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DETERMINATION OF TOTAL FLAVONOID LEVELS OF BAJAKAH WOOD EXTRACT (SPATHOLOBUS LITTORALIS HASSK) ON VARIOUS ETHANOL CONCENTRATIONS AND ANTIBACTERIAL ACTIVITY TEST OF STAPHYLOCOCCUS AUREUS

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ABSTRACT

The bajakah plant is one of the biological plants used in traditional medicine. Bajakah is also a medicinal herbal plant that can be used in all its parts. This plant can be found in the depths of Kalimantan Province, but it has not spread to other regions. There has been no effort to cultivate bajakah because of the lack of understanding of the local population about the use of this plant. This research includes the stages of processing plant materials, making ethanol extracts, characterization examinations, phytochemical screening, and determining total flavonoid levels of ethanol extract of Bajakah wood using the visible spectrophotometry method and antibacterial activity. Bajakah wood extract was made by maceration method using ethanol concentrations of 96%, 70%, and 50%, and the extract obtained was concentrated with a rotary evaporator, then the total flavonoid level was determined using the visible spectrophotometry method and antibacterial activity tests were carried out against Staphylococcus aureus As a positive control using chloramphenicol and negative control using dimethyl sulfoxide. The results were obtained by determining the maximum wavelength of quercetin and operating time, measuring the quercetin calibration curve, and calculating the total flavonoid level using the visible spectrophotometry method. The results obtained in the determination of total flavonoid levels in Bajakah wood ethanol extract at a concentration of 96% were 349.888 ± 4.5022 mg QE/g, at a 70% concentration of 307.137 ± 6.7626 mg QE/g and a 50% concentration of 6.15173 ± 2.9148 mg QE/g. The results of antibacterial activity in ethanol extract of 96% timberbaja against Staphylococcus aureus bacteria had an average inhibition of 19.3 mm.

KEYWORDS *Bajawood, Flavonoids, Visible Spectrophotometry, Antibacterial, Staphylococcus aureus*

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INTRODUCTION

Indonesia's natural resources are very diverse (Ekananda, 2022). Many plants grow in Indonesia (Chien et al., 2022). Through forests spread across several regions of Indonesia, forest products in the form of plants are often used by the Indonesian people as traditional medicine (Donovan & Puri, 2004; Hasna et al., 2021). One of the plants used as traditional medicine is Bajakah wood, which is known among the Dayak people as a medicine and cures various diseases (Novriyanti et al., 2021; Rujehan et al., 2024).

The bajakah plant is one of the biological plants used in traditional medicine. Bajakah is also a medicinal herbal plant that can be used in all its parts (Mahfudh et al., 2024). This plant can be found in the depths of Kalimantan Province, but it has not spread to other regions (Yulianti et al., 2024). There has been no effort to cultivate bajakah because of the lack of understanding of the local population about the use of this plant (Saputera & Ayuchecaria, 2018).

The temple steel plant *(Spatholobus littorilis* Hassk*)* contains phenolics, flavonoids, tannins, and saponins. The content of these secondary metabolite compounds can treat various degenerative diseases, such as diabetes, cancer, tumors, and others (Fitriani & Saputra, 2020).

Bajakah plants contain secondary and primary metabolites. Metabolites are produced from glycolysis, as well as the TCA (*tricarboxylic acid*) cycle, or shikimate pathway (Arifin et al., 2021). Meanwhile, secondary metabolites are non-essential natural metabolic products for the vegetative growth of product-producing organisms. Secondary metabolites are considered compounds that provide adaptive roles, for example, functioning as defense or signaling, symbiosis, transportation, protecting against harmful things, or combating pathogens (Aguirre-Becerra et al., 2021; Divekar et al., 2022).

The plant of the Bajakah tempala can be used to treat the wound healing process, and the decoction of the bajakah stem can be used as a remedy for dysentery.

In addition to phytochemical testing on the bark extract and root plant of the bajakah wood, this plant has secondary metabolite content, such as alkaloids, flavonoids, and terpenoids (Theresa et al., 2021). The compound has the potential to be an antibacterial (Karnwal & Malik, 2024) Based on the results of Saparudin's research*., et al* About the Antibacterial Activity Test of Bajakah Wood *(Spatholubus littoralis* Hassk) on growth *Staphylococcus aureus*, in this study, Bajakah wood ethanol extract has flavonoid compounds, and has the potential to have an antibacterial effect in inhibiting bacterial growth *Staphylococcus Aureus.*

This study aims to determine the total flavanoid levels of steelwood extract (*Spatholobus littolaris* Hassk) at various ethanol concentrations using the UV-Vis Spectrophotometer method and to determine whether the ethanol extract of steelwood (*Spatholobus littoralis* Hassk) has antibacterial activity of *Staphylococcus aureus*.

RESEARCH METHODS

This research is a type of laboratory experimental research. The method used to extract the chemical content in the stem of tampala steel (*Spatholobus littoralis* Hassk) is by maceration method using ethanol solvents. The antibacterial activity test was carried out using *Staphylococcus aureus bacteria* to determine the effect on the fraction of steel logs (*Spathoobus littoralis Hassk*). The research was carried out at the Integrated Pharmacy Laboratory of Muslim Nusantara University (UMN) Al-Washliyah Medan. The research plan is for January–May 2023. The sample used in this study is steel wood obtained from Pontianak City, West Kalimantan Province. The material used in this study is steel wood that is dried into simplisia. Chemicals used for the determination of total flavonoid levels include 96% ethanol, anhydrous acetic acid, nitric acid, sulfuric acid, amyl alcohol, iron(III) chloride, bismuth(III) nitrate, iodine, potassium iodide, magnesium powder, mercury(II) chloride, alpha-naphthol, lead(II) acetate, toluene, chloroform, n-hexane, aquadest, hydrochloric acid, quercetin, aluminum chloride, sodium acetate, quercetin. The chemicals used for the antibacterial test are sterile aquatics (Otsuka) and physiological NaCl (Widatra). The test bacteria used was *Staphylococcus aureus*. The bacterial medium used for rejuvenation is a pure culture, Nuttrient Agar (Merck). The bacterial medium used in the diffusion method (disc) is Mueller Hinton Agar (MHA) (Himedia). The anti-bacterial comparator used as a positive control in this experiment was chloramphenicol 30µg. The tools used in this study were analytical scales (Sartonius CP224S), blenders, ovens (Memmert), a series of *rotary evaporators* (Heidolph), a set of glassware, metal spatula, cotton, tissues, dark bottles, tweezers, test tube racks, ose needles, *hot plates,* bunsen, *microchips*, *microtubes,* vortex (Labnet), aluminum foil (Klin-pak), filter paper, *water bath*, autoclave (ALP), *micropipettes* (SOCOREX ASBA S.A), *swabs*, calipers, *air flow valves* (Airtech) and incubators (Gallenkamp). The data analysis used in this study is the results of secondary metabolites obtained from the saponin test, Alkaloids, flavonoid tests, triterpenoid tests, essential oil tests, and tannin tests were then analyzed using theoretical and descriptive approaches.

RESULTS AND DISCUSSION

Plant Identification

The results of plant identification were carried out at the Medanense Herbarium of the University of North Sumatra with the name of the plant Bajakah Wood (*Spatholobulus littoralis* Hassk). The results obtained showed that the plant used in this researcher was indeed Bajakah Wood (*Spatholobulus littoralis* Hassk*)* and the results of identification to Appendix 1.

Sample Processing Results

The sample used in this study was 3 kg of steel wood, then the weight after the display was 2.9 kg, and the weight of simplicia powder was 2,850 kg and the powder results were seen in appendix 4.

Results of Simplisia Characteristics

a. Results of Macroscopic Examination of Bajakah Wood

The macroscopic examination is carried out by observing the shape, size,

color, and smell of Bajakah Wood. The results of the macroscopic examination can be seen in appendix 7.

Table 1. Macroscopic timber Bajakah

It	Organolepti c Parameters	Information
1	Shape	Length: 5.8– 6.5 cm Width: 1–1.5 cm
2	Color	Light brown
3	Smell	Typical timber

b. Results of Microscopic Examination of Simplicia

Microscopic examination of Bajakah wood leaf simplicia powder is seen the presence of parenchyma cells, sclerenchyma fragments contain oxalate crystals, xylem wood vessels with thickening of noctins. The results of microscopic examination of simplicia can be seen in appendix 10.

2. Simplisia Characteristic Test

Table 2. Results of Examination of Symplisia Characteristics

It	Parameters	Rate Acquisition	MMI
1	Moisture content	6,666%	< 10
2	Water-soluble pollen rate	4,9766%	>3
3	Soluble pollen content in ethanol	6,46%	>3
4	Total ash content	2,548%	<4
5	Ash content insoluble in acids	0,07166%	<0.2

The purpose of this characterization test is to find out the properties of the samples used. The moisture content obtained is 6.666%, and this result meets the requirements for moisture content below 10%. Determining the moisture content is very important to provide a maximum limit on the water content in the steel wood simplicia because a large amount of water can damage the compounds contained in the simplicia. For the water-soluble juice content and ethanol soluble juice content of 4.9766% and 6.46%, these results also show that the sample meets the requirements. The determination of water and ethanol-soluble pollen levels is carried out to provide an initial overview of the number of compounds that can be at the pollen content with water and ethanol solvents from a symposia. The total ash content was 2.548%. The determination of total ash content is carried out with the aim of providing an overview of the internal and external mineral content from the initial process to the formation of simplicial related to organic and inorganic compounds obtained internally and

externally. While the acid insoluble ash content was obtained, which was 0.07166%, this met the requirements for acid insoluble ash content, which was less than 0.2%; this acid insoluble ash content test was carried out with the aim of finding out the amount of ash obtained from external factors such as sand or soil.

3. Results of Making Bajakah Wood Extract

Bajakah Wood Simplicia powder as much as 500 grams with maceration using ethanol solvents of 96%, 70%, 50%, as much as 4 liters, filtrate from maceration using a rotary evaporator at a temperature of 500C obtained a thick extract of Bajakah wood ethanol with a concentration of 96% which is 67.1688 grams, 70% concentration of 66.5252 and 50% concentration obtained 67.1688 (Latu., 2023; 110)

4. Phytochemical Screening Results of Bajakah Wood Powder and Extract

The results of the phytochemical screening examination of Bajakah wood Simplicia powder can be seen in Attachment 15.

Table 3. Phytochemical screening results from Bajakah Wood Simplicia powder

It	Compound class	Powder Results	Extract Results
1	Alkaloids	+	+
2	Flavonoids	+	+
3	Saponins	+	+
4	Tannins	+	+
5	Triterpenoids/steroids	+	+
6	Glycosides	+	+

Information:
+=Positive
- = Negative

Table 3 Above shows that from the results of phytochemical screening, there is the same group of secondary metabolite chemical compounds in simplicia powder and bajakah wood extract of flavonoids, saponins, tannins, glycosides, alkaloids and triterpenoids.

In the flavonoid test of Simplicia powder and jackfruit wood extract, a red ring layer was formed on the amyl alcohol layer, which showed a sign marked by the formation of foam more than 1 cm high for no less than 10 minutes and did not disappear after the addition of hydrochloric acid. In the identification test, tannins, simplicia powder, and bajakah wood extract formed a blackish-green color caused by the reaction of the addition of FeCl3 with one of the hydroxyl groups contained in tannin compounds. In the glycoside test, a purple ring is formed. In the alkaloid test using the Mayer reagent, white or yellow precipitates are formed, while in the Bouchardat reagent, brown to black precipitates are formed, and the Dragendrof reagent is red or orange precipitate. Steroids/terpenoids in simplicial powder and steel wood extract were obtained

by the formation of green color in the vapor dish that had been dripped with anhydrous acetic acid and concentrated sulfuric acid.

5. **Preparation of Kuercetin Raw Solution**

Weighed 25 mg of quercetin, dissolved in a flask with 25 ml of methanol until the limit mark into the raw parent solution (C= 1000 µg/ml) LIB I Then pipetted 5 mL from LIB I put into a measured flask 50 ml filled with methanol to the limit mark (C= 100 µg/ml.) LIB II

6. **Results of Determination of Maximum Wavelength of Quercetin**

The maximum wavelength has to be determined, and the wavelength of the measurement where the complex between quercetin and AICI3 provides optimum absorbance. The determination of maximum wavelength is an important factor in chemical analysis using the spectrophotometry method. Measurements at the maximum wavelength will provide the greatest change in absorbance for each rate unit. So that if remeasurement and replication are carried out, measurement errors will be minimized (Suharyanto, 2020).

Flavonoid testing began with the measurement of the wavelength of the fmaximum test of quercetin solution with a concentration of 4 ug/ml with the addition of 0.1 ml of AICI3, 10%, 0.1 ml of sodium acetate, and the addition of 2.8 ml of aquades and methanol to the limit mark by the visible light spectrophotometry method.

The compound used as a standard in determining the level of this flavonoid is quercetin because quercetin is the largest component in plants. Quercetin is a flavonol group that has a ketone group at the C-4 atom and a hydroxy group at the C-3 and C-5 atoms which are neighbors of flavones and flavonols (Yulistian, et al., 2015).

Image Reaction of Yellow Color Formation of Flavonoids and AICI3

The flavonoid content in the extract was quantitatively measured by the aluminum chloride method using a standard solution of quercetin so that the results were calculated as milligrams of QE (Quercetin Equivalent) per gram dw (dry weight) of the ethanol extract sample of Bajakah wood. Quercetin is used as a standard because quercetin is a flavonoid that has high reactivity compared to rutin, daflon, diosmin and morin (Mir *et. al*., 2014). The color of visible rays can be related to their wavelengths in the table. The result of this solution is yellow. The wavelength results obtained in the study are acceptable because they are still in the wavelength range when the solution obtained is yellow. The results obtained were at a wavelength of 438 nm. This indicates that the sample contains flavonoids.

Operating Time Results

Operating time is used to determine the measurement time of a compound obtained when absorption is most stable. Operating time is carried out by measuring the measurement time with the absorbance of the solution. Setting the operating time needs to be done to minimize the occurrence of measurement errors. This is because the compound whose absorption will be measured in this study is a complex compound between ACI3 quercetin. This complex compound takes time for the reaction to be stable, showing the result that the absorbance value is carried out at minute 5 for the reason that it starts from minute 5, then the measurement of absorbance at minute 5, this shows that from minute 5 the flavonoid compound has

finished reacting with the AICI3 signal, which is marked by a reading of a stable absorption value of 0.422, This reading is done on the three numbers after the comma because the fourth number is a pseudo-number, so its existence can be ignored.

Results of Measuring the Cursetin Calibration Curve

Calibration curve measurements were performed with different concentrations of solution pipetted from a concentration solution of 100 µg/ml. Pipetted 0.2 ml, 0.3 ml, 0.4 ml, 0.5 ml and 0.6 ml, so a concentration of 2 µg/ml, 3 µg/ml, 4 µg/ml, 5 µg/ml and 6 µg/ml. Put in a measured pumpkin 10 ml, and add methanol until the limit mark. Then pipette 1 ml of each of these concentrations into a 10 ml flask, add 0.1 ml of AICI3, 10%, and 0.1 ml of sodium acetate, and add 2.8 ml of equates and methanol to the limit mark. Then, it was left for 5 minutes and measured at a wavelength of 438 nm. From the measurement results, the absorbance of each raw solution was obtained, which was then converted into a linear regression equation. The function of adding ACI3 reagents with flavonoid groups forms a complex between groups neighboring hydroxyl and ketone or with neighboring hydroxyl groups.

Table 4. Absorbance Value of Quercetin Raw Solution

Concentration	Absorbance	egression Equation
0	0.000	
2	0.277	
3	0.378	Y= 0.1232x +0.0
4	0.480	041
5	0.625	
6	0.753	

The regression equation obtained from the quercetin raw solution is y = 0.1232x + 0.0041 with a correlation coefficient obtained of 0.999.

Results of Analysis of Total Flavanoid Levels of Bajakah Wood Extract (*Spatholobus littoralis* Hassk) at Various Ethanol Concentrations

Analysis of flavonoid levels using the UV-Vis spectrophotometry method is an analysis that uses an ultraviolet electromagnetic radiation source with a wavelength (λ) of 190-380 nm and visible light at a wavelength (λ) of 380-780 nm. The working principle of UV-Vis spectrophotometry is the interaction between matter and light that has a certain wavelength (Hardjono, 1991).

The principle of determination of flavonoids by the spectrophotometric method is defined by the AICI3 reagent, which is the formation of a complex between AICI3, with a keto group on the C-4 atom and also with a hydroxy group on the C-3 or C-4 atom that is a neighbor of flavones and flavonols. In addition, aluminum chloride forms a stable acid complex with an orthohydroxyl group in the A- or B- ring of flavonoid compounds. The tested sample showed a change in color to yellow. This shows a positive test for the presence of flavonoid compounds in the sample. A sample containing flavonoids, when reacted with AICI3, will form a yellow color. This occurs due to the formation of complex compounds between

flavonoids and AIC3 (Harbome 1987).

The compound used as a standard in determining the level of this flavonoid is quercetin. The choice of quercetin as the standard solution is because quercetin is the most widely distributed compound found in plants. Quercetin and its glycosides are in the amount of about 60-75% of flavonoids. Quercetin is also one of the flavonoid compounds that can react with AICI3, forming complexes (Kelly, 2011).

In the measurement of total flavonoid compounds, the sample solution is added Ah, which can form complex colors, so that there is a shift in the wave in the visible directionthat is Marked with a color that is more yellow. The addition of potassium acetic aims to maintain a long wave in the visible region (Chang 2002). The determination of total flavonoid levels was calculated by using the linear regression grease equation y- ax+b obtained from the cursetin calibration curve so that the concentration (x) was obtained, the value of x was obtained then Substituted deep formula for calculating total flavonoid levels. Rate setting Flavonoids Total Done with Repeated 6 times and taken on average as presented in the following table.

Table 5. Average Value of Actual Flavonoid Levels of Total Ethanol Extract of Bajakah Wood (*Spatholobus littoralis* Hassk)

Methanol Concentration	Actual Rate (mg QE/)
96%	349.888 ± 4.5022 mg QE/g
70%	307.137 ± 6.7626 mg QE/g
50%	6.15173 ± 2.9148 mg QE/g

It can be seen that the results of the research on ethanol extract are positive for flavonoids. This is evidenced by the results of analysis by the visible ray spectrophotometry method with six replications. The results of the study showed that the average value of the actual level of total flavonoids in the sample of bamboo grass herbal ethanol extract was 223.4188+ 0.6749 mg. It can be concluded that a methanol concentration of 96% results in the highest flavonoid content.

Results of Bacterial Identification Using Selective Media

Staphylococcus aureus bacteria are shaped like grapes. This bacteria produces a golden yellow pigment called aureus (meaning gold, like the sun). These bacteria can grow with or without the help of oxygen (Radjdi and Biomed, 2009: 97).

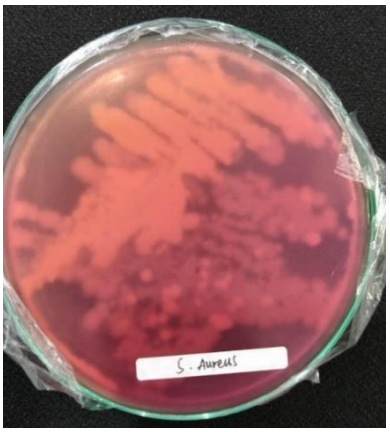

Figure 1. *Staphylococcus aureus*

Test Results of Antibacterial Activity of Bajakah Wood Extract (*Spatholobulus littoralis* Hassk) at 96% ethanol concentration

An inhibition test of ethanol at a concentration of 96% was carried out against *Staphylococcus aureus* with the inhibition of the formation of a clear zone in the area around the paper disk (Latu *et al.,* 2023: 112)

In the antibacterial test of 96% ethanol extract, the highest flavonoid levels were obtained, followed by testing the activity of bacteria; the positive control used was chloramphenicol, which aimed to find out the inhibition as killed bacteria; the negative control used was DMSO; it was known that DMSO did not have an effect on bacteria. The results of the measurement of ethanol extract of Bajakah wood with a concentration of 96% can be seen in Table 4.6 (*Spatholobulus littoralis* Hassk).

Table 6. Antibacterial Activity Test of Bajakah Wood Extract (*Spatholobulus littoralis* Hassk)

Test substance	Average Inhibition	Category
(+) Control	21.37 mm	Very Strong
Ethanol extract 96%	19.3 mm	Strong
Control (-)	0	Not Inhibit

The results obtained from ethanol extract of bajakah wood (*Spatholobus littoralis Hassk*) at a concentration of 96% have antibacterial activity. The results of the diameter of the inhibitory power *of Staphylococcus aureus* bacteria were repeated 3 times, namely 19.75 mm, 19.75 mm, 18.4 mm with the inhibitory power categorized as strong. In the test, Bajakah wood extract at a concentration of 96% has the ability to be an antibacterial against gram-positive bacteria, namely *Staphylococcus aureus bacteria.*

CONCLUSION

Total flavonoid content of Bajakah wood extract **(***Spatholobulus littoralis* Hassk) at a 96% ethanol concentration of 349,888 ± 4.5022 mg QE/g At 70% ethanol concentrations of 307,137 ± 6.7626 mg QE/g at 50% ethanol concentrations of 6.15173 ± 2.9148 mg QE/g. Bajakah wood extract **(***Spatholobulus littoralis* Hassk) at an ethanol concentration of 96% provides an inhibition against *Staphylococcus aureus* bacteria of 19.3 mm.

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