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# Determination of Total Flavonoid Content of Raru Bark Extract: *Cotylelobium Melanoxylon Pierre* at Various Methanol Concentrations and Antibacterial Activity Against Staphylococcus

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**Abstract**

*Raru bark (Cotylelobium melanoxylon (Hook.f) Pierre) is a plant with antidiabetic properties. Raru bark plants contain flavonoids, saponins, and tannins. The purpose of this study was to determine the class of secondary metabolites contained in the sample, determine flavonoid levels, and determine antibacterial activity against Staphylococcus aureus bacteria. This research includes processing samples made simplisia, then extracted and thickened using rotary evaporatory. Simplisia was tested for phytochemical screening to determine the content of metabolite compounds, in addition to testing its characteristics. The next test was the determination of total flavonoid content using UV-Vis spectrophotometry. Followed by testing antibacterial activity against Staphylococcus aureus. The results of phytochemical screening obtained flavonoids, tannins, and saponins. Flavonoid levels were 42.4308 ± 2.4739 mgQE/g; 38.1813 ± 1.5799mgQE/g; and 23.8153 ± 3.049276 mgQE/g, respectively. In testing antibacterial activity using concentrations of 10%, 40%, 70%, and 100%, and the highest concentration is found at a concentration of 100% which is 19.83mm.*

## Introduction

Indonesia is a country that has biodiversity that is utilized as a medicinal plant. The utilization of medicinal plants in Indonesian society is from the stems, flowers, leaves, seeds, and roots of these plants. Medicinal plants contain secondary metabolite compounds, namely flavonoids, alkaloids, tannins, terpenoids (Julianto, 2019; Anulika et al., 2016; Hanafiah et al., 2017).

One of the plants used as a treatment is raru bark (Cotylelobium melanoxylon (Hook.f) Pierre) which is widely used by the people of Tapanuli. Besides as a treatment, raru bark is added to palm wine to increase the alcohol content of the drink. The metabolite content contained in raru bark is flavonoids, tannins, and saponins (Winahyu et al., 2019).

The flavonoid content is the largest phenol compound and flavonoids are secondary metabolite compounds that are widely found in plants. Phenol compounds which have effective properties in inhibiting the growth of fungi, viruses, and bacteria. Flavonoids have properties as antibacterials, antioxidants, as drugs for infections in wounds, as anti-cancer, and as anti-allergic (Andika et al., 2020; Zawawi et al., 2025).

Wound is a state of disconnection or damage to the continuity of body tissue that causes disruption of body function (Wintoko et al., 2020). One type of wound, namely diabetic

wounds. Diabetic wounds are chronic wounds of diabetes mellitus, initially known as ordinary wounds, which if mishandled cause infection (Damsir et al., 2018; Ottolino-Perry et al., 2017).

Infectious disease is a disease caused by pathogenic bacteria that enter and multiply in the body. One of the pathogenic bacteria that often attack the body is the staphylococcus aureus bacteria (Damayanti et al., 2022; Lake et al., 2019) states that staphylococcus aureus bacteria are often found in the urinary tract, mouth, upper respiratory tract, wound infections, and skin tissue from within purulent boils.

Raru bark is used by the community as anti-diabetes, which in diabetes causes wounds that are difficult to heal, causing infection. According to Lake et al. (2019) Staphylococcus aureus bacteria are found in wound infections. In addition, the flavonoid content in raru bark has antibacterial properties. So from the explanation above, the author conducted research on determining the total flavonoid content of raru bark extract (Cotylelobium melanoxylon (Hook.f) Pierre) at various methanol concentrations and antibacterial activity against Staphylococcus aureus.

This study aims to identify the secondary metabolites contained in the powder and methanol extract of raru bark (Cotylelobium melanoxylon (Hook.f) Pierre), measure the total flavonoid content in raru bark using UV-Vis spectrophotometric method, and evaluate the antibacterial activity of raru bark methanol extract against Staphylococcus aureus bacteria.

This research is useful in providing scientific information related to the potential of raru bark (Cotylelobium melanoxylon (Hook.f) Pierre) as a source of natural materials with antibacterial activity against Staphylococcus aureus. This finding can be the basis for the development of natural antimicrobial agents that have the potential to replace or complement synthetic antibiotics, especially in dealing with the problem of bacterial resistance. In addition, the results of this study are expected to be a valuable reference for future researchers who want to further explore the active components in raru bark or develop their applications in the pharmaceutical and health fields.

## Methods

The research was conducted with experimental research method, where the independent variable in this research is the concentration of methanol extract of raru bark (Cotylelobium melanoxylon (Hook.f) Pierre) and the dependent variable in this research is the examination of simplisia characterization, phytochemical screening, determination of total flavonoid content of raru bark methanol extract (Cotylelbium melanoxylon (Hook.f) Pierre), antibacterial inhibition and data analysis. The design includes collection and processing of samples, phytochemical screening, examination of simplisia characterization, preparation of methanol extract of raru bark (Cotylelbium melanoxylon (Hook.f) Pierre), determination of flavonoid content and antibacterial inhibition test.

The research was conducted at the Research Laboratory of the Faculty of Pharmacy, Al-Washliyah Muslim Nusantara University Medan. The research was conducted from January to May 2023.

### Tools and Materials

The tools used are laboratory tools, analytical scales, glassware, rotary evaporator, aluminum foil, water bath, UV-Vis spectrophotometry, wooden stirrer, tube rack, round ose, tweezers, spatula, autoclave, oven, incubator, paper discs, caliper, Bunsen, micropipette, belender, filter paper, vortex, swab, *laminar airflow*.

The materials used were raru bark (*cotylelobium melanoxylon* (Hook.f) pierre), quercetin, methanol, glacial acetic acid, sulfuric acid (p), hydrochloric acid (p), potassium iodide, iodine, iron (III) chloride, bismuth (II) nitrate, astetic acid anhydride, mercury (II) chloride, nitric acid,

lead (II) chloride, chloralhydrate, sodium hydroxide, magnesium powder, MSA media, NA media, media, MHA media, Staphylococcus aureus bacteria colonies, 70% ethanol, mercury (III) chloride, sterile distilled water, physiological NaCl, chloramphenicol

**Sample Preparation**

The raru bark was cleaned of dirt and washed thoroughly and then drained. After that, the wet weight is weighed. Then dry it in a drying cabinet. After the sample is considered dry, it is pulverized using a blender until it becomes a fine powder. Sifted and weighed the powder. And stored in a container.

**Characteristics of Simplisia**

Macroscopic examination was carried out on raru bark (*Cotylelobium melanoxylon* (Hook.f) Pierre) by paying attention to color, shape, smell and size. The examination was carried out by observing the fragments in the simplisia using a microscope.

**Preparation of Raru Bark Methanol Extract**

The extract was made by maceration, as much as 10 parts of simplisia put into the vessel added 75 parts of methanol in a tightly closed container for 5 days. Protected by sunlight, stirring occasionally. Then squeezed using flannel cloth. Then wash the pulp using methanol until 100 parts are obtained. after that it is stored in a tightly closed vessel and protected from sunlight for 2 days. Filtered and filtered. then concentrated using a rotary evaporator until a thick extract is obtained. The temperature should not be more than 50°C.

**Phytochemical Screening**

Phytochemical screening was carried out on simplisia powder and raru wood extract to determine the alkaloid, flavonoid, tannin, steroid/tripenoid groups. The simplisia powder and methanol extract were weighed as much as 0.5gram and added 1ml of 2 N HCl and 9 ml of distilled water and heated in a water bath for 2 minutes. After it was cooled and filtered, the filtrate was used for alkaloid testing: 1) Filtrate as much as 3 drops added with 2 drops of mayer reagent then formed a white or yellow clumpy precipitate; 2) Filtrate as much as 3 drops added with 2 drops of reagent drangendroff will terbentu brown or orange; 3) Filtrate as much as 3 drops added with 2 drops of bouchardat reagent then formed a brown to black precipitate.

If a precipitate or turbidity occurs in at least two of the three trials, the sample is positive for alkaloids.

**Flavonoid Screening**

10 grams of simplisia powder and extract were weighed and added to 100 ml of hot distilled water. boiling for 5 minutes, after which it was filtered in a hot state. Into 5 ml of filtat added magnesium powder and 1 ml of concentrated HCl and 2 ml of amyl alcohol, shaken vigorously allowed the solution to separate. It is said to be positive for flavonoids in the presence of red, yellow, or orange color in the amyl alcohol layer.

**Steroid and Tripenoid Screening**

A total of 1 gram of simplisia powder and extract were macerated with 20ml of n-hexane for 2 hours. The macerate was filtered, the filtrate was evaporated in a vaporizer cup. The rest is added to Lieberman-Burchard reagent solution. If a purple or red color forms and then turns blue or blue green, it indicates the presence of steroids or tripenoids.

**Saponin Screening**

A total of 0.5gram of powder and extract added 10 ml of hot distilled water, then shaken vigorously for 10 minutes. If foam is formed with a height of 1 to 10 cm which is stable for not

less than 10 minutes and does not disappear when added 1 drop of HCl 2N indicates the presence of saponins.

**Tannin Screening**

1 gram of powder and extract is extracted with 10 ml of distilled water and then filtered. the filtrate is diluted using distilled water until clear. The solution was taken as much as 2 ml and then added 1% iron (III) chloride reagent as much as 1 to 2 drops if a blue-black or green-black color occurred, negating the presence of tannins.

**Measurement of Total Flavonoid Content of Raru Bark Extract (*Cotylelobium melanoxylon* Pierre)**

***Preparation of Quercetine Solution***

25 mg of quercetin in a 25ml volumetric flask using methanol until the limit mark in the mother liquor (C: 1000µg/ml) LBI, then pipet 5 ml of LBI solution then put it in a 50 ml volumetric flask and add methanol until the limit mark (LB II).

***Quersetine Maximum Wavelength Preparation***

pipette 0.4 ml of LB II solution into a 10 ml volumetric flask (C= 4µ g/ml), then add 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M sodium aetate, and add 2.8 ml of distilled water then add methanol to the limit. Then let stand for 30 minutes. Measure the absorbance at a wavelength of 400-800. The running result shows the maximum wavelength is 437nm.

***Determination of Operating Time***

Pipetted 0.4 ml of standard mother liquor II (LIB II) into a 10 ml volumetric flask (C = 4µ g/ml), added 0.1 ml of 10% $AlCl_3$, 0.1 ml of 1 M sodium acetate, and added 2.8 ml of distilled water, then added methanol to the limit mark, then measured the *operating time of* quercetin for 60 minutes at a wavelength of 437 nm.

***Measurement of Quercetin Calibration Curve***

Weighed 25 mg of quercetin, put into a 25 ml volumetric flask and added methanol to the limit (C = 1000µ g/ml) (LIB I). Then pipetted 5 ml of the standard mother liquor I into a 50 ml volumetric flask and filled with methanol to the limit (C = 100µ g/ml) (LIB II). Then made a series of levels and then put into a 10 ml flask each pipetted 0.2 ml; 0.3ml; 0.4ml; 0.5ml and 0.6ml of LIB II with a concentration of 2µg / ml; 3µg / ml; 4µg / ml; 5µg / ml; 6µg / ml and then added methanol to the limit mark. Pipetted as much as 1 ml from each flask with various concentrations and put into a 10 ml flask then added 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M sodium acetate, and added 2.8 ml of distilled water, added methanol to the limit, homogenized and allowed to stand for 6-9 minutes. The absorbance was measured at the maximum wavelength of 437 nm.

**Determination of Total Flavonod Level of Methanol Extract of Raru Bark (*Cotylelobium melanoxylon* (Hook.f) Pierre)**

Methanol extract solution of raru bark (*Cotylelobium melanoxylon* (Hook.f) Pierre) 1000 ppm was pipetted as much as 1 ml, then add 0.1ml $AlCl_3$ 10% and 0.1 ml sodium acetate 1 M then add methanol until the limit mark let stand for 1 minute then measure the wavelength obtained.

**Antibacterial Activity Testing**

***Tool Sterilization***

Before the tool is used, it is necessary to sterilize the tool. Tools that are sterilized are Petri dishes, test tubes, beaker glass, Erlenmeyer, volume pipettes, measuring cups, wrapped in paper and then put into an autoclave for 15 minutes at 121 $^0$C. for tools such as ose needles are

sterilized by burning directly on a Bunsen flame. Then for the media used, it is sterilized using an autoclave for 15 minutes at a temperature of 121$^0$C.

**Preparation of *Nutien Agar* (NA) Media**

Composition:

| | |
|---|---|
| NA | 20 grams/L |
| Peptone from meat | 5 grams |
| Agar-Agar | 12 grams |
| Aquadest | 1 L |
| Meat Extract | 3 grams |

A total of 6.72 grams of nutrient agar was weighed and put into a 250 ml erlenmeyer and added with distilled water and heated until dissolved, closed the mouth of the erlenmeyer with cotton and aluminum foil and sterilized in an autoclave for 15 minutes at 121 $^0$ C.

**Preparation of Mannitol Salt Agar (MSA)**

Composition:

| | |
|---|---|
| Mannitol | 10 grams |
| Peptone | 10 grams |
| Sodium cloride | 75 grams |
| Phenol red | 0.0025 gram |
| Agar | 15 grams |
| Beef Extract | 1 gram |
| Aquadest | 1 L |

MSA as much as 40 grams was dissolved with 1000 ml of distilled water then heated until dissolved while stirring and closed the erlenmeyer mouth using cotton and aluminum then sterilized using an autoclave at 121 $^0$ C for 15 minutes.

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

Composition:

| | |
|---|---|
| Acid hydrolysate of casein | 17.5 grams |
| Beef Extract | 2 |
| Starch | 1.5 grams |
| Agar | 17 grams |
| Aquadest | 1 L |

A total of 38 grams of MHA media was weighed and put into an erlenmeyer and dissolved with 1 L of distilled water and then heated until dissolved. Then sterilized using an autoclave at 121 $^0$ C for 15 minutes.

**Breeding Bacteria**

Colonies of *staphylococcus aureus* bacteria are taken from the culture using an ose needle and then planted on *salt agar media* (MSA) by scratching the media. Then incubate 1x24 hours at 37 $^0$ C. then observe the growing colonies, if the growing colonies are golden yellow in color, it shows that it is *staphylococcus aureus.* Then inoculate on NA beveled agar by scratching the colony on an ose needle aseptically. Then incubated for 1x24 hours at 37 $^0$ C. The growing colonies were used as antibacterial activity tests.

Staphylococcus bacteria that have been incubated using an ose needle are taken and then put into a test tube that has contained 10 ml of 0.9% NaCl solution. Then homegenized using a vortex. The turbidity results were compared with Mc Farland turbidity, which has a

concentration of 10 $^{8}$ CFU / ml. Then pipet 0.1 ml of bacterial culture and then put it into a tube containing 9.9 ml of sterile 0.9% NaCl and vortexed. Then obtained a colony suspension with a concentration of $10^{6}$ CFU/ml.

The antibacterial activity test was conducted using the disc method. *Staphylococcus aureus* bacterial suspension was taken using a sterile cotton bud. Sterile cotton buds are inserted into the bacterial suspension by squeezing at the edge of the tube. Then the cotton bud is scratched onto MHA media. Then place the disk that has been soaked with the test solution on the media. The positive control used was chloramphenicol and the negative control used was DMSO using sterile tweezers. Then put the Petri dish into the incubator and incubate for 24 hours at 37℃ and measure the inhibition zone using a caliper.

## Result and Discussion

### Sample Determination Results

Determination is carried out to determine the truth of the sample used, so that there is no error in the use of the sample used. From the results of the determination of the samples used, it was found that the samples were true raru bark plants (*Cotylelobium melanoxylon* (Hook.f) Pierre*)*. Determination testing was carried out at Herbarium Medanense (MEDA), University of North Sumatra.

### Simplisia characteristic test

Table 1. Macroscopic Results

| No. | Parameters | Results |
|---|---|---|
| 1. | Color | Chocolate |
| 2. | Smell | Typical |
| 3. | Size | The length of the skin is 21 cm, and the width is 3 cm. |

The macroscopic characteristics test of raru (*Cotylelobium melanoxylon*) bark simplisia showed that the simplisia had a brown color which is a visual characteristic of the bark of the plant. A characteristic odor was also identified, reflecting the presence of volatile compounds or volatile oils that might contribute to its biological activity. In terms of size, the length of the simplified bark was 21 cm with a width of 3 cm, indicating a relatively consistent physical shape and suitable for use in further processing, such as extraction.

### Characteristic Inspection Results

Table 2. Characteristic test results

| No. | Testing Parameters | Characteristic Results | Requirements |
|---|---|---|---|
| 1 | Determination of moisture content | 6% | < 10% |
| 2 | Water soluble essence content | 16,79% | ≥ 10,5 % |
| 3 | Ethanol soluble juice content | 26,52% | ≥ 16,5 % |
| 4 | Total ash content | 5,825% | ≤ 9,5 % |
| 5 | Acid insoluble ash content | 5,825% | ≥ 1,5 % |

Testing water and ethanol soluble juice content has the aim of providing an initial description of the number of compounds that can be extracted using ethanol and water solvents from a simplisia. In the table above, it can be seen that the ethanol soluble juice content has a value of 26.52% and for the water soluble juice content it has a value of 16.79%, which is more ethanol soluble than water soluble juice. This shows that the content of active compounds in simplisia is easier to extract using ethanol solvents, because this solvent is universal so that it can attract polar and nonpolar compounds (Islamiyah, 2019) . The results obtained meet the requirements of Materia Medica Indonesia (MMI), namely for ethanol soluble juice content ≥10.5% and for water soluble juice content ≥16.5%.

Total ash content testing aims to determine the content of inorganic and organic compounds, both internal and external (Islamiyah, 2019) . The requirement for total ash content based on Indonesian Medical Materia (MMI) is ≤ 9.5% and in the tested sample it was found that 5.825% and met the requirements. If the ash content obtained is large, the amount of minerals contained is greater.

The acid insoluble ash content aims to determine the presence of impurity compounds from outside such as sand or soil. The requirement from materia medica Indonesia (MMI) is ≥ 1.5%, from the sample tested, it meets the requirements of 5.825%. For the examination of water content is done using the azeotropy method. Determination of water content aims to determine the limit of water content in simplisia, in order to prevent contamination of microorganisms. For the requirements of water content in simplisia < 10% and the test found that the water content in simplisia was 6% which meets the requirements.

**Extraction**

The method used is maceration. This maceration method is a simple method which is without heating so that it can be used on compounds that are not resistant to heat (Badaring et al., 2020). because in flavonoid compounds if heating is done it will reduce the levels of flavonoids (Dalming, 2022).

Maserat as much as 5000 ml is thickened by using a rotary evaporator to get a thick extract. The yield of each concentration of 100% concentration is 9.805%; then 75% concentration is 9.505%; and 50% concentration is 9.233%.

**Phytochemical Screening**

Table 3. Phytochemical screening

| No. | Parameters | results |
|---|---|---|
| 1 | Alkaloids | + |
| 2 | Flavonoids | + |
| 3 | Tannins | + |
| 4 | Saponins | + |
| 5 | Triterpenoids | + |
| 6 | Glycoside | - |

Description:

(+) = shows the results obtained contain metabolite compounds

(-) = this sign indicates that it does not contain metabolite compounds

In the phytochemical screening test conducted, it was found that the chemical compounds contained in the sample were flavonoids, tannins, saponins. In the flavonoid test there is the addition of Mg powder and concentrated HCl where the flavonoid compound will be reduced to produce a red, yellow or orange color, the results in the orange sample test indicate the presence of flavonoid content in the sample. Flavonoid compounds can act as antioxidants, anticancer, anti-inflammatory, antiallergic, and antihypertensive (Winahyu et al., 2019; Guven e al., 2019; Ullah et al., 2020).

In the saponin test, it was found that the foam which was 1 cm and did not disappear in less than 10 minutes, also did not disappear in the addition of HCl. The function of adding HCl to the saponin examination is to form a more stable foam. Saponin compounds have a function that can kill cells of bacteria by touching complex compounds with sterols so that they can damage the membrane of bacteria (Badaring et al., 2020; Pratiwi et al., 2013).

In the examination of tannin compounds, there is the addition of $FeCl_3$ reagent so that a blackish green color is formed which shows positive for tannins. Color changes occur due to

tannins are polar compounds that have OH groups. Alkaloid examination is carried out using three reagents, namely dragendrof, mayer, bouchardat. the mayer reagent shows positive results when the solution has a yellowish white precipitate, then for the dragendrof solvent the sample shows positive alkaloids when a brown, orange, or orange precipitate is formed.

**Wavelength**

Wavelength measurements using quercetin samples because quercetin is a flavonoid compound that has the widest distribution in plants. In the measurement of flavonoid wavelengths there is the addition of $AlCl_3$, which functions as a wavelength shift effect towards visible light which is characterized by a yellow solution. In addition, there is also the addition of sodium acetate which functions as a stabilizer (Winahyu et al., 2019).

Measurements were made by pipetting 4 ml of LIBII solution and adding $AlCl_3$, sodium acetate, aqudest and methanol to the limit mark. Wavelength measurements were carried out with a range between 400-800 nm and the maximum wavelength was 437 nm. The wavelength results can be seen in Figure 1.

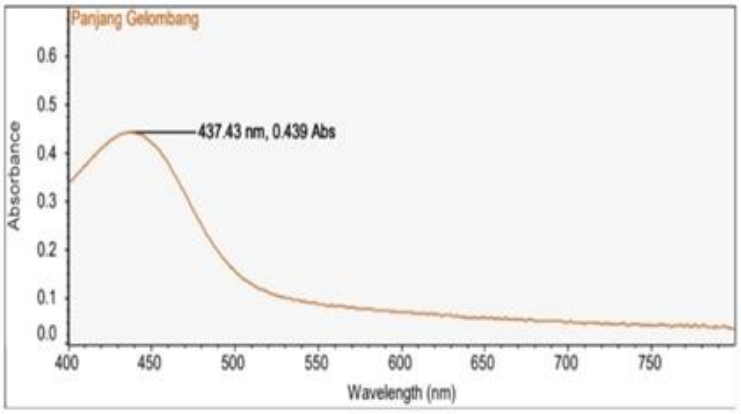


*Figure 1. Quercetin Wavelength*

**Operating Time**

Operating time is done to determine the stable time of the solution by observing the absorbance at a certain time. And where the absorbance does not decrease. The operating time test was carried out for 60 minutes. Measurement of operating time using quercetin solution with a concentration of 40µg/ml, and measured at a wavelength of 437 nm. The operating time results were stable at minute 34 to minute 37.

**Calibration Curve Results**

Calibration curve measurements were made by pipetting 0.2ml; 0.3ml; 0.4ml; 0.5ml; 0.6ml of LIB II solution with a concentration of 100µg/ml. then obtained concentrations of 2µg/ml; 3µg/ml; 4µg/ml; 5µ/ml; 6µg/ml and methanol to the limit mark. After that, 1 ml each was taken and added 0.1ml $AlCl_3$ 0.1 ml and 0.1 ml of 1 M sodium acetate and also added 2.8 ml of distilled water after that it was sufficient with methanol to the limit. Using a 10 ml volumetric flask. Furthermore, measurements were taken at a wavelength of 437 nm. For the measurement results obtained absorbance can be seen in table 4 below

Table 4. Absorbance Value of Calibration Curve

| Concentratio | Absorbanc |
|---|---|
| 0 | 0 |
| 2 | 0,241 |
| 3 | 0,320 |
| 4 | 0,419 |

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| | |
|---|---|
| 5 | 0,554 |
| 6 | 0,634 |

On the calibration curve, a linear regression equation is obtained, namely y = 0.1057 x + 0.0091 and the correlation coefficient value is 0.9982. The correlation coefficient value is a number that is used as a parameter to determine the level of the relationship between the variables being tested. In the correlation coefficient there are levels, namely strong, moderate or weak. In the results of the correlation coefficient obtained that the relationship between the variables is very strong, because the value obtained is close to 1. Can be seen in Figure 2 below.

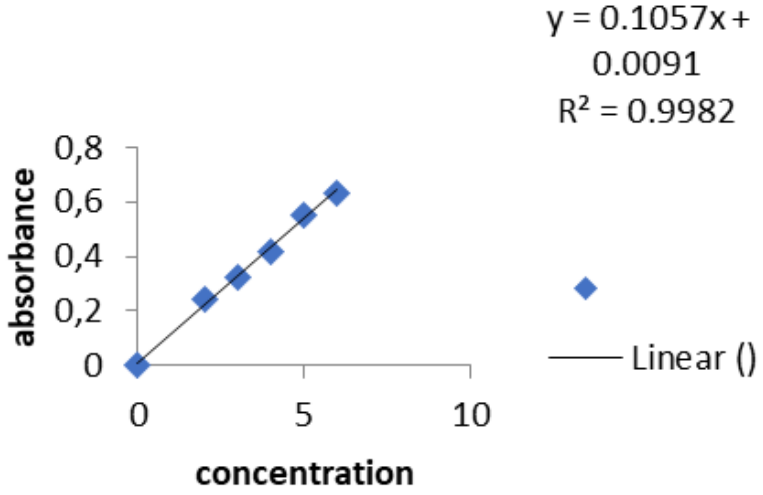


*Figure 2. Calibration curve*

**Total Flavonoids Result**

Determination of total flavonoid content was carried out using uv-vis spectrophotometric method. In flavonoid testing, the compound used as a comparator is quercetin because this compound is a flavonoid group that has a ke group at the c-4 atom and has a hydroxyl group at the c-3 or c-5 atom that neighbors flavones and flavonols.

In the measurement, the sample is reacted with $AlCl_3$ which forms a complex and stable compound with ketone groups at C-4 atoms and hydroxyl groups at C-3 or C-5 atoms neighboring flavones and flavonols. In the sample added $AlCl_3$ will form a complex compound so that there is a change in wavelength towards visible (visible light), which is characterized by a yellow solution. Then there is the addition of sodium acetate which makes the solution remain stable at visible wavelengths (Winahyu et al., 2019)

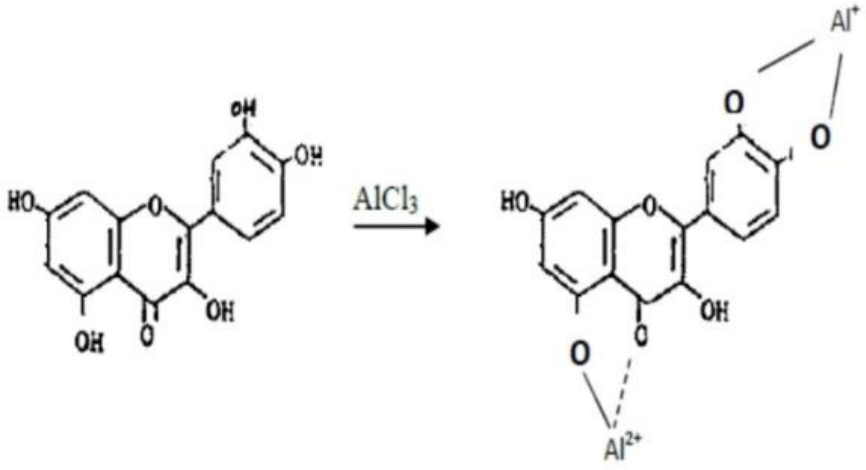


*Figure 3. Reaction of Flavonoid Complex Compound with Alcl₃*

In processing the total flavonoid data by finding the x value in the liner equation. Then the x value is substituted into the total flavonoid formula. The results of the total flavonoid content can be found in table 5.

Table 5. Total Flavonoid Content

| No. | Concentratio | Results |
|---|---|---|
| 1. | 100 % | 42.4308± 2.4739 mgQE/g |
| 2. | 75% | 38.1813± 1.5799 mgQE/g |
| 3. | 50% | 23.8153± 3.049276 mgQE/g |

The table above shows the results of the total flavonoid content in raru wood (*cotylelobium melanoxylon* (Hook.f) Pierre) which of the three samples tested, it can be seen that the highest total flavonoid results are at 100% concentration, namely 42.4308 ± 2.4739 mgQE/g and for the lowest total flavonoid results at 50% concentration, namely 23.8153 ± 3.049276 mgQE/g.

**Antibacterial Activity Testing**

Testing antibacterial activity on *staphylococcus aureus bacteria* using the diffusion method using discs. which is where this method sees a clear zone around the disk. And for the negative control used is DMSO. because DMSO is not bactericidal, which does not inhibit bacteria. And DMSO is also a solvent that can dissolve polar and nonpolar compounds. And the positive control used is chloramphenicol because chloramphenicol is a broad-spectrum antibiotic that is active against gram-positive and gram-negative bacteria (Putri et al., 2023; Danesh et al., 2017).

Table 6. Antibacterial Activity Testing

| Bacteria | Concentration | Repetition1 | Repetition 2 | Repetition3 | Average |
|---|---|---|---|---|---|
| *Staphylococcus aureus* | 100 % | 19,5 | 19,6 | 20,4 | 19,83 |
| | 70 % | 17,15 | 17,3 | 16,75 | 17,066 |
| | 40 % | 15,3 | 15,75 | 14,55 | 15,2 |
| | 10 % | 11,8 | 12,5 | 12,35 | 12,216 |
| | Control (+) | | | | 20,6 |
| | Control (-) | | | | 0 |

In testing antibacterial activity against staphylococcus aureus using concentrations of 100%, 70%, 40% and 10%. Of the four concentrations, the smallest clear zone diameter was obtained at a concentration of 10% and the highest at a concentration of 100%. At a concentration of 100%, the inhibitory power was categorized as strong, namely 19.83. The positive control used was chloramphenicol which had an inhibition of 20.6.

The results showed that the total flavonoid content in raru wood (*Cotylelobium melanoxylon* (Hook.f) Pierre) correlated with the concentration of methanol solvent used in the extraction process. The 100% methanol concentration produced the highest flavonoid content of 42.4308 ± 2.4739 mgQE/g, while the 50% concentration showed the lowest flavonoid content, which was 23.8153 ± 3.049276 mgQE/g. This indicates that solvents with high polarity such as pure methanol have the optimal ability to dissolve flavonoids, phenolic compounds that have a high affinity for polar solvents. The extraction efficiency decreases with decreasing methanol concentration, which can be caused by a decrease in the solubility of flavonoid compounds due to an increase in water content in the solvent.

Antibacterial activity testing against *Staphylococcus aureus* showed that the diameter of the inhibition zone increased along with the concentration of the extract solution. The 100% concentration produced the largest zone of inhibition, at 19.83 mm, which was categorized as strong antibacterial activity. In contrast, the 10% concentration produced the smallest inhibition zone diameter, reflecting the positive relationship between total flavonoid content and

antibacterial effectiveness. In comparison, the positive control using chloramphenicol showed an inhibition of 20.6 mm, which was slightly higher than the inhibition of the extract at 100% concentration. This suggests that raru wood extract has potential as a natural antibacterial agent that is almost equivalent to standard antibiotics.

This research is relevant to a previous study by Fikayuniar et al. (2022) , which showed that flavonoids have antibacterial mechanisms through the inhibition of enzymes important in the synthesis of bacterial cell walls and damage the integrity of cell membranes. In another study (Fahmi et al., 2019) on garlic reported that higher extract concentrations resulted in larger inhibition zone diameters against *Staphylococcus aureus*, supporting the result that flavonoid content and antibacterial activity have a close correlation.

In addition, compared to the study by Rahmawati et al. (2022) on *Psidium guajava* plant extracts, it was found that extracts with high flavonoid content also showed significant antibacterial activity against various gram-positive bacteria, including *Staphylococcus aureus*. However, the difference in inhibition zone diameter may be due to the specific flavonoid composition of each plant. Flavonoids such as quercetin, rutin, and kaempferol are known to have varying antibacterial activities depending on their chemical structure.

The significant antibacterial activity at high concentrations of raru wood (*Cotylelobium melanoxylon*) extract supports the potential use of flavonoid compounds as natural active ingredients in antimicrobial therapy. The high flavonoid content at 100% concentration most likely contributes to the extract's ability to disrupt the bacterial cell wall, inhibit protein synthesis, or damage the bacterial cell membrane. This mechanism is consistent with previous findings of (Sidik & Mambang, 2021) on other plants containing flavonoids as major secondary metabolites. For example, flavonoids such as quercetin are known to have the ability to interact with bacterial cell wall proteins, causing fatal structural damage.

The antibacterial inhibition found in this study provides opportunities for the development of clinical applications, especially in the treatment of infections caused by *Staphylococcus aureus*. This bacterium is known to be the main cause of various infections, including skin infections, abscesses, and pneumonia (El Mubarokah, 2023), so the exploration of natural antibacterial sources is important to overcome resistance to conventional antibiotics. Compared to chloramphenicol, although raru wood extract showed slightly lower inhibition, its advantages lie in the potential for less side effects and the sustainability of the raw material source.

In the context of the research method, the difference in inhibition zones between different concentrations of extracts indicates that the concentration of the active substance greatly affects the antibacterial effectiveness. This study confirms the importance of solvent selection and optimal concentration for secondary metabolite extraction, as evidenced by the higher flavonoid levels at 100% methanol solvent concentration. This method can serve as a reference for other studies in optimizing the extraction of active compounds from plants with similar metabolite characteristics.

This study is also in line with previous research that discusses the relationship between flavonoid content and antibacterial activity. Research by Utami & Damayanti (2022) showed that plant extracts with high flavonoid content have a significant ability to inhibit the growth of gram-positive bacteria such as *Staphylococcus aureus*. However, the variation in antibacterial activity in this study suggests the need for further testing to understand the role of other secondary metabolites, such as saponins and tannins, which may have synergistic effects.

From a pharmaceutical perspective, raru wood extract has the potential to be developed into natural antimicrobial formulations, both as topical creams and active ingredients in oral medicines. To support this, it is necessary to test the toxicity and stability of the extract in various dosage forms. In addition, antibacterial spectrum testing against gram-negative bacteria

can provide more complete information regarding the broad potential of this extract as a therapeutic agent.

Further research could focus on the identification and isolation of major flavonoid compounds in raru wood using chromatographic and spectroscopic techniques. An in-depth understanding of the chemical structure and mechanism of action of these compounds can open up opportunities for the development of plant-based products that are more effective and can compete with synthetic antibiotics. This can also support efforts to conserve biodiversity by raising the economic value of local plants such as raru wood.

## Conclusion

This study concluded that raru wood (Cotylelobium melanoxylon (Hook.f) Pierre) contains major secondary metabolites in the form of flavonoids, saponins, and tannins, which contribute to its biological activity. The measured flavonoid levels showed results of 42.4308 ± 2.4739 mgQE/g, 38.1813 ± 1.5799 mgQE/g, and 23.8153 ± 3.049276 mgQE/g, depending on the extraction conditions. The highest antibacterial activity was observed at 100% concentration with an inhibition zone diameter of 19.83 mm, which was categorized as strong antibacterial activity. For future research, it is recommended to explore the use of different solvents in the determination of flavonoid content and conduct antidiabetic activity tests to explore other therapeutic potentials of raru bark.

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