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New Method for Isolation of Naringin Compound from *Citrus maxima*

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Abstract

This research aims to know the proper isolation method to isolate naringin compound from *Citrus maxima* for natural product chemistry laboratory. This natural product chemistry laboratory provides students with the essential skills and knowledge required to perform the extraction, isolation, and structural identification. The isolation began with sample preparation, extraction with methanol, partition with n-hexane, purification by crystallization using isopropanol, by analyzing the purity with Thin Layer Chromatography (TLC) tested, and the presence of functional groups with Fourier Transform Infrared (FT-IR) spectrophotometer test. The result of FT-IR spectrophotometer analysis contains hydroxy group (−OH), aromatic cyclic groups (C=C), CH_2, CH_3, and ether groups.

Keywords

Isolation, Naringin, *Citrus maxima*

1. Introduction

Indonesia is a country with abundant biodiversity. The diversity of animals and plants is diverse. The diversity of plants is a source of secondary metabolite compounds that have been known to have many benefits [1]. Indonesian people also have long known that some plants are processed into herbs. Secondary metabolite compounds contained in plants can be obtained by the implementation of isolation method [2] [3]. The biodiversity is a source of chemical compounds. The primary metabolite compounds such as proteins, carbohydrates, fats used by plants for their growth, and secondary metabolites such as terpenoids, steroids, polyphenols, flavonoids and alkaloids serve as a protector of plants [4]. Secondary metabolites are produced from various organisms such as plants, bacteria, and fungi. Chemical compounds as a result of secondary metabolites in var-

ious types of plants have been widely used as dyes, toxins, food scents, drugs and so forth [5].

Secondary metabolite compounds such as steroids, alkaloids, terpenoids, phenolics, flavonoids, polyphenols, saponins, etc. are of great benefit to humans [2]. Naringin, dihydroflavones, are especially present in the peels and fruits of grapefruit, pomelo and other citrus fruits. It has been shown to be highly beneficial such as preventing cancer, inhibiting microbial growth and activity of many enzymes, anti-oxidation, lowering blood cholesterol and triglyceride levels and maintaining normal capillary permeability of the blood, thus demonstrating potential drug applications. Pomelo (*Citrus maxima* Merr) expressed contains flavonoids (naringin) on the fruit skin and flesh. The thickness of the fruit skin depends on the variety. The skin of citrus fruit is divided into three layers, namely the outer skin, the middle skin and the inner skin. The outer skin is green or yellow. Middle fruit skin is pure white and the inner skin is pink. Naringin is isolated from the middle skin and the inner skin of pomelo.

2. Research Method

The tools and materials used in this research are rotary evaporator, tool sets of infrared spectrophotometer, extraction flask, hot plate, thin layer chromatography plate, middle skin and inner skin (albedo) of pomelo, methanol solvent, n-hexane, isopropanol and chloroform. Pomelo peeled, part albedo cut into small pieces. Dried in the sun for 3 days. Next, oven for 24 hours. After completely dry the pieces are smoothed to produce a powder. Albedo powder 50 grams macerated using 550 ml of methanol for 3 days.

The filtrate is separated and evaporated by a rotary evaporator to produce a dry methanol extract. The dry methanol extract was then added 50 ml of water and heated to a constant temperature of 70˚C for 30 minutes. The solution was extracted with 20 ml of n-hexane, and was allowed to stand for 3 - 4 days, extraction with n-hexane was performed twice. The liquid phase of the extraction was added with 25 ml of isopropanol and heated to half the volume. Cool the refrigerator to form a crystal. The crystals that have been produced are recrystallized with isopropanol solvent, the recrystallization is done repeatedly until the crystals are felt to be free from impurities. The compounds were tested with two tests: Thin Layer Chromatography (TLC) with 3 variations of eluent, and Fourier Transform Infrared (FT-IR).

3. Result and Discussion

Determination of isolation steps is taken from a journal by modifying the isolation steps from the journal. Modification is carried out on the use of solvents for liquid extraction and recrystallization processes. Steps in the reference [6] before being modified are in the form of maceration and liquid extraction, whereas in the step the modification results are added recrystallization. Excess modification steps with added recrystallization allow the crystals produced to be purer be-

cause the recrystallization process is carried out to free crystals from impurities.

The isolation step began with the sample preparation. The sample was made into a powder. The powder form has a wider surface so that the reaction can run faster [7]. The maseration was carried out using methanol. Used polar solvent makes the compound soluble in methanol. Evaporation used rotary evaporator to produce a dry methanol extract, and in liquid extraction with n-hexane. Non-polar solvents are used in extraction for polluting to dissolve on n-hexane. Extraction was performed twice, for the impurity to be maximally dissolved in n-hexane so that recrystallization takes place at a faster time without too much repetition. Then purified by recrystallization serves to remove impurities to produce pure crystals. Then the crystals are tested qualitatively by using TLC and FT-IR. The isolation procedure can be seen in **Figure 1**.

Thin Layer Chromatography (TLC) is conducted to determine the purity of the resulting compound [8]. If the color stain produced one then there is the possibility of the compound tested purely. To test and reassure that the compound is truly pure, the TLC is performed three times with three different eluents. Results of TLC with the following three eluents can be seen in **Figure 2**.

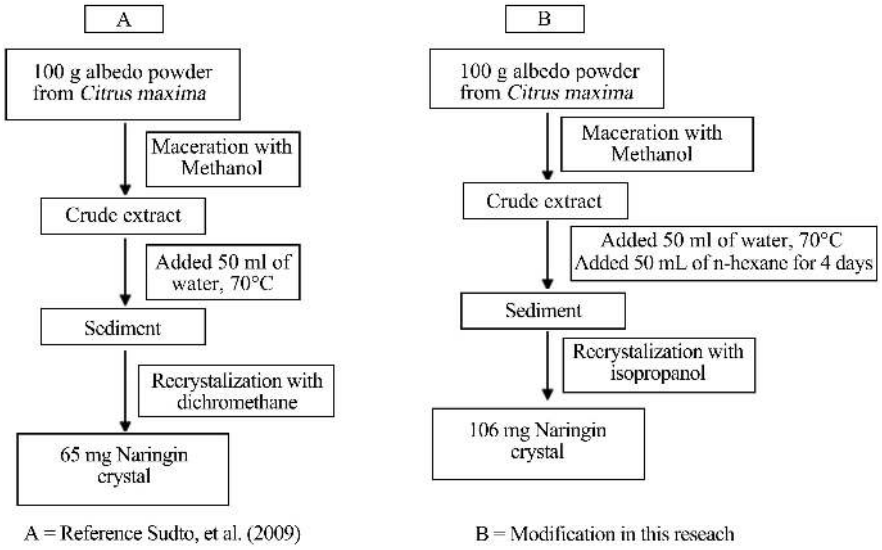

Figure 1. Isolation of naringin from *Citrus maxim*.

(a) (b) (c)

Figure 2. The result of TLC.

Based on purification test results using TLC obtained one color stain on the variation of eluent/solvent used. The color stain spacing corresponds to the nature of the solvent polarity and the resulting compound, where the naringin compounds tested for their purity are polar. In Figure 2(a) it can be seen that the result is a spot with a semipolar and polar eluent. Furthermore Figure 2(b) is added with a non-polar eluent, n-hexane showing a spot with a distance close to the starting line. Then the last one Figure 2(c) is added with the polar eluent *i.e.* isopropanol shows a spot of color near the finish line. Difference in stain spots on klt plate is influenced by eluent polarity. The more polar eluen the further the spot distance and the greater the Rf value. Infra red (IR) spectrum provided important information about the various functional groups possessed by the molecule can be seen in Figure 3.

IR spectrum showed absorption band at wave number region 3404.91 cm^{-1} for the shifting vibration of hydroxy group (OH). This was reinforced by the vibration of the recut at 1264.53 cm^{-1} for the –OH group. The absorption band in the wavelength region of 2933.92 cm^{-1} was a C-H group of CH_3. While the absorption band at the wave number 1519.95 cm^{-1} shows the C=C bond of the aromatic ring. The absorption band in the 1410.64 cm^{-1} wave region was a C-H bond in the CH_2, then the absorption band number in the wave number region 1371.36 cm^{-1} was the C-H bond of the CH_3. The absorption bands 1076.33 cm^{-1} and 1057.03 cm^{-1} are the C-O-C bonds of the ether. From these infrared spectral data it can be estimated that the tested compound contains a hydroxy group (–OH), aromatic cyclic groups (C=C), CH_2, CH_3, and ether groups. Structure of naringin can be seen in Figure 4.

According to the naringin structure can be matched with the result of IR spectrum analysis. Based on analysis of IR spectrum results showed that the tested compound contained a hydroxy group (–OH), aromatic cyclic groups (C=C), CH_2, CH_3, and ether groups. The functional groups are also present in the naringin structure, it is increasingly convinced that the isolated compound is the naringin compound.

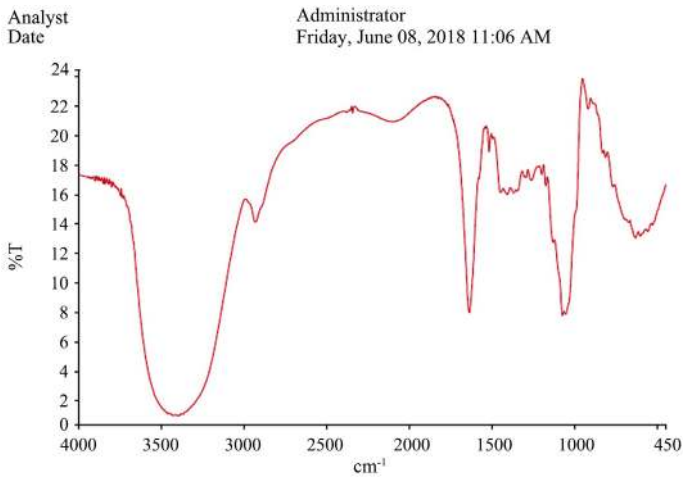

Figure 3. IR spectrum of naringin.

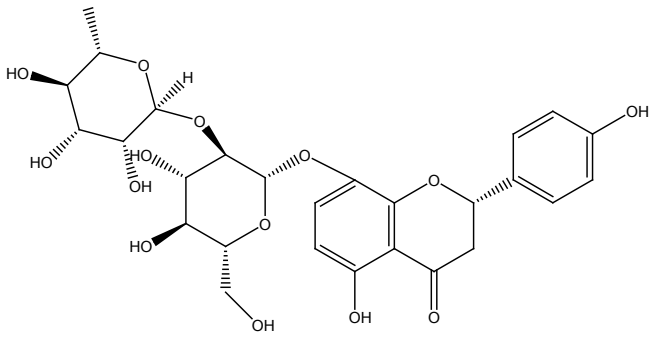

Figure 4. Structure of naringin.

4. Conclusion

The isolated compound exhibits a pure compound as it produces one color stain from the TLC using 3 eluent variations, and the isolated compound is naringin. The IR spectrum shows that the functional groups by the isolated compound are similar to the functional group naringin. Further research on identification of naringin compounds isolated by other spectrophotometer tests such as MS, NMR and UV-Vis is required.

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Conflicts of Interest

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

References

[1] Hakim, A., Liliasari, Kadarohman, A. and Syah, Y.M. (2016) Making a Natural Product Chemistry Course Meaningful with a Mini Project Laboratory. *Journal of Chemical Education*, **93**, 193-196. https://doi.org/10.1021/ed500930s

[2] Raharjo, T.J. (2013) Kimia Hasil Alam. Pustaka Pelajar, Yogyakarta.

[3] Hakim, A., Liliasari, Kadarohman, A. and Syah, Y.M. (2016) Effects of the Natural Product Mini Project Laboratory on the Students Conceptual Understanding. *Journal of Turkish Science Education*, **13**, 27-36.

[4] Hakim, A., Liliasari, Kadarohman, A. and Syah, Y.M. (2016) Improvement of Student Critical Thinking Skills with the Natural Product Mini Project Laboratory Learning. *Indonesian Journal of Chemistry*, **16**, 315-321. https://doi.org/10.22146/ijc.21149

[5] Hakim, A. and Jufri, A.W. (2017) Applications of Isolation and Structure Elucidation of Secondary Metabolites in Natural Product Chemistry Laboratory. In: *Advances in Chemistry Research*, NOVA Publisher, New York, 5.

[6] Sudto, K., Surachai, P. and Supason, W. (2009) An Efficient Method for the Large Scale Isolation of Naringin from Pomelo (*Citrus grandis*) Peel. *International Journal of Food Science and Technology*, **44**, 1737-1742.

https://doi.org/10.1111/j.1365-2621.2009.01989.x

[7] Hakim, A., Andayani, Y. and Rahayuan, B.D. (2018) Isolation of Ethyl P-Methoxy Cinnamate from *Kaemferia galangal* L. *IOP Conference Series*: *Journal of Physics*, **1095**, Article ID: 012039. https://doi.org/10.1088/1742-6596/1095/1/012039

[8] Hakim, A. and Jufri, A.W. (2018) Natural Products Laboratory Project: Isolation and Structure Elucidation of Piperin from *Piper nigrum* and Andrographolide from *Andrographis paniculata*. *Journal of Turkish Science Education*, **15**, 15-28.