

# Evaluation of Total Flavonoid Content in *Nephelium lappaceum* L. Leaves from Different Altitudes Using UV-Vis Spectrophotometry

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## ABSTRACT

Rambutan (*Nephelium lappaceum* L.) is a widely distributed plant in various regions and is known to contain flavonoid compounds. In pharmaceutical development, the geographical location of plant growth can influence the levels of secondary metabolites. This study aims to determine the total flavonoid content in rambutan leaves based on differences in altitude using UV-Visible spectrophotometry at a wavelength of 435 nm. The leaves were extracted via maceration using ethanol p.a. as the solvent. The total flavonoid content was determined using AlCl<sub>3</sub> reagent with quercetin as the standard. The results showed that rambutan leaves collected from lowland areas (Komo Luar Subdistrict, Wenang District, Manado City) had a higher total flavonoid content (42.7 ppm) compared to those of highland regions (Kampung Jawa Subdistrict, Tomohon City), which contained 30.4 ppm. These findings indicate that plants grown at lower altitudes produce higher levels of flavonoids, possibly due to increased light intensity and climatic conditions, which promote the synthesis of secondary metabolites such as flavonoids.

**Kata kunci:** Flavonoids; Maceration; Quercetin; UV-Vis Spectrophotometry

## INTRODUCTION

Rambutan (*Nephelium lappaceum* L.) has traditionally been utilized for medicinal purposes; however, its leaves are often overlooked despite their potential pharmacological value. The rambutan plant has compound leaves arranged pinnately with 5–9 leaflets. Each leaflet is ovate with a pointed apex and base and pinnate venation and varies in color from yellowish-green to dark green or sea green. The leaf veins form slanted, parallel lines and often exhibit signs of drying due to limited water availability (Purbasari Karlina, 2017).

Several studies have reported that the content of secondary metabolites in plants is influenced by the altitude at which the plant grows. For example, galangal (*Alpinia galanga* L.) grown at altitudes of 20, 1093, and 1200 meters above sea level (masl) showed flavonoid contents of 1.09±0.08 mg QE/g, 5.47±0.24 mg QE/g, and 1.16±0.3 mg QE/g, respectively, with LC<sub>50</sub> values of 332.48 ppm, 447.14 ppm, and 518.57 ppm (Lallo et al., 2022). Similarly, *Chromolaena odorata* exhibited flavonoid contents of 5.63±0.26 mg QE/g, 10.28±0.28 mg QE/g, and 26.57±0.24 mg QE/g at altitudes of 223, 618, and 1012 masl, respectively, along with antioxidant activity values of 61.74±0.59 ppm, 73.06±0.97 ppm, and 91.54±0.817 ppm (Lia, 2022).

To date, no studies have investigated the influence of altitude on the flavonoid content in rambutan leaves. Therefore, this study aims to explore the effect of growing altitude on the total flavonoid content of rambutan leaves using UV-Visible spectrophotometry.

UV-Visible spectrophotometry is an analytical technique used to quantify compounds containing chromophore and auxochrome groups (Sahumena dkk., 2020) such as flavonoids. This method is widely used for quantitatively analyzing flavonoid content in plant samples due to its advantages, including sensitivity, efficiency in sample use, and the ability to detect small amounts of analyte based on light absorption within a specific wavelength range. (Risna (2023)

## METHOD

This study employed an experimental design to examine the influence of plant growth altitude on the total flavonoid content of ethanol extracts from *Nephelium lappaceum* L. leaves. Flavonoids

were quantified using UV-visible spectrophotometry at a wavelength of 435 nm. The test concentrations for the extract were 20, 40, 60, 80, and 100 ppm, while standard quercetin solutions were prepared at 2, 4, 6, 8, and 10 ppm.

### **Instruments and Materials**

The instruments used included a UV-Vis spectrophotometer (Rittun), water bath (Joanlab), blender (Philips), 40-mesh sieve (GB/T6003), analytical balance (Sojilab), rotary evaporator (Ika HB 10), glass jars, test tubes (Iwaki), test tube racks, dropper pipettes (Pyrex), micropipettes (Joanlab), volumetric flasks (Herma), measuring cylinders (Pyrex), beakers (Pyrex), and porcelain crucibles.

The materials used in this study were *Nephelium lappaceum* L. leaves, 70% ethanol, distilled water (aqua dest), quercetin powder (rofa), aluminum chloride (AlCl<sub>3</sub>) (rofa), and sodium acetate (rofa).

### **Study Period and Location**

The research was conducted from April to May at the Pharmacognosy-Phytochemistry Laboratory, Diploma Program in Pharmacy, Faculty of Health Sciences, Muhammadiyah University of Manado.

### **Preparation of Plant Materials**

Samples were taken from two different locations in the highlands of Tomohon City, South Tomohon District, Kampung Jawa Village at one in the afternoon, while the lowlands in Komo Luar Village, Wenang District at 9 in the morning. Two kilograms of fresh rambutan leaves were collected and subjected to wet sorting to remove dirt and impurities. The leaves were washed three times with clean water and sliced to facilitate drying. Drying was performed under direct sunlight until completely dry, followed by dry sorting to eliminate any remaining foreign matter. The dried leaves were ground using a blender and passed through a 40-mesh sieve. The resulting powder was stored in a sealed container, protected from sunlight.

### **Preparation of Ethanol Extract**

Four hundred grams of powdered rambutan leaves were macerated with 3000 mL of 70% ethanol in a 1:7.5 ratio for 72 hours with occasional stirring. The extract was then filtered, and the residue was re-macerated with 1000 mL of 70% ethanol (1:2.5 ratio) for 48 hours. The combined filtrates were concentrated using a rotary evaporator at 40°C–60°C and thickened using a water bath to obtain a viscous extract. The yield was then calculated.

### **Determination of Total Flavonoid Content in Rambutan Leaf Extract**

Ten milligrams of thick rambutan leaf extract were dissolved in 10 mL of ethanol p.a. and diluted in a 100 mL volumetric flask to obtain 20, 40, 60, 80, and 100 ppm concentrations. For each concentration, 1 mL of extract was mixed with 1 mL of 10% AlCl<sub>3</sub> and 1 mL of 1 M sodium acetate. The solutions were homogenized and incubated for 10–14 minutes. Absorbance was measured using a UV-Vis spectrophotometer at a wavelength of 435 nm.

## **RESULTS AND DISCUSSION**

This study aimed to determine the total flavonoid content in rambutan (*Nephelium lappaceum* L.) leaf ethanol extract. The samples were collected from two different altitudes: highland (Kelurahan Kampung Jawa, South Tomohon District, Tomohon City) and lowland (Kelurahan Komo Luar, Wenang District, Manado City, North Sulawesi Province).

The preparation of simplicia began with washing the rambutan leaves to remove dirt and impurities. This step was crucial to ensure that the extracted compounds were solely derived from the plant material and not contaminated by external substances. The leaves were then sun-dried to reduce moisture content, facilitating drying. Once dried, the leaves were ground into a fine powder using a blender and sieved through a 40-mesh sieve to obtain uniform particle size. Smaller particle sizes improve extraction efficiency by increasing surface area for solvent penetration (Puji & Della, 2022).

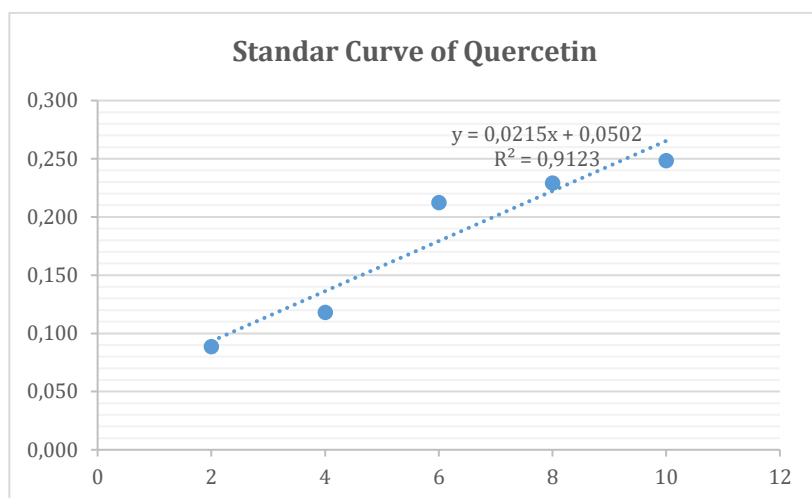


Figure 1. Quercetin standar curve at a wafelength of 435 nm

Table I. Yield of Rambutan Leaf Extract (*Nephelium lappaceum* L.)

Sample weight	Extract Weight (g)	Yield (%)
600	37,321	6,22%

Table II. Absorbance Measurements of Rambutan Leaf Extract (Lowland Sample)

Concentration (ppm)	Replication	Absorbansion	Average	x	Average	Total flavonoid (ppm)
20	1	0,121		3,293		16,31008
	2	0,12	0,12	3,247	3,262	
	3	0,12		3,247		
40	1	0,122		3,34	3,34	16,69767
	2	0,122	0,122	3,34		
	3	0,122		3,34		
60	1	0,146		4,456	4,456	22,27907
	2	0,146	0,146	4,456		
	3	0,146		4,456		
80	1	0,195		6,735	6,735	33,67442
	2	0,195	0,195	6,735		
	3	0,195		6,735		
100	1	0,234		8,549	8,549	42,74419
	2	0,234	0,234	8,549		
	3	0,234		8,549		

The extraction process employed maceration, a simple and effective method especially suitable for leaf samples, as it avoids heat that could potentially degrade thermolabile active compounds (Azlin, Wahyu & Asiska, 2023). A total of 600 grams of powdered simplicia were macerated with 4.5 liters of 70% ethanol (ratio 1:7.5) for four days. The filtrate was then separated and concentrated using a rotary evaporator at 90°C and 280 rpm for two days, followed by further evaporation using a water bath at 40°C to yield a thick extract. The final extract weight was 37.32 grams, from which the yield was calculated.

The standard curve for quercetin was established using UV-Vis spectrophotometry at 435 nm. Absorbance readings from the standard solutions were averaged and used to generate a linear regression equation  $y = 0.0215x + 0.0502$  with a correlation coefficient ( $R^2$ ) of 0.9123, indicating a strong linear relationship. This equation was then used to determine the total flavonoid content of

**Table III. Absorbance Measurements of Rambutan Leaf Extract (Highland Sample)**

Concentration (ppm)	Replication	Absorbansion	Average	x	Average	Total flavonoid (ppm)
20	1	0,041	0,041	1,906977	1,906977	9,5348837
	2	0,041		1,906977		
	3	0,041		1,906977		
40	1	0,049	0,049	2,279	2,264	11,317829
	2	0,048		2,232558		
	3	0,049		2,27907		
60	1	0,075	0,075	3,488372	3,488372	17,44186
	2	0,075		3,488372		
	3	0,075		3,488372		
80	1	0,092	0,092	4,27907	4,27907	21,395349
	2	0,092		4,27907		
	3	0,092		4,27907		
100	1	0,131	0,131	6,093023	6,093023	30,465116
	2	0,131		6,093023		
	3	0,131		6,093023		

the samples based on their absorbance values. The maximum absorbance was observed at a wavelength of 435 nm, confirming the presence of flavonoid compounds in the extracts.

The determination of total flavonoid content was conducted by dissolving 10 mg of the thick extract in ethanol p.a. and diluting to 100 mL in a volumetric flask to achieve stock solutions. These were used to prepare five test concentrations: 20 ppm, 40 ppm, 60 ppm, 80 ppm, and 100 ppm. Each concentration was mixed with 1 mL of 10% AlCl<sub>3</sub> and 1 mL of 1 M sodium acetate. The mixtures were incubated for 10–14 minutes before absorbance was measured using a UV-Vis spectrophotometer at 435 nm. Each measurement was performed in triplicate, and the results are presented in Tables II and III.

Based on the results presented in Tables 2 and 3, the total flavonoid content in rambutan leaves was found to be higher in samples collected from lowland areas compared to those from highland regions. This difference is reflected in the higher absorbance values observed in the lowland extracts. The altitude of the growing location significantly influences the flavonoid content in rambutan leaf extracts. As elevation increases, the total flavonoid content tends to decrease. This may be attributed to variations in environmental factors such as temperature, light intensity, and stress conditions, which can affect the biosynthesis of secondary metabolites, including flavonoids.

## CONCLUSION

This study demonstrates that the geographical location where a plant grows can significantly influence the content of its secondary metabolites. In the case of *Nephelium lappaceum* L. leaf extract, samples collected from lowland areas contained higher levels of flavonoids compared to those from highland regions. Altitude affects various environmental parameters such as temperature, light intensity, and rainfall, all of which can impact the biosynthesis of flavonoid compounds in plants. These findings are expected to contribute to the standardization of medicinal plants and support the development of pharmaceutical preparations containing optimal levels of bioactive metabolites.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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