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Analysis of Tanin Content in Bajakah Tampala Root (*Spatholobus Littoralis* Hassk) Infusion from East Kalimantan with Variation of Boiling Time Using Uv-Visible Spectrophotometry Method

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A B S T R A C T
One of the plants that is empirically used by the people of the interior of Kalimantan as a traditional medicine is the bajakah plant *(Spathholobus littoralis* Hassk). This plant is empirically used by the people of the interior of Kalimantan as a traditional medicine. In addition to phenolic or polyphenolic compounds, this plant also contains flavonoids, saponins, terpenoids, steroids and tannins. These compounds have properties and pharmacological effects that can prevent or cure various diseases. The potential of tannins in the health sector is as an antioxidant. The purpose of this study was to determine the tannin content qualitatively, and to determine the tannin levels in the infusion of bajakah wood roots based on variations in boiling time using the UV-Visible spectrophotometry method. The results of the phytochemical screening carried out obtained positive infusion of bajakah tampala wood roots containing alkaloids, flavonoids, tannins and saponins. Tannin levels in bajakah tampala root infusion with various boiling times of 5, 10, 20, and 25 minutes were 1.20 ppm, 3.16 ppm, 2.97 ppm, 2.23 ppm respectively. The highest tannin levels were with a boiling time of 10 minutes.

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INTRODUCTION

One of the plants empirically utilized by the indigenous communities of the Kalimantan (Borneo) interior as traditional medicine is the Bajakah plant, specifically *Spatholobus littoralis* Hassk (Ayuchecaria et al., 2020). This forest plant has been widely used, often through traditional healing ceremonies, to cure various diseases (Nastiti & Nugraha, 2022). Previous studies have demonstrated the promising pharmacological activities of the red Bajakah variety, including anticancer and antidiabetic properties.

The observed bioactivities are attributed to its rich phytochemical content, which includes phenolic compounds (polyphenols), flavonoids, saponins, terpenoids, steroids, and tannins (Nastiti & Nugraha, 2022; Anggraito et al., 2018). These secondary metabolites possess significant pharmacological effects essential for preventing or curing diseases (Anggraito et al., 2018). Tannins, in particular, are large molecular weight polymeric phenolic compounds synthesized within the plant (Hidayah, 2016). They are recognized for their therapeutic potential, notably their antioxidant activity (Sulasiyah et al., 2018).

LITERATURE REVIEW

Traditionally, the root bark of *Spatholobus littoralis* (Bajakah Tampala) is consumed as a decoction (boiled water extract) to treat various ailments. The infusion method was therefore selected for laboratory extraction as it mimics the traditional preparation and is effective in extracting polar active compounds (secondary metabolites) such as tannins (Widyani et al., 2019). Infusion, an extraction process using heat and water as a polar solvent, is a relatively simple method for optimal yield of water-soluble components. However, the traditional boiling method often lacks control over the temperature and duration of heating. Elevated temperatures and prolonged boiling times are critical factors that can significantly affect the chemical stability of sensitive compounds, particularly tannins (Widyani et al., 2019). Furthermore, these parameters can influence the rate and efficiency of the tannin extraction process.

Based on these considerations, it is crucial to investigate the effect of preparation variables on the active compound content. Therefore, this research aims to determine the Tannin Content Analysis in the Infusion of *Spatholobus littoralis* Hassk (Bajakah Tampala) Root from East Kalimantan using UV-Visible Spectrophotometry with varying Boiling Times.

METODOLOGY

Materials

The primary material used was Bajakah Tampala root (*Spatholobus littoralis* Hassk) obtained from a distributor in Lempake, Samarinda City, East Kalimantan. Reagents and solvents included Folin-Ciocalteu reagent, 15% Na_2CO_3 solution, 96% Ethanol, Gallic acid *pro analysis* (p.a.), Oxalic acid p.a., Dragendorff's reagent, Mayer's reagent, $FeCl_3$ solution, and distilled water (aquadest).

Research Methodology

1. Simplisia Preparation

The raw Bajakah root sample was collected from the Lempake sub-district, Samarinda City, and subsequently authenticated via determination. The root wood was then shaved and subjected to oven drying at a maximum temperature of 50° C. Following drying, the *simplisia* (dried crude drug) was pulverized into a fine powder using a grinder.

2. Preparation of Bajakah Tampala Root Infusion

A total of 75 grams of the Bajakah Tampala root *simplisia* was used for the infusion, conducted in replicates of 25 grams each. The *simplisia* was initially dampened with water equivalent to twice its weight (50 mL), followed by the addition of 100 mL of water. The infusion process was carried out at a temperature of 90°C for varying time intervals: 5, 10, 15, 20, and 25 minutes. The time count started once the temperature reached 90°C. The mixture was stirred occasionally (maximum of 4 times) during the process. The resulting infusion was immediately filtered while hot using flannel cloth and the filtrate volume was adjusted to 100 mL using pre-heated aquadest.

3. Phytochemical Screening of Bajakah Tampala Root Infusion

Qualitative phytochemical screening was performed on the Bajakah root infusion to detect the presence of key secondary metabolites, specifically alkaloids, saponins, tannins, polyphenols, and flavonoids.

4. Alkaloid Test

Two milliliters of the test solution were evaporated over a porcelain dish to obtain a residue. The residue was dissolved in 5 mL of 2N HCl. This solution was then divided into three test tubes. The first tube served as a blank (acid only). The second tube was treated with 3 drops of Dragendorff's reagent, and the third tube was treated with 3 drops of Mayer's reagent. The formation of an orange precipitate in the second tube and a yellow precipitate in the third tube indicates the presence of alkaloids.

5. Saponin Test

A volume of 10 mL of the test solution was placed into a test tube and shaken vertically for 10 seconds, followed by a rest period of 10 seconds. The formation of stable foam measuring 1-10 cm high for not less than 10 minutes suggests the presence of saponins. A key confirmation is that the foam does not disappear upon the addition of 1 drop of 2 N HCl

6. Tannin and Polyphenol Test

Two milliliters of the test solution were divided into two parts. Tube A served as the blank, and Tube B was reacted with 10% Iron(III) Chloride solution. The appearance of a deep blue or greenish-black color indicates the presence of tannins and polyphenols.

7. Flavonoid Test

For the flavonoid examination, 1 mL of the test solution was moistened with acetone, followed by the addition of small amounts of finely powdered boric acid and oxalic acid. The mixture was heated on a water bath, avoiding excessive heating. The resulting residue was then mixed with 10 mL of (Ether P) and

observed under UV light at 366 nm. The observation of an intensive yellow fluorescence indicates the presence of flavonoids

Identification and Quantification of Total Tannin Content

1. Preparation of Gallic Acid Standard Solution (100 ppm)

10 mg of Gallic acid (*pro analysis*) was accurately weighed and dissolved in aquadest to a final volume of 100 mL, establishing a 100 ppm stock standard solution.

2. Determination of Maximum Wavelength

A 4 ppm Gallic acid solution was accurately pipetted into a 10 mL volumetric flask. 1 mL of Folin-Ciocalteu reagent was added, shaken, and allowed to stand for 5 minutes. Subsequently, 2 mL of 15% Na_2CO_3 solution was added, shaken until homogeneous, and allowed to stand for another 5 minutes. Aquadest was then added to adjust the volume to 10 mL. The solution was scanned using the UV-Vis Spectrophotometer across the wavelength range of 600-800 nm to determine the maximum absorbance wavelength.

3. Preparation of the Gallic Acid Standard Curve

Specific amounts of the Gallic acid stock solution were accurately pipetted into 10 mL volumetric flasks to prepare standard solutions at concentrations of 1, 2, 3, 4, 5, 6, and 7 ppm. To each flask, 1 mL of Folin-Ciocalteu reagent was added, shaken, and allowed to stand for 5 minutes. Then, 2 mL of 15% Na_2CO_3 solution was added, shaken until homogeneous, and the mixture was allowed to stand for a fixed time of 90 minutes to complete the color development. The absorbance was measured at the Maximum Wavelength. The procedure was repeated to obtain seven concentration points for the Gallic acid standard curve.

4. Spectrophotometric Determination of Total Tannin Content in Bajakah Root Infusion

The total tannin content in the Bajakah Tampala root infusion, prepared at various boiling times (5, 10, 15, 20, and 25 minutes), was determined using UV-Vis Spectrophotometry. One milliliter of the infusion sample was accurately pipetted into a 10 mL volumetric flask. To this, 1 mL of Folin-Ciocalteu reagent was added, and the mixture was shaken and allowed to stand for 5 minutes. Subsequently, 2 ml Na_2CO_3 15 % solution was introduced, shaken until homogeneous, and allowed to stand for another 5 minutes. Distilled water (aquadest) was then added to adjust the final volume to 10 mL. The solution was incubated in the dark for 90 minutes to ensure complete color development. The absorbance was then measured at the determined maximum wavelength. The entire procedure was performed with three replications (triplo) for each boiling time variation

5. Determination of Limit of Detection (LoD) and Limit of Quantitation (LoQ)

` To evaluate the sensitivity and accuracy of the analytical method, the Limit of Detection (LoD) and Limit of Quantitation (LoQ) were determined. Five concentrations of the previously prepared serial standard solutions were used. From each standard concentration, 0.5 mL was accurately pipetted and mixed with 7.5 mL of aquadest and 0.5 mL of Folin-Ciocalteu reagent, followed by an incubation period of approximately 5 minutes. Subsequently, 1.5 mL of saturated Na_2CO_3 solution was added. The absorbance was measured at the maximum

wavelength. The LoD and LoQ values were calculated using the following equations:

$$LoD = 3 \times SD_{slope}$$

$$LoQ = 10 \times SD_{slope}$$

RESULT AND DISCUSSION

Phytochemical Screening Test

The extraction method in this study was the Infusion method. The dried *simplisia* was heated using aquadest (purified water) as the solvent for specific time intervals, with the duration counted once the temperature reached 90°C. This temperature increase enhances the extraction yield by improving the solubility of secondary metabolite compounds within the plant matrix (Yuliani et al., 2015). The infusion method was specifically chosen because it is simple, utilizes water—the solvent traditionally used by the local community—and is thus highly representative of the traditional preparation method (Kuncoro et al., 2022). The primary objective of using aquadest as the polar solvent is to optimally extract the polar active compounds (Yuliani et al., 2015).

Table 1. Phytochemical Screening of Bajakah Root Infusion

No.	Compound	Reagent	Result	Conclusion
1.	Alkaloid Wagner Dragen-drof	Wagner Dragendrof	Brown precipitate Orange precipitate	Positive (+) Positive (+)
2.	Flavonoids	Magnesium powder +concentrated HCl	Orange solution	Positive (+)
3.	Taninn	$FeCl_3$	Greenish-brown color	Positive (+)
4.	Saponins	Distilled water + shaken	Stable foam	Positive (+)

The results of the phytochemical screening performed on the Bajakah root infusion are summarized in Table 1, confirming the positive presence of alkaloids, flavonoids, saponins, and tannins. This finding aligns with previous research conducted by Saputera & Ayuchecaria (2018). The presence of tannins, in particular, confirms that this secondary metabolite is a major constituent of the Bajakah Tampala root wood.

Determination of Total Tannin Content

1. Determination of Maximum Wavelength

The maximum wavelength required for the spectrophotometric analysis was determined by scanning the Gallic Acid standard solution. The analysis

revealed that the maximum absorbance was successfully obtained at a wavelength of 784 nm. The result of the maximum wavelength determination is illustrated in Figure 1.

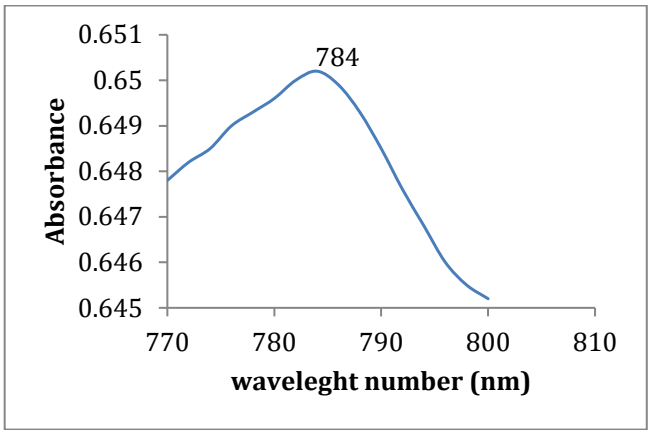

Figure 1. Determination of Gallic Acid Maximum Wavelength

The maximum wavelength was determined by scanning the Gallic Acid solution across the range of (600-800 nm). While previous research by Noviyanty *et al*. (2020) reported a maximum wavelength of nm, the results obtained from this specific analysis fall within the expected (600-800) range, with the maximum absorbance recorded at 784 nm.

2. Determination of Gallic Acid Standard Calibration Curve using Folin-Ciocalteu Reagent

The construction of the Gallic Acid standard calibration curve using the Folin-Ciocalteu reagent yielded the linear regression equation: y = 0.1035x + 0.1334. The linearity of the curve was validated by a high correlation coefficient (r^2) of 0.9907. The absorbance values used for plotting the standard curve, along with the visual representation of the curve, are shown in Figure 2.

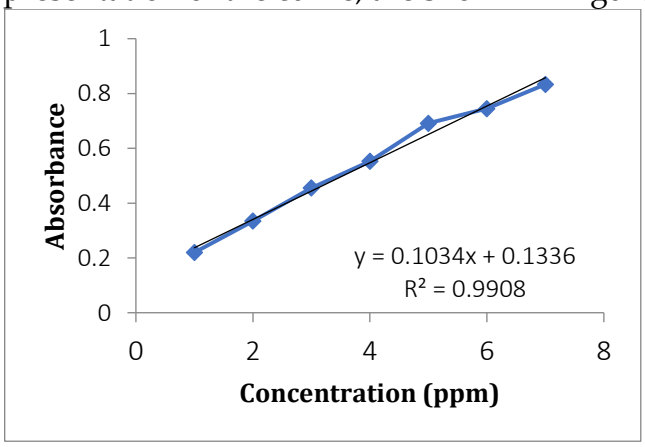

Figure 2. Gallic Acid Calibration Curve

The quantitative determination of total tannin content was achieved via UV-Vis Spectrophotometry utilizing the Folin-Ciocalteu method. This analytical technique is based on the redox reaction that results in the formation of a molybdenum-tungsten blue complex.Specifically, the hydroxyl groups -OH present in the phenolic compounds (including tannins) react with the Folin-Ciocalteu reagent. The resulting complex is then detected spectrophotometrically

at the previously established maximum wavelength of 784 nm. Because the phenolic compounds require an alkaline medium to react with the Folin-Ciocalteu reagent, Sodium carbonate Folin-Ciocalteu was added to ensure the necessary basic environment. Gallic acid was chosen as the reference standard (comparative) due to its defined phenolic structure, high stability, purity, and cost-effectiveness. The primary objective of constructing the standard calibration curve was to confirm the required linearity between the concentration of the Gallic acid standards and their measured absorbance values.

The resulting regression equation from the standard curve was subsequently used to calculate the total tannin content in the Bajakah Tampala root infusion samples. The curve was generated by measuring the absorbance of seven Gallic acid concentrations (1, 2, 3, 4, 5, 6, and 7 ppm) at a wavelength of 784 nm. The measured absorbance values for the standard series ranged from (0.2196 to 0.8333), which is in compliance with the Lambert-Beer Law's optimal linearity range (typically 0.2-0.8). As presented in Figure 2, the plot of concentration versus absorbance yielded the linear regression equation: $y = 0.1035x + 0.1334$. Furthermore, the curve exhibited an excellent correlation coefficient (r) of 0.9907, confirming a highly satisfactory degree of linearity for the quantification method.

3. Quantification of Total Tannin Content

Following the qualitative phytochemical screening, a quantitative analysis was performed on the Bajakah Tampala root infusion with varying boiling times. The results obtained from the calculation of Gallic Acid Equivalents (GAE), which represent the total tannin content in the Bajakah root infusion, are presented in **Table 2**.

Table 2. Total Tannin Content

Boiling Time	**Absorbance**	**Concentration (ppm)**	**Average**	**Standard Deviation**	**%RSD**
5 minute	0,256	1,184	1,203	0,025	0,02128
	0,257	1,193			
	0,261	1,232			
10 minute	0,454	3,099	3,166	0,060	0,01975
	0,466	3,215			
	0,463	3,186			
20 minute	0,449	3,050	2,976	0,120	0,04037
	0,427	2,838			
	0,448	3,041			
25 minute	0,362	2,209	2,23	0,044	0,01999
	0,370	2,286			
	0,362	2,209			

The variation in boiling time significantly influenced the resulting tannin content. An increase in the measurable concentration (C) was observed from the 5-minute interval to the 10-minute interval. However, the tannin content subsequently decreased at the 20-minute and 25-minute intervals. This reduction suggests that excessive boiling time may affect the stability of the tannin

compounds, as prolonged heating influences the speed and rate of absorption of tannin content in the decoction. This observation aligns with previous research; for instance, a study on *Physalis viscosa* showed that a minimum boiling time of 15 minutes was required to significantly reduce its tannin content (Essack et al., 2017). Furthermore, the trend of decreasing tannin content through boiling is consistent with the findings of Singh *et al.* (2015), which reported that boiling reduced the tannin content of *Eryngium foetidum* by 3.6 %.

4. Determination of Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The Limit of Detection (LoD) represents the smallest measurable parameter of an analyte that an instrument can detect, while the Limit of Quantitation (LoQ) defines the lowest concentration or amount of analyte that can be reliably determined with acceptable accuracy and precision. Based on the calculations derived from the validation data the following values were obtained LoD: 0.75 ppm, LoQ: 2.26 ppm,

Table 3. Calculated Results for LoD and LoQ

Type of Test	**Concentration (ppm)**
LOD	0,754
LOQ	2,260

The calculated Limit of Detection (LoD) value of 0.75 ppm signifies that the UV-Vis Spectrophotometer utilized in this study is capable of generating a distinguishable response/result for tannin analysis with a minimum amount of analyte still quantifiable at 0.75 ppm. Conversely, the Limit of Quantitation (LoQ) value of 2.260 ppm represents the lowest concentration of the analyte that can be determined with the best acceptable accuracy and precisions

CONCLUSION AND RECOMMENDATION

Based on the research findings, the following conclusions can be drawn:

1. Phytochemical screening of the *Spatholobus littoralis* (Bajakah Tampala) root infusion demonstrated the positive presence of key secondary metabolites, specifically alkaloids, flavonoids, tannins, and saponins.
2. The duration of boiling time significantly influences the total tannin content of the Bajakah Tampala root infusion. The infusion boiled for 10 minutes yielded the highest tannin concentration at 3.166 ppm, with the concentration subsequently decreasing as the boiling time was further extended.

The analytical method validation yielded an LoD value of 0.75 ppm and an LoQ value of 2.26 ppm. This LoD value confirms that the UV-Vis Spectrophotometer is capable of providing a reliable response for tannin analysis for analyte concentrations as low as 0.75 text ppm.

FURTHER STUDY

This research still has limitations so further research on this topic is still needed "Analysis of Tanin Content in Bajakah Tampala Root (*Spatholobus Littoralis* Hassk) Infusion from East Kalimantan with Variation of Boiling Time Using Uv-Visible Spectrophotometry Method"

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