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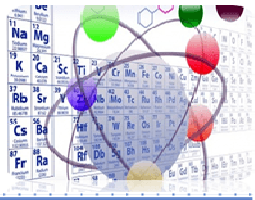

Analysis of Total Flavonoid Content in the Extract of Bajakah Kalalawit Root (*Uncaria gambir* Roxb) Infunded Results

Nurul Reza Alfani, Rizki Febriyanti*, Wilda Amananti

Politeknik Harapan Bersama, Jl. Mataram No. 9, Kel. Pesurungan Lor, Margadana, Tegal City, Central Java 52147

**Corresponding author : rizkifebriyanti.phb@gmail.com*

ABSTRACT

Bajakah kalalawit is one of the plants that has been empirically used in traditional medicine. The roots of Bajakah kalalawit (Uncaria gambir Roxb) have phenol and antibacterial content and have a high enough content of flavonoid compounds so that they have pharmacological activity as an anticancer. This article examines the content of flavonoid compounds contained in the extract of Bajakah Kalalawi root and also examines the total levels of flavonoids contained in the roots of Bajakah Kalalawi. Bajakah kalalawit root extract was obtained by the infundation extraction method using a water solvent (1:10). Examination of the characteristics of the sample is carried out by macroscopic and microscopic identification. The qualitative test of flavonoids was carried out by color reaction tests in two treatments. Determination of flavonoid levels in the root extract of bajakah kalalawi was carried out by the UV-Vis spectrophotometry method. From the results of the study showed that the levels of flavonoids in the root extract of Bajakah Kalalawi in sample A were 33.2% and in sample B by 44.8%. The results showed that the roots of Bajakah Kalalawi were shown to contain flavonoid compounds.

Keywords: *Bajakah Kalalawit Root, Flavonoids, Infundation, UV-Vis Spectrophotometry*

1. INTRODUCTION

Indonesia is known for its abundant natural resources due to its fertile soil. This makes Indonesia one of the countries with the second largest tropical forest in the world and has more than 20,000 types of medicinal plants[1]. Medicinal plants have long been used by the people of Indonesia as traditional medicine to cure various types of diseases. One of the plants that is empirically used as an alternative to traditional medicine is the Bajakah kalalawit plant.

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Bajakah kalalawit (*Uncaria gambir* Roxb) or known as the gambir plant is a shrub plant that comes from the Fabaeae tribe. This plant is commonly found in forest and plantation areas and is widespread in various regions in Indonesia such as West Sumatra, Riau, South Sumatra, North Sumatra and West Kalimantan. Bajakah kalalawit generally grows on land with an altitude of 650 m to 800 m above sea level[2]. Bajakah kalalawit is one of Indonesia's export commodities which has high economic value. Indonesia is the largest supplier of kalalawit steel in the world, namely 80%, and 90% of Indonesia's supply of kalalawit steel is produced from West Sumatra[3].

Bajakah kalalawit has been widely used by the community as a complement to betel nut, skin tanner, cosmetics, and herbal medicine. In the field of pharmaceuticals this plant can be used as a styptic, astringent, antiseptic and medicine for stomach ache. Modernly, bajakah kalalawit has been used in the pharmaceutical industry, such as the Zyma company from Switzerland which isolated catechins from the leaves of bajakah kalalawit which is used to cure liver disease with the patent name "Catergen"[4].

In 1986, Carrick et al conducted an extensive review of the chemical composition of Malay plants and reported on the phytochemistry of the kalalawit plant. They identified that gambier contains alkaloid compounds. With continuous improvement in medicinal plant research methods, an increasing number of compounds including alkaloids, flavonoids and tannins have been discovered in the kalalawit plant. Modern studies show that this plant has pharmacological activities such as anticancer, enzyme inhibitors, and hypoglycemic activity due to flavonoid compounds[5].

In addition, the extract of bajakah kalalawit contains several components including catechins (1%-33%), catechin tanic acid (20%-55%), pyrocatecol (20%-30%), gambir fluoresce (1%-2%), catechins red (3%-5%), quercetin (2%-4%), fixed oil (1%-2%), wax (1%-2%), and small amounts of alkaloids(4). Catechins are compounds that belong to the class of flavonoids. Flavonoids are one of the secondary metabolite compounds and are a large group of antioxidants called polyphenols which are evenly distributed in plant tissues.

This article examines the content of flavonoid compounds contained in the root extract of the bajakah kalalawit (*Uncaria gambir* Roxb). In this study, the root of the kalalawit bajakah was extracted by the infundation method using water as a solvent. The infundation extraction method is a common extraction method used to extract water-soluble active substances. In addition, the infundation method was chosen based on the way consumers consume or process the roots of Bajakah as a treatment, namely by boiling. The UV-Vis spectrophotometry method was carried out to quantitatively determine the levels of flavonoids in the root extract of the kalalawit bajakah. This method can be used to analyze a substance in small quantities, has high sensitivity, provides accurate results, and the processing process is faster.

2. EXPERIMENTAL

2.1. Chemicals, Equipment and Instrumentation

The tools used were 250 mL beaker glass, 10 mL and 100 mL measuring cup, stir bar, porcelain cup, glass funnel, object glass, deck glass, flannel cloth, test tube, test tube rack, wooden clamp, thermometer, pipette. drops, volume pipettes, micro pipettes, volumetric flasks, cuvettes, infusion pans, electric stoves, microscopes, and uv-vis spectrophotometry. The materials used were the root powder of the kalalawit root,

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aquadest, magnesium, 32% HCl, concentrated sulfuric acid (H_2SO_4), methanol, quercetin, 5% $NaNO_2$, 10% $AlCl_3$, and 1 M NaOH.

2.2. Research Procedure

Examination of Simplicia Characteristics

Examination of simplicia characteristics was carried out by macroscopic and microscopic identification. Macroscopic identification by observing the organoleptic simplicia includes shape, color, smell, and taste. Meanwhile, microscopic identification is by observing the visible tissue fragments in simplicia using a microscope[6].

Making Bajakah Root Extract Using the Infundation Method

Weighing the simplicia of the root of the Bajakah is 10 grams each. Mix 10 grams of simplicia with 100 mL of distilled water, then heat over a water bath for 15 minutes starting at 90°C while occasionally stirring. Filter the extract while hot using a flannel cloth. Add enough hot water through the dregs to obtain the desired extract volume[7].

Color Reaction Test

Shinoda test

A total of 1 mL of sample extract was added with 0.1 gram of magnesium and 2 drops of concentrated hydrochloric acid (HCl). The presence of flavonoid compounds is indicated by the formation of orange (flavones), pink (flavonols), and red (2,3 dihydroflavanol) colors[8].

H2SO4 test

As much as 1 mL of sample extract was added with 3 drops of concentrated sulfuric acid (H_2SO_4), producing a dark yellow solution, a bluish-red solution (chalcone, auron), orange-red (flavonols) indicating the presence of flavonoid compounds[8].

UV-Vis Spectrophotometry Test

Preparation of Blank Solution

Take 5 mL of methanol and put methanol into the cuvette

Preparation of Quercetin Mothers Solution

Weigh 10 mg of quercetin, then dissolve it in methanol to obtain a mother liquor of 1 mg/mL. Prepare quercetin solutions (from mother liquor) in a 10 mL volumetric flask to obtain various concentrations of 20, 40, 60, 80, and 100 µg/mL, then add each solution with methanol up to the mark. Pipette 0.5 mL of each mother liquor into a pipette and add 2 mL of distilled water and 150 µL of 5% $NaNO_2$. After 6 minutes, then added with 150 µL $AlCl_3$ 10%. And 6 minutes later, 2 mL of 1 M NaOH was added and added aquadest until 5 mL was measured by UV-Vis spectrophotometry at the maximum wavelength[9].

Determination of Maximum Wavelength (λmax)

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Determination of the maximum wavelength is done by pipetting a certain volume of mother liquor in the cuvette and then checking the wavelengths of 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410 and 420 nm. Then record the absorbance produced by each wavelength and make a curve between the wavelength and absorbance relationship.

Determination of Total Flavonoid Compounds in Extract Samples

a. Preparation of 1000 ppm Extract Mother Solution

Weigh 10 mg of the extract of the root of the plant, then dissolve it in 10 mL of methanol, the volume is added up to the mark.

b. Determination of Total Flavonoid Compounds

Pipette 0.5 mL of a standard extract solution into a test tube, then add 2 mL of distilled water and 150 µL of 5% $NaNO_2$. After 6 minutes, then added with 150 µL $AlCl_3$ 10%. And 6 minutes later, 2 mL of 1 M NaOH was added and added distilled water to a volume of 5 mL. the solution was shaken until homogeneous, then the absorbance was measured at the maximum wavelength obtained. Absorbance measurements were carried out three times. Then the total flavonoid content in the Bajakah Kalalwit extract was calculated using the following formula.

$$\frac{\frac{(\text{absorbansi sampel} - \text{intersep})}{\text{slope}} \times FP \times 100\%}{\text{konsentrasi awal}}$$

3. RESULTS AND DISCUSSION

This article examines the content of flavonoid compounds contained in the extract of the root of the bajakah kalalawit (*Uncaria gambir* Roxb) and also examines the total levels of flavonoids contained in the root of the bajakah kalalawit.

3.1. Examination of Simplicia Characteristics

Examination of simplicia characteristics can be done by macroscopic and microscopic identification. Macroscopic tests are carried out to see the shape and characteristics of the physical or organoleptic appearance of a preparation by direct observation using the five senses. Meanwhile, this microscopic test was carried out to see the identification fragments contained in plant samples using a microscope with a certain magnification[10].

Table 1. Simplicia Macroscopic Test

Observation	**Sample of Bajakah Kalalawit Root Powder**		**References**[7]
	A	**B**	
Bentuk	Fine Powder	Fine Powder	Fine Powder

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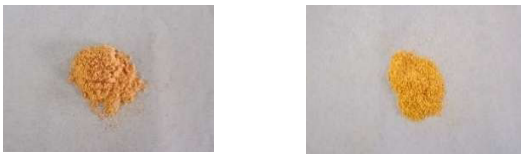
Color	Light Brown	Yellowish-brown	Brown
Scent	No smell	No smell berbau	No smell rbau
Flavor	Slightly bitter, ending with a sweet taste	Slightly bitter, ending with a sweet taste	Sepat slightly bitter, ending with a sweet taste

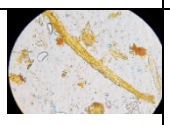
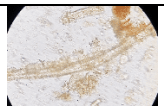
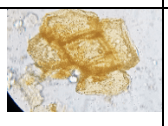
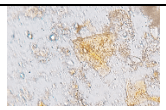
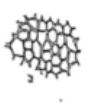


(A) (B)

Figure 1. The Simplisia root of Bajakah Kalalawit brand A and brand B

The results of the macroscopic test in table 1 show that the root of the kalalawit bajakah in sample A is in the form of powder, light brown in color, has a distinctive odor and tastes slightly bitter and ends with a slightly sweet taste. Whereas in sample B, the powder is yellowish brown in color, has a distinctive odor and tastes slightly bitter and ends with a sweet taste. This shows that there is a slight difference in terms of the color of the powder, but these results are in accordance with the literature so that macroscopically, the sample used in this study is the root powder of Bajakah Kalalawit.

Table 2. Simplicia Microscopic Test

No	**Sample of Bajakah Kalalawit Root Powder**		**References**[11]	**Keterangan**
	A	**B**		
1.				Serabut Sklerenkim
2.				Sel gabus

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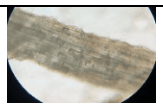
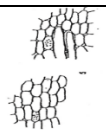
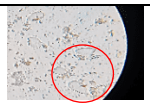
3.				Jaringan Parenkim
4.				Hablur pasir kalsium oksalat

The results of the microscopic test in table 2 show that there is a match between the powder samples and the literature on the Indonesian Medika Materials I-IV. Where in sample A and sample B, typical fragments were found in the form of sclerenchyma fibers, cork cells, parenchyma tissue, and calcium oxalate sand crystals. Observation of tissue fragments in the root simplicia of the Bajakah kalalawit root was carried out using a microscope with a magnification of 40x.

3.2. Preparation of Bajakah Kalalawit Root Extract

Furthermore, the manufacture of the extract of the root of the bajakah kalalawit is carried out using the infundation method where the method is related to the way consumers consume the root of the plant as a treatment, namely by boiling. The infundation method is a hot method of extraction which is very economical, easy to do and does not take a long time. The infundation method was carried out by boiling the root simplicia of the Bajakah kalalawit root using a water solvent with a ratio of 1:10. Where the weight of the Bajakah Kalalawit simplicia is used as much as 10 grams so that the water used is 100 mL. The choice of water solvent is because flavonoids are polar compounds so that the extraction of flavonoids from plants is carried out using polar solvents[12]. The infundation method was carried out at 90°C for 15 minutes. The temperature during the extraction process must be kept stable because flavonoids have properties that are not resistant to high temperatures.

3.3. Color Reaction Test

Qualitative analysis of flavonoid compounds was carried out through a color reaction test with two different treatments, namely the first treatment using the Shinoda test and the second treatment with the addition of concentrated sulfuric acid (H_2SO_4).

Table 3. Color Reaction Test

Sample	Treatment	Observation result	References
Sample A	1 ml sample extract + 0.1 gr Mg + 2 drops HCl P (32%)	Orange, there is foam (+)	Orange or brick red in color

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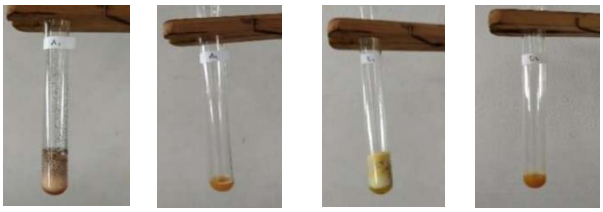
	1 ml sample extract + 3 drops of H2SO4	Orange in color (+)	Dark yellow, orange-red (flavonol), red-bluish (kalkon, auron)
Sample B	1 ml sample extract + 0.1 gr Mg + 2 drops HCl P (32%)	Yellow-orange in color, there is foam (+)	Orange or brick red in color
	1 ml sample extract + 3 drops of H2SO4	Orange in color (+)	Dark yellow, orange-red (flavonol), red-bluish (kalkon, auron)



(A1) (A2) (B1) (B2)

Figure 2. Test the color reaction of the root extract of Bajakah Kalalawit brand a and brand b

From table 3, the results of the study showed that both the bajakah kalalawit extract, sample A and sample B, both contained flavonoid compounds. This is evidenced by the color change in sample A with the first treatment being orange with foam on it and the second treatment being orange after dropping H_2SO_4. The color change that occurred in sample B with the first treatment being a yellow-orange color accompanied by foam and the second treatment being an orange color after being dripped with H_2SO_4.

In the Shinoda test, the addition of Mg and HCl metal aims to reduce the benzopirone nucleus contained in the flavonoid structure so that flavilum salts are formed and the color changes to orange or red. Likewise in the H_2SO_4 test, the addition of H_2SO_4 causes an electrophilic substitution reaction to occur resulting in an orange-red color[13].

3.4. Maximum Wavelength Determination

Quantitative analysis of flavonoids was carried out using the UV-Vis spectrophotometry method by first preparing a blank solution in the form of methanol which is useful for making a zero point concentration from the calibration chart[14]. Then determine the maximum wavelength of the quercetin solution to obtain the maximum absorbance. The standard solution used in determining the levels of flavonoids is quercetin.

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Quercetin is the best flavonoid compound that is often found in plants and is known to have biological activities such as antioxidants. So that the measurement of the wavelength of the quercetin standard solution is obtained as follows.

Table 4. Wavelength and absorbance data

Wavelength (nm)	Absorbance
300	0,435
310	0,757
320	0,959
330	1,259
340	1,645
350	2,248
360	2,899
370	3,049
380	2,965
390	2,306
400	1,067
410	0,317
420	0,069

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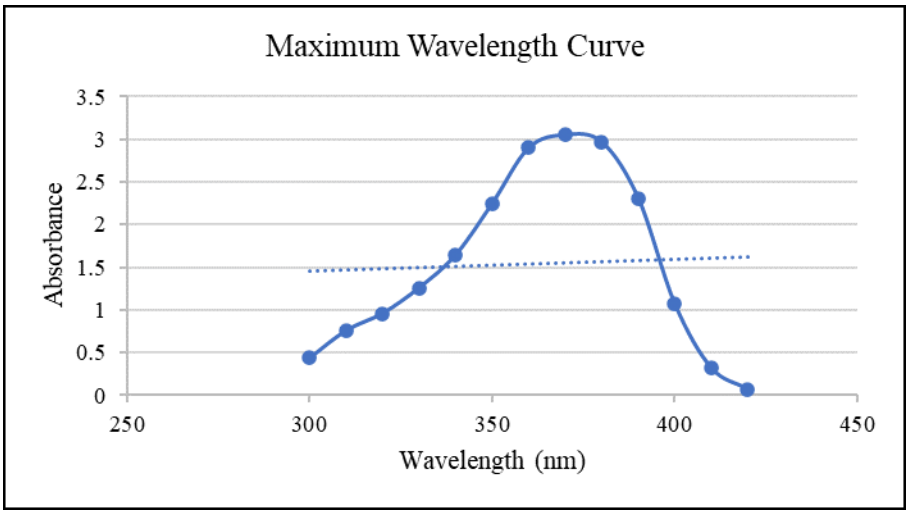

Figure 3. Maximum Wavelength Curve

Based on the graph above, it shows that the maximum wavelength obtained is at a wavelength of 370 nm with an absorbance of 3.049. Determination of the maximum wavelength aims to obtain the maximum sensitivity and absorption at which the change in absorbance for each concentration unit is the greatest[14].

3.5. Quercetin Standard Curve Determination

Quercetin standard curve was prepared by making 5 series quercetin solutions with concentrations of 20, 40, 60, 80, and 100 ppm. A standard curve is made to determine the relationship between the concentration of the solution and the absorbance value. The absorbance of the serial solution was measured at the maximum wavelength obtained, namely 370 nm.

Table 5. Concentration and absorbance of quercetin standard solution

Concentration (ppm)	Absorbance			Average absrobance
	I	II	III	
20	0,416	0,412	0,411	0,413
40	0,623	0,612	0,613	0,616
60	0,771	0,769	0,768	0,769
80	0,855	0,852	0,850	0,852
100	1,282	1,279	1,275	1,279

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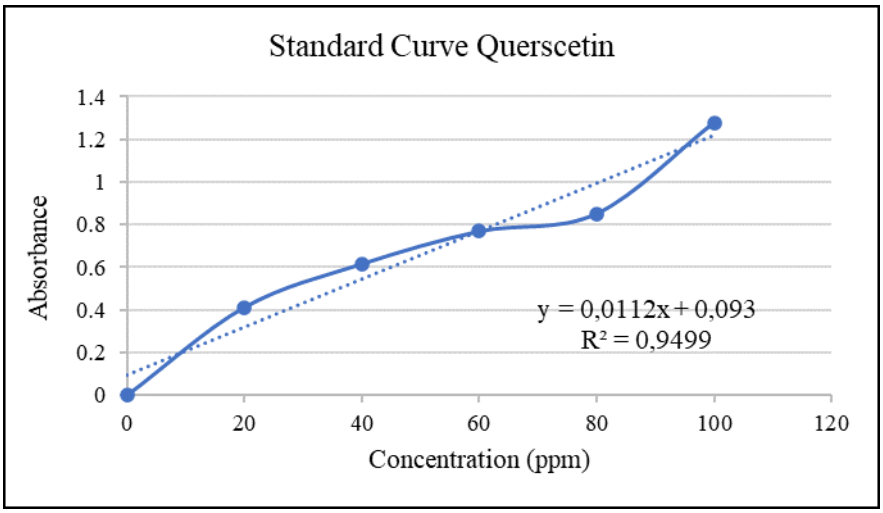

Figure 4. Standard Curve Querscetin

The results of observations on the quercetin calibration curve above, obtained a linear regression equation y = 0.0112x + 0.093 with a relative coefficient price (r) of 0.9499. This equation is used to calculate the total flavonoid content in the sample.

3.6. Determination of Total Flavonoid Content Using UV-Vis Spectrophotometry Method

Furthermore, the determination of the levels of flavonoids in each sample was carried out by the UV-Vis spectrophotometric method using a blank solution as a blank for compounds that did not need to be analyzed. Determination of total flavonoid content in the extract of bajakah kalalawit sample A and sample B was determined by the absorbance at a wavelength of 370 nm.

Table 6. Extract Total Flavonoid Levels

Sample	Replication	Absorbance	Average Absorbance	Total Flavonoid Levels (%)
Sample A	I	0,467	0,465	33,2%
	II	0,465		
	III	0,464		
Sample B	I	0,595	0,595	44,8%
	II	0,594		
	III	0,595		

Based on the results of calculating the levels of flavonoids in the Bajakah kalalawit extract in table 5, it shows that the average level of flavonoids in sample A was 33.2% and in sample B it was 44.8%. So from these results it can be seen the comparison between the Bajakah Kalalawit sample A and the Bajakah Kalalawit sample B, where the Bajakah Kalalawit extract from sample B has a higher content of flavonoid compounds compared to the Bajakah Kalalawit sample A.

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4. CONCLUSION

From the research results obtained, it can be concluded that the root of the kalalawit (*Uncaria gambir* Roxb) is proven to contain flavonoid compounds. The total flavonoid content contained in the extract from the infundation of the root of the kalalawit root in sample A was 33.2% and in sample B was 44.8%.

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