Flavonoid Compound from Rambutan Bark (*Nephelium lappaceum* L.)

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Keywords: Isolation, *Nephelium lappaceum* L., Flavonoids, Flavonol.

Abstract: Flavonoids included polyphenol compounds that are found in many plants which are important compounds in human food.The flavonoid group, including flavanone, flavone, dihydroflavonol, catechin, flavonol, flavan-3-ols, isoflavones, auron, anthocyanidins, proanthocyanidins and chalcones, has a general structure of C6-C3-C6. The aims of this study is to isolate and identify the group of flavonoid compound contained in rambutanbark (*Nephelium lappaceum* L.). Rambutan bark powder was extracted maceration with methanol. Methanol extract was dissolved with ethyl acetate repeatedly until the solution was negative flavonoids. Ethyl acetate extract was dissolved with methanol and partitioned with *n*-hexane. The methanol extract which is a total of flavonoids separated by column chromatography using chloroform: methanol (90:10; 80:20 and 70:30 (v/v)). The isolates obtained were purified by preparative thin layer chromatography and producing flavonoid glycoside, yellow amorphous with Rf value of 0.25 using the chloroform: ethyl acetate eluent (50:50) v/v. The pure isolate obtained were analyzed by UV-Visible Spectropometer, FT-IR, and H-NMR. Based on the interpretation of spectroscopic data, the flavonoid compound isolated from rambutan bark wasflavonol group.

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1 INTRODUCTION

Rambutan (*Nephelium lappaceum* L.) is an evergreen tree (Sukmandari et al., 2017), a plant that identical with Southeast Asian countries, in some areas of Indonesia (Wahini et al., 2018). The dried rambutan fruit peel is used in traditional medicine, cooking and in the manufacture of soap. The roots bark and rambutan leaves have various uses in medicine and in the production of dye (Suganthi & Josephine, 2016). Rambutan fruit peel contains flavonoids, tannins and saponins (Hariana, 2006).

Flavonoids are the most extensive groups of phenolics Flavonoids are secondary with low molecular weight that have bioactivity (Weston & Mathesiu, 2013). Flavonoids are polyphenol compounds composed of 15 carbon atoms, with two aromatic rings connected by a bridge consisting of three carbon atoms (Crozier et al., 2006). The flavonoid group, including flavanone, flavone, dihydroflavonol, catechin, flavonol, flavan-3-ols, isoflavones, auron, anthocyanidins, proanthocyanidins and chalcones, has a general structure of C6-C3-C6 (Rosa et al., 2010).

Flavonoids have a positive effect on human and animal health. Flavonoids are widely used as a therapy for disease and chemoprevention (Panche et al., 2016). Bioactivities of these phenolic, polyphenolic acids or essential oil are potential as new leads for the development of pharmaceutical (Saranya et al., 2017), antibacterial (Sembiring et al., 2019)and agricultural products to improve human health and nutrition (Khadem & Marles, 2010) .Flavonoids are able to treat diseases, such as cancer and heart disease (Zhang et al., 2015) can be used to protect the human body from free radicals (Megawati et al., 2015), (Molyneux, 2004) and can reduce the risk of cancer and inflammation (Kumar & Pandey, 2013).

Pangalinan et al., (2012) mentioned that rambutan bark are effective as antifungal against *Candida albicans*. The purpose of this study was to isolated and identified the flavonoid group contained in the bark of the rambutan (*N. Lappaceum* L.). Flavonoid compounds were isolated by maceration extraction and column chromatography methods. Flavonoid compound identification was carried out by FT-IT, UV-Visible and HNMR spectroscopy.

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2 METHODS

2.1 Material

Rambutan bark was obtained from Gelugur Rimbun Village, Pancur Batu District, Deli Serdang, North Sumatra, Indonesia. Identification of plant was done at Herbarium Medanense (MEDA) Universitas Sumatera Utara. All chemicals used such as Silica Gel (70 – 230 mesh), for column chromatography, $FeCl_3$, NaOH, Serbuk Mg, $HCl_{(p)}$, $H_2SO_{4(p)}$, kloroform, silika gel 60 F_{254} for thin layer chromatography, KLT Preparative 60 F_{254} and methanol were from E Merck, methanol and ethyl acetate as solvent were distilled before used (Saldanha et al., 2013).

2.2 Instrument

The ^{1}H NMR spectra were recorded with a (Agilent 500 MHz, Frekuensi 500 MHz), spectrometer instrument with CD_3OD as a solvent and TMS as an internal standard and chemicalshifts are given in δ (ppm). IR spectra were recorded on FT-IR (type Mb3000, 485 – 8500 cm^{-1}), UV spectra were recorded on Spektrofotometer UV-Visible (Type UV – 1800 Shimadzu, 190 – 1100 nm), evaporation of solvents with rotary evaporator (Heidolph), spotting monitoring with lights of UV(254nm/356nm, UVGL 58).

2.3 Procedure

Isolation flavonoid compounds were done based on (Megawati et al., 2015) and (Hostettmann et al., 1995) with a slight modification. Rambutan bark powder (1900 g) was macerated for 2 days using 11 L methanol (until all samples were submerged with methanol). Maserat is accommodated and the solvent is evaporated with a rotary evaporator and dried with a water bath until a dry methanol extract was obtained. Dry methanol extract was re-extracted with ethyl acetate to separate tannins. The filtrate obtained was evaporated with a rotary evaporator and water bath until all the ethyl acetate solvent evaporated. Ethyl acetate extract was redissolved with methanol and repartitioned with *n*-hexane until the *n*-hexane layer was colorless. The methanol layer was re-concentrated with a rotary evaporator and dried with a water bath to obtain a dry extract of methanol.

The dried methanol extract (5g) was added to the column chromatographic containing silica gel slurry, eluted with chloroform: methanol (90:10; 80:20; 70:30 v / v) slowly. The isolates were collected in vials every 10 ml, then analyzed with TLC. The fractions that have the same Rf value are combined. Fractions of 41-85 have the same Rf value, combined then purified with preparative TLC with cloroform: etil asetat (40:60) (v/v),) and produced one band spot at the Rf 0.25. The band spot was crushed, eluted with metanol: etil asetat (1:1) $^v/_v$, evaporated to obtain 7.9 mg pure isolate in the form of yellow amorphous. The pure isolate was identification by UV-Vis, FT-IR and ^{1}H-NMR spectroscopy.

3 RESULTS AND DISCUSSION

The sample used in this study was the rambutan bark, *Nephelium lappaceum* L. (Figure 1A), family Sapindaceae. The pure isolate isolated from the bark rambutanis a yellow amorphous (Figure 1B) and identified by using UV-Vis, FT-IR and ^{1}H-NMR spectroscopic analysis.

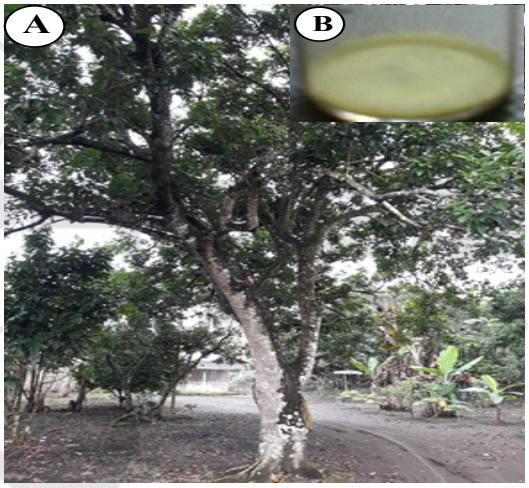

Figure 1: A. Rambutan plant; B. isolate.

The UV-Visible (CH_3OH) spectra was shown in Figure 2. Based on the spectra, the isolate isolated from rambutan bark was fllavonol group, because presence of spectraat λ_{max}280,00 nm. Absorption band II at maximum wavelength (max λ) 280.00 nm is a flavonoid of the flavonol group (Andersen & Markhan, 2006). FT-IR spectra of pure isolated was shown in Figure 3. The FT-IR spectra for pure isolates showed (KBr, ν max, cm^{-1}) 3433.29(O-H), 2854,65 and 2924,09 (C-H sp^3 stretching),1627.92 (C=O),1527.62(C=C), 1381.03 (C-Hsp^3bending) and 1273,02(C-O).All of these vibrations are common vibrations found in flavonoid compounds. The stretching vibration of the C-H sp^2 bond is not detected because it overlaps with the broad vibration of the O-H bond (Pavia et al., 2001).

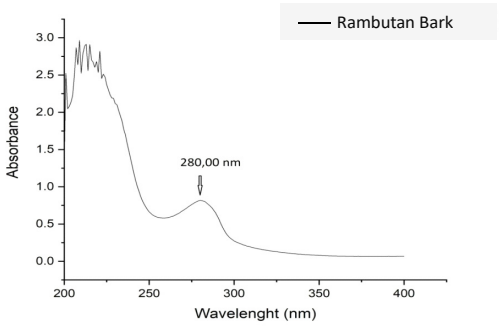

Figure 2: The UV-Visiblespectra of isolate.

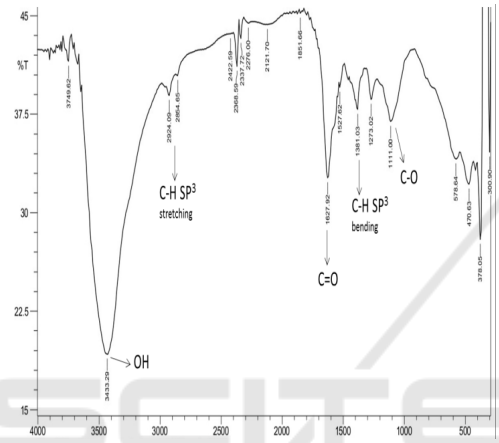

Figure 3: The FT-IR Spectra of pure isolate.

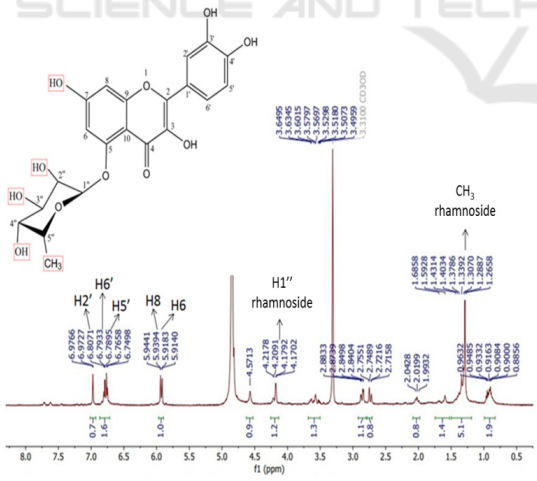

Figure 4: The [1]H NMR spectra of pure isolate.

[1]H NMR spectra of pure isolate wasdepected in Figure 4. Based on 1H NMR spectra (Methanol-D6, 500 MHz, (ppm)), chemical shift (δ (ppm)): δ 5.914 and 5.918 (1H, d, H-6), δ 5.939 and 5.944 (1H, d, H-8), δ 6.750 and 6.766 (1H, d, H-5'), δ 6.790 and 6.807 (1H, d, H-6') and δ 6.972 and δ 6.977 (1H, d, H-2'),)), the isolate had five aromatic protons and eight rhamnose protons. The type and number of protons in this isolate are the same as the type and number of protons of quercetin compounds reported by Huang et al.,(2013) But it has a slight difference in chemical shift and splitting pattern, due to differences in the frequency of the spectroscopic used. Substituent -OH bind toC-3, C-7, C-3', dan C-4'. However, C-5 has a glycoside bond because no -OH peaks are found at a chemical shift of 12.22 ppm (Claramunt et al., 2006). Rhamnose is thought to bind to flavonoids because of the presence of peaks δ (ppm) 4,1702 dan δ 4,1792 (1H, d, H-1''), δ 3,6495 (4H, m, H-2'',H-3'',H4'',H-5'') and δ 1.289 and 1.307 (3H, d, -CH_3rhamnoside)ofrhamnose. The chemical shift of rhamnose protons are similar to the chemical shift of rhamnoseprotons reported by Plazonić et al, 2009)

Based on interpretation data of the UV-Visible, FT-IR and 1H-NMR spectra, the flavonoid compound isolated from rambutan bark was flavonol group bound torhamnose at C-5with the structure shown as in Figure5.

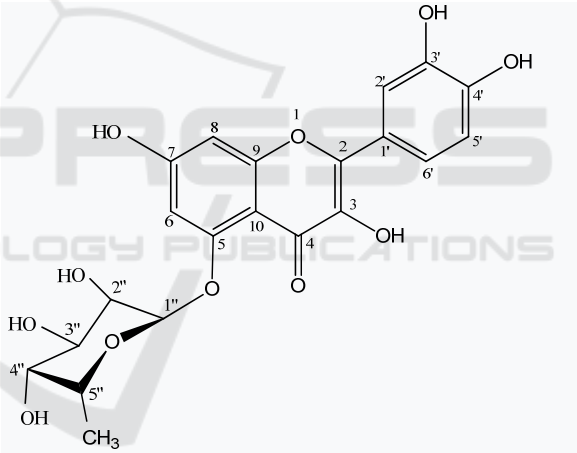

Figure 5: Structure of isolate.

4 CONCLUSIONS

Isolate obtained from 1900 g rambutan bark (*N. lappaceum* L.) is a flavonoid 7.9 mg yellow amorphous with an Rf value of 0.25 using the chloroform: ethyl acetate eluent (50:50) v / v. Based on the spectra and interpretation data the flavonoid compound isolated from rambutan bark was flavonol group.

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