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Isolation and Identification of Phenolic Compounds from Sea Grape (*Coccoloba uvifera (L.) L*)

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ABSTRACT
Isolation and identification of phenolic compounds from Sea Grapes (*Coccoloba uvifera* (*L.*) *L.*) leave, where as much as 1700 g have been macerated using methanol. The concentrated methanol extract was dissolved in water and partitioned with ethyl acetate. Ethyl acetate concentrated extract was separated by column chromatography with chloroform as eluents: methanol 90:10; 80:20; 70:30; 60:40 v/v. The compound obtained was purified by column again using chloroform: ethyl acetate 70:30 v/v with the resulting was 10 mg of brownish yellow paste with a value of Rf = 0.5. The resulting compounds were then analyzed using a UV-Vis, FT-IR, and ^{1}H-NMR spectrophotometer. Based on the data analysis, the results showed phenolic compounds in Sea Grape.

Keywords: Isolation, Phenolic Compound, Sea Grape, Spectrophotometer,

ABSTRAK
Isolasi dan identifikasi senyawa fenolik dari daun Anggur Laut (*Coccoloba uvifera* (*L.*) *L.*) dimana sebanyak 1700 g telah dimaserasi dengan pelarut metanol. Ekstrak metanol pekat dilarutkan dalam air kemudian dipartisi dengan etil asetat. Ekstrak pekat etil asetat dipisahkan dengan kromatografi kolom dengan kloroform sebagai eluen: metanol 90:10; 80:20; 70:30; 60:40 v/v. Senyawa yang diperoleh dimurnikan kembali dengan alat kolom menggunakan kloroform:etil asetat 70:30 v/v, menghasilkan pasta berwarna kuning kecoklatan sebanyak 10 mg dengan nilai Rf = 0,5. Senyawa yang dihasilkan kemudian dianalisis menggunakan spektrofotometer UV-Vis, FT-IR dan ^{1}H-NMR. Berdasarkan hasil analisis data menunjukkan adanya senyawa fenolik terhadap Anggur Laut.

Keyword: Anggur Laut, Isolasi, Senyawa Fenolik, Spektrofotometer

1. Introduction

Indonesia has the third-largest tropical forest in the world (after Brazil and Zaire). Biodiversity is the basis for future medicinal and pharmaceutical industry discoveries. The number of medicinal plants in Indonesia is estimated to be around 1,260 plant species. Plants produce secondary metabolites that have potential as antioxidants, dyes, food aroma enhancers, perfumes, insecticides and drugs. 150,000 secondary metabolites have been identified, and there are 4,000 secondary metabolites [1].

Phenolic compounds have one or more hydroxyl groups attached to an aromatic ring. In other words, phenolic compounds have at least one phenol group [2]. Phenolic compounds are also the largest group of secondary metabolites in plants. Phenolic compounds generally have the potential as a bactericidal, antiseptic, antioxidant, and so on [3]. Some compounds included in the phenolic group are simple phenols, coumarins, saponins, and flavonoids. These compounds are usually in the form of glycosides or esters in plants [4].

Spectroscopy is the study of the interaction between light energy and matter. The colours that appear are due to the absorption of energy by organic and inorganic compounds. The spectroscopy technique is a chemical-physical analysis technique that observes the interaction of atoms or molecules with electromagnetic radiation. There are two kinds of instruments in spectroscopic techniques: spectrometers and spectrophotometers. A spectrometer is an instrument that uses a fixed slit monochromator at the focal plane. If the spectrometer is equipped with a photoelectric detector, it is called a spectrophotometer [5].

Sea grapes are evergreen shrubs, or sometimes trees, varying in height and habits according to their environment. In more exposed conditions, it can become a spreading shrub only 1 meter tall, while in good soil under sheltered conditions, it can sometimes grow up to 15 meters tall. Some are straight and up to 40 cm in diameter on the more giant trees. This plant is often harvested from the wild as food, medicine, and a source of wood when large enough is available, and this wood is highly valued for cabinet work and furniture. Sometimes cultivated for its edible fruit and often grown as an ornamental, it is advantageous as a hedge in maritime areas [6].

In 2011 Iuri Bezzera DB et al. researched the leaves and roots of *Coccoloba mollis,* often used in medicine in Londrina, Brazil. In its identification using pharmacognostic methods that produce the presence of flavonoids and tannins. Then [7] also conducted research with the ethanol extract of *Coccoloba acrostichoides* which was tested for in vitro antimicrobial activity, which can inhibit fungal growth and is active against Fusarium oxysporum. The literature shows that a plant called sea grapes does not live in the sea but on land. This plant is very suitable to grow in the tropics. And it is known that this plant is still rare and cultivated by Indonesians. The community has used the bark and fruit of the sea grapes as a medicine for diarrhea and cough medicine. Ten g of Sea grapes were used to cure diarrhea; it was washed and boiled with one glass of water until boiling, cooled, and filtered. The filtered results are drunk at once.

So far, research on the content of the leaves of the Sea Grape (*Coccoloba uvifera* (L.) L) plant has not been in the literature. Therefore, researchers are interested in researching the phenolic content of the leaves of the Grapefruit plant. Based on the phytochemical screening test carried out with 5% $FeCl_3$ showed that the methanol and ethyl acetate extracts of the leaves of the Grapefruit plant positively contained phenolic compounds.

2. Materials and Methods

2.1. Equipment

The tools used in this study were a UV-Vis spectrophotometer, FT-IR spectrophotometer, ^{1}H-NMR spectrophotometer, chromatography column, TLC vessel, TLC plate silica gel 60 F_{254}, measuring cup, beaker glass, Erlenmeyer glass, glass funnel, separating funnel, test tube, chamber, rotary evaporator, bottom flask, stand and clamp, spatula, stir bar, analytical balance, pipette, blender, water bath, vials, steaming water bath.

2.2. Materials

The materials used are Sea grape leaves, methanol, ethyl acetate, aquadest, *n*-hexane, silica gel 40, $FeCl_3$, NaOH, Mg powder, HCl, H_2SO_4, cotton, Chloroform, KLT Plate Silica Gel 60 F_{254}, aluminium foil, and filter paper.

2.3. Sample Provision

The samples studied were the leaves of the Sea grape plant. Sea grape leaves (*Coccoloba uvifera* (*L.*) *L.*) obtained from the Medan Sand paper area, North Sumatra. The sea grape leaves are dried in the open air, chopped into small pieces, and then crushed using a blender until 1700 g of Sea grape leaf powder is obtained.

2.4. Preliminary Test on Sea Grape Leaf Extract

The mashed Sea grape leaves were then identified using a phytochemical screening method. In order to prove the presence of phenolic compounds in the leaves of Sea grapes, a preliminary qualitative test was carried out. Ten g of Sea grape leaves were mashed into the Erlenmeyer, added 100 mL methanol into Erlenmeyer, set aside for one night, filtered, and added $FeCl_3$ 5%. The same treatment was carried out using ethyl acetate solvent, and the same results were obtained, namely, producing a black solution.

2.5. Extraction of Sea Grape Leaves

As much as 1700 g of crushed Sea grape leaves were weighed and then macerated with ± 10 L of methanol until all samples were submerged and left for 24 hours. Soaking was carried out until the sample was negative for 5% $FeCl_3$.The maceration was accommodated and concentrated using a rotary evaporator to obtain a concentrated methanol extract. Then evaporated with a water bath until all the methanol solvent evaporated extract of the methanol layer of 153.97 g.

2.6. Thin Layer Chromatography Analysis

Thin Layer Chromatography analysis was performed on the methanol extract using Merck's 60F $_{254}$ silica gel stationary phase. This analysis is intended to find the appropriate solvent system and ratio for column chromatography. The mobile phase used was a mixture of chloroform: methanol solvents with a percentage of 90:10; 80:20; 70:30; 60:40; 50:50 (v/v). The 90:10 (v/v) chloroform: methanol mobile phase solution was then saturated in the chromatography vessel. 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. 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; 60:40; 50:50 (v/v).

2.7. Analysis of Phenolic Compounds by Column Chromatography

Phenolic compounds were isolated by column chromatography of concentrated methanol extracts. The stationary phase used was silica gel 40 (70-230 mesh) ASTM, 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). Firstly, slurry silica gel 40 (70-230 mesh) using chloroform, stirred until homogeneous and then put into the chromatography column. They were then eluted using 100% chloroform until the silica gel was solid and homogeneous. 5 g of concentrated methanol extract was added with 15 g of silica gel, then added with methanol solvent, and then dried to form a powder. Then put it into the chromatography column, which already contains silica gel slurry, then add the mobile phase of chloroform: methanol 90:10 (v/v) slowly and adjust it so that the flow of the phase-out of the column is the same as the addition of the mobile phase from above. The polarity was increased by adding chloroform: methanol as the mobile phase in a ratio of 80:20 (v/v), 70:30 (v/v), 60:40 (v/v), and 50:50 (v/v). The results were collected in vials every ± 10 mL, then in TLC, combined with fractions with the same Rf value, and then tested with $FeCl_3$ 5 %. Then evaporated to form a paste.

2.8. Purification by Return Column Chromatography

The paste obtained from isolation by column chromatography was redissolved with methanol and then analyzed by TLC to determine whether the compound obtained was pure or not. Then carry out column chromatography again with chloroform mobile phase: ethyl acetate 70:30 (v/v). The isolated results were purified using ethyl acetate and n-hexane to obtain a pure compound proven by a single spot on the TLC test.

2.9. Test the Purity of Isolation Results by Thin Layer Chromatography (TLC)

Thin Layer Chromatography (TLC) was carried out for the paste purity test using silica gel 60 F $_{254}$ as a stationary phase with chloroform as a mobile phase: ethyl acetate 70:30 (v/v). Then 10 mL of mobile phase solution was put into a thin layer chromatography vessel, then saturated. Spotted the paste, which was previously dissolved with methanol, on the TLC plate. Insert the TLC plate into the saturated thin-layer chromatography vessel. After the mobile phase solvent seeped up to the mark limit, the TLC plate was removed from the vessel, dried, observed under UV light, and fixed using $FeCl_3$ 5% in methanol to produce black spots indicating the presence of phenolic compounds and the Rf obtained was calculated.

2.10. Identification of isolated compounds

Analysis of pure compounds obtained from the isolation results was identified with UV-Visible Spectrophotometer, Infrared Spectrophotometer (FT-IR), and Proton Nuclear Magnetic Resonance Spectrophotometer (^{1}HNMR).

2.11. Identification with a UV-Visible Spectrophotometer

A UV-Visible Spectrophotometer was characterized at the UGM Integrated Research and Testing Laboratory (LPPT) Jl. Kaliurang Km. 4 Sekip Utara Yogyakarta with using methanol as a solvent.

2.12. Identification with a Proton Nuclear Magnetic Resonance Spectrophotometer (^{1}HNMR)

The ^{1}H-NMR spectrophotometer analysis was characterized at the UGM Integrated Research and Testing Laboratory (LPPT) Jl. Kaliurang Km. 4 Sekip North of Yogyakarta with using with methanol as a solvent.

3. Results and Discussion

Based on the results of phytochemical screening of methanol and ethyl acetate extracts from the leaves of the sea grape (*Coccoloba uvifera* (L.) L.) using $FeCl_3$ 5%, it showed that the methanol and ethyl acetate extracts positively contained phenolic compounds. The elution results from a solvent ratio of chloroform: methanol

90:10 (v / v) for fraction 46-47 were separated again on the column with the eluent chloroform: ethyl acetate 70:30 (v / v) and purified with chloroform and methanol to obtain the compound pure. In order to obtain a pure compound in the form of a brownish-yellow paste weighing 10 mg and an Rf value = 0.5. The UV-Vis spectrum of the isolated compounds using methanol solvent is shown in Figure 1 below :

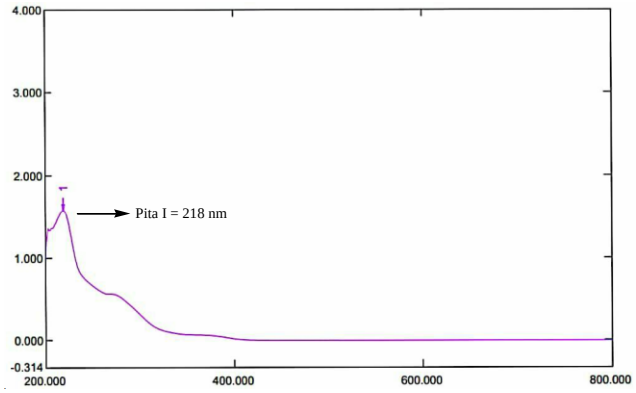

Figure 1. The UV-Visible spectrum of the isolated compound results

The FTIR analysis result of the compounds isolated using KBr pellets can be seen in Figure 2.

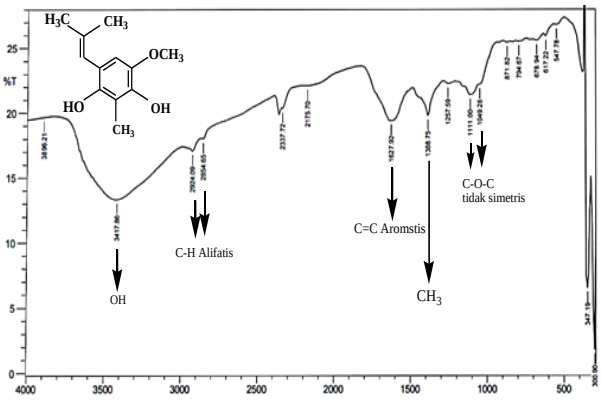

Figure 2. The FTIR spectrum of isolated compounds

From the analysis of the FTIR analysis, the absorption bands in the wave number region (cm^{-1}) are the wave number 3417.86 cm^{-1}, and the medium peak indicates the presence of OH stretching vibrations. Next, the moderate peaks in wave numbers 2924.09-2854.65 cm^{-1} indicate C-H bending vibrations. The band at 1627.92 cm^{-1} shows a stretching vibration of the C=C double bond. While the band at 1388.75 cm^{-1} indicates the presence of CH_3 bending vibrations.

Following are the results of the Proton Nuclear Magnetic Resonance Spectrophotometer (^{1}H-NMR) analysis of isolated compounds using methanol and TMS solvents as internal standards, as shown in Figure 3.

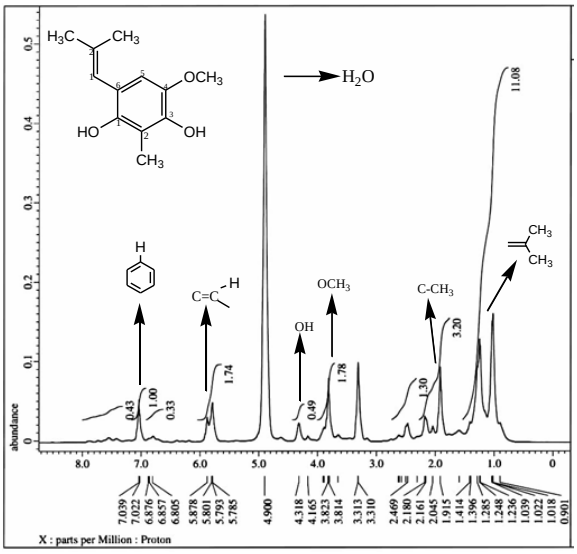

Figure 3 The ^{1}H-NMR spectrum of the isolated compounds

From the results of the analysis of the Proton Nuclear Magnetic Resonance Spectrophotometer (^{1}H-NMR), the isolated compound using methanol solvent gives a chemical shift in the area (ppm) as follows :

1. A chemical shift in the region δ = 7.039 ppm with a singlet peak indicating one proton on H-5 in the ring of phenolic compounds.
2. A chemical shift in the δ region = 3.823 ppm with a singlet peak indicating a proton from OCH_3 on the phenolic ring.
3. A chemical shift in the region δ = 5.801 ppm with a singlet peak indicating protons – C=C.
4. A chemical shift in the region δ = 1.915 ppm with a singlet peak indicating a proton from $–CH_3$ on the phenolic ring.
5. A chemical shift in the region δ = 1.285-1.018 ppm with a singlet peak indicating a proton from the dimethyl gem on the phenolic ring.

Data analysis and interpretation were carried out on the UV-Visible spectrum, FTIR spectrum, and ^{1}H-NMR. They concluded that the paste isolated from the leaves of the sea grape plant may be a phenolic compound, as shown in Figure 4, whose substituent positions cannot be ascertained because it can still change.

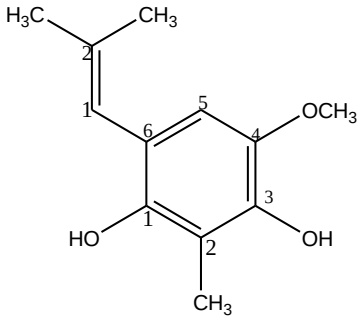

Figure 4. The structure of the phenolic compounds obtained from the isolation

4. Conclusion

Isolation of 1700 g of phenolic compounds from Sea grape (*Coccoloba uvifera* (*L.*) *L.*) leaves, in which resulting a 10 mg brownish yellow paste with chloroform as eluent: ethyl acetate (70:30) v/v Rf = 0.5. The analysis of isolated compounds from Sea grape leaves (*Coccoloba uvifera* (*L.*) *L.*) using UV-Vis, FT-IR, and ^{1}H-NMR spectrophotometer yielded phenolic compounds.

5. Acknowledgements

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6. Conflict of Interest

Authors declare no conflicts of interest

References

[1] Saifudin A. 2014. Natural Compounds of Secondary Metabolites Theory, Concepts, and Purification Techniques. Yogyakarta. Deeppublish/publisher.
[2] Nicholas W. 2006. Social Research Methods. Sage Publications Ltd. New Delhi
[3] Pangelly, S. 2006. Occupational Therapy in Housing. Whurr Publisher. London
[4] Vermerris W and Nicholson R. 2006. Phenolic Compound Biochemistry. Published by Springer. The Netherlands
[5] Muldja MH. 1995. Instrumental Analysis. First Print. Airlangga Press University. Surabaya
[6] Dawes, CJ. 1998. Marine Botany. John Wiley. New York
[7] Bethany, BC. 2003. Antimicrobial Activity and Constituents of Coccoloba Acrosticoides. Brazil