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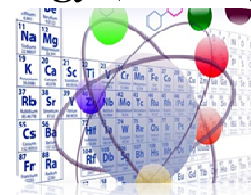
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Determination Of Total Flavonoid Content Of Bajakah Tampala And Kalalawit Roots Using The Reflux

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ABSTRACT

Bajakah is one of the plants found in the forest around Muara Badak. Bajakah root contains flavonoids which have potential as natural ingredients that have medicinal properties. Currently, there are two types of bajakah root on the market, namely tampala and kalalawit. This study aims to determine the content and total flavonoid content of the extract of the Bajakah root from the two types of Bajakah root. The research procedure was initiated by the process of extracting the roots of the Bajakah root using the reflux method using 96% ethanol. The reflux extraction results are then carried out by an evaporation process to obtain a thick extract. The second step is to test the flavone content through a color test. And the quantitative test for determining total flavonoid levels was carried out by UV-Vis Spectrophotometry. Based on the results of a qualitative test using a color reaction, it shows that the roots of the kalalawit and tampala bajakar contain flavonoids. Where the total flavonoid content in the roots of Bajakah Tampala was 59.38% and in Bajakah Kalalawi roots was 47.14%.

Keywords: total flavonoid, reflux, tampala bajakah root, kalalawit bajakah root.

1. INTRODUCTION

Bajakah is a plant originating from Kalimantan. It was first discovered in 1842 by a German botanist named Justus Karl Hassk. (1) As written by detikhealth from the official website of the Bogor Agricultural Institute (IPB), there are 3 types of pirated plants, namely bajakah tampala, kalalawit, and lamei. Bajakah is included in the Spatholobus genus category which is a plant from the Phaseoleae tribe and grows creeping on a tree. This plant grows in tropical forests that are not exposed to much sunlight.¹

Based on research conducted by researchers at the University of Lambung Mangkurat, Bajakah wood has a high antioxidant content, especially for cancer healing. Several laboratory test results found phenolic compounds, flavonoids, steroids, tannins, alkaloids, saponins, and terpenoids in the roots of Bajakah.² The

content of secondary metabolites contained in Bajakah roots can treat degenerative diseases such as diabetes, cancer, tumors, and others. -other. The Bajakah Tampala plant can be used to help the wound healing process and the boiled water from the stems can be used as a medicine for dysentery.³

At present, many online shopping sites sell various types of pirated roots, one of which is the bajakah root used in this study, namely the tampala and kalalawit root types. One of the secondary metabolites contained in the roots of Bajakah is flavonoids. Where Flavonoids are the largest group of phenolic compounds found in nature.⁴ Flavonoid compounds are polyphenolic compounds that have 15 carbon atoms arranged in a C6-C3-C6 configuration, meaning that the carbon skeleton consists of two C6 groups (substituted benzene rings) connected by three-carbon aliphatic chain.⁵ These phenolic compounds have antioxidant activity from various pharmacological effects such as anti-inflammatory, anticancer, antibacterial, and antiviral. This pharmacological effect has also been widely claimed in the packages of bajakah root circulating in the market.² In this study, we tried to identify flavonoid compounds in bajakah root with the title Determination of total flavonoid content Determination of total flavonoid content of Bajakah root extract using reflux extraction method from several brand root of the root circulating in the market.

There are several types of extraction that can be used to identify flavonoids such as maceration, reflux, infudation and soxhletation methods.⁶ In the process of extracting the roots of the pirates using the reflux method. This method was chosen because it can extract coarse-textured samples and easily isolate compounds from samples in solution at high temperatures above room temperature because energy assistance in the form of heat in the reflux process will help speed up the isolation process.⁷ This article examines the presence of flavonoids and their determination. Total flavonoid content of Bajakah root extract by measuring UV-Vis spectrophotometric absorbance.

2. EXPERIMENTAL

2.1. Material

The tools used in this study were analytical balances, UV-Vis spectrophotometer, cuvettes, measuring cup microscopes, vials, glass funnels, dropping pipettes, volume pipettes, test tubes, test tube racks, beaker glass, stir bar, object glass, glass deck , flannel cloth, filter paper, wooden clamps, micro pipettes, measuring flasks, waterbaths, round bottom flasks, condensers, clamps, statives, hoses.

The materials used in this study were bajakah tampala root, bajakah kalalawit root, 96% ethanol, magnesium, concentrated HCl, concentrated H₂SO₄, methanol, 10% AlCl₃, aquadest, NaNO₃, quercetin, acetic acid. (AAS; Variant SpectrAA-400 spectrometer).

2.2. Procedure

Preparation simplicia

The samples used in this study were the roots of the tampala and kalalawit types that are circulating in the market.

Examination of simplicia characteristics

Examination of simplicia characteristics is carried out by macroscopic test through organoleptic test which includes color, texture, shape and microscopic test through visible fragments using a microscope.

Preparation of extract by reflux method

The method of making the extract was carried out by extracting 40 grams of Bajakah root samples by refluxing using 240 mL of 96% ethanol for 3 hours. The results obtained from the extraction process are then evaporated using a water bath until a thick extract is obtained.

Ethanol free test

The test is 1 ml of extract then added 2 drops of acetic acid and 2 drops of concentrated H₂SO₄ then heated. Observing the change in odor, namely if it does not smell of esters, then the extract is free of ethanol and if it still smells of esters, it needs to be evaporated again.⁸

Qualitative test of flavonoid compounds

It was carried out using 2 tests, namely the Shinoda test and with H₂SO₄. In the Shinoda test, the test is 1 mL of extract added to 0.1 gram of magnesium and 2 drops of concentrated HCl. Observe the color changes to orange, pink, and red. In testing using H₂SO₄ the test is 1 mL of extract then added 2 drops of concentrated H₂SO₄. Observing the color change that occurs until it becomes brick red to blackish brown indicates the presence of flavonoid compounds.⁹

Quantitative Analysis

1. Preparation of blank solution

Take 5 mL of methanol and put it into the cuvette.⁸

2. Preparation of Comparison Mains Solution

Weigh 10 mg of quercetin, dissolve in methanol to obtain a mother liquor of 1 mg/mL. Various concentrations of quercetin solution (from mother liquor) were made in a 10 mL volumetric flask to obtain various concentrations of 20, 40, 60, 80, and 100 µg/mL, then adding each solution with methanol up to the mark mark. Pipette 0.5 mL of each mother liquor into a pipette and add 2 mL of distilled water and 150 µL of 5% NaNO₂. After 6 minutes, 150 µL AlCl₃ 10% was added. And 6 minutes later, 2 mL of 1 M NaOH was added and added distilled water until 5 mL was measured by UV-Vis spectrophotometry at the maximum wavelength.⁸

3. Determination of Maximum Wavelength

This is done by pipetting a certain volume of mother liquor and then checking the wavelengths of 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410 and 420 nm. Record the resulting absorbance and construct a curve between the wavelength and absorbance relationship.⁸

4. Determination of Total Flavonoid Compounds in Extract Samples

a. Preparation of 1000 ppm Extract Mother Solution

Weigh 10 mg of extract, dissolve in 10 ml of methanol, volume up to mark limit.

b. Determination of Total Flavonoid Compounds



Pipette the standard extract solution as much as 0.5 mL, then add 2 mL of distilled water and 150 μ L of 5% NaNO₂. After 6 minutes, then added with 150 μ L AlCl₃ 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 is shaken until homogeneous, then the absorbance produced at the maximum wavelength is measured and a relationship curve is made between the standard concentration and the absorbance.⁸

3. RESEARCH RESULT

3.1. Preparation simplicial

Macroscopic Test

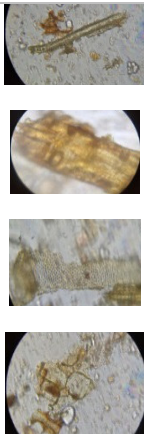
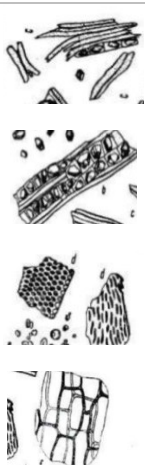

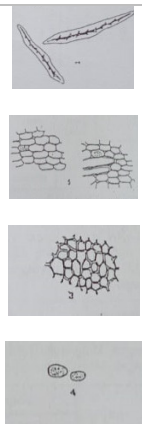
Table 1. Simplicia Macroscopic Test

Simplicia Name	Organoleptic Results	Observation Result	Picture
Bajakah Tampala Roots	Forms Color Textures	Round Chocolate Rather rough	
Bajakah Kalalawit Roots	Forms color Textures	Flat Chocolate Smooth	

The results of simplicia research on the root of Bajakah Tampala are brown in color, round in shape, rather rough in texture. Whereas in the simplicia, the root of the kalalawit root is brown, flat in shape, smooth in texture.

Microscopic Test

Table 2. Simplicia Macroscopic Test

Sample	Observation Result	Reference	Description
Bajakah Tampala Roots			sclerenchyma fiber fragments Empulur fragments Yellowish fragments/vessels Cell fragments contain starch
Bajakah Kalalawit Roots			sclerenchyma fiber fragments Fragment parenchyma cell fragments cork Calcium oxalate sand crystal

Microscopic examination was carried out using a microscope with a magnification of 40x. The results obtained in the root of the tampala pirate were fragments of sclerenchyma fibers, pith, vessels, and starch-containing cell fragments. In the roots of Bajakah Kalalawit there are fragments of sclerenchyma fibers, parenchyma, cork cells, and calcium oxalate crystals. Both results are in accordance with the 1977 *Materia Medika* literature

3.2 Ethanol Free Extract Test

Table 3. Ethanol Free Extract Test





Sample	Test Treatment	Results
Bajakah Tampala Root Extract	1 ml of extract + 2 drops of acetic acid + 2 drops of concentrated H ₂ SO ₄ + heated	(+)
Bajakah Kalalawit Root Extract	1 ml of extract + 2 drops of acetic acid + 2 drops of concentrated H ₂ SO ₄ + heated	(+)

Description (+) = no ester odor

The results showed that both extracts were free from ethanol characterized by the absence of an ester odor.

3.3 Flavonoid Qualitative Test

Table 4. Flavonoid Qualitative Test

Method	Extract	Test Treatment	Results	Description	Picture
Shinoda test	Bajakah Tampala	1 ml of extract + 0.1 gram of concentrated HCl	Brick red	(+)	
	Bajakah Kalalawit		Red	(+)	
H ₂ SO ₄ test	Bajakah Tampala	1 ml of extract + 2 drops of concentrated H ₂ SO ₄	Red	(+)	
	Bajakah kalalawit		Red	(+)	

Description: (+) = give a positive reaction

Based on the tests that have been carried out, each positive sample contains flavonoid compounds due to a red or brick red color change after the addition of the reagent. It can be concluded that both pirated samples contain flavonoids.

3.4 Quantitative Test

Maximum Wavelength Determination

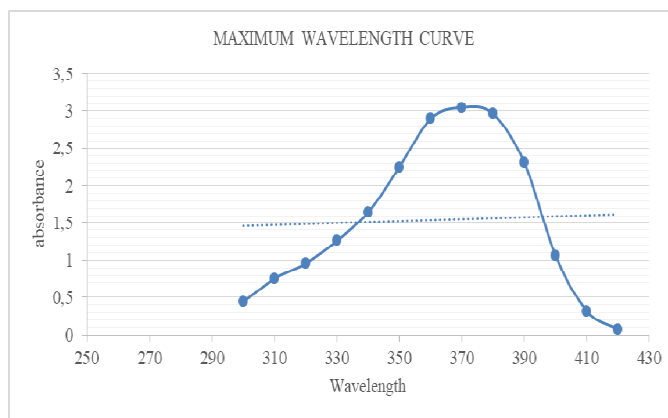


Figure 1. Maximum Wavelength Curve

Quercetin Standard

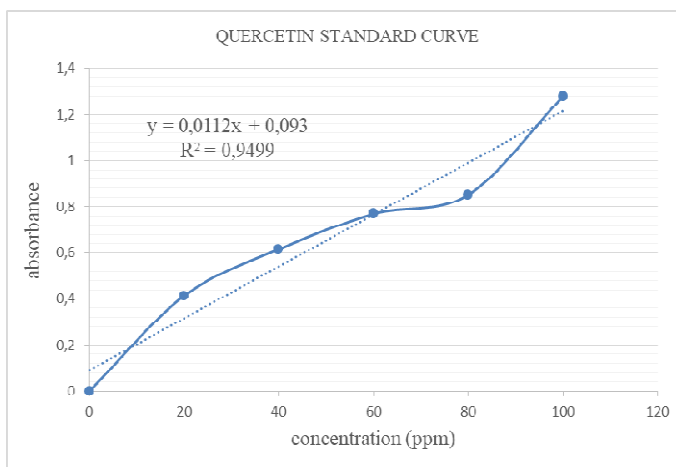


Figure 2. Quercetin Standard Curve

From the research results obtained the maximum wavelength of 750nm. From the results obtained, a quercetin standard curve can be made as shown in Figure 2 which aims to determine the relationship between the concentration of the solution and the absorbance value. From the observations, the quercetin regression equation is $y=0.0112x+0.093$ with a correlation coefficient of 0.9499. This equation is used to calculate the levels of flavonoids in the sample.

Total Flavonoid Levels

Table 5. Flavonoid Qualitative Test

Sample	Replication	Absorbance	Absorbance Average	Total Flavonoid Levels (%)
Bajakah Tampala Roots	I	0,764	0,758	59,38%
	II	0,755		
	III	0,755		
Bajakah Kalalawit Roots	I	0,621	0,621	47,14%
	II	0,622		
	III	0,620		

Based on the results of Table 5, the results of calculating the levels of flavonoids were obtained by entering the absorbance value of the standard quercetin so that the results of the average total flavonoid content in the roots of Bajakah Tampala were 59.38% and in Bajakah Kalalawi roots were 47.14%. The higher the flavonoids, the higher the benefits of flavonoids as antioxidants.

4. CONCLUSION

Based on the results of the study, samples of the roots of the tampala and kalalawit root contained flavonoids. Where the total flavonoid content in the root extract of Bajakah Tampala was 59.38% and in Bajakah Kalalawit root was 47.14%.

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