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Natural Product Chemistry Laboratory: Isolation Phenobarbitone from Mangrove Bark (*Avicennia marina*)

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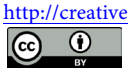

Abstract

Natural product chemistry laboratory in this research carried out a project-based laboratory related to the isolation of secondary metabolites from medicinal plants. The students learn the essential skills required to perform the extraction, fractionation, purification, and structural elucidation of secondary metabolites from medicinal plant. This study was conducted to isolate phenobarbitone from the bark of *A. marina.* The modified method uses maceration and recrystallization. This study obtained phenobarbitone 0.102 g from 100 g dried powder of bark of *A. marina*. Identified of phenobarbitone based on chromatogram of TLC, FT-IR, and UV-Vis.

Keywords

Isolation, Phenobarbiton, Mangrove (*Avicennia marina*)

1. Introduction

Natural product chemistry is one of the compulsory courses taken in third years by chemistry education students (Hakim & Jufri, 2018). Natural product chemistry courses studied secondary metabolites, classification, structure, biosynthesis, and methods of isolation of terpenoid, steroids, flavonoids, polyphenols, alkaloids (Hakim & Jufri, 2017). Natural product chemistry course cannot be separated from laboratory activities (Hakim et al., 2016). In natural product chemistry laboratory, the students are given the opportunity to complete laboratory activities independently, but still under the guidance of lecturers. Before carrying out the laboratory activities, students have done a literacy journal related to plants that will be isolated as a reference. Students design a modification method from the journal.

One type of plant that can be used as a sample to be isolated in natural product chemistry is mangrove (*Avicennia marina*). Mangrove plants are scattered throughout Indonesia and are available abundantly (Mahera et al., 2011). Mangroves can grow and develop in coastal areas and have unique adaptations to deal with environmental pressures. High salinity and ultraviolet radiation can cause oxidative damage to plant cells. Plants that can live in extreme areas like this, certainly have compounds that protect it from damage. This is the reason that *A. marina* is very good as a sample in this study. According to Kordi (2012), mangrove plants contain secondary metabolite compounds, such as alkaloids, flavonoids, phenols, terpenoids, steroids and saponins. Based on research conducted by Darminto et al. (2009), *A. marina* contains alkaloid compounds which have a fairly high inhibitory activity with a low level of toxicity. Generally, alkaloids are found in flowers, seeds, leaves, twigs, roots and bark. Darminto et al. (2012) have succeeded in isolating the 5-ethyl-5-phenilpirimidin-2,4,6-trion from the bark of mangrove. Another name of 5-ethyl-5-phenylprimidine-2,4,6-trion is phenobarbitone (Connors et al., 1986).

Phenobarbitone contains two nitrogen atoms. Phenobarbitone is included in pyrimidine alkaloids. Pyrimidine is a heterocyclic compound that has six ring members consisting of four carbon atoms and two nitrogen atoms one and three in the ring (Trease & Evans, 1983). The structure of phenobarbitone can be seen in **Figure 1**.

The molecular formula of phenobarbitone is $C_{12}H_{12}N_2O_3$. The form is white crystal powder, bitter taste, difficult to dissolve in water. The melting point is 174˚C - 178˚C (Irianto, 1987). The phenobarbitone is an anti-convulsant derivative of barbiturates. According to Darminto et al. (2009), *Avicenia* sp. potentially developed for handling MAS (*Motile Aeromonads Septicemia*) disease or often called red spot disease (*red spot disease*). The main cause of this disease is the bacterium *A. hydrophyla*. Based on the description above, it can be said that the phenobarbitone compound contained in the bark of mangrove has a property that can be utilized by the community.

2. Isolation of Phenobarbitone

There were several steps undertaken to isolate phenobarbitone from the bark of a mangrove *A. marina*. The first step, bark of the dried mangrove *A. marina* has been macerated using the right solvent. Darminto et al. (2012) used ethanol as the solven. The selection of the solvent follows the principle of "like dissolve like" based on polarity differences. Polar compounds will dissolve in polar solvents and non-polar compounds will dissolve in non-polar solvents. Based on these principles, the desired compound can be separated from the mixture selectively in the solvent used. Whereas in the modified method, the solvent used during maceration is n-hexane. N-hexane is a non-polar solvent. When compared with the principle of *like dissolve like*, the expected compound, phenobarbitone, cannot be dissolved in n-hexane solvent. However, in the modified method, additional treatment is carried out before screening, which is heating the

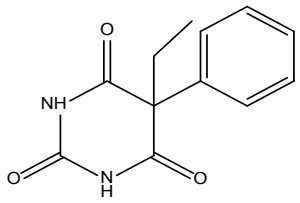

Figure 1. The structure of phenonabarbitone.

sample with the solvent until it boils and then filtered hot. This is done because at high temperatures compounds that cannot be dissolved in non-polar solvents will be completely dissolved and at low temperatures the compound will form a precipitate at the bottom of the container used.

The concentration process was carried out until the maserat remains half of the filtering result. This process used to remove impurities present in the maserat. The next step was maserat evaporation at room temperature. In this condition, non-polar compounds will be dissolved in the n-hexane solvent and will evaporate while polar compounds will settle to the bottom of the bottle used. Recrystallization was carried out using the same solvent, n-hexane. Recrystallization is a technique of purifying chemical compounds from impurities by recrystallizing the substance after it has been dissolved with the appropriate solvent. The principle of recrystallization is based on the difference in solubility between the purified compound and its impurity component. After recrystallization several times, a white powder weighing 0.102 grams was obtained (**Figure 2**).

After getting white precipitate (isolate), the next step is to prove the purity of the isolates obtained using Thin Layer Chromatography (TLC). In the study of Darminto et al. (2012), TLC was carried out using n-hexane: ethyl acetate eluent 7: 3 with an Rf value of 0.35, whereas in this study using n-hexane eluent: chloroform 8: 2 with an Rf value of 0.37.

The next step is spectroscopic analysis. Spectroscopic analysis used was FT-IR and UV-Vis analysis. This analysis was carried out to ensure that the isolates obtained were phenobarbitone compounds. The results of the FT-IR analysis Darminto et al. (2012) showed strong data in the presence of absorption bands being widened in areas of 3410.15 cm^{-1} indicating N-H bonds, absorption bands at 1427.32 cm^{-1} showing C=C aromatic absorption bands, sharp absorption bands in the area of 1681.93 cm^{-1} indicates the C=O carbonyl group, the absorption band is weak at 292.09 cm-1 for methylene and in the region of 2846.93 cm^{-1} for C-H. The following are the results of IR phenobarbitone obtained by researchers: (**Figure 3**).

From the isolation phenobarbitone IR spectrum, it can be seen several functional groups which are functional groups found in phenobarbitone. The absorption band is widening in the area 3454.08 cm^{-1} indicating the N-H bond, the absorption band C-H in the region 2849.7 cm^{-1}, the sharp absorption band in the region of 1735.8 cm^{-1} indicates the C=O carbonyl group and the absorption band at 1463.40 cm^{-1} shows the band C=C aromatic uptake. Based on the analysis of IR Darminto et al. (2012) and IR data analysis researchers have similarities.

The next analysis is the UV-Vis analysis to determine the maximum wavelength. UV-Vis analysis is usually used to prove the existence of conjugated double bonds in the structure of compounds characterized by maximum absorption occurring at wavelengths ≥ 250 nm. The following spectrum of UV-Vis phenobarbitone analysis results from the isolation of researchers: (Figure 4).

From the UV-Vis spectrum of phenobarbitone it is produced that the maximum absorption occurs at a wavelength of 274.80 nm indicating that the structure of phenobarbitone contains conjugated double bonds. The results of this analysis reinforce that the isolates obtained were phenobarbitone compounds.

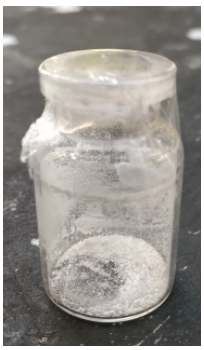

Figure 2. Phenobarbitone compoud.

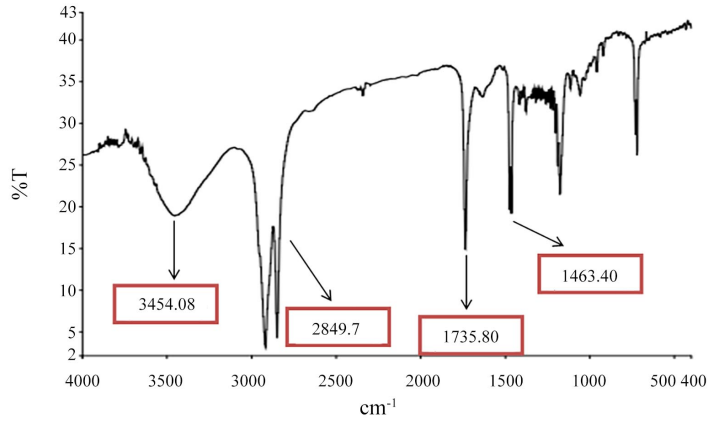

Figure 3. Isolated phenobarbitone IR spectrum.

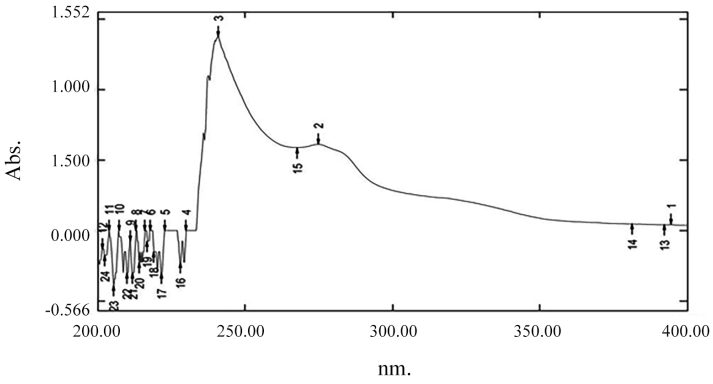

Figure 4. Phenobarbitone UV-Vis spectrum from isolation.

3. Conclusion

The method of isolation of phenobarbitone from the bark of *A. marina* has been modified. It can be used as a reference in the natural product chemistry laboratory. This method is simpler and the materials are easily available. This method also produced more phenobarbitone compounds.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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