JOURNAL OF CHEMICAL EDUCATION
Laboratory Experiment
pubs.acs.org/jchemeduc

Making a Natural Product Chemistry Course Meaningful with a Mini Project Laboratory

Aliefman Hakim,*[,†] Liliasari,[‡] Asep Kadarohman,[‡] and Yana Maolanas Syah[§]

[†]Study Program of Chemistry Education, University of Mataram, Jalan Majapahit 62, Mataram-Lombok 83125, Indonesia
[‡]Science Education Program, Graduate School, Indonesia University of Education, Jalan Setiabudi 229, Bandung 40173, Indonesia
[§]Organic Chemistry Division, Faculty of Mathematics and Natural Sciences, Institut Teknologi Bandung, Jalan Ganesha 10, Bandung 40132, Indonesia

Supporting Information

ABSTRACT: This paper discusses laboratory activities that can improve the meaningfulness of natural product chemistry course. These laboratory activities can be useful for students from many different disciplines including chemistry, pharmacy, and medicine. Students at the third-year undergraduate level of chemistry education undertake the project to isolate secondary metabolites from medicinal plants. This project provides opportunities for students to design their own activities to isolate secondary metabolites from medicinal plants. Students were exposed to skills as extraction, fractionation, purification, and structural elucidation of secondary metabolites. In this project, two secondary metabolites were successfully isolated from medicinal plants. The implementation of this project improved students' understanding of natural product chemistry.

KEYWORDS: *Upper-Division Undergraduate, Organic Chemistry, Problem Solving/Decision Making, Natural Products, Student-Centered Learning, Laboratory Instruction, Plant Chemistry, Separation Science, Thin Layer Chromatography, Spectroscopy*

■ INTRODUCTION

The purpose of this laboratory is first to provide the students with the experience to design laboratory activities in order to isolate secondary metabolites from medicinal plants. Second, the students learn the essential skills required to perform the extraction, fractionation, purification, and structural elucidation of secondary metabolites. This laboratory is useful for third or fourth year undergraduate students who have a basic understanding of the chromatographic and spectroscopic techniques used in the identification of natural compounds. Many activities in this laboratory may be appropriate for other courses such as structure elucidation of organic compounds course and organic chemistry course.

Secondary metabolites are isolated by the extraction, fractionation, purification, and structural elucidation of secondary metabolites.[1] Strategies to assess secondary metabolites from plants are described according to the following scheme (Figure 1).

Herbs are used by people as traditional medicines.[2] Examples of medicinal plants widely used in Indonesia are rhizome of *Curcuma xanthorrhiza*; rhizome of *Kaemferia pandurata*; rhizome of *Curcuma aeruginosa*; leaf of *Artocarpus altilis*, heartwood of *Hopea odorata*; and rind, pulp, and seed of *Cassia grandis*. Various properties of these plants relate to levels of the chemical content of the plants. Major compounds contained in *K. pandurata* consists of cardamonin (**1**), pinocembrin (**2**), pinostrobin (**3**), 4-hydroxipanduratin (**4**), and panduratin A (**5**), while the major compounds contained in *C. xanthorrhiza* consists of curcumin (**6**), demetoxicurcumin (**7**), and bisdemetoxicurcumin (**8**).[3,4]

In Figure 2 compounds **1**, **2**, **3**, **4**, **5** are flavonoid derivatives, and compounds **6**, **7**, and **8** are curcuminoid derivatives. *K. pandurata* and *C. xanthorrhiza* are widespread in tropical and subtropical regions such as Myanmar, Malaysia, Indonesia, China, India, Japan, Korea, United States, and some European countries.[5]

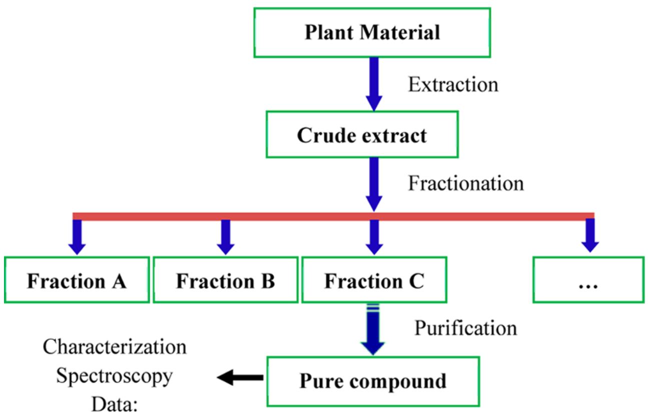

Figure 1. General stages of isolation of secondary metabolites.

■ METHODOLOGY

This study uses a quasi-experimental research with a one group pretest−posttest design. Participants in this study consisted of 31 third year students (teacher preparation) from the chemistry education department at one of the state universities in West Nusa Tenggara, Indonesia. Participants were divided into 8 groups (3−4 participant per group). Each group worked on one plant sample. Plant samples used in this study consisted of rhizome of *C. xanthorrhiza*; rhizome of *K. pandurata*; rhizome

DOI: 10.1021/ed500930s
J. Chem. Educ. XXXX, XXX, XXX−XXX

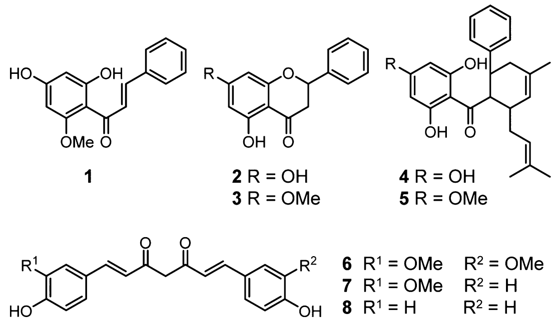

Figure 2. Major compounds contained in *K. pandurata* and *C. xanthorrhiza*.

of *C. aeruginosa*; leaf of *A. altilis*; heartwood of *H. odorata*; and rind, pulp, and seed of *C. grandis*. The plant samples were selected based on factor local herbs popularly and the existence of major compounds in the plant.

The goal of the project is to design laboratory activities in order to isolate secondary metabolites from medicinal plants. Implementation of laboratory activities took place during second semester of the 2012/2013 academic years. The laboratory sessions met 180 min per week for an entire semester (16 weeks). In this article, two secondary metabolites isolated by the groups investigating *K. pandurata* and *C. xanthorrhiza* are discussed. Six secondary metabolites isolated by other groups are not discussed because the structure of these compounds could not be identified.

This project was called the natural product mini project laboratory (NP-MPL), and it began with a problem to be solved by the students. The problem was entitled "how do you isolate one of the secondary metabolites from medicinal plant to be studied further?" The structure of this project can be seen in Table 1.

The instruments were used to assess between instruments and were precondition test containing 15 questions and NPC concept test containing 35 questions. A questionnaire was used to determine students' perception of the implemented laboratory activities. The validity of the instrument was determined by using content validity experts.

Analysis of qualitative data was done by interpretive descriptive analysis, and quantitative data analysis was done by calculating the percentage of normalized gain scores (%g) using the formula in eq 1[6]

$$\%g = \frac{S_{post} - S_{pre}}{S_{max} - S_{pre}} \times 100\% \quad (1)$$

where S_{post} and S_{pre} are the posttest and pretest scores, respectively, and S_{max} is the maximum possible score. Values of

Table 1. Structure of the Natural Product Mini Project Laboratory

Component	Description
Introduction	1. An explanation of the research and the schedule. 2. Precondition test performance (diagnostic tests to determine the basic understanding of the chromatographic and spectroscopic techniques. For example, students understand how to interpret NMR spectra). 3. Pretest performance (diagnostic test before the learning process that aims to determine which natural product chemistry (NPC) core material can be mastered by students. For example, students understand about the distribution of secondary metabolites on plants).
Laboratory activities training	1. Formation of groups based on the results of the pretest and precondition test (3–4 students per group). 2. Lecturer discussed concepts that had a high percentage of wrong answers on the preconditions test. Students practiced in groups to isolate a secondary metabolite from the rhizome of *Curcuma longa*. 3. Lecturer and assistants provided guidance to students about the procedures to isolate a secondary metabolite from the rhizome of *Curcuma longa*.
Orientation problem	1. Students were given the problem. 2. Each group worked on one plant sample. 3. Lecturer give explanation about mini project laboratory which will be carried out.
Designing laboratory activities	1. Students undertook a literature review of various sources and made project proposals. 2. Lecturer acted as facilitator and provided time to receive questions from students.
Presenting laboratory activities proposal	1. Students communicated proposals to the other groups. Communication was done through a presentation. 2. Students from other groups obtained information about procedures to isolate a secondary metabolite from investigated plant sample and give some questions or suggestions about the proposal for improvement. Lecturer acted as facilitator of the various problems that arose during class discussions.
Implementation of laboratory activities	1. Students implemented the proposal and collected data from sample preparation, extraction, fractionation, purification, structural elucidation of secondary metabolites. 2. Lecturer acted as facilitator in addition to guiding the investigation.
Results reporting and presentation	1. Students made a project report of their investigation. 2. Students communicated their project report to other groups. Communication was done through presentations. 3. Students from other groups obtained information and give some questions or suggestions about the project report for improvement. Lecturer acted as facilitator of the various problems that arose during class discussions.
Evaluation of the laboratory activities and analysis of the complex concepts	1. Students evaluated the laboratory activities that have been performed. Evaluation of the laboratory activities carried out by assessing their own procedures and giving the corrective solutions. 2. Students concluded the complex concepts from information that has been obtained during laboratory activities such as secondary metabolite nomenclature, common properties of secondary metabolites, characteristics of secondary metabolite structures, separation of chemical components, and identification of secondary metabolite structures. 3. Posttest

DOI: 10.1021/ed500930s
J. Chem. Educ. XXXX, XXX, XXX–XXX

%g were then characterized as high for %g > 70%, medium for 30% ≤ %g ≤ 70%, and poor for %g < 30%.

■ HAZARDS

This project uses some potentially carcinogenic substances and flammable solvents. The use of carcinogenic substances must be carried out in a fume hood. The use of flammable solvents should not utilize direct flame.

■ RESULTS

All groups implemented their own design laboratory activities, but only two groups succeeded in structural elucidation of their isolated secondary metabolites. Pinostrobin (**3**) was successfully isolated by the group investigating *K. pandurata*, and curcumin (**6**) was isolated by the group investigating *C. xanthorrhiza*. Detailed procedures developed by both groups can be seen in the Supporting Information.

Other groups failed to identify the structure of the isolated secondary metabolites due to the low purity of isolated compounds. Although the majority of the student groups failed to isolate a secondary metabolite that could be characterized, they still have gained experience in designing laboratory activities and conducting isolation of secondary metabolites.

Effect of the Natural Product Mini Project Laboratory on the Students

In Table 2, it can be seen that NPC conceptual understanding of students falls into the medium category and high category.

Table 2. Comparative Scores for Student Assessment on the Natural Product Chemistry Laboratory Concepts

Concepts	Pretest Mean Scores[a]	Posttest Mean Scores[a]	Normalized Gain, %
Secondary metabolites nomenclature	34	80	69.70
Common property of secondary metabolites	32	76	64.71
Characteristic of secondary metabolite structures	31	74	62.32
Separation of chemical components	27	69	57.53
Identification of secondary metabolite structures	32	73	60.29
Average	31	74	62.91

[a]N = 31.

The highest increase of students' mastery of NPC concept occurred in secondary metabolites nomenclature by 69.70% (medium category), and the lowest increase of students' mastery of NPC concept occurred in separation of chemical components by 57.53% (medium category). Acquisition of the average normalized gain (%g) is 62.91% (medium category). This suggests that this project improved students' understanding of NPC concept.

Letting the students design the project led to an increase in learning gains. This was seen in the student activities during research implementation. As an example case, the students determined the methods for purification which used high-performance liquid chromatography based on the literature, but in the implementation process, the result of fractionation showed a crystalin fraction that can be purified through the recrystallization. Therefore, the students tried recrystallization with some solvent in order to get a pure compound. This experience will increase students' understanding of purification methods.

Student responses to the application of this project were captured through a questionnaire summarized in Table 3. The students gave a positive response to NP-MPL. The majority of students contended that the project could answer their curiosity about isolated secondary metabolites and motivate them to read more learning resources related to secondary metabolites isolation in order to design a good project.

Table 3. Student Responses to Survey Items about Applied Aspects of the Natural Product Mini Project Laboratory

	Student Survey[a] Responses, %[b]		
Measured Aspects[a]	Very Positive	Positive	Negative
The ability to motivate learning	84	16	0
The ability to improve the mastery of NPC concepts	90	10	0
The ability to improve life skills	71	29	0

[a]The Supporting Information includes the survey instrument translated into English with data by item; data here are averages of responses to three or four related items. [b]N = 31.

This project stimulated students' critical thinking to determine the isolated secondary metabolites, and gave responsibility to students to work in groups and express their opinions in class discussions. It showed that NP-MPL could increase life skills for the students.

The NP-MPL has similarities and differences from other natural product laboratories published in *Journal of Chemical Education* (*JCE*).[7−14] Similarities can be found in the basic procedures for isolation of secondary metabolites consisting of extraction, fractionation, purification, and characterization of secondary metabolites, and the differences can be seen in the students' activities such as that in NP-MPL where the students were strongly directed to design their own procedures of secondary metabolites isolation. In the other natural product laboratories published in *JCE*, the instructor designed the procedures of secondary metabolites isolation.[7−14]

■ SUMMARY

This project has given the students experience to design their own laboratory activities and gain firsthand experience in isolating secondary metabolites from medicinal plants. Students gave a positive response to this project, and this project improved their understanding of NPC concept. These results showed that NP-MPL has made a NPC course meaningful. Additionally, this type of laboratory can be implemented using different plants.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/ed500930s.

- Student handouts (PDF, DOC)
- Instructor notes (PDF, DOC)
- Example questions test (PDF, DOC)
- Student responses to NP-MPL (PDF, DOC)
- Experimental section (PDF, DOC)

DOI: 10.1021/ed500930s
J. Chem. Educ. XXXX, XXX, XXX−XXX

AUTHOR INFORMATION

Corresponding Author

*E-mail: Aliefmanhakim27@gmail.com.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We wish to thank Suzany DE from Department of Chemistry, Institut Teknologi Bandung, Indonesia, for the measurement of UV and IR spectra of isolated compounds in this research.

REFERENCES

(1) Belofsky, G.; Aronica, M.; Foss, E.; Diamond, J.; Santana, F.; Darley, J.; Dowd, P. F.; Coleman, C. M.; Ferreira, D. Antimicrobial and Antiinsectan Phenolic Metabolites of *Dalea searlsiae*. *J. Nat. Prod.* **2014**, *77* (5), 1140−1149.

(2) Balganesh, T.; Kundu, T. K.; Chakraborty, T. K.; Roy, S. Drug Discovery Research in India: Current State and Future Prospects. *ACS Med. Chem. Lett.* **2014**, *5* (7), 724−726.

(3) Shindo, K.; Kato, M.; Kinoshita, A.; Kobayashi, A.; Koike, Y. Analysis of antioxidant activities contained in the *Boesenbergia pandurata* Schult. Rhizome. *Biosci., Biotechnol., Biochem.* **2006**, *70* (9), 2281−2284.

(4) Cahyono, B.; Huda, M. D. K.; Limantara, L. Pengaruh Proses Pengeringan Rimpang Temulawak (*Curcuma xanthorriza* Roxb.) terhadap Kandungan dan Komposisi Kurkuminoid. *Reaktor* **2011**, *13* (3), 165−171.

(5) Rukmana, H. R. *Temu-Temuan: Apotek Hidup di Pekarangan*; Penerbit Kanisius: Yogyakarta, Indonesia, 2004. http://www.kanisiusmedia.com/product/detail/025260/TEMU-TEMUAN-Apotek-Hidup-di-Pekarangan (accessed Oct 2015).

(6) Hake, R. Interactive engagement vs. traditional methods: A six-thousand-student survey of mechanics test data for introductory physics courses. *Am. J. Phys.* **1998**, *66* (1), 64−74.

(7) Griffin, R. W. Natural products. An independent study project. *J. Chem. Educ.* **1974**, *51* (9), 601−602.

(8) Cannon, J.; Li, D.; Wood, S. G.; Owen, N. L.; Gromova, A.; Lutsky, V. Investigation of secondary metabolites in plants - A general protocol for undergraduate research in natural products. *J. Chem. Educ.* **2001**, *78* (9), 1234−1237.

(9) Doyle, A. M.; Reilly, J.; Murphy, N.; Kavanagh, P. V.; O'Brien, J. E.; Walsh, M. S.; Walsh, J. J. Nature's Sedative: Isolation and Structural Elucidation of Valtrate from *Centranthus ruber*. *J. Chem. Educ.* **2004**, *81* (10), 1486−1487.

(10) Kavanagh, P. V.; Douglas, R. H.; Muldowney, C. A.; Mohamed, R.; Keohane, F.; Shanahan, C.; Walsh, J. J. Detection and Quantification of Valerenic Acid in Commercially Available Valerian Products. *J. Chem. Educ.* **2007**, *84* (5), 829−831.

(11) Halpin, C. M.; Reilly, C.; Walsh, J. J. Nature's Anti-Alzheimer's Drug: Isolation and Structure Elucidation of Galantamine from *Leucojum aestivum*. *J. Chem. Educ.* **2010**, *87* (11), 1242−1243.

(12) Walsh, E. L.; Ashe, S.; Walsh, J. J. Nature's Migraine Treatment: Isolation and Structure Elucidation of Parthenolide from *Tanacetum parthenium*. *J. Chem. Educ.* **2012**, *89* (1), 134−137.

(13) Nazri, M. M.; Samat, F. D.; Kavanagh, P. V.; Walsh, J. J. Nature's Cholesterol-Lowering Drug: Isolation and Structure Elucidation of Lovastatin from Red Yeast Rice-Containing Dietary Supplements. *J. Chem. Educ.* **2012**, *89* (1), 138−140.

(14) Carroll, A. M.; Kavanagh, D. J.; McGovern, F. P.; Reilly, J. W.; Walsh, J. J. Nature's Chiral Catalyst and Anti-Malarial Agent: Isolation and Structure Elucidation of Cinchonine and Quinine from *Cinchona calisaya*. *J. Chem. Educ.* **2012**, *89* (12), 1578−1581.

DOI: 10.1021/ed500930s
J. Chem. Educ. XXXX, XXX, XXX−XXX