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Isolation and Identification of Phenolic Compounds from Ebony Plant Leaves (*Diospyros celebica* Bakh.)

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Abstract. Isolation of phenolic compounds from Ebony plant leaves (*Diospyros celebica* Bakh .) was done by maceration technique with methanol solvent. The concentrated extract of methanol dissolved with distilled water and partition was extracted with ethyl acetate. The concentrated ethyl acetate extract dissolved with methanol solvent and partitioned with n-hexane. Then Thin layer chromatography was analyzed before column chromatography, and the eluent suitable for separation is chloroform: methanol 90:10, 80:20, 70:30,60:40,50:50 (v/v) as mobile phase. The compounds were purified with a thin layer preparative and crystallization yielding yellow paste weighing 7.5 mg with Rf = 0.45. The test purity of compounds is done by thin layer chromatography showing a single spot with eluent chloroform: ethyl acetate (50:50) v/v and n-hexane: ethyl acetate (80:20) v/v. The following compound was further indicated by Ultraviolet-Visible Spectrophotometer (UV-Vis), Fourier Transform Infra Red Spectrophotometer (FT-IR), and Proton Nuclear Magnetic Resonancy Spectrophotometer (^{1}H-NMR) was estimated as a phenolic methyl gallate, phenolic acid.

Keywords: Ebony Leaves (*Diospyros celebica* Bakh.), Phenolic, Methyl Gallate

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1 Introduction

Secondary metabolites are products of metabolism found in plants. Secondary metabolites are divided into several parts, including phenolic compounds. Phenolic compounds are plants' most widely distributed secondary metabolite compounds (Cheynier et al., 2013). These phenolic compounds have a basic carbon framework consisting of a benzene ring (C6) attached to the end of the propane carbon chain (C3). This group of phenolic compounds is found in many higher plants (Kristanti et al., 2002). Phenolic compounds dissolve quickly in water because they generally bind to sugars as glycosides. Phenol is a compound characterized by the attachment of the hydroxyl group directly to the aromatic ring (Harborne, 1987). The physiological activities of phenolic compounds in plants are many and varied. While some phenolic compounds have essential functions in the plant, they are made of antioxidants, antibacterials, and so on (Robinson,

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1995). Blackwood leaves are taken two times a day, in the morning and night before bed (Sam, 2016). So researchers want to know what compounds are contained in the leaves of the ebony plant.

The previous researcher Syam (2016), conducted an acute toxicity test of ebony ethanol extract (*Diospyros celebica* Bakh) on male mice (Mus musculus), which obtained an LDSO value with acute toxicity of 5.168 mg/Kg BW.

Then Alwi et al. (2010) conducted a Fungidisa Test on *Phytophthora palmivora* Butler from Blackwood sawdust extract. A concentration of 1% to 5% can potentially inhibit the growth of the *Phytophthora palmivora*. Based on the results of the Phytochemical Screening, it was shown that the ebony sawdust waste extract contained several secondary metabolite compounds. This causes the Ebony sawdust extract to function as a fungicide against *Phytophthora palmivora* Butler.

However, until now, there has been no research on isolating phenolic compounds from ebony plants. Therefore researchers are interested in researching the leaves of ebony plants, particularly regarding the isolation and identification of phenolic compounds contained in the plant leaf black wood.

2 Materials and Methods

2.1 Equipment

In this study, the tools used were the ^{1}H-NMR spectrophotometer (Agilent 2NMR 500MHz), the FT-IR Spectrophotometer (Shimadzu), the UV-Vis spectrophotometer (Hewlett Packard Agilent), Column Chromatography (pyrex), rotary evaporator (Heidolph), UV lamp (254nm/356nm), UVGL5 8), analytical balance (Mettler AE 200), chamber, extractor, distillation apparatus, and glassware.

2.2 Materials

The materials used in this study were powdered ebony leaves (1800gr), methanol (technical), ethyl acetate (technical), aquadest, n-hexane (technical), silica gel (70 – 230 mesh, E-Merck.kgA), $FeCl_3$ 5%, NaOH 10%, Mg powder, $HCl_{(p)}$, $H_2SO_{4(p)}$, cotton, chloroform (E.Merck), TLC plate silica gel 60 F_{254} (E.Merck.Art 554), Preparative TLC Plate 60 F 254, Benzene Pa Merck), acetone (Merck).

2.3 Sample Provision

The sample studied was the leaves of the ebony plant obtained from the garden area of the USU Rector's Bureau, Padang Bulan, Medan, North Sumatra. Ebony plant leaves are dried in the open air, then pulverized until 1800 g of Ebony plant leaf powder is obtained.

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2.4 Preliminary Test on Ebony Plant Leaf Extract

The fine dry powder of ebony leaves was a preliminary test using a phytochemical screening test of 10 grams of dried ebony leaves powder put into an Erlenmeyer glass. Then, 100 mL of methanol was added to the Erlenmeyer glass and left to stand for one night. 10 mL of sample extract was put into a test tube. Three drops of 5% $FeCl_3$ reagent were added to produce a black opposite solution.

2.5 Ebony Plant Leaf Extraction

Blackwood plant leaf powder was macerated with 8L of methanol, weighed 1800 g, then macerator, and left for 24 hours. The macerate is collected and concentrated using a rotary evaporator to obtain methanol extract.

The methanol extract was evaporated over a water bath until the methanol solvent evaporated, and a concentrated methanol extract was formed in the form of a paste. The concentrated methanol extract was dissolved using aquadest. The aquadest filtrate was partitioned repeatedly using ethyl acetate to separate the tannins until the ethyl acetate layer was negative when tested with the $FeCl_3$ reagent. The ethyl acetate fraction was then put in the rotary evaporator until all the ethyl acetate solvent evaporated. Then the concentrated ethyl acetate fraction was dissolved with methanol, and the partition was extracted repeatedly using n-hexane until the n-hexane layer was transparent. The methanol layer was separated from the n-hexane layer and then concentrated again using a rotary evaporator to obtain a concentrated extract of the methanol layer. Thin Layer Chromatography analyzed the concentrated methanol extract with the stationary phase of silica gel 60 F 254 Merck and with the mobile phase of a mixture of chloroform: methanol with a ratio of 90:10, 80:20, 70:30, 60:40, 50:50 (v/v) to find the appropriate eluent on column chromatography.

2.6 Separation of Phenolic Compounds by Column Chromatography

Separation of phenolic compounds by column chromatography was carried out on the concentrated methanol extract that had been obtained. The stationary phase used was silica gel 40G (70-230 mesh), and the mobile phase was 100% chloroform, a mixture of chloroform: methanol solvents with a ratio of 90:10, 80:20, 70:30, 60:40, 50:50 (v /v). The column chromatography apparatus was assembled, and the silica gel was pulverized first using chloroform solvent, then stirred until homogeneous and put into the chromatography column. Elution was carried out using 100% chloroform on a chromatography column containing silica gel slurry until the silica gel was solid and homogeneous. Silica gel was added to the methanol extract and evaporated until the methanol had evaporated, then put into the chromatography column containing silica gel slurry, then added the mobile phase of chloroform: methanol 90:10 (v/v) slowly and adjusted so that the flowing phase The amount that came out of the column was the same as the addition of the mobile phase from above. The polarity was increased by adding the

mobile phase chloroform: methanol at a ratio of 90:10, 80:20, 70:30, 60:40, and 50:50 (v/v). The results obtained were collected into vials every 10 ml, then in TLC, combined for each fraction with the same Rf value, and then tested using $FeCl_3$. Evaporate until paste forms.

2.7 Purification of Isolated Compounds

The isolated paste was redissolved with methanol solvent. Then a TLC test was carried out to find out the purity of the compound, whether it was pure or not, and at the same time, it was carried out to find the appropriate mobile phase in preparative TLC. The mobile phase of chloroform: ethyl acetate 50:50 (v/v) was obtained, which showed good separation. The paste dissolved with methanol is daubed slowly and evenly along the lower boundary of the activated TLC plate. Then the plate is put into a vessel containing a previously saturated solvent mixture and closed. After the plate was eluted, it was removed from the vessel, dried, and the results were examined under UV light. Each zone is marked and dredged to be eluted with methanol: ethyl acetate (1:1) v/v. Then the elution results were evaporated and crystallized using methanol and n-hexane solvents to obtain yellow crystals. Crystals were tested for purity by thin layer chromatography using silica gel 60 F_{254} stationary phase with chloroform as mobile phase: ethyl acetate 50:50 (v/v) and n-hexane: ethyl acetate 80:20 (v/v).

2.8 Identification of Isolated Compounds

Analysis of the isolated compounds using a UV-Visible Spectrophotometer, Infrared Spectrophotometer (FT-IR), and Proton Nuclear Magnetic Resonance Spectrophotometer (1 HNMR) was carried out at the Analytical Chemistry Laboratory and organic laboratory Bandung Institute of Technology Jl. Genesha 10 Bandung 40132

3 RESULT AND DISCUSSION

The results of the phytochemical screening of the methanol extract of ebony leaves using $FeCl_3$ reagent 5% indicate that the methanol extract from ebony leaves positively contains phenolic compounds. Isolation of the phenolic compounds from the leaves of the ebony plant began with the maceration extraction process, and 209.63 g of concentrated methanol extract was obtained. Then the concentrated methanol extract was dissolved using distilled water to separate nonpolar compounds. The aquadest filtrate was partitioned using ethyl acetate solvent to obtain a concentrated extract of 32.25 g of ethyl acetate. The concentrated ethyl acetate extract obtained was then dissolved with methanol and then partitioned again using n-hexane solvent to obtain a concentrated methanol extract of 12 g. The results of thin-layer chromatography show that a good solvent ratio for separating phenolic compounds from ebony leaves is chloroform: methanol 80:20 (v/v), which shows the presence of four stains with different spacing. Separation was carried out by column chromatography and using chloroform: methanol as eluent with a ratio of 90:10, 80:20, 70:30, 60:40, 50:50 (v/v), then TLC analysis was performed to combine the fractions and obtained

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180 fractions, where the fraction that is continued is the fraction 38 – 92 this is because the weight of the paste is more, namely 153 mg while the fraction 17 – 37 is only 43 mg, the fraction 93 – 102 weights 52 mg, the fraction 103-112 weights 66 mg and 113 – 180 has the weight of 115 mg, fractions 38 – 92 were used as much as 100 mg and then analyzed by TLC again with a solvent system of chloroform: ethyl acetate 80:20 (v/v), the furthermore in Chromatography Lapis Thin Preparative with a suitable solvent system, namely chloroform: ethyl acetate 50:50 (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 compound obtained was then purified again by crystallization using acetone and n-hexane 50:50 (v/v), which showed one stain on the resulting compound with an Rf value of 0.45. Then it was evaporated, and 7.5 yellow crystals were obtained. g.

The results of characterization and elucidation using an Ultraviolet-Visible (UV-Vis) spectrophotometer show a maximum absorption wavelength (λ max) of 290nm. It is shown in Figure 1.

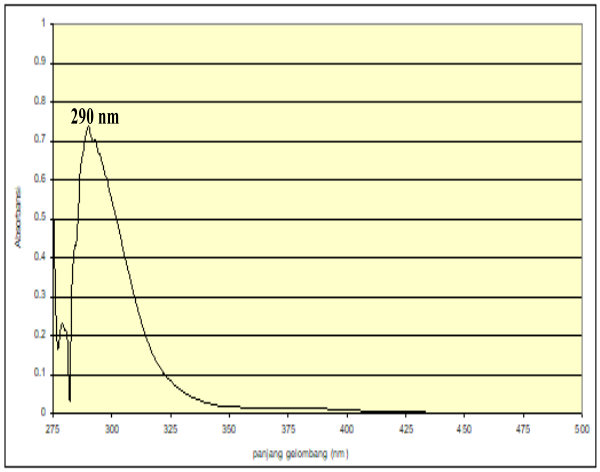

Figure 1. Ultraviolet-Visible (UV-Vis) Spectra of the isolated compounds

The results of the analysis of the Infrared Spectrophotometer (FT-IR) from the isolated paste produce absorption bands in the region wave number (cm -1) is shown in Figure 2. At wave number 3468.01 cm $^{-1}$, sharp peaks indicate–OH stretching vibrations. At wave number 3311.78 cm^{-1}, it shows aromatic CH stretching vibrations supported by aromatic CH bending vibrations at wave number 767.6 cm^{-1}. At wave number 2953.02 cm^{-1}, the sharp peak indicates an aliphatic – CH stretching vibration supported by CH buckling at 1440.83 cm^{-1}. At wave number 1695 cm^{-1}, a sharp peak indicates a stretching vibration of the C=O double bond from carbonyl. At wave number 1618.28 cm$^{-1,}$ a moderate peak indicates a stretching vibration of C= C Aromatic. At wave number 1251.80 cm^{-1}, the moderate peak indicates a stretching vibration of CO alcohol.

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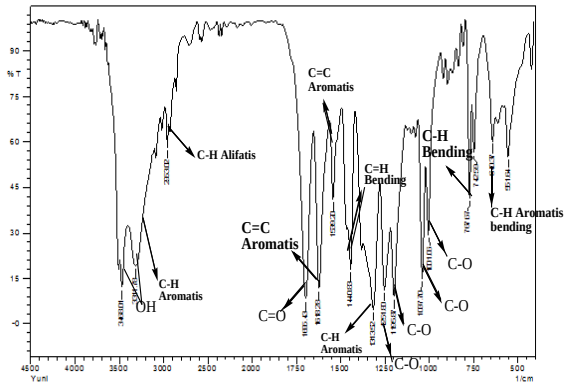

Figure 2. FTIR spectrum of the isolated compounds

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

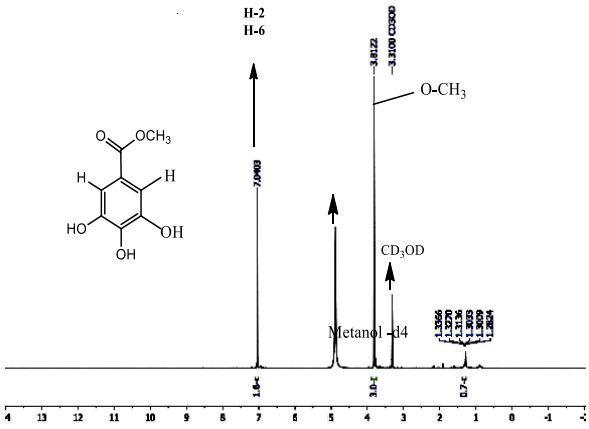

Figure 3. ^{1}H-NMR spectrum of the isolated compound

From the results of the Proton Nuclear Magnetic Resonance Spectrometer (^{1}H-NMR) analysis, the isolated compound using Methanol -D4 solvent gives a chemical shift in the area (ppm). The chemical shift in the region δ (ppm) 7 .0403 with the Singlet peak showing H-2 and H-6 protons. The chemical shift in the δ (ppm) 3.8122 z region with the singlet peak indicates the H proton of OCH_3.

Based on the interpretation of the data analysis carried out on the UV-Visible spectrum, infrared spectrum (FT-IR), and ^{1}H-NMR spectrum, it was concluded that it is most likely that the compound isolated from the leaves of the Sawo Kecik plant (*Manilkara kauki* (L.) Dubard) is a flavonoid compound belonging to the Flavones.

Nevertheless, the authors acknowledge that the ^{1}H-NMR data is still not pure because of a mixture of other isolated compounds. Figure 4 below shows the structure of the flavone obtained from the isolated compound:

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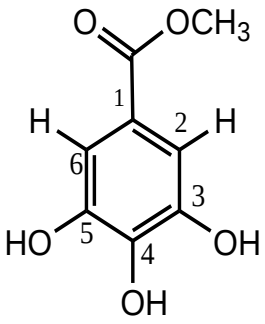

Figure 4. The structure of the isolated compound (phenolic)

4 Conclusion

Phenolic compounds from the leaves of Ebony plants were isolated by maceration extraction with methanol, Extract methanol is dissolved with aquadest and distilled water filtrate partitioned with ethyl acetate. The ethyl acetate extract is dissolved with methanol and then partitioned with n-hexane. The methanol extract (total phenolic) was separated by column chromatography using chloroform: methanol as eluents with ratios of 90:10, 80:20, 70:30, 60:40, and 50:50 (v/v). The compound obtained was purified by preparative thin layer chromatography with chloroform: ethyl acetate 50: 50 (v/v) as the eluent, producing 7.5 mg of yellow crystals with an Rf value of 0.45. From the results of the isolation of Ebony leaves and identification of compounds with a UV-Visible Infrared Spectrophotometer (FT-IR) and Proton Nuclear Magnetic Spectrophotometer (^{1}H-NMR) which showed phenolic compounds, namely, it was suspected that the Methyl Galat compound belonged to the phenolic acid group.

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