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Determination of Tannin in Ethanol Extract of Bajakah Tampala Root (*Spatholobus littoralis* Hassk) as an Anticholesterol

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ABSTRACT: The root of Bajakah Tampala (*Spatholobus littoralis* Hassk.) is known to contain several bioactive secondary metabolites, including saponins, flavonoids, phenolics, and tannins, which contribute to its broad pharmacological potential. Among these compounds, tannins play an important role in various therapeutic applications such as hemostasis, burn treatment, and the management of diarrhea, dysentery, and tumors. In addition, tannins possess anticholesterol properties by precipitating mucosal proteins on the surface of the small intestine, thereby reducing the absorption of cholesterol and lipids into the bloodstream. This study aimed to determine the tannin content in the ethanol extract of Bajakah Tampala root as part of an investigation into its potential as a natural anticholesterol agent. The extraction process was carried out using the maceration method with 96% ethanol as the solvent to ensure efficient recovery of phenolic compounds. Quantitative analysis of tannin content was performed using the UV-Visible spectrophotometric method at a wavelength of 750 nm, with gallic acid employed as the standard reference compound. The results showed that the tannin concentration in the ethanol extract of Bajakah Tampala root was 91.699 mg GAE/g extract (milligrams of gallic acid equivalent per gram of extract). These findings suggest that Bajakah Tampala root possesses a high tannin content, supporting its potential development as a natural anticholesterol agent and contributing to the growing evidence of its pharmacological benefits in traditional medicine.

KEYWORDS: Anticholesterol; extraction; phytochemical; spectrophotometry; tannin.

1. INTRODUCTION

Indonesians have a strong habit in Indonesians have a strong habit in their daily lives, which is to consume traditional medicine. In general, traditional medicine is made from plants that contain secondary metabolites that contain secondary metabolites (Fawwaz et al., 2024; Arifin et al., 2025). One plant that is widely used as traditional medicine is bajakah root (S*patholobus littoralis* Hassk) (Hasna, Sehkhaemi and Aviciena, 2021).

Bajakah wood (*Spatholobus littoralis* Hassk) is a plant native to Borneo, found in the interior forests of the region. This plant has a creeping and knotty stem and is known to grow in the forests of Central Kalimantan, although in limited numbers. limited quantities. The people of Kalimantan recognize bajakah wood as a medicinal plant and is referred to as bajakah wood tampala (Nastati and Nugraha, 2022).

Based on qualitative research, the bajakah tampala plant contains secondary compounds such as saponins, flavonoids, phenolics, and tannins (Mochtar *et al.*, 2022). Tannins are very complex compounds and are found equally in various types of plants, almost every plant species contains tannins. This compound has several benefits, including stopping bleeding and treating burns, and can form a protective layer on wounds and kidneys (Khasanah, Sutaryono and Addin, 2021).

In addition, the activity of tannin compounds may also prevent the disruption of oxidant balance and antioxidant production associated with free radical consumption, thus inhibiting LDL oxidation. Tannins also reduce fat absorption in the intestine by reacting with mucosal proteins and intestinal epithelial cells, which can reduce the accumulation of cholesterol in the blood and accelerate the excretion of cholesterol in the feces. In addition, tannins can precipitate mucosal proteins on the surface of the small intestine, thereby reducing the absorption effectiveness of cholesterol and fat (Hasanah *et al.*, 2023).

Based on the above description, it is necessary to conduct research on the determination of tannin content of ethanol extract of bajakah tampala root (*Spatholobus littoralis* Hassk) which has potential as anticholesterol using UV-Vis spectrophotometry method. UV-Vis spectrophotometry is one of the most widely used instrumental methods in chemical analysis to detect compounds (solid/liquid) based on photon absorption (Irawan, 2019).

2. EXPERIMENTAL SECTION

2.1. Materials and sample

Bajakah Tampala root from Central Kalimantan Province, Palangkaraya City. The sample used is the extract of ethanol of the bajakah tampala root. The materials used in this study were bajakah root (*Spatholobus littoralis* Hassk), 96% ethanol, distilled water, potassium ferricyanide, ammonia, $FeCl_3$ 1%, gallic acid, saturated Na_2CO_3, and Folin reagent (sodium tungstate, phospholybdic acid, phosphoric acid).

2.2. Sample preparation

Samples of Bajakah Tampala roots (*Spatholobus littoralis* Hassk.) were first washed thoroughly with running waterto remove any adhering soil and impurities. The cleaned samples were then air-dried at room temperature until completely free of moisture, followed by dry sorting to ensure uniform quality. The dried roots were subsequently ground into a fine powder using a blender to obtain simplicia powder suitable for extraction. The resulting powdered material was stored in a tightly sealed container, protected from direct sunlight and moisture, to prevent degradation of active compounds and maintain sample stability prior to the extraction process.

2.3. Extraction

The extraction process was carried out using a cold extraction method, specifically the maceration technique (Fawwaz *et al*., 2017). A total of 100 g of Bajakah Tampala (*Spatholobus littoralis* Hassk.) root simplicia powder was macerated with 96% ethanol for 3 × 24 hours, followed by re-maceration until the solvent became clear, indicating complete extraction. During the maceration process, the mixture was occasionally stirred to enhance solvent penetration and compound diffusion. The resulting filtrate was then collected and concentrated using a rotary evaporator at 60°C to obtain a thick ethanol extract. The extract yield was subsequently calculated using the following formula:

$$\%\ yields\ extract = \frac{total\ weight\ of\ the\ extract}{total\ simplisia\ powder\ wight} \times 100\%$$

2.4. Qualitative Analysis

A thick extract weighing 0.5 g was dissolved in 10 mL of distilled water, followed by the addition of a few drops of 1% $FeCl_3$ solution. The formation of a dark blue or greenish-black color indicated a positive reaction for tannins(Indriyani et al., 2023). In a complementary test, 200 mg of the thick extract was placed into a test tube and treated with potassium ferricyanide and ammonia. The appearance of a dark brown coloration further confirmed the presence of tannins in the extract (Magfira, 2018).

2.5. Determination of tannin content by UV-Vis Spectrophotometric method

2.5.1. Preparation of 1000 ppm Gallic Acid Standard Solution

A total of 10 mg of gallic acid was weighed and then dissolved with distilled water in a 10 mL volumetric flask until the limit mark, then the solution was used as a 1000 ppm stock solution. The 1000 ppm stock solution was diluted to 100 ppm by pipetting as much as 1 mL and sufficed with distilled water using a 10 mL volumetric flask (Mulyani, Herlina and Suci, 2022).

2.5.2. Preparation of Gallic Acid Standard Solution

The 100 ppm stock solution was pipetted in 0.2, 0.4, 0.6, 0.8, and 1 mL volumes into different 10 mL volumetric flasks and diluted with distilled water to the mark, then shaken until homogeneous to obtain gallic acid standard solution with concentrations of 2, 4, 6, 8, and 10 ppm, respectively.

2.5.3. Determination of the maximum wavelength

The maximum wavelength was obtained by measuring the absorbance of a stock solution of gallic acid at a concentration of 4 ppm. From the solution, 1 mL was pipetted, then 1 mL of *Folin-Ciocalteu* reagent was added and incubated for 5 minutes, then 2 mL of sodium carbonate (Na_2CO_3) was added and incubated for 65 minutes, then the absorbance was measured in the wavelength range of 400-800 nm to obtain the maximum wavelength of 750 nm. Then the operating time was determined from 0-100 minutes at a wavelength of 750 nm and the stable time was determined at 65 minutes (Mulyani, Herlina and Suci, 2022).

2.5.4. Measurement of gallic acid standard solution

Pipette 1 mL of each standard solution of gallic acid. Then 1 mL of *Folin-Ciocalteu* reagent was added and incubated for 5 minutes, followed by 2 mL of sodium carbonate (Na_2CO_3) and incubated for 65 minutes, and the absorbance was measured at a wavelength of 750 nm using a UV-Vis spectrophotometer (Mulyani, Herlina and Suci, 2022).

2.5.5. Analysis of Tannin Content in Ethanolic Extract of Bajakah Tampala

A 10 mg thick extract of Bajakah Tampala root (Spatholobus littoralis Hassk) was weighed and dissolved with distilled water to 10 mL, giving a 1000 ppm stock solution. Next, 5 mL was pipetted into a 10 mL volumetric flask to give a 500 ppm stock solution, from which 1 mL was pipetted and then transferred to a 10 mL volumetric flask to give a sample solution with a concentration of 50 ppm. After pipetting 1 mL of the sample solution, 1 mL of *Folin-Ciocalteu* reagent was added and incubated for 5 minutes. After incubating for 5 minutes, 2 mL of sodium carbonate (Na_2CO_3) was added and incubated for 65 minutes. The absorbance was then measured by UV-Vis spectrophotometry at a wavelength of 750 nm. Then replication was performed 3 times (Mulyani, Herlina and Suci, 2022).

2.6. Data Analysis

Calculated levels using the linear equation of the calibration curve, namely y = a + bx, so that the concentration of the sample is known. using quantitative analysis methods. calculating the tannin content using the following formula:

$$total\ tannin = \frac{c.v.fp}{g}$$

Description:
c = tannin concentration (x value)
v = volume of extract used (mL)
Fp = dilution factor
g = weight of sample used (g)

3. RESULTS AND DISCUSSION

This study utilized samples of Bajakah Tampala root (*Spatholobus littoralis* Hassk.), a plant species widely distributed in the interior forests of Central Kalimantan and traditionally used by local communities as a herbal remedy. The woody roots of Bajakah are reputed to possess diverse pharmacological activities, including antifungal, antimalarial, and anticholesterol properties, which are attributed to their rich content of secondary metabolites such as flavonoids, tannins, and saponins. The extraction process was carried out using the maceration technique with 96% ethanol as the solvent for 3 × 24 hours, followed by re-maceration to ensure complete extraction of bioactive compounds. The combined filtrates were then concentrated using a rotary evaporator at 60°C, resulting in a thick ethanol extract. The percentage yield of the ethanol extract obtained from Bajakah Tampala root was approximately 10%, as shown in **Table 1**.

Table 1. Extraction result of ethanol extract of bajakah tampala root (*Spatholobus littoralis* Hassk).

Sample	Solvent Ethanol 96%	Weight of simplisia (g)	Extract weight (g)	Extract yield (%)
Bajakah tampala root (*Spatholobus littoralis* Hassk)	2.900	300	30.3	10.1

To identify the presence of tannin compounds in the ethanol extract of bajakah tampala root (*Spatholobus littoralis* Hassk.), qualitative phytochemical tests were conducted. These tests aimed to confirm the existence of tannins based on characteristic color reactions observed after the addition of specific reagents. The results of the qualitative analysis of tannin compounds in the ethanolic extract of Bajakah Tampala root are presented in **Table 2**.

Table 2. Qualitative test results of tannin compounds in the ethanolic extract of bajakah tampala root

Sample	Reagent	Test Result	Description
Bajakah Tampala Root (Spatholobus littoralis Hassk)	$FeCl_3$ 1%	Blackish green	+
	Kalium ferrisianida & Ammonia	Dark Brown	+

Description: + = positive

Before the measurement of tannin content, the maximum wavelength of gallic acid, which served as the standard solution, was first determined to identify the wavelength at which gallic acid exhibits maximum absorbance (Basri, Abidin, and Pratama, 2023). Determining this wavelength ensures the accuracy and sensitivity of subsequent quantitative analyses. The measurement results indicated that the maximum absorption wavelength (λmax) of the gallic acid standard occurred at 750 nm. Furthermore, a series of five standard concentrations—2, 4, 6, 8, and 10 ppm—was prepared to

construct the standard calibration curve, which was later used to determine the tannin concentration in the ethanol extract of Bajakah Tampala root as shown in **Table 3** and **Figure 1**.

Table 3. Absorbance measurement results of gallic acid standard solution

Concentration (ppm)	Absorbance (nm)
2	0.389
4	0.504
6	0.642
8	0.724
10	0.83

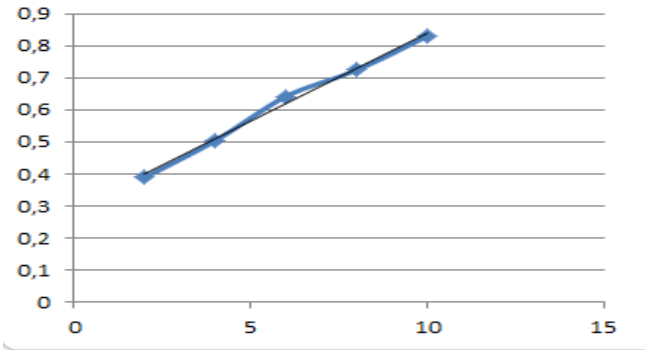

Figure 1. Calibration curve of gallic acid at 750 nm wavelength.

Based on the measurement data of the concentration series of gallic acid standard solution, a standard curve between concentration and absorbance was established (**Table 4**), and a linear equation y = 0.0551x + 0.2872 with a coefficient value of R2 = 0.9938 and a correlation coefficient value of r = 0.9968 was obtained. The resulting value met the linearity requirements, namely the value of r ≥ 0.995 (Tahir *et al.*, 2024).

Table 4. Calculation results of total tannin content of bajakah tampala roots with a concentration of 50 ppm

Replication	Absorbance (Y)	Extract weight (mg/mL)	Total tannin content (mgGAE/g extract)	Average Total Tannin Content (mgGAE/g extract)
1	0.544	0.0104	89.615	
2	0.546	0.103	91.184	91.699
3	0.547	0.0100	94.3	

The measurement results of the sample solution were obtained, namely replication 1 of 89.615 mg GAE/g extract, replication 2 of 91.184 mg GAE/g extract, replication 3 of 94.3 mg GAE/g extract. Then the three replicates were averaged to obtain the average value of total tannin content in the ethanol extract of Bajakah Tampala root (*Spatholobus littoralis* Hassk) which amounted to 91.699 mgGAE/g extract which means that each gram of ethanol extract of Bajakah Tampala root (Spatholobus littoralis Hassk) is equivalent to 91.699 mg gallic acid.

Based on the results of tannin content, the ethanol extract of bajakah tampala root (*Spatholobus littoralis* Hassk) has the potential as an anticholesterol where tannin has several properties, namely as an astringent, anti-diarrhea, anti-diarrhea and anti-diarrhea. astringent, anti-diarrhea, antibacterial and antioxidant. Other antioxidant compounds that can reduce cholesterol levels in the blood is tannin. The tannin compound acts as an antioxidant that can prevent increase in total cholesterol levels in the blood (Mutia, Fauziah and Thomy, 2018).

4. CONCLUSION

Based on the results of this study, it can be concluded that the ethanol extract of bajakah tampala root (*Spatholobus littoralis* Hassk.) contains a tannin concentration of 91.699 mg GAE/g extract. This relatively high tannin content indicates that the extract possesses significant bioactive potential, particularly as an anticholesterol agent. The presence of tannins is known to reduce cholesterol absorption by forming complexes with mucosal proteins in the intestinal lining, thereby supporting the traditional use of *S. littoralis* as a natural therapeutic candidate for managing hypercholesterolemia. Further research involving in vivo and clinical evaluations is recommended to confirm its efficacy and safety for potential pharmaceutical applications.

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