extract of Bajakah wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 using disc method

No.	Concentrations	Replication (mm)			Mean
		I	II	III	(mm)
1.	Kontrol (+)	23	-	-	23
2.	5%	10,5	12,1	10,9	11,1
3.	15%	-	-	-	-
4.	25%	-	-	-	-
5.	50%	-	-	-	-
6.	100%	-	-	-	-

B. Test of antibacterial effectiveness of bajakah wood (*Spatholobus sp*.) against the growth of *S. aureus* ATCC 25923 by the well method

The results of this method in three repetitions of the inhibition zone that arose were still at the lowest concentration, namely 5% concentration with an average diameter of 16.8 mm (in table 4.). The zone of inhibition produced in the wells method is larger than the disc method. However, in the first repetition, concentrations of 25%; 50% and 100% produced inhibition zone diameters of 9.4 mm; 11.3 mm and 11.7 mm (In table 4.). The resulting data cannot be used because it is not reliable, that is, it does not appear in the next repetition.

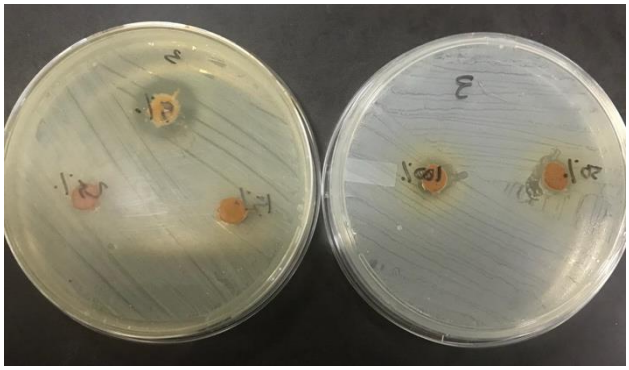

Figure 2. The results of the diameter of the inhibition zone of the ethanol extract of Bajakah Wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 in the second repetition using the well method. Left media 5% (17,3 mm); 15% and 25% concentration and right media 50% and 100% concentration

Table 4. Measurement of inhibition zone of ethanol extract of Bajakah wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 using well method

No.	Concentrations	Replication (mm)			Mean
		I	II	III	(mm)
1.	5%	17,2	16	17,3	16,8
2.	15%	-	-	-	-
3.	25%	9,4	-	-	-
4.	50%	11,3	-	-	-
5.	100%	11,7	-	-	-

C. Test of antibacterial effectiveness of bajakah wood (*Spatholobus sp*.) against bacterial growth of *S. aureus* ATCC 25923 by *Whatman* paper method

In this study, the results of testing antibacterial activity with the *Whatman* paper method remained the same, only at the lowest concentration, 5% concentration with an average inhibition zone of 10.8 mm (in table 5.). Other higher concentrations did not produce inhibition zones at all. When viewed from the response of the inhibition zone produced, the 5% concentration with the *Whatman* paper method has a moderate response in inhibiting the growth of *S. aureus* ATCC 25923 bacteria.

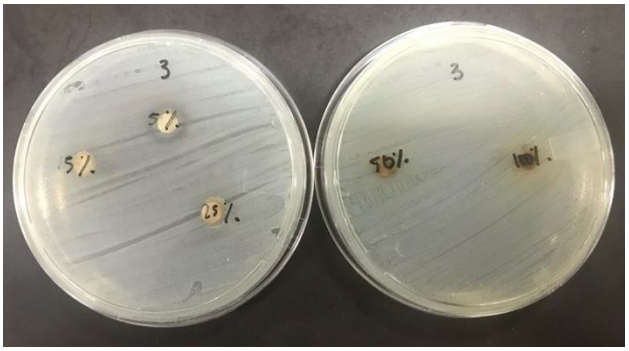

Figure 3. The results of the diameter of the inhibition zone of the ethanol extract of Bajakah Wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 in the second repetition using the *Whatman* paper method. Left media 5% (12,8 mm); 15% and 25% concentration and right media 50% and 100% concentration

Table 5. Measurement of inhibition zone of ethanol extract of Bajakah wood (*Spatholobus sp*.) on *S. aureus* ATCC 25923 using *Whatman* paper method

No.	Concentration	Replication (mm)			Mean
		I	II	III	(mm)
1.	5%	10,1	9,5	12,8	10,8
2.	15%	-	-	-	-
3.	25%	-	-	-	-
4.	50%	-	-	-	-
5.	100%	-	-	-	-

4. Discussion

A. Phytochemical Screening of Bajakah Wood (*Spatholobus sp.)*

The content of secondary metabolite compounds contained in Bajakah Wood (*Spatholobus sp.*) based on the results of phytochemical screening (in table) detected

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alkaloid compounds, tannins and glycosides. Alkaloid compounds have antibacterial properties by slowing down the cellular respiration process and have a function during the intercalation process in DNA. Alkaloids inhibit the biosynthesis of bacterial nucleic acids, thus inhibiting bacterial growth and ultimately causing bacterial death[7]. Tannin compounds can be antibacterial by disrupting the stability of reverse transciptase and DNA topoimerase thus inhibiting the formation of bacterial cells[16]. Glycoside compounds become antibacterial by penetrating the cell wall and damaging bacterial cell wall components[17]. In this study, by referring to the phytochemical screening results obtained (in table), Bajakah wood (*Spatholobus sp*.) was able to inhibit bacterial growth.

B. Antibacterial Effectiveness of bajakah wood (*Spatholobus sp*.) against the growth of *S. aureus* ATCC 25923 bacteria

The study was conducted by Weldy et al. (2022) that the average inhibition zone of ethanol extract of Bajakah Wood with concentrations of 5%, 15%, 25%, 50%, and 100% tested on Pseudomonas aeruginosa bacteria using the disc method was 11.1 mm; 13.3 mm; 16.7 mm; 20.0 mm; and 22.4 mm. From the results obtained, it shows that the ethanol extract of Bajakah wood has antibacterial effectiveness on *Pseudomonas aeruginosa*. The study was conducted by Saputera et al. (2019), namely testing the ethanol extract of Bajakah wood with a concentration of 3.12%; 6.25%; 12.5%; 25% and 50% on *Eschericia coli* bacteria with the well method producing an average inhibition zone of 9.8 mm; 11.71 mm; 15.83 mm and 20.32 mm. Based on the study conducted by Utami et al. (2019), namely testing orange leaf extract (*Citrus maxima*) on *Shigella dysenteriae* bacteria with concentrations of 30%; 35% and 40% using the *Whatman* paper method produces an average inhibition zone of 8.1 mm; 8.47 mm and 9.15 mm.

From the results of this study, the greater concentration has a low inhibitory ability (in the table). There are several factors that affect the diameter of the inhibition zone, namely the thickness of the agar media. The effective thickness of agar media is about 4 mm, if it is less than the diffusion of the extract will be faster while if it is more than the diffusion of the extract will be slower. In this study, no measurements were made on the agar media so it is not known exactly the thickness of the MHA media used. In addition, another possibility that can affect the diameter of the inhibition zone is the limited diffusion of the extract into the media. The higher the concentration, the lower the solubility, thus causing a decrease in the diffusion rate of the active metabolites of the extract into the media. This results in the limitation of extracts with high concentrations in inhibiting the growth of *S. aureus* ATCC 25923 bacteria in MHA agar media[18].

Another factor that affects the diameter of the inhibition zone in this study compared to previous studies is the type of bacteria used. In this study, the bacteria used were *S. aureus* ATCC 25923. *S. aureus* bacteria are gram-positive bacteria, where gram-positive bacteria have a thicker cell wall structure compared to gram-negative. Gram-positive bacteria have a thickness of 20-80 nm with >50% peptidoglycan layer, while gram-negative bacteria only have a thickness of 10 nm with 10-20% peptidoglycan only. Peptidoglycan is the main component of the cell wall that is rigid and maintains cell integrity and determines cell shape[19]. Because of this difference in cell wall structure, secondary metabolite compounds in the extract become more difficult to enter the bacteria. Therefore, this may affect the effectiveness of the ethanol extract of Bajakah Wood (*Spatholobus sp.)* in inhibiting the growth of S. aureus ATCC 25923.

C. Analysis Test Results

After the antibacterial activity is tested and the inhibition zone measurement is carried out, then perform a normality test using Shapiro-wilk and homogeneity test with the Test of Homogeneity Variances method as a fulfilment of the One-Way ANOVA test requirements. Based on the normality test, the data is normally distributed with the condition that p> 0.05 and based on the homogeneity test, the data is homogeneous if it shows a significance value of p> 0.05.

The normality test results show that the diameter of the inhibition zone measurement with the disc method is normally distributed as seen from the significance of 0.078, while the homogeneity test results show a significance value <0.001 which means it does not meet the requirements of p>0.05. The normality test results of the wells method are normally distributed with a significance of 0.50, while the homogeneity test results show a significance value of 0.005 which means it does not meet the requirements, namely p> 0.05. The normality test results of the *Whatman* paper method showed that the data were not normally distributed with a significance value of <0.001. Because the data obtained did not meet the requirements of One Way ANOVA, the test of ethanol extract of Bajakah Wood (*Spatholobus sp*.) could not be continued

5. Conclusion

Based on the results of the study that has been done, it can be concluded that the ethanol extract of Bajakah Wood (*Spatholobus sp*.) with a concentration of 5% can inhibit the growth of S. aureus ATCC 25923 bacteria using the disc method, the well method and the *Whatman* method. With an average inhibition zone of 5% concentration, the disc method was 11.1 mm; the well method was 16.8 mm; and the *Whatman* paper method was 10.8 mm.

Ethical Approval

This study was conducted after obtaining ethical approval from the Ethics Commission of the Faculty of Medicine, University of North Sumatra with letter number 839/KEPK/USU/2023.

Conflicts of Interest

There were no conflicts of interest in this study.

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Author Contributions

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