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Isolation of Flavonoid Compounds from Bangun-bangun Leaves (*Plectranthus amboinicus* (Lour.) Spreng.)

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Abstract. Isolation of flavonoid compounds from leaves of Bangun-bangun (Plectranthus amboinicus (Lour.) Spreng.) has been conducted by maceration process with methanol solvent, added with ethyl acetate, and then partially extracted with n-hexane, acided by HCl 6% and partition extracted with chloroform. The concentrated extract of chloroform was separated using column chromatography with eluent n-hexane: ethyl acetate. The resulted tawny paste yielding was 10 mg with Rf= 0.38. The resulted compounds were characterized using UV-Vis, FT-IR, and ^{1}H-NMR which was estimated as a flavonoid is a flavanone.

Keywords: Isolation, Flavonoids, Chromatography, *Plectranthus amboinicus* (Lour.) Spreng.

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1 Introduction

Bangun-bangun leaves, the term commonly used by the Batak people, is one of Indonesian ethnobotany which has been used by the people of North Sumatra for generations as a daily vegetable menu and especially served for mothers who have just given birth. This plant is of unknown origin, the stem is round and slightly hairy, rarely blooms (the color is purple and white) but is easily propagated by cuttings and quickly takes root in the soil (Heyne, 1987).

Bangun-bangun plants were used as the basis for traditional medicine several years ago in India, Nigeria, China, and Indonesia. These groups of plants produce a diverse array of more than 500,000 types of high and low-molecular-weight natural substances known as secondary metabolites. The best-known examples are flavonoids, phenols, saponins, and cyanogenic glycosides (Shahidi et al, 2008). It is traditionally used to cure coughs and fevers, sore throats, and shortness of breath, but is also used to help in other treatments such as infections,

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rheumatism, and epilepsy (Bhattagcharjee, 2010). Because many drug activities have been identified, Bangun-bangun plants are said to be traditional medicinal plants.

Flavonoids are natural compounds with structural characteristics having two aromatic hydroxyl rings A and B, which are linked by three carbon atoms (Torssell, 1981).

Previous researches were conducted on the species Plectranthus amboinicus Lour. Spreng., including by Christin Santosa in 2005 proved that the aqueous extract of the Bangun-bangun leaves containing polyphenolic compounds, saponins, flavonol glycosides, and essential oils, and administration of extracts of the leaves of Bangun-bangun for 60 days was able to increase the phagocytic properties of neutrophil cells in white mice. , by Chiu 2011 who investigated the analgesic and anti-inflammatory activity of the aqueous extract of Plectranthus amboinicus (Lour.) Spreng. Furthermore, Seham El-hawary in 2012 regarding the content of polyphenolic compounds and the biological activity of Plectranthus amboinicus (Lour.) Spreng, the polyphenolic compounds found from the ethyl acetate extract of roots and stems are caffeic acid, eriodictiol, rosmarinic acid, cumaric acid, luteolin, chryseoriol, and quercetin, while the alcoholic extract from the leaves has shown antioxidant activity by the Beutler method, anti-inflammatory activity, and analgesic activity.

From the preliminary test that the researchers carried out, namely the phytochemical screening test with $H_2SO_{4(p)}$, $FeCl_3$ 5%, NaOH 10%, and Mg-HCl reagents, it was shown that the methanol and ethyl acetate extracts of the leaves of the plants contain flavonoid compounds.

Based on the explanation above and based on the literature regarding the content contained in the plant, the researchers are interested to research Bangun-bangun leaves, especially regarding the flavonoid compounds contained in them.

2 Materials and Methods

2.1 Equipments

In this study, the equipments used were: graduated cylinder, beaker glass, Erlenmeyer glass, funnel, separating funnel, extractor, test tube, dropper, capillary tube, spatula, Buchi B-480 rotary evaporator, distillation apparatus, bottom flask, column chromatography, 254/356 nm UV lamp, analytical balance, water bath, vial, chamber, stative and clamps, magnetic bar, a set of ultraviolet-visible (UV-Visible),fourier transform infrared (FT-IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectrophotometry.

2.2 Materials

The materials used were Bangun-bangun leaves, methanol, n-hexane, ethyl acetate, distilled water, silica gel 40, $FeCl_3$ 5%, NaOH 10%, Mg powder, HCl 37%, H_2SO_4 96% 6%, HCl,

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chloroform, cotton, silica gel TLC plate 60 F254, preparative TLC plate 60F254, benzene, ether, acetone and Benedict's reagent.

2.3 Sample Preparation

The samples studied were Bangun-bangun leaves obtained from the area of Jalan Pelajar Timur Ujung, Gang Kelapa, Medan Denai Sub-district, North Sumatra. The leaves of Bangun-bangun were dried in the open air, then mashed until 330 g of leaf powder was obtained.

2.4 Preliminary Test on Bangun-bangun Plant Leaves Extract

In order to determine the presence of flavonoid compounds in the Bangun-bangun plant leaves were carried out a qualitative preliminary test. As much as 10 g Bangun-bangun leaf powder was put into two Erlenmeyer glasses, then added 100 mL of methanol into the Erlenmeyer I, and 100 mL of ethyl acetate into the Erlenmeyer II, allowed to stand for 1 night, filtered, divided each sample extract into 4 test tubes, each reagent was added.

- For methanol extract samples

a. Tube I: added FeCl 35% to produce a black solution

b. Tube II: added Mg powder, and HCl to produce a pink solution

c. Tube III: added NaOH 10% to produce a turquoise solution

d.Tube IV: added H_2SO_4 to produce a yellowish orange solution

- For ethyl acetate extract samples

a. Tube I: added FeCl 35% to produce a black solution

b. Tube II: added Mg powder, and HCl to produce a pink solution

c. Tube III: added NaOH 10% to produce a turquoise solution

d. Tube IV: added H_2SO_4 to produce a yellowish orange solution

2.5 Extraction and Fractionation of Bangun-bangun Plant Leaves

As much as 330 g leaf powder was macerated with ± 3 L of methanol until all samples were immersed and left for ± 24 hours. The macerate was collected and concentrated using a rotary evaporator to obtain a concentrated methanol extract. Then evaporated until all the methanol solvent evaporates. After that, the tannin was separated by dissolving the concentrated methanol fraction with ethyl acetate and filtered.

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The filtrate was then evaporated using a rotary evaporator. The concentrated ethyl acetate fraction was dissolved with methanol and repeated partition extraction with n-hexane. The methanol layer was separated from the n-hexane layer, then concentrated again with a rotary evaporator and re-evaporated to obtain a concentrated extract of the methanol layer. The methanol fraction was tested for sugar content with Benedict's reagent, then hydrolyzed using HCl 6%. Next, filtered and the filtrate obtained was partition extracted with chloroform 3 times. The chloroform extract obtained was concentrated with a rotary evaporator and re-evaporated to obtain concentrated chloroform. The concentrated chloroform obtained was 0.58 g.

2.6 Thin Layer Chromatography Analysis

Thin Layer Chromatography (TLC) analysis was performed on chloroform extract using 60F. silica gel as stationary phase254Merck. This analysis was intended to find a suitable solvent system and ratio for column chromatography. The mobile phase used was a mixture of n-hexane: ethyl acetate solvent with a ratio of 90:10, 80:20, 70:30, and 60:40 (v/v), respectively.

As much as 10 mL n-hexane: ethyl acetate 90:10 (v/v) mobile phase solution was added into the chromatography vessel, then saturated. The concentrated chloroform extract was tested on the activated TLC plate. The TLC plate was put into a vessel containing a solvent mixture that has been saturated, then closed and elution. The eluted plate was removed from the vessel, then dried. The stains formed were observed under UV light, then fixed with $FeCl_3$ 5% reagent. The color of the spots that appear is observed and the Rf value obtained is calculated. The same treatment was carried out for the ratio of n-hexane: ethyl acetate with a ratio of 80:20, 70:30, and 60:40 (v/v).

As much as 10 mL n-hexane: ethyl acetate 90:10 (v/v) mobile phase solution was added into the chromatography vessel, then saturated. The concentrated chloroform extract was tested on the activated TLC plate. The TLC plate was put into a vessel containing a solvent mixture that has been saturated, then closed and elution. The eluted plate was removed from the vessel, then dried. The stains formed were observed under UV light, then fixed with$FeCl_3$ a 5% reagent. The color of the spots that appear was observed and the Rf value obtained was calculated. The same treatment was carried out for the ratio of n-hexane: ethyl acetate with a ratio of 80:20, 70:30, and 60:40 (v/v).

2.7 Isolation of Flavonoid Compounds by Column Chromatography

Isolation of flavonoid compounds by column chromatography was carried out on the concentrated chloroform extract that had been obtained. The stationary phase used was silica gel 40 (70-230 mesh) ASTM and the mobile phase was n-hexane 100%, a solvent mixture of n-hexane: ethyl acetate with a ratio of 90:10, 80:20, 70:30, 60:40 (v/v).

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The chromatography column was assembled. Firstly, silica gel 40 (70-230 mesh) ASTM was slurried using n-hexane, stirred until homogeneous, and then put into the chromatographic column. Then it was eluted using n-hexane 100% until the silica gel was solid and homogeneous. Next, as much as 0.58 g of concentrated chloroform extract was slurried with silica gel with acetone as a solvent, then put into a chromatographic column that already contained silica gel slurry, then the mobile phase n-hexane: ethyl acetate 90:10 (v/v) was slowly added. and adjusted that the phase flow out of the column is equal to the addition of the mobile phase from above. The polarity was increased by adding the mobile phase n-hexane: ethyl acetate with a ratio of 80:20, 70:30, and 60:40 (v/v), respectively. The results obtained are accommodated in vials every ± 13 mL, then in TLC and combined with fractions with the same Rf value, and then tested with $FeCl_3$ 5%. Then evaporated to form a paste.

2.8 Purification

The paste obtained from isolation by column chromatography was redissolved with ethyl acetate and then analyzed by TLC to determine whether the compound obtained was pure or not, while at the same time looking for a mobile phase suitable for TLC preparations. N-hexane: ethyl acetate 70:30 (v/v) was the mobile phase that showed the best separation and was subsequently used to saturate the preparative TLC vessel. Meanwhile, the diluted paste was applied slowly and evenly along the lower edge of the activated TLC plate. The plate was placed in a vessel containing a saturated solvent mixture, then closed. After eluting, the plate was removed from the vessel, dried, and the result was examined under UV light. Each zone was marked and dredged and then eluted with methanol: ethyl acetate (1:1). The elution results were evaporated to obtain a brownish-yellow paste.

2.9 Purification Test of Isolation Results with Thin Layer Chromatography

The paste purity test was carried out by thin layer chromatography using silica gel 60 F254 as a stationary phase with n-hexane: ethyl acetate 80:20 (v/v), chloroform: ethyl acetate 60:40 (v/v) as mobile phase, ethyl acetate: methanol 90:10 (v/v).

As much as 10 mL of the mobile phase solution was added into a thin layer chromatography vessel, then saturated. The paste was spotted which was previously dissolved in chloroform on a TLC plate. The TLC plate was inserted into the thin layer chromatography vessel which had grown dull. After the mobile phase seeps up to the mark, the TLC plate was removed from the vessel, dried, observed under UV light, and fixed using a 5% FeCl3 reagent in methanol-produced black spots which indicated the presence of flavonoid compounds.

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2.10 Identification of Isolated Compounds

The isolated compounds were characterized by UV-Visible Spectrophotometry, fourier transform infrared (FT-IR), spectrophotometry, and proton nuclear magnetic resonance (^{1}H-NMR) spectrophotometry.

3 RESULT AND DISCUSSION

The phytochemical screening of methanol and ethyl acetate extracts from the Bangun-bangun plant leaves (*P. amboinicus* (Lour.) Spreng.) using flavonoid reagents showed that the samples were positive for flavonoids.

The elution results from the solvent ratio of n-hexane: ethyl acetate 70:30 (v/v) in the 44-51 fraction, preparative TLC was carried out with n-hexane: ethyl acetate 70:30 (v/v) eluent to obtain the pure compound, in term of this fraction was chosen due to it produced fewer stains than other fractions, namely 2 spots. So that the pure compound was obtained in the form of a brownish-yellow paste, the weight was 10 mg, and the Rf value was 0.38.

The UV-Visible spectrophotometry analysis results on paste isolated with methanol solvent presented in figure 1 show that band I at 328 nm and band II at 299.5 nm, which showed the maximum wavelength.

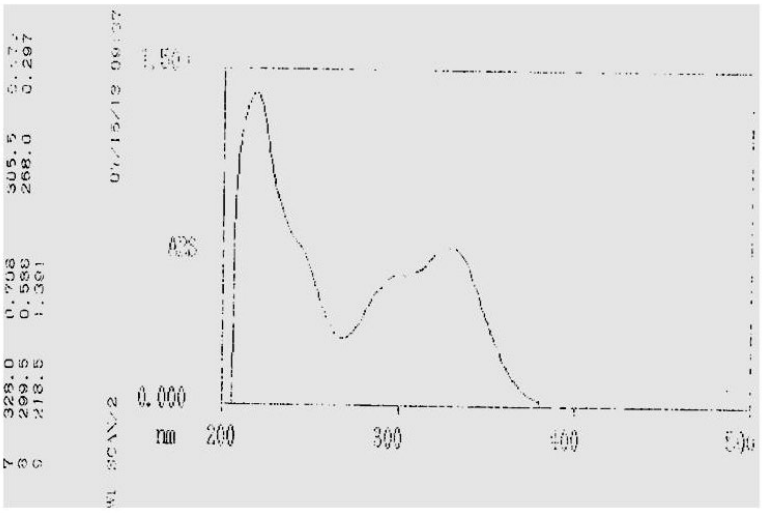

Figure 1. The UV-Visible spectrum of isolation result compound

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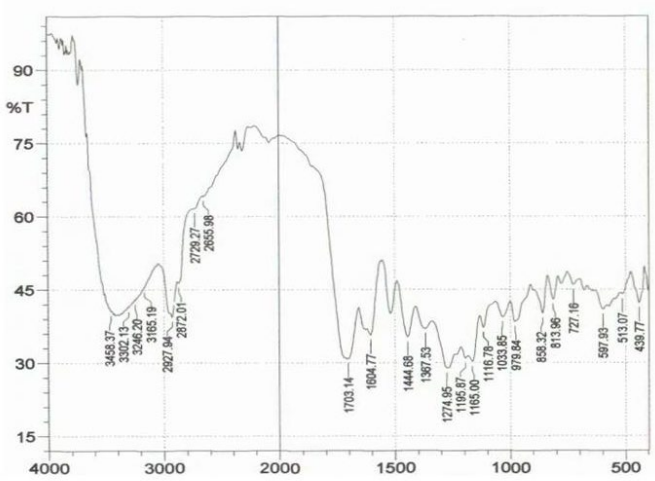

Figure 2. FT-IR spectrum of isolation result compound

In addition, the FT-IR test results of the isolated paste indicated in figure 2 show that the band at 3458.37 to 3302.13 cm^{-1}, which indicated the presence of OH groups, and the band at 3246.20 to 3165.19 cm^{-1}, which assigned aromatic CH bonds, the band at 2927.94 to 2872.01 cm^{-1}, which showed aliphatic CH bonds, the band at 1703.14 cm^{-1}, which indicated C=O bonds from ketone, the band at 1604.77 cm^{-1}, which showed aromatic C=C bonds, the band at 1500 to 1444.68 cm^{-1}, which assigned aliphatic C=C, the band at 1367.53 cm^{-1}, which indicated CH_2 bonds, the band at 1274.53 cm^{-1}, which assigned CO bonds from alcohol, the band at 1165.00cm^{-1}, which –C-C-O-C from ketone, the band at 1033.85 cm^{-1}, which symmetrical COC bonds, the band at 1116.78cm^{-1}, which assigned asymmetric COC, the band at 979.84 cm^{-1}, which showed aromatic CH.

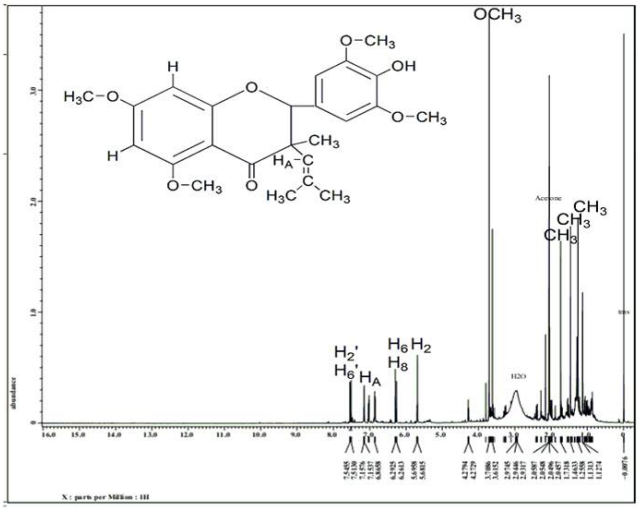

Figure 3. ^{1}H-NMR spectrum of isolation result compound

Furthermore, the ^{1}H-NMR test results displayed in figure 3 show that the chemical shift in the δ = 1.2558 ppm singlet peak which indicated a proton from $–CH_3$ in the aliphatic chain, the chemical shift in the δ = 1.4633 ppm singlet peak which indicated a proton from $–CH_3$ in the

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aliphatic chain, the chemical shift in the δ = 1.7318 ppm singlet peak which indicated a proton from $–CH_3$ in the aliphatic chain, the chemical shift in the δ = 3.7086 ppm singlet peak which showed the protons from the methoxy group $–OCH_3$ in rings A and B of the flavonoid structure, the chemical shift in the δ = 5.6815 ppm singlet peak which assigned the H-2 proton in ring C of the flavonoid structure, the chemical shift in the δ = 6.2613 to 6.2925 ppm doublet peaks which showed the protons from H- 6 and H-8 in ring A of the flavonoid structure, the chemical shift in the δ = 7.1537 ppm singlet peak which showed the protons in the HA ring C flavonoid structure, the chemical shift in the δ = 7.5130 to 7.5455 ppm doublet peaks which indicated the protons of H-2' and H-6' in ring B of the flavonoid structure.

Based on data analysis results were carried out using UV-Vis, FT-IR, and ^{1}H-NMR spectrophotometry to show that the paste isolated from the Bangun-bangun plant leaves (*P.amboinicus* (Lour.) Spreng.) was flavonoid compound of the flavanone group with the substituents in the form of vinyl and methyl groups. The vinyl shift in the 1H-NMR spectrum is in the area of about 5-6 ppm, but because the proton position of the vinyl is closed to the carbonyl (C=O) of the C ring on the flavonoid, the vinyl proton shift will shift in a downward direction (away from TMS) due to the influence of electronegativity in the area of 7.1537 ppm. The methyl shift in the area was around 0.9-1.7 ppm is the type of methyl that was bound to the open chain, so the position of methyl is a branch of the vinyl compound (Pavia et al, 1979). However, the position between the methyl substituent and the shift in the ^{1}H-NMR spectrum has not been determined, so further ^{13}C-NMR and MS analysis are necessary.

From the ^{1}H-NMR data, the final compound showed data that supported that the isolated compound was a flavonoid compound of the flavanone group. This was evidenced that in the ^{1}H-NMR data there was a single peak in the area of 5.6815 ppm for protons at C-2 and this shift area is a characteristic of flavanone compounds, namely in the area around 5.0 to 5.6 ppm for protons at C-2 (Markham, 1988).

In addition, phytochemical screening test results indicated that the isolated obtained was flavanone compounds which were supported by Uv-Vis, FT-IR spectrophotometry, and compared to 1H-, it can be said that it is very likely that the paste isolated from the leaves of Bangun-bangun is a flavonoid compound of the flavanone group.

The following was the structure of flavanones that were suspected from isolated compounds as shown in figure 4.

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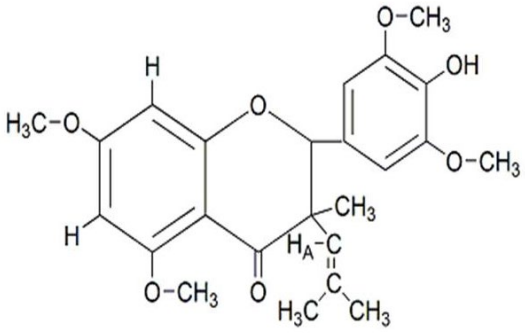

Figure 4. Flavanone structure

4 Conclusion

In conclusion, the isolated results from 330 g of Bangun-bangun plant leaves (*P.amboinicus* (Lour.) Spreng.) were a brownish yellow paste was obtained as 10 mg with Rf = 0.38 using eluent n-hexane: ethyl acetate 80:20 (v/v) show that the isolated compound was positive for flavonoid compounds which supported by UV-Visible spectrophotometry, FT-IR, spectrophotometry and ^{1}H-NMR spectophotometry and the isolated produced showed flavonoid compound of the flavanone group.

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