Isolation of trymiristin from *Myristica fragrans* for natural product chemistry laboratory

A Hakim*, Jamaluddin, S W Al Idrus
University of Mataram, Jalan Majapahit 62, Mataram-Lombok 83125, Indonesia

*Corresponding author: Aliefmanhakim27@gmail.com

**Abstract.** This study aims generated natural products chemistry project laboratory for student in order to isolation trymiristin from *Myristica fragrans*. The skill of isolating trimyristin will be useful for students to obtain natural whitening raw materials derived from nutmeg. Various studies on the isolation can be used in natural products laboratory project. Students are directed to study literature related to trymiristin isolation procedures. Students practice skills of extraction, fractionation, purification, and structure elucidation of trymiristin. They were asked to design the laboratory activities. Manipulation of the liquor by soxhletation and recrystallization techniques proved to be a simple method to isolate trymiristin in low resource laboratory setting. The trymiristin was treated with structure elucidation NMR and infrared spectrometry. The laboratory method, comprising for 3 hours laboratory sessions, was a simple way for the students to understand the process of isolating trymiristin.

1. **Introduction**

Project based laboratories of active compounds can provide opportunities for students to learn secondary metabolite isolation procedures which include extraction, fractionation, and purification of secondary metabolite compounds. In addition, students will have experience in determining the structure of secondary metabolite compounds based on spectroscopic data. This laboratory can be useful for students from various disciplines such as chemistry, pharmacy, and medicine.

Natural chemistry learning demands high standards in laboratory activities [1-4]. One alternative to natural chemical laboratories is the isolation of active compounds [5]. The active compounds which are the study of the chemistry of natural substances can be found in higher plants [6-7]. Active compounds or secondary metabolites do not come from the main metabolic pathway, but these compounds have a role to protect plants from environmental disturbances [8]. Like several active compounds that have toxic properties that are useful for plants as a weapon against predators. There are many other interesting bioactivities of secondary metabolites which are useful in the health and medicinal fields such as antimicrobial [9], antimalarial [10], anti-viral [11], and cytotoxic [8] activities. Currently, it is noted that quite a lot of drugs used in the health sector are derived from active compounds [7]. Based on this fact, there have been many studies conducted by universities or the pharmaceutical industry that have studied secondary metabolites and their bioactive properties.

Various researches on active compounds have the potential to be integrated in learning the chemistry of natural products in the form of laboratory activities. This article will describe some of the project-based laboratory activities for isolating trimyristin from *Myristica fragrans* in Low Resource
Laboratory Setting. Traditionally *Myristica fragrans* (nutmeg) has been used as a medicine for diarrhea, mouth sores, and insomnia [12]. In addition, nutmeg is also used as a medicine for stomach pain, stimulant, inflammation, and controls flatulence [13]. Nutmeg oil products are used topically as analgesics, rheumatism and anti-inflammatory drugs. Chemically, the main components contained in nutmeg are fatty acid derivatives such as myristic acid, trimyristin, lauric acid, stearic and palmitate by 20-40%. Trimyristin, along with myristic acid, myristicin and elemicin have antioxidant, analgesic, anti-inflammatory, anti-diabetes, anti-bacterial and anti-fungal activities. Trimyristin can also be used as a precursor for the synthesis of its derivative compounds, namely myristic acid and myristyl alcohol for various industrial uses.

![Figure 1. Structure of Trimyristin](image)

2. Materials and Methods
The type of this research was a quasi-experimental. The number of participants in this study was 29 third-year students from the Study Program of Chemistry Education at one of the state universities in Mataram, Indonesia. Participants worked in eight groups (3-4 participants per group). All of the groups worked on the sample of *Myristica fragrans*.

The test instrument containing 10 questions was used to assess students' abilities in the isolation process. Content validity experts were used to determine the validity of the instrument. Data analysis was carried out by calculating the percentage of normalized gain score (%g) using the formula in equation 1 (Hake, 1998)

$$\%g = \frac{S_{post} - S_{pre}}{S_{max} - S_{pre}} \times 100\%$$

\(S_{post}\) and \(S_{pre}\) are the posttest and pretest scores, respectively, and \(S_{max}\) is the maximum possible score. The %g was characterized as high for %g > 70%, moderate for 30% ≤ %g ≤ 70%, and poor for %g < 30%.

Laboratory reports were used at the end of laboratory activities which were compiled in groups. Student surveys were used to show students' involvement with the experiment.

3. Results and Discussion
The laboratory activities, conducted over four 3-hour laboratory sessions, were useful for third year undergraduate students. The students gained first-hand experience of various techniques involved in the extraction, isolation and purification of trimyristin from *Myristica fragrans* seeds. In general, the isolation procedures used by successful students are as follows.

10 g of *Myristica fragrans* seeds (nutmeg) was crushed and put into a soxhlet tube. 40 mL of n-hexane was added. Soxlet was carried out for 30 minutes. Furthermore, the extract was concentrated by distillation method to form a sediment residue. The precipitate was separated with a Buechner funnel and washed with a cold acetone-methanol (1:1). In addition, allow the crystals to dry. IR and detailed NMR spectroscopic analyses confirmed that the substances isolated were trimyristin (Figure 1). The characteristic of the IR spectrum is that there is a specific strong absorption for the carbonyl group at the wave number 1729 cm⁻¹ and the absorption for the group (C-O-C) at the wave number 1093 cm⁻1.
In addition, for identification using NMR there is a typical signal for the three esters in the trimyristin structure at δC 173 ppm.

Seven groups succeeded in obtaining a single compound, based on the purity of the TLC chromatogram compared with standard trymyristin, from eight groups that followed this laboratory. In the structural elucidation stage, all groups were still given the IR and NMR spectrum which has been provided by the instructor to be interpreted by all groups, so each student gains spectrum reading experience. All groups were able to determine the functional groups in trymyristin compounds based on IR data. However, students still have difficulty interpreting the NMR spectrum. There was only one group that could be almost correct in the interpretation of NMR data. The other group still showed poor NMR interpretation ability. Quantitative analysis of the student's n-gain percentage value was 63.51% (medium category). The advantage of the natural product chemistry laboratory of isolation trym riskin from Myristica fragrans is the students get real experience as scientists, while the disadvantage is the high cost.

4. Conclusions
This experiment isolated trimyristin from Myristica fragrans over four 3 hours of laboratory sessions. Students work in small groups and practice skills of extraction, fractionation, purification, and structure elucidation of secondary metabolites. Various studies on the isolation of secondary metabolites can be used in laboratory projects. Isolated compounds can be replaced from another plant species. This laboratory project can increase student awareness of plants.

References
[1] Hakim A, Jufri A W, Jamaluddin, Supriadi, Mutmainnah P A 2020 Understanding the Uniqueness of Artocarpus Flavonoids: Isolation and Structure Elucidation of Cycloartocarpin from the Roots of Artocarpus altillis. J. Chem. Educ. 97 4133–4136 (2020).
[2] Hakim A, Jufri A W, Jamaluddin 2019 Innovative natural product chemistry laboratory: Isolation of artelastin from Artocarpus scortechinii AIP Conference Proceedings 2199, 050001
[3] Hakim A, Andayani Y, Rahayuan B D 2018 Isolation of Ethyl P-Methoxy Cinnamate from Kaemferia galanga L. Journal of Physics: Conference Series 1095(1) 012039
[4] Hakim A, Sahmadesti I, Hadisaputra S 2020 Promoting students’ argumentation skill through development science teaching materials based on guided inquiry models Journal of Physics: Conference Series 1521(4) 042117
[5] Hakim A & Jufri AW 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 (2018).
[6] Hakim A, Liliasari, Kadarohman A, 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 (2016).
[7] Hakim A, Jufri A W, Jamaluddin 2021 Isolation of artelasticine for student practice in low-resource laboratory settings. IOP Conference Series: Earth and Environmental Sciencethis link is disabled 712(1) 012050
[8] Blair L M, & Sperry J 2013 Natural Products Containing a Nitrogen–Nitrogen Bond. Journal of Natural Products, 76, 794-812 (2013).
[9] Gu J Q, Graf T N, Lee D, Chai H B, Mi Q, Kardono L B S, Setyowati F M, Ismail R, Riswan S, Farnsworth N R, Cordell G A, Pezzuto J M, Swanson S M, Kroll D J, Falkingham J O, Wall M E, Wani M C, Kinghorn A D, & Oberlies N H 2004 Cytotoxic and Antimicrobial Constituents of the Bark of Diospyros maritima Collected in Two Geographical Locations in Indonesia. Journal of Natural Products, 67, 1156-1161 (2004).
[10] Angawi R F, Calcinai B, Cerrano C, Dien H A, Fattorusso E, Scala F, & Scafati O T 2009 Dehydroconicasterol and Aurantoic Acid, a Chlorinated Polyyne Derivative, from the Indonesian Sponge. Theonella swinhoei. Journal of Natural Products, 72, 2195-2198 (2009).
[11] Kosela S, Hu L H, Rachmatia T, Hanafi M, & Sim K Y 2000 Dulxanthones F–H, Three New Pyranoxanthones from *Garcinia dulcis*. *Journal of Natural Products*, **63**, 406-407 (2000).
[12] Somani R S, Singhai, A K 2008 Hypoglycaemic and antidiabetic activities of seeds of *Myristica fragrans* in normoglycaemic and Alloxan-induced diabetic rats. *Asian J. Exp. Sci*. **22**, 95-10 (2008).
[13] Min B S, Cuong T D, Hung TM, Min B K, Shin B S, Woo M H 2011 Inhibitory Effect of Lignans from *Myristica fragrans* on LPS-induced NO Production in RAW264.7 Cells. Bull. *Korean Chem. Soc.* **32**, 4059 (2011).