

Isolation and Identification of Flavonoid Compound from the Rambutan Stem Bark (*Nephelium lappaceum L.*)

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ABSTRACT

Flavonoids derived from the stem barks of Rambutan (*Nephelium Lappaceum L.*) have been extracted. The extraction of Rambutan stem bark was performed by maceration using a methanol solvent. The concentrated methanol extract was diluted in ethyl acetate until the solution became devoid of all positive and flavonoid compounds. The highly concentrated ethyl acetate extract was subsequently dissolved using methanol and separated into different parts using an n-hexane. The methanol content was determined using thin-layer chromatography and separated using column chromatography using eluent chloroform: methanol in various ratios (90:10, 80:20, 70:30) v/v. The chemicals were purified using preparative thin-layer chromatography (TLC) and yielded 7.9 mg of amorphous solids with an R_f value of 0.25. The eluent was a chloroform and ethyl acetate mixture in a 50:50 volume-to-volume ratio. The substance was analyzed using Spectrophotometer Ultraviolet Visible (UV-Vis), Fourier Transform Infra Red Spectrophotometer (FT-IR), and Proton Nuclear Magnetic Resonance Spectrophotometer (¹H-NMR). The analysis identified the chemical as a flavonoid of the flavonol.

Keywords: Rambutan Steam Bark, Flavonoid, Flavonol.

ABSTRAK

Flavonoid yang berasal dari kulit batang Rambutan (*Nephelium Lappaceum L.*) telah diekstraksi. Ekstraksi kulit batang rambutan dilakukan dengan cara maserasi menggunakan pelarut metanol. Ekstrak metanol pekat diencerkan dalam etil asetat sampai larutan bebas dari semua senyawa positif dan flavonoid. Ekstrak etil asetat dengan konsentrasi tinggi selanjutnya dilarutkan menggunakan metanol dan dipisahkan menjadi beberapa bagian menggunakan n-heksana. Kadar metanol ditentukan dengan menggunakan kromatografi lapis tipis dan dipisahkan menggunakan kromatografi kolom menggunakan eluen kloroform:metanol dengan berbagai perbandingan (90:10, 80:20, 70:30) v/v. Bahan kimia tersebut dimurnikan menggunakan kromatografi lapis tipis preparatif (TLC) dan menghasilkan 7,9 mg padatan amorf dengan nilai R_f = 0,25. Eluen yang digunakan adalah campuran kloroform dan etil asetat dengan perbandingan volume terhadap volume 50:50. Zat tersebut dianalisis menggunakan Spektrofotometer Ultraviolet Visible (UV-Vis), Spektrofotometer Fourier Transform Infra Red (FT-IR), dan Spektrofotometer Resonansi Magnetik Nuklir Proton (¹H-NMR). Analisis mengidentifikasi bahan kimia tersebut sebagai flavonoid flavonol.

Kata kunci: Rambutan (*Nephelium lappaceum L.*), Flavonoida, Flavonol.



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1. Introduction

Flavonoids belong to the extensive group of natural phenols. It is present in several components of plants, such as roots, bark, wood, nectar, pollen, flowers, fruits, and seeds (Markham, 1988). Flavonoids, which encompass algae, are also present in certain types of lower plants (Connolly, 1986). Flavonoids are polyphenolic chemicals consisting of 15 carbon atoms, characterized by two aromatic rings linked by a three-carbon bridge [1].

Flavonoid chemicals are considered highly advantageous in diet due to their classification as phenolic compounds, which possess strong antioxidant properties. Flavonoids possess the ability to eradicate harmful

oxidizing species, therefore making meals abundant in flavonoids crucial for the treatment of disorders like cancer and heart disease. Flavonoids, found in plants, have the ability to shield the human body from free radicals and reduce the risk of inflammation and cancer [2].

Rambutan (*Nephelium lappaceum L.*) is a plant that can be utilized for medicinal purposes. The fruit of the rambutan plant is extensively grown in Indonesia (Haryanto, 2012). The peel of the rambutan fruit has medicinal properties that can be utilized in treating fever and diarrhea. The leaves possess medicinal properties that can effectively alleviate symptoms of diarrhea and darken the hair. Roots are used to alleviate fever. According to Dalimartha (2005) [3], the stem bark has medicinal properties that can be used to treat thrush. Saponins and tannins are present in the leaves and skin of rambutan fruit. Simultaneously, the stem bark comprises flavonoids, saponins, tannins, and iron [4].

[5] reported the rambutan stem bark (RSB) is beneficial by impeding the proliferation of *Candida albicans*, a species of fungus. Flavonoids, saponins, and tannins are believed to possess antifungal properties. Flavonoids can disrupt cell membranes by breaking protein links, leading to membrane lysis. This allows the flavonoids to enter the cell nucleus and prevent the growth of the fungus. [6] conducted a study to assess the antioxidant activity of an ethanol extract derived from the peel of rambutan fruit.

The study utilized the linoleic-thiocyanate method to measure the extract's ability to suppress the generation of linoleic acid-free radicals. The capacity to suppress the creation of linoleic acid-free radicals was determined by calculating the absorbance of 499 nm wavelength. The data indicated that the ethanol extract derived from the peel of rambutan fruit possesses equivalent efficacy to vitamin E in preventing the generation of linoleic acid-free radicals. The initial experiments performed by researchers, specifically the phytochemical screening test using the reagents FeCl_3 5%, H_2SO_4 , NaOH 10%, and $\text{Mg(s)} - \text{HCl}$, indicated that the methanol and ethyl acetate extracts of the stem bark of this plant exhibited the presence of flavonoid components. Based on the given description and existing literature on rambutan stem bark, researchers want to extract and identify the specific flavonoid components and categories present in the bark of rambutan.

2. Materials and Methods

2.1. Equipment

This study used the following instruments: an analytical balance (Mettler AE 200), a chamber, an extractor, a distillation apparatus, a separator funnel (Pyrex), glass tools, capillary pipes, and clamps. In addition, $^1\text{H-NMR}$ spectrophotometer (Agilent 2NMR 500MHz), FT-IR spectrophotometer (Shimadzu), UV-Vis spectrophotometer (Hewlett Packard Agilent), Chromatography Column (pyrex) were also used.

2.2. Materials

The materials used in this study include RBS powder, methanol, ethyl acetate, n-hexane, Silica Gel (70 - 230 mesh, E-Merck.kgA), FeCl_3 5%, Mg powder, HCl, H_2SO_4 , cotton, chloroform (E.Merck), KLT plate silica gel 60 F₂₅₄ (E.Merck.Art 554), Preparative KLT plate 60 F₂₅₄, Benzene (P.a Merck), Acetone (P.a Merck).

2.3. Research Procedures

2.3.1. Sample Provision

The examined specimen consisted of rambutan bark sourced from Gelugur Rimbun Village, Pancur Batu District, Deli Serdang, North Sumatra. The rambutan bark was dehydrated outdoors and subsequently crushed into a fine powder, resulting in 1900 g of rambutan bark powder.

2.3.2. Preliminary Test of Rambutan Stem Bark Extract

RSB powder was performed with a qualitative preliminary test using a color reaction. A total of 10 g of the dried powder was put into an Erlenmeyer glass, and then 100 mL of ethyl acetate allowed it to stand after filtration. The sample extract is partitioned into four test tubes and introduced into each reagent.

Tube I: FeCl_3 5% produces a black solution

Tube II: with Mg-HCl produces a pink solution

Tube III: with H_2SO_4 produces a yellowish orange solution

2.3.3. Extraction of Rambutan Stem Bark

A 1900 g of RSB powder was measured and then soaked in 11 liters of methanol until fully immersed. The samples were then left undisturbed for approximately 2 days. The macerate was gathered and condensed using a rotary evaporator to get a concentrated methanol extract, which was further evaporated in a water bath until all the methanol solvent dissipated. Next, the tannin was isolated by dissolving the methanol extract in ethyl acetate and filtering it. The ethyl acetate filtrate was thereafter subjected to rotational evaporation until complete evaporation of the ethyl acetate solvent. The ethyl acetate fraction was dissolved again using methanol and subjected to many extractions by partitioning with n-hexane until the n-hexane layer became nearly transparent. The methanol layer was isolated from the n-hexane layer, further concentrated using a rotary evaporator, and evaporated once more to yield the concentrated methanol extract.

2.3.4. Thin Layer Chromatography Analysis

The thin layer chromatography analysis used a stationary phase of silica gel 60F254 Merck on the methanol extract. This investigation aimed to determine the optimal solvent system and ratio for column chromatography. The mobile phase consisted of a blend of chloroform and methanol solvents in various volume-to-volume ratios: 90:10, 80:20, 70:30, and 60:40.

Add 10 milliliters of a mobile phase solution mixture containing chloroform and methanol in a ratio of 90:10 (volume/volume) to the chromatography vessel, then saturate. The methanol extract was concentrated and then placed on an active KLT plate. Place the plate into a container that has previously held a fully saturated combination of solvents, then seal it and perform elution. The plate separated from the vessel is subsequently subjected to a drying process. Examined the stain that developed when exposed to UV light, then treated it with a 5% FeCl₃ reagent. The color of the dots was observed, and the R_f value was determined. The chloroform: methanol solvent ratio of 80:20, 70:30, and 60:40 (v/v) underwent identical treatment.

2.3.5. Separation of Flavonoid Compounds by Column Chromatography

Flavonoid components were separated using column chromatography with a methanol-concentrated extract. The silica gel 60 (70-230 mesh) was employed as the stationary phase, while the mobile phase consisted of 100% chloroform and a mixture of chloroform: methanol solvents in ratios of 90:10, 80:20, and 70:30 (v/v).

A column chromatography apparatus was constructed, and the initial dissolved silica gel 60 (70-230 mesh) was mixed with chloroform until it formed a uniform mixture. The mixture was then introduced into the chromatography column and eluted with 100% chloroform until the silica gel became solid and uniform. First, I dissolved 5.0 grams of a concentrated methanol extract using methanol as the solvent. Then, I placed this solution into a chromatographic column filled with a silica gel slurry. Next, I slowly added the mobile phase, which consisted of a mixture of chloroform and methanol in a ratio of 90:10 v/v. I gradually increased the polarity of the mobile phase by adding more chloroform and methanol in ratios of 80:20 and 70:30 v/v, respectively. The results were gathered in a vial container, with each container holding 10 ml. The contents were then separated using thin-layer chromatography (KLT) and merged fractions with identical R_f values. These fractions were then subjected to a test using a 5% solution of FeCl₃. Finally, the mixture was evaporated until a paste-like consistency was achieved.

2.3.6. Purification of Isolated Compounds

The paste obtained from the separation process of column chromatography was mixed with methanol and subsequently examined using thin-layer chromatography (TLC) to ascertain the purity of the compounds obtained and identify the suitable mobile phase for preparative TLC. The mobile phase consisting of chloroform and ethyl acetate in a 40:60 (v/v) ratio is ideal for achieving the most effective separation. This mobile phase can be used to saturate the preparative KLT vessel. The previously dissolved paste is carefully and uniformly poured into the KLT plate, starting from the bottom edge. The plate is placed into a jar that contains a mixture of solvents that have been fully absorbed and then sealed. Following the elution process, the plate is extracted from the vessel and subjected to drying. Subsequently, the outcomes are scrutinized using ultraviolet (UV) light. Every zone was clearly identified and carefully treated with methanol: ethyl acetate (1:1) v/v to extract the desired compounds. Subsequently, the elution outcomes were subjected to evaporation until a brownish-yellow paste was obtained. The individual compounds were examined using thin layer chromatography, employing a stationary phase of silica gel 60 F254 and a mobile phase consisting of chloroform and ethyl acetate at a ratio of 40:60 (v/v). Subsequently, the separated molecules underwent

analysis using three spectrophotometers: a UV-visible spectrophotometer, an Infrared Spectrophotometer (FT-IR), and a Proton Core Magnetic Resonance Spectrophotometer ($^1\text{H-NMR}$).

3. Results and Discussion

The ethyl acetate extract of RSB was subjected to phytochemical screening with 5% of FeCl_3 , H_2SO_4 , and Mg-HCl reagents. This screening showed the presence of flavonoid components. The flavonoid compounds were isolated from the stem bark of rambutan using a maceration extraction process. A methanol-concentrated extract weighing 294.86 g was obtained and subsequently dissolved in ethyl acetate solvent to separate the suspected tannin compounds. This resulted in a concentrated ethyl acetate extract weighing 33 g. The concentrated ethyl acetate extract was subsequently dissolved using ethyl acetate solvent. The concentrated extract of ethyl acetate was dissolved in methanol and subsequently separated into different parts using n-hexane. Next, the methanol layer was evaporated until it yielded 8 grams. A little portion of the concentrated methanol layer was extracted and subjected to a test using a benedict reagent. This resulted in the formation of a brick-red solid, indicating the presence of sugar. Due to its tiny volume, the methanol-concentrated layer was not subjected to hydrolysis. If hydrolysis had been performed, the resulting chemicals would have been insufficient, making it difficult to interpret the UV, FT-IR, and $^1\text{HNMR}$ spectroscopic data.

The thin-layer chromatography research conducted before column chromatography revealed that the most effective solvent ratio for isolating flavonoid components from rambutan stem bark was chloroform: methanol (80:20) v/v. This particular ratio demonstrated superior separation of the resultant stains. This is demonstrated using TLC analysis, which reveals the existence of four distinct stains with a significant separation distance between them. After separating the mixture using column chromatography, the fractions were analyzed using TLC with a mixture of chloroform and ethyl acetate (40:60) v/v as the eluent. Three fractions were obtained. The stains produced on the thin layer chromatography plates for fractions 77-86 showed the best separation when treated with a 5% FeCl_3 reagent. The R_f distance between the stains was measured to be 0.24 and 0.60. The weight of the sample used for analysis was 150 mg. The TLC analysis was repeated using the same eluent mixture. Preparative thin-layer chromatography was then performed using the appropriate solvent system, which was chloroform and ethyl acetate (40:60) v/v. The resulting plate was observed under a UV lamp. The first stain from the upper limit was collected by scraping the silica gel and eluting it with a solvent mixture of methanol and ethyl acetate (1:1) v/v in a small column. The chemical was assessed for purity using TLC using chloroform: ethyl acetate (40:60) v/v eluent. The TLC analysis revealed a single spot for the compound, with a retention factor (R_f) value of 0.25.

The examination conducted using an Ultraviolet-Visible Spectrophotometer (UV-Vis) with methanol solvent revealed the existence of a single wavelength absorption (λ_{max}) in band II, with a precise wavelength of 280.00 nm, as depicted in Figure 1.

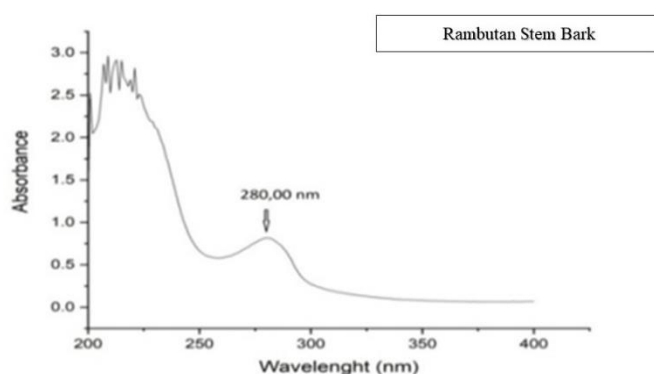


Figure 1. UV-VIS spectra of isolated compounds

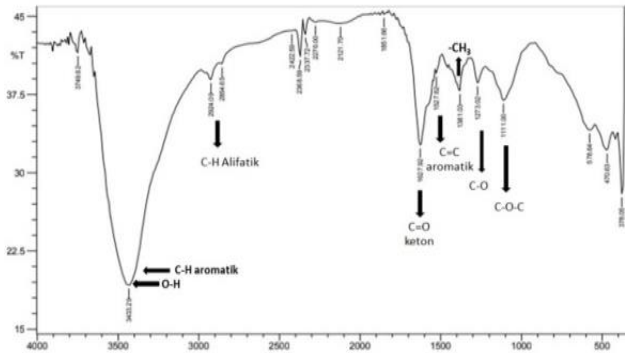
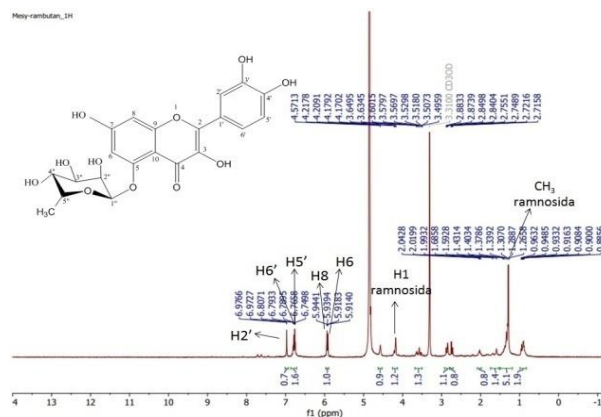


Figure 2. Infrared Spectrum (FT-IR) of Isolated Compounds

The results of the FT-IR analysis of the isolated amorphous solids provide absorption in the wave number region (cm^{-1}) shown in Figure 2. The results of the FT-IR analysis of the isolated amorphous solids provide absorption in the wave number region (cm^{-1}) shown in Figure 2. At wave number 3433.29cm^{-1} , the broad peak shows the presence of -OH stretching vibrations that overlap with aromatic C-H. At wave numbers $2854.65 - 2924.09\text{cm}^{-1}$, the medium peak shows the presence of aliphatic C-H vibrations. At wave number 1627.92cm^{-1} , the sharp peak shows the presence of C=O ketone double bond vibration. At wave number 1527.62cm^{-1} , the medium peak shows C=C stretching vibrations in the aromatic system. At wave number 1381.03cm^{-1} , the sharp peak shows the bending vibration of -CH₃ from C-H aliphatic. At wave number 1111.00cm^{-1} , the medium peak shows stretching vibrations in the C-O-C ether group.

Figure 3. ¹H-NMR spectrum of isolated compound $\delta = 0 - 14.0\text{ ppm}$

The results of ¹H-NMR analysis of isolated compounds using Methanol-d₆ solvent and TMS chemical shifts in the region (ppm) as a standard, as shown in Figure 3. The spectrum of ¹H-NMR derived isolated compounds using Acetone-d₆ solvent reveals doublet peaks at chemical shifts of $\delta = 5.9140\text{ ppm}$ and $\delta = 5.9183\text{ ppm}$, indicating the presence of protons on H-6. Chemical shifts in the $\delta = 5.9394\text{ ppm}$ and $\delta = 5.9441\text{ ppm}$ doublet peaks indicate the proton of H-8. Chemical shifts at $\delta = 6.7498\text{ ppm}$ and $\delta = 6.7658\text{ ppm}$ doublet peaks indicate protons of H-5'. Chemical shifts at $\delta = 6.7895\text{ ppm}$ and $\delta = 6.8071\text{ ppm}$ doublet peaks indicate the H-6 proton. Chemical shifts at $\delta = 6.9727\text{ ppm}$ and $\delta = 6.9766\text{ ppm}$ doublet peaks indicate protons from H-2' in ring B of flavonoid compounds. At C-5, it is thought to be bound to a sugar group because the chemical shift in the area $\delta = 3.5073\text{ ppm}$ to $\delta = 3.6495\text{ ppm}$ shows the presence of four protons from sugar. Chemical shifts at $\delta = 1.2887\text{ ppm}$ and $\delta = 1.3070\text{ ppm}$ with doublet peaks show the -CH₃ ramnoside proton, so the sugar binds to C-5 is ramnoside sugar. Chemical shift at $\delta = 4.1702\text{ ppm}$ and $\delta = 4.1792\text{ ppm}$ with doublet peak show the proton of H-1 ramnoside.

Based on the data analysis carried out on the UV-visible spectrum, Infrared Spectrum (FT-IR), and ¹H-NMR spectrum, it is concluded that the possible isolated compounds from rambutan stem bark are flavonoid compounds of flavonol type bound to O-ramnoside with the structure as in Figure 4.

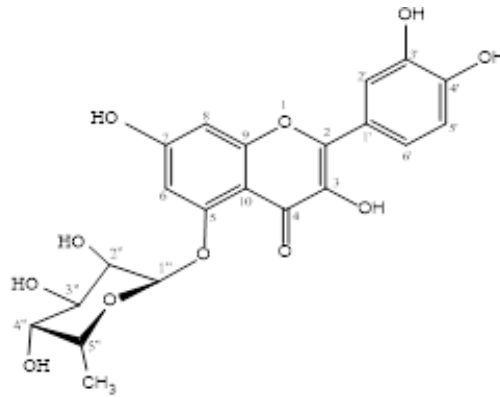


Figure 4. Flavonol structure isolated from RSB

4. Conclusion

According to the research findings, the following conclusions can be drawn: The isolation process yielded a brownish-yellow paste weighing 7.9 mg from 1900 g of rambutan (*Nephelium lappaceum* L.) stem bark. The isolation was achieved using chloroform: ethyl acetate (40:60) v/v eluent, resulting in a $R_f = 0.25$. The UV-Vis, FT-IR, and $^1\text{H-NMR}$ analysis results show that the flavonoid compound isolated from the RSB was revealed and believed to belong to the flavonol group and bound to O-rhamnoside.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

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