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Isolation and Identification of Flavonoids from Mundu Plant Leaves *(Garcinia Dulcis* (Roxb.) Kurz)

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Abstract. Isolation and identification of the flavonoid compounds found in the leaves of the mundu plant *(Garcinia dulcis* (Roxb.) Kurz) have been carried out. The leaves of the mundu plant were extracted by maceration with methanol solvent. The concentrated methanol extract was added to distilled water and then filtered. Aquadest filtrate partitioned with ethyl acetate. Concentrated ethyl acetate extract was dissolved with methanol and partitioned with n-hexane solvent. The concentrated methanol extract was analyzed by thin layer chromatography and separated by column chromatography with chloroform as eluent: methanol (90:10) v/v ; (80;20) v/v ; (70:30) v/v ; (60:40) v/v. The compounds obtained were purified by preparative thin layer chromatography, producing a yellow amorphous solid of 8.4 mg with a value of Rf = 0.35 using chloroform: ethyl acetate (40:60) v/v as the eluent. The compounds obtained were analyzed using UV-Visible Spectrophotometer, Infrared Spectrophotometer (FT-IR), and Proton Nuclear Magnetic Resonance Spectrophotometer (^{1}H-NMR). Based on the analysis and interpretation of spectroscopic data, it is suspected that the isolated compounds obtained are flavonoid compounds, namely the biflavonoid group.

Keywords: Biflavonoids, Flavonoids, Mundu (*Garcinia dulcis* (Roxb.) Kurz)

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1 Introduction

Flavonoid a is a compound polyphenol arranged on 15-atom carbon-carbon, with two aromatic rings linked by a bridge of three carbon atoms (Crozier et al. 2006). Flavonoids can prevent growth cell cancer, and antioxidants can counteract free radicals and prevent premature ageing (Tyas, 2011).

Plant flavonoids are the largest class of natural materials widely distributed in higher plants but are also found in several lower plants, including algae. Flavonoid a is found in different plant parts, including roots, bark, leaves, flowers, fruits and seeds (Connolly, 1986).

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(Garcinia dulcis (Roxb.) Kurz) belongs to the high-level plant of the mangosteen family, *Gutiferae or Clusiaceae*. This species grows a lot in the tropics, there are many in Southeast Asia, for example, in Indonesia, Malaysia, Thailand, Cambodia, the Philippines, Burma and Vietnam, and some are in Sri Lanka, southern India, Brazil and Central America. (Heyne, 1987).

The plant *(Garcinia dulcis* (Roxb.) Kurz), with the local name mundu, is found and grows in many areas in Indonesia, such as the islands of Java, Kalimantan and Papua. The community widely uses this plant, the leaves and seeds of mundu, to treat lymphatics, parotitis and goitre, while the fruit parts are used as juice which is helpful as an expectorant. (Kosela et al. 2000).

Muharni et al . (2011) tested the isolation of bioflavonoid compounds from gamboge (Garcinia xanthochymus) stem bark. They concluded that the methanol extract of Garcinia xanthochymus stem bark produced a biflavonoid compound (+)-morello flavone using a spectrophotometer ^{1}HNMR, ^{13}C-NMR, H-MQC, H-MBC.

Santosa, (2015) has tested antioxidant activity against (*Garcinia dulcis* (Roxb.) Kurz), B *lumea-mollis* (D.Don) Merr., Siegesbeckia Orientalis L. and Salvia Riparia HBK stated that among the four methanol extracts, the extract (*Garcinia dulcis* (Roxb.) Kurz) had highest antioxidant activity. Analysis of qualitative methanol extract (*Garcinia dulcis* (Roxb.) Kurz) using thin layer chromatography (TLC) showed that the methanol extract (Garcinia dulcis (Roxb.) Kurz) contains phenolic and flavonoid compounds.

Widodo (2010) tested the antimalarial activity of the ethyl acetate extract of the bark of mundu (Garcinia dulcis (Roxb.) Kurz) on mice and concluded that the ethyl acetate extract of the bark of mundu (Garcinia dulcis (Roxb.) Kurz) showed the highest antimalarial activity against mice. The compounds identified in the ethyl acetate extract and thought to play a role in antimalarial activity are flavonoids, saponins and tannins.

The preliminary tests the researchers carried out, namely the phytochemical screening test with $FeCl_3$ 5 %, $H_2SO_{4\,(p)}$, NaOH 10%, and Mg-$HCl_{\,(p)}$ reagents, showed that the methanol and ethyl acetate extracts of the leaves of this plant positively contained these flavonoids compounds.

From the description above and some literature related to the Mundu plant, the researchers are interested in researching the leaves of the Mundu plant, especially regarding the isolation and determination of the structure of the flavonoid compound class.

2 Materials and Methods

2.1 Equipment

The tools used in this study were: Spectrophotometer ^{1} H-NMR (Agilent 2NMR 500MHz), FT-IR Spectrophotometer (Shimadzu), UV-Vis Spectrophotometer (Hewlett Pack-

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ard Agilent), Column Chromatography (pyrex), rotary evaporator (Heidolph), UV lamp (254nm/356nm), UVGL58), Analytical Balance (Mettler AE 200), Chamber, Extractor, distillation apparatus, and glassware.

2.2 Materials

The materials used were oil palm empty fruit bunch (EFB), NaOH, H_2O_2, aquadest, HNO_3, H_2SO_4, $NaNO_2$, $NaSO_3$, NaOCl aquabidest, and dialysis membrane of 0.22 µm.

2.3 Procedure

2.3.1 Sample Provision

The sample studied was the leaves of the Mundu plant obtained from the vicinity of the Rector's Office at the University of North Sumatra. The leaves of the Mundu plant are dried indoors and then crushed to obtain powder Mundu plant leaves as much as 1.8 kg.

2.3.2 Phytochemical Screening Test

A preliminary qualitative test was carried out to determine the presence of flavonoid compounds in the leaves of the Mundu plant. Put as much as 10 grams of dried Mundu plant leaf powder into the Erlenmeyer, add 100 mL of ethyl acetate, leave for one night, filter, divide each sample extract as much as 2 mL into 3 test tubes, and add each reagent.

a. Tube I: with three drops of $FeCl_3$ 5% to produce a black solution

b. Tube II: with 0.1 mg of Mg powder and three drops of $HCl_{(p)}$ to produce a pink solution

c. Tube III: with three drops of H_2SO_4 *(p)* to produce a yellowish orange solution.

2.3.3 Extraction of Mundu Plants Leaves

Mundu plant leaf powder was weighed as much as 1.8 kg, then macerated with 13 L of technical methanol until the sample was completely submerged and left for 24 hours. Soaking was carried out until the sample was negative for $FeCl_3$ 5%. The macerate was collected and concentrated using a rotary evaporator to obtain a concentrated methanol extract and tested with $FeCl_3$ 5%. Then evaporated with a water bath until all the methanol solvent evaporated. Then, tannins were separated by dissolving the concentrated methanol extract with distilled water and then partitioning it repeatedly with ethyl acetate until it was negative for flavonoids. tested with $FeCl_3$ 5%. Filtrate has then rotated evaporator and then evaporated with a water bath until all the ethyl acetate solvent evaporated. Then the concentrated ethyl acetate extract was tested with 5% $FeCl_3$. Ethyl acetate concentrated extract was dissolved with methanol and partition extracted repeatedly with n-hexane until the n-hexane layer was clear. The methanol layer was separated from the n-

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hexane layer, then tested with $FeCl_3$ 5% and concentrated again using a rotary evaporator and evaporated again to obtain a concentrated extract of the methanol layer.

2.3.4. Thin Layer Chromatography Analysis

Thin Layer Chromatography analysis was performed on the methanol extract using silica gel 60 F_{254} E. Merck.Art 554 as the stationary phase. The mobile step was a mixture of chloroform solvents: methanol with comparison - a 90:10; 80:20; 70:30; 60:40 (v/v). 10 ml of 90:10 (v/v) chloroform: methanol mobile phase mixture was added to the chromatography vessel, then saturated. The concentrated methanol extract was dotted on the activated TLC plate. The plate is inserted into a saturated solvent mixture vessel, then closed and eluted until the solvent reaches a predetermined limit. The eluted plates were removed from the vessel and then dried. The stains formed under UV light were observed and then fixed with $FeCl_3$ 5%. Observe the colour of the spots that appear and calculate the Rf values obtained. The same treatment was carried out for the chloroform: methanol solvent ratio with a ratio of 80:20, 70:30, and 60:40 (v/v).

2.3.5 Separation of Flavonoid Compounds by Column Chromatograph

Separation of flavonoid compounds was separated by column chromatography of concentrated methanol extracts. The stationary phase used was silica gel 40 (70-230 mesh) ASTM E.Merck KgA, and the mobile step is chloroform 100%, mixture solvent chloroform: methanol with ratios 90:10, 80:20, 70:30, 60:40 (v/v).

Column chromatography apparatus was assembled. Then slurry ASTM silica gel 40 (70-230 mesh) using chloroform, stir until homogeneous and put into column chromatography and then eluted using 100% chloroform until the silica gel was solid and homogeneous. A dissolved 6 g concentrated methanol extract was added with silica gel, dissolved with methanol solvent, and dried to form a slurry. Then it was put into the column chromatography containing silica gel slurry, then added the mobile phase chloroform: methanol 90:10 (v/v), 80:20 (v/v), 70:30 (v/v), 60:40 (v/v). The results were collected in vials every 10 mL, then in TLC, tested with 5 % $FeCl_3$, and then evaporated to form a solid. The solids obtained were analyzed by thin-layer chromatography with chloroform as eluent: ethyl acetate (80:20) (v/v).

Then in preparative TLC with silica gel stationary phase and chloroform: ethyl acetate (40:60) (v/v) mobile phase, dried and irradiated under a UV lamp. Crushed from the plate and dissolved with a mixture of methanol: ethyl acetate 1:1, then filtered and evaporated.

2.3.6 Purification of Isolated Compound Results

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The solid obtained from separation by column chromatography was redissolved with methanol and then analyzed by TLC using several solvents in specific ratios. Chloroform: ethyl acetate 4 0: 6 0 (v/v) is the mobile phase which shows the best separation for further use to saturate the preparative TLC vessel. Then The dissolved solid is dappled slowly and evenly along the bottom edge of the activated TLC plate. The plate is inserted into a saturated solvent mixture vessel and then closed. After elution, the dish was removed from the vessel, dried, and the results were examined under UV light. Each zone is marked and dredged and then eluted with methanol: ethyl acetate (1:1) v / v . The elution results were evaporated to obtain a yellow amorphous solid. The isolated results were purified using acetone and n-hexane solvents to obtain a pure compound, as evidenced by a single stain on the TLC plate.

3 RESULT AND DISCUSSION

Based on the results of the phytochemical screening of the ethyl acetate extract of the leaves of the Mundu plant using $FeCl_3$ 5%. It showed that the ethyl acetate extract from the leaves of the Mundu plant positively contains phenolic compounds. Isolation of flavonoid compounds from the leaves of the Mundu plant started with the maceration extraction process and obtained a concentrated methanol extract of 203.35 g. Then the concentrated methanol extract was dissolved using distilled water to separate the nonpolar compounds. The aquadest filtrate was partitioned using ethyl acetate solvent to obtain 10 g of concentrated ethyl acetate extract. The concentrated ethyl acetate extract obtained was then dissolved in methanol and then partitioned again using n-hexane solvent to obtain a concentrated methanol extract of 6.90 g. From the results of thin layer chromatography, it is known that a good solvent ratio for separating flavonoid compounds from the leaves of the Mundu leaf plant is chloroform: methanol 80:20 (v/v), which shows the presence of three stains with different spacing. Separation was carried out by column chromatography and using chloroform: methanol as eluent comparison - a 90:10, 80:20, 70:30, 60:40 (v/v), then TLC analysis was performed to combine the fractions and obtained 238 fractions, where the fractions that were continued were fractions 7 – 75 with FeCl 3 5 reagent % produced the best stain separation with an Rf distance between stains of 0.51 with a weight of 9.7 g, then analyzed by TLC again with a solvent system of chloroform: ethyl acetate 40:60 (v/v), the furthermore in Chromatography Lapis Thin Preparative with a suitable solvent system, namely chloroform: ethyl acetate 40:60 (v/v), observed with a UV lamp, then stains were taken from the boundaries that had been marked, then silica gel was scraped and eluted with a solvent ratio of methanol: ethyl acetate 1:1 (v/v). The compounds obtained were then purified again using acetone and solvents.

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From the results of the UV – Visible spectrophotometer with methanol solvent gives maximum wavelength (λ_{max}) 339,500 nm and 288,500 nm, the Ultraviolet-Visible (UV-Visible) spectrum shows that the group of flavonoid structures obtained belongs to the flavone group, the Ultraviolet-Visible (UV-Visible) spectrum Visible) the isolated compounds can be seen in Figure 1.

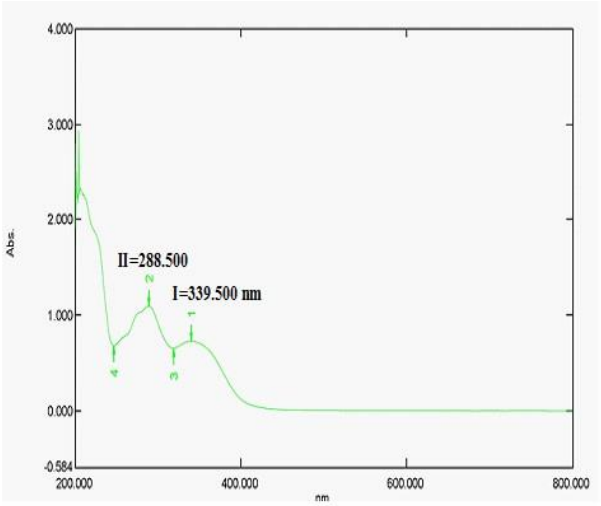

Figure 1 . Ultraviolet-Visible (UV-Vis) spectrum of the isolated compound.

Figure 1 shows that the UV-Vis spectrum of the isolated compounds produces absorptions in the wave number region (cm $^{-1}$), which can be seen in Figure 2. with the following explanation :
On number wave, 3 379.29 cm^{-1} width indicates the presence of –OH stretching vibrations that overlap with the vibrations that hold out CH aroma. At number wave 2854.65-2924.09 cm^{-1} peak shows aliphatic –CH stretching vibrations. A sharp peak at wave number 1 635.64 cm-1 indicates a bond stretching vibration double C=O from ketones. On bil - wishful g waves 1 458.18 – 1512.19 cm$^{-1,}$ the moderate peak indicates a double bond stretching vibration from C= C Aromatic. At wave number 1 365.60 cm^{-1}, a sharp peak indicates the presence of –CH 3 bending vibrations from aliphatic CH. At wave number 1165.00 cm^{-1}, the sharp peaks indicate the presence of C- O stretching vibrations. At wave number 1087.85 cm$^{-1,}$ the medium peak shows stretching vibrations COC from the ether.

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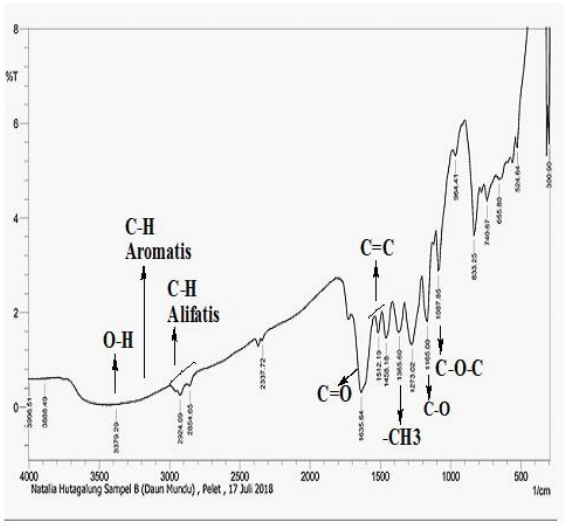

Figure 2. FT-IR spectrum isolated compound results.

The Proton Nuclear Magnetic Resonance Spectrophotometer (1H-NMR) analysis results for the compounds isolated using Methanol-D4 and TMS chemical shift in the area (ppm) as standard, as shown in Figure 3 and Figure 4.

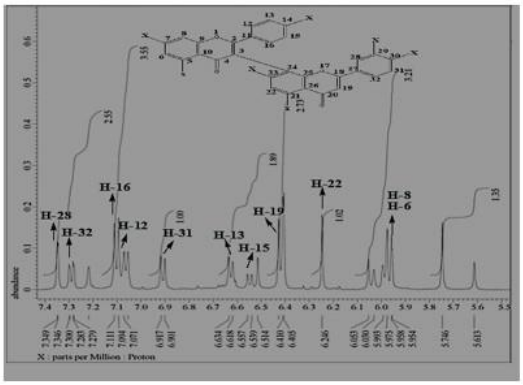

Figure 3 . [1]H-NMR spectra compound isolation results on δH = 5.5.

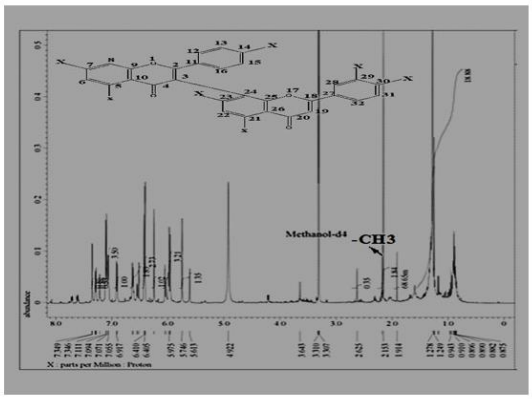

Figure 4. [1]H-NMR spectra compound isolation results on δH = 0 -8.0 Ppm

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The [1]H-NMR) isolated compounds using solvent M ethanol -D 4 mem -give chemical shift in the area (ppm) as follows: The chemical shift in the δ (ppm) 2.153 region with a singlet peak indicates CH 3 protons. The chemical shift in the δ (ppm) region 5.958 – 5.975 with a doublet peak indicates H -6 & H-8 protons in ring A of the flavonoid structure. The chemical shift in the δ (ppm) 6.246 region with a singlet peak indicates the H-22 proton in ring A of the flavonoid structure. Chemical shift on area δ (ppm) 6.427 with singlet peaks show protons H-19 in ring C structure of flavonoids. The chemical shift in the region of δ (ppm) 6.557 with peak doublet pointed - the H-15 proton in the B ring of the flavonoid structure. The chemical shift in the δ (ppm) 6.634 region with a doublet peak indicates the H-13 proton in ring B of the flavonoid structure. A chemical shift in the region of δ (ppm) 6.917 with doublet peaks indicating protons H-31 in the ring B structure of flavonoids. Chemical shifts in the δ (ppm) region 7.071-7.111 with doublet peaks showing H-12 & H-16 protons in ring B of the flavonoid structure. A chemical shift in the δ (ppm) 7.300 region with doublet peaks indicating protons H-32 in the ring B structure of flavonoids. The chemical shift in the δ (ppm) 7.349 region with a singlet peak indicates the H-28 proton in ring B of the flavonoid structure.

Based on the interpretation of the data on the UV-Visible spectrum, FT-IR spectrum, and [1]HNMR spectrum large to the possibility amorphous solid isolated from Mundu plant leaves (Garcinia dulcis (Roxb.) Kurz) is a flavonoid and bioflavonoid compound with the structure in Figure 5.

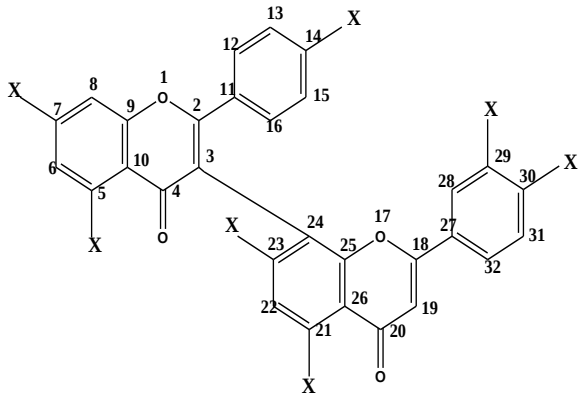

Figure 5. The structure of the resulting compounds Isolation (Biflavonoids)

4 Conclusion

Flavonoid compounds were isolated from 1.8 kg of leaves of the Mundu plant (*Garcinia dulcis* (Roxb.) Kurz) by maceration extraction and partition extraction by column chromatographic separation using chloroform as eluent: methanol (90:10) v/v; (80:20) v/v; (70:30) v/v; (60:40) v/v. The compounds obtained were purified by preparative thin layer chromatography, resulting in 8.4 mg of a yellow amorphous solid with a value of Rf = 0.35 using chloroform as eluent: ethyl acetate (40:60) v/v. Analysis results with a UV-Visible spectrophotometer, FT-IR spectrophotometer,

and ^{1}HNMR spectrophotometer showed that the isolated compounds were suspected to be flavonoid compounds belonging to the biflavonoid group.

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