

Natural Products Laboratory  
Project: Isolation and Structure  
Elucidation of Piperin from *Piper  
nigrum* and Andrographolide  
from *Andrographis paniculata*  
*by* Wahab Jufri

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## Natural Products Laboratory Project: Isolation and Structure Elucidation of Piperin from *Piper nigrum* and Andrographolide from *Andrographis paniculata*

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### ABSTRACT

This study aims to review the implementation of a project-based laboratory in a natural product chemistry course in order to isolate secondary metabolites from medicinal plants. Various studies on the isolation and structure elucidation of secondary metabolites can be used in natural products laboratory project. Students are directed to study literature related to secondary metabolites isolation and structure elucidation procedures. Furthermore, students practice skills of extraction, fractionation, purification, and structure elucidation of secondary metabolites. Once students have an understanding of secondary metabolites isolation and structure elucidation procedure, they were asked to design the isolation procedure of secondary metabolites. In addition, students implemented their own design to isolate secondary metabolites. This study used a quasi-experimental research. Data was collected done through observation and qualitatively analysis was conducted on the collected data. Participants in this study comprised 32 third-year students from the chemistry education department at one of the state universities in West Nusa Tenggara, Indonesia. Participants were divided into eight groups. Each group worked on one plant sample that comprised roots of *Artocarpus heterophyllus*, fruit of *Piper nigrum*, rhizome of *Curcuma xanthorrhiza*, rhizome of *Kaempferia pandurata*, rhizome of *Curcuma aeruginosa*, rhizome of *Kaempferia galanga*, fruit of *Cassia grandis*, and seeds of *Cassia grandis*. In this laboratory project, two groups succeeded in structural elucidation of their isolated secondary metabolites, piperin (1) from *Piper nigrum* and andrographolide (2) from *Andrographis paniculata*.

**Keywords:** Project-based laboratory, natural product chemistry, secondary metabolites.

### INTRODUCTION

Various studies have isolated secondary metabolites of biodiversity in the world (Hakim, et al., 1999; Thompson, et al., 2016). These studies could be useful in natural product laboratory project. The secondary metabolite isolation procedures of the various studies can be used as references in this laboratory project. The purpose of this laboratory activity is first to provide third-year students from the chemistry education department 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 from medicinal plant. Many of secondary metabolites that can be isolated from Indonesian biodiversity showed interesting biological activities such as cytotoxicity (Blair, et al., 2013; Kardono, et al., 1991; Syah, et al., 2004), antimalarial (Linn, et al., 2005; Elfahmi, et al., 2006; Angawi, et al., 2009; Zainuddin, et al., 2007), antiviral (Handayani, et al., 1997; Kosela, et al., 2000), antifungal (Nakazawa, et al., 2007; Yasman, et al., 2003, and antimicrobial (Gu, et al., 2004). Various bioactivities show potential lead compound as useful for industrial drug or pesticide industries.

Laboratory activities that give opportunities for students to isolate secondary metabolites from medicinal plants provide an opportunity for students to prove the concept that polar compounds will be soluble in polar solvents and nonpolar compounds will dissolve in nonpolar solvents, to invent the concept such as the distribution of secondary metabolites in each species of plant, or to connect new concepts with the knowledge that has been previously owned by learners through scientific measures to rationalize various phenomena such as the properties of plants that can be used by humans for treatment. Students who understand the concept properly will be able to generalize their knowledge better than learners who just memorize definitions. Generalized capability can be used as a basis for generating original ideas (Sevim, 2013; Özdemir, 2015; Bezen, et al., 2016). These activities enhance the students' understanding of the concepts learned in class. The concepts of separation of chemical components and identification of secondary metabolite structures were difficult to learn without engaging in laboratory activities (Hakim, et al., 2016<sup>a</sup>; Hakim, et al., 2016<sup>b</sup>; Hakim, et al., 2016<sup>c</sup>; Hakim & Jufri, 2017)

#### Reviews of Natural Product Laboratory

Much research has been conducted to improve the quality of learning of chemistry through laboratory activities (Herrington, et al., 2016; Çelik, et al., 2015; Çibik & Yalçın, 2013, Yiğit & Bilgin, 2013). Griffin (1974), in his article "Natural Products. An Independent Study Project," described natural product independent study project suitable for students in detail. This article discussed students' activities that consist of field collection and plant identification, laboratory screening procedures and isolation of large quantities, library work, and presentation of results as oral and written reports. Cannon, et al. (2001), in their study "Investigation of secondary metabolites in plants – A general protocol for undergraduate research in natural products" outlined typical experimental procedures that can be used to extract and isolate chemical constituents from a plant, suggested some simple procedures to test for selected bioactivity and explained how the molecular structures of the natural products may be determined using spectroscopic techniques. Doyle, et al. (2004) in his article entitled "Nature's Sedative: Isolation and Structural Elucidation of Valtrate from *Centranthus ruber*" exposed variety of chromatographic techniques employed in the isolation of Valtrate from *Centranthus ruber* for the college students of chemistry laboratory. Douglas, et al. (2007), in their research "Detection and Quantification of Valerenic Acid in Commercially Available Valerian Products," described that several valerian-containing products sold in pharmacies were evaluated to verify the content of *Valeriana officinalis* by identifying the presence of valerenic acid. Halpin, et al. (2010) allowed students in his article "Nature's Anti-Alzheimer's Drug: Isolation and Structure Elucidation of Galantamine from *Leucojum aestivum*" to follow the isolation procedure of galantamine from *L. aestivum* for students accessing chemistry laboratory.

Walsh, et al. (2012) in his research "Nature's Migraine Treatment: Isolation and Structure Elucidation of Parthenolide from *Tanacetum parthenium*" afforded the student opportunity to follow the entire procedure of parthenolide isolation from *T. parthenium*. Nazri, et al. (2012) reported his study "Nature's Cholesterol-Lowering Drug: Isolation and Structure Elucidation of Lovastatin from Red Yeast Rice-Containing Dietary Supplements" that explained the procedure for isolation and structure elucidation of lovastatin from red

yeast rice. Carroll, et al. (2012), in his article “Nature’s Chiral Catalyst and Anti-Malarial Agent: Isolation and Structure Elucidation of Cinchonine and Quinine from *Cinchona calisaya*,” explained various techniques in the extraction, isolation, and purification of medicinally important natural products from the crude plant material. Hakim, et al. (2016), in his article “Making a Natural Product Chemistry Course Meaningful with a Mini Project Laboratory,” afforded the student to the project isolation secondary metabolites from medicinal plant.

## METHODS

This study used a quasi-experimental research. Participants in this study consisted of 32 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 eight groups (4 participant per group). The division of students into groups was done considering the heterogeneity of the results of the precondition test. Each group worked on one plant sample. Students follow the steps initiated in project-based laboratory that begins with a problem to be solved by the students (Hakim, et al., 2016<sup>a</sup>), the problem being “How can I isolate secondary metabolite from medicinal plant?” Plant samples used in experiment class consisted of roots of *Artocarpus heterophyllus*, fruit of *Piper nigrum*, rhizome of *Curcuma xanthorrhiza*, rhizome of *Kaempferia pandurata*, rhizome of *Curcuma aeruginosa*, rhizome of *Kaempferia galanga*, fruit of *Cassia grandis*, and seeds of *Cassia grandis*. The plant samples were selected based on factor local herbs popularly and the existence of major compounds in the plant. The structure of project-based laboratory project can be seen in Table I (Hakim, et al., 2016<sup>a</sup>).

**Table I. Structure of Project-Based Laboratory**

Component	Description
Introduction	<ol style="list-style-type: none"><li>1. An explanation of the research and the schedule</li><li>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).</li></ol>
Laboratory activities training	<ol style="list-style-type: none"><li>1. Formation of groups based on the results of the precondition test (4 students per group).</li><li>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 <i>Curcuma longa</i>.</li><li>3. Lecturer and assistants provided guidance to students about the procedures to isolate a secondary metabolite from the rhizome of <i>Curcuma longa</i>.</li></ol>
Orientation problem	<ol style="list-style-type: none"><li>1. Students were given the problem.</li><li>2. Each group worked on one plant sample.</li><li>3. Lecturers explained about mini project laboratory that had to be carried out.</li></ol>
Designing laboratory activities	<ol style="list-style-type: none"><li>1. Students undertook a literature review of various sources and made project proposals.</li><li>2. Lecturers acted as facilitators and provided time to receive questions from students.</li></ol>
Presenting laboratory	<ol style="list-style-type: none"><li>1. Students communicated proposals to the other groups. Communication was done through a presentation.</li></ol>

activities proposal	2. Students from other groups obtained information about procedures to isolate a secondary metabolite from the investigated plant sample and posed some questions or gave suggestions about the proposal for improvement. Lecturers acted as facilitators of the various problems that arose during class discussions.
<sup>1</sup> Implementation of laboratory activities	1. Students implemented the proposal and collected data from sample preparation, extraction, fractionation, purification and structural elucidation of secondary metabolites. 2. Lecturer acted as facilitator in addition to guiding the investigation.
<sup>1</sup> Results reporting & 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 posed some questions or gave suggestions about the project report for improvement. Lecturers acted as facilitators of the various problems that arose during class discussions.
Evaluation of the laboratory activities	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.

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 the second semester of the academic year 2015–2016. The laboratory sessions were held for 180 min every week for an entire semester (16 weeks). In the experiment class, two secondary metabolites, piperin and andrographolide, isolated by the groups investigating *Piper nigrum* and *Andrographis paniculata* are discussed. Six secondary metabolites isolated by other groups are not discussed because the structure of these compounds could not be identified.

## <sup>2</sup> RESULTS and DISCUSSION

All groups have implemented their own design of laboratory activities but only two groups succeeded in structural elucidation of their isolated secondary metabolites. The difference between successful and failed groups' laboratory designs was the complexity of its isolation procedures. In general, the design of secondary metabolite isolation procedures of the failed groups is more complex than the successful groups. The differences were also seen in the characteristics of the group in that successful group has very active characteristics, while the failed group has less active characteristics.

This laboratory project trained the students who have not experienced isolation of secondary metabolites. So, we choose herbs for which the active constituent is already known. It also confirmed that the students' failures were not because of the herbs used in the research. The fact that the active constituent was already known does not necessarily make the exercise of finding the isolation procedure easy for the student. It happened because the students were asked to develop a procedure that has been designed in accordance with the results of each stage of isolation process. This laboratory is useful for third-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.

Piperin (<sup>1</sup>) was successfully isolated by the group investigating *Piper nigrum*, and andrographolide (<sup>2</sup>) was isolated by the group investigating *Andrographis paniculata*. Structure of piperin (<sup>1</sup>) and andrographolide (<sup>2</sup>) can be seen in Figure 1. Other groups failed to identify

The structure of the isolated secondary metabolites due to less pure 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.

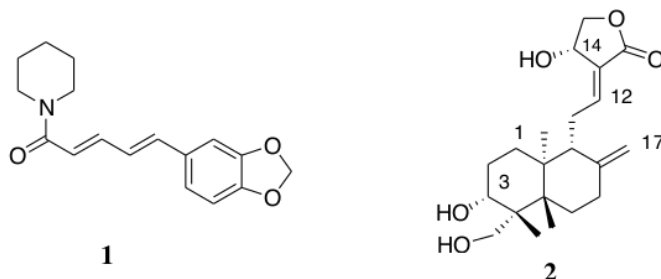
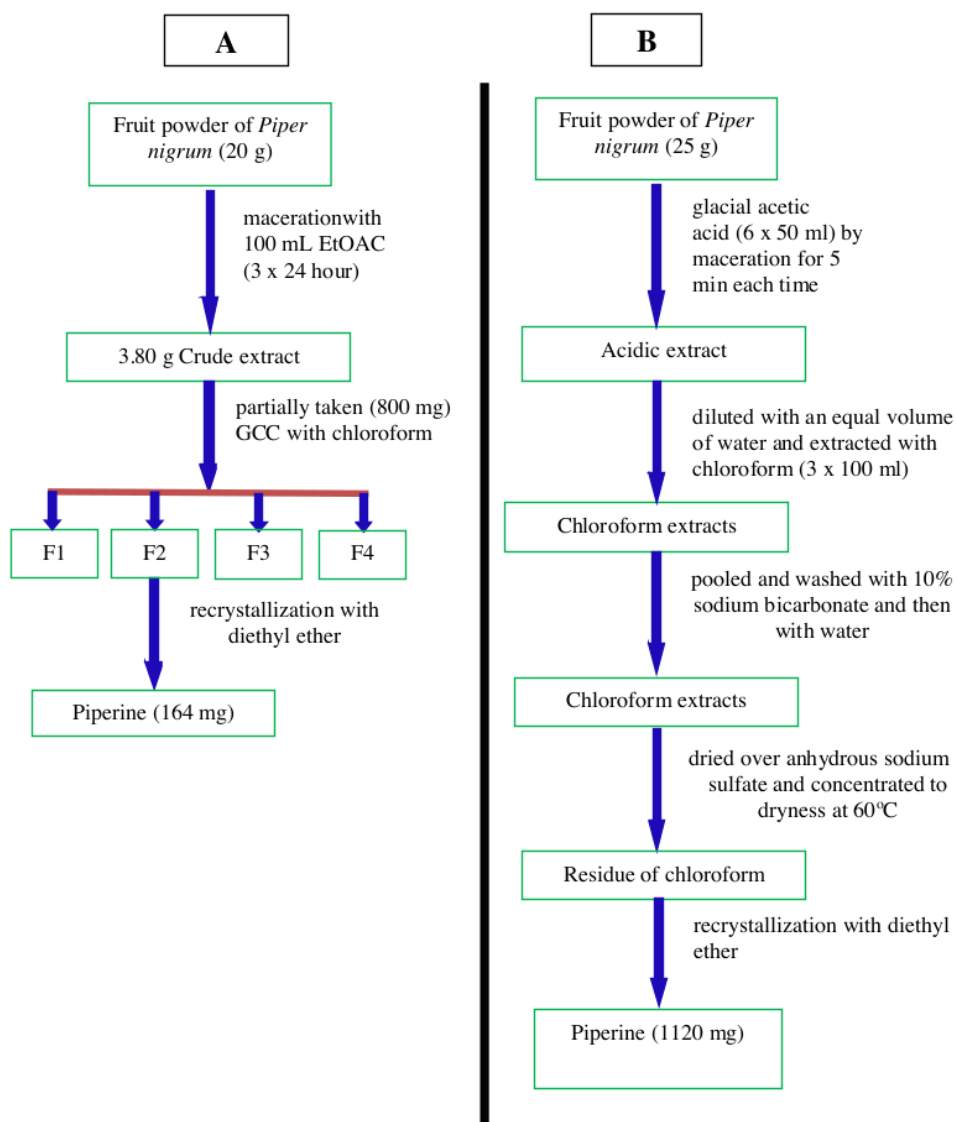


Figure 1. Structure of piperin (1) and andrographolide (2)

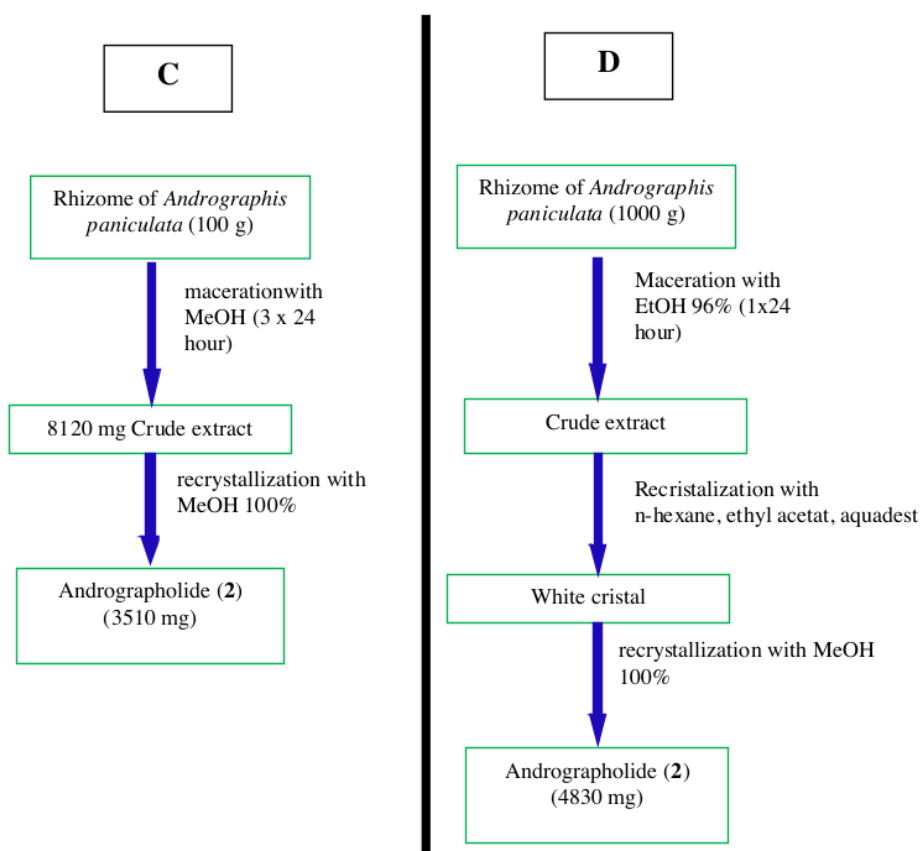
The students' group investigating *Piper nigrum* extracted fruit powder of *Piper nigrum* (20 g) with 100 mL ethyl acetat during 3 x 24 hours, and 3.8 g of crude extract was produced from these extraction processes. Furthermore, students partially took 800 mg of crude extract of *Piper nigrum* for Gravity Column Chromatografi with chloroform as eluent. Four main fractions resulted from this fractionation. Fraction 2 was purified with recrystallization using diethyl ether as solvent. Piperine (164 mg) was successfully isolated by students. These procedures were different from those in the literature (Kanaki, et al., 2008), as shown in Figure 2. It happened because the students were asked to develop a procedure that has been designed in accordance with the results of each stage of the isolation process.



A = Students' group investigating fruit of *Piper nigrum*, B = Literature (Kanaki, et al., 2008)

Figure 2. Procedures to isolate piperin from Fruit powder of *Piper nigrum*

Similarly, the students' group investigating *Andrographis paniculata* also produces a different procedure than there is in the literature (Warditiani, et al., 2012), as shown in Figure 3. Rhizome of *Andrographis paniculata* (100 g) was macerated with MeOH during 3 x 24 hours to produce 8.12 g crude extract. Furthermore, the students used MeOH for recrystallization process. Andrographolide (**2**) (3510 mg) was successfully isolated by students.



A = Students' group investigating rhizome of *Andrographis paniculata*, B = Literature (Warditiani, et al., 2012)

Figure 3. Procedures to isolate Andrographolide from rhizome of *Andrographis paniculata*

### SUMMARY

This laboratory project has given the students a chance to experience being a scientist as well as an opportunity to solve project problems using scientific measures. This laboratory can help students with active characteristics to be successful, but it has also hindered students with passive characteristics from being successful. The students have designed laboratory activities and gained firsthand experience of isolating secondary metabolites from medicinal plants. Two of the eight groups succeeded in getting the pure compound. Both groups produced different procedures from literatures. It indicates that these laboratory activities enabled students to develop original ideas.

### SUPPLEMENTAL MATERIAL

UV, IR, and NMR spectra of piperin (1) and andrographolide (2) are available in appendix.



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Alefman-mumi2\_1H

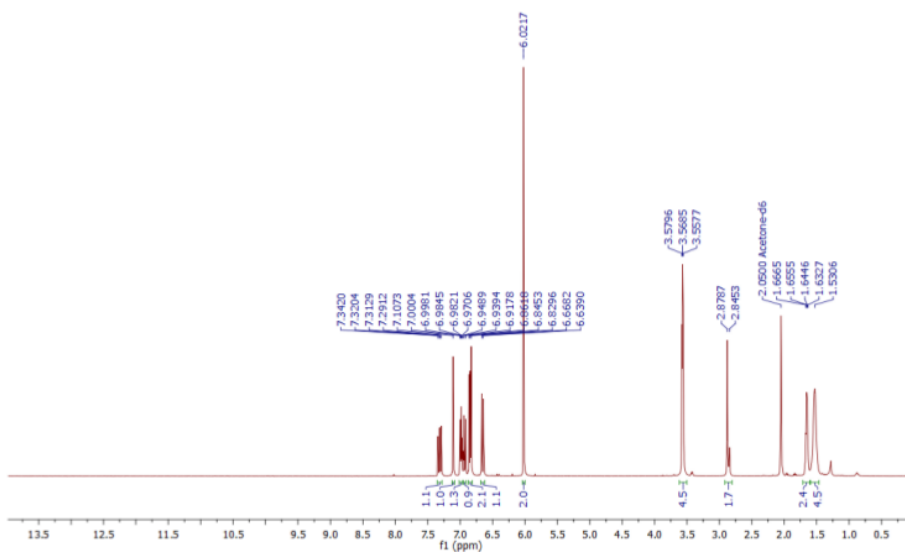


Figure 4. <sup>1</sup>H-NMR spectra of piperin

Alefman-mumi2\_13C

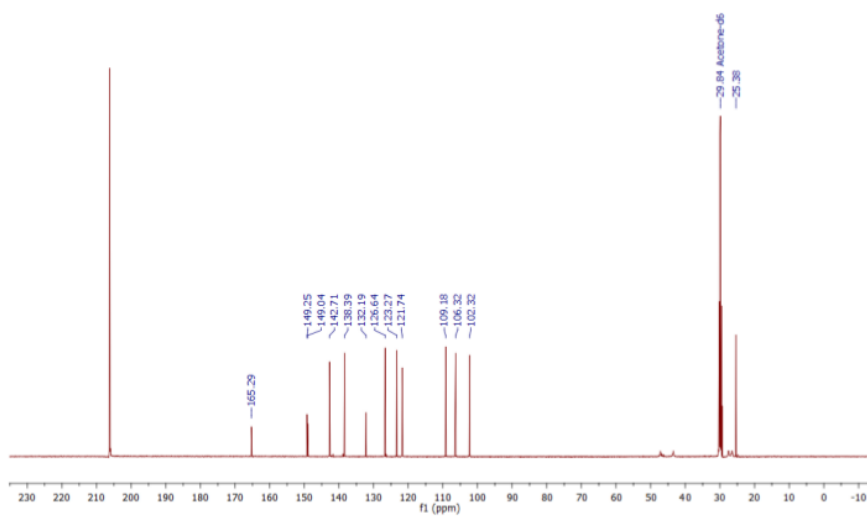
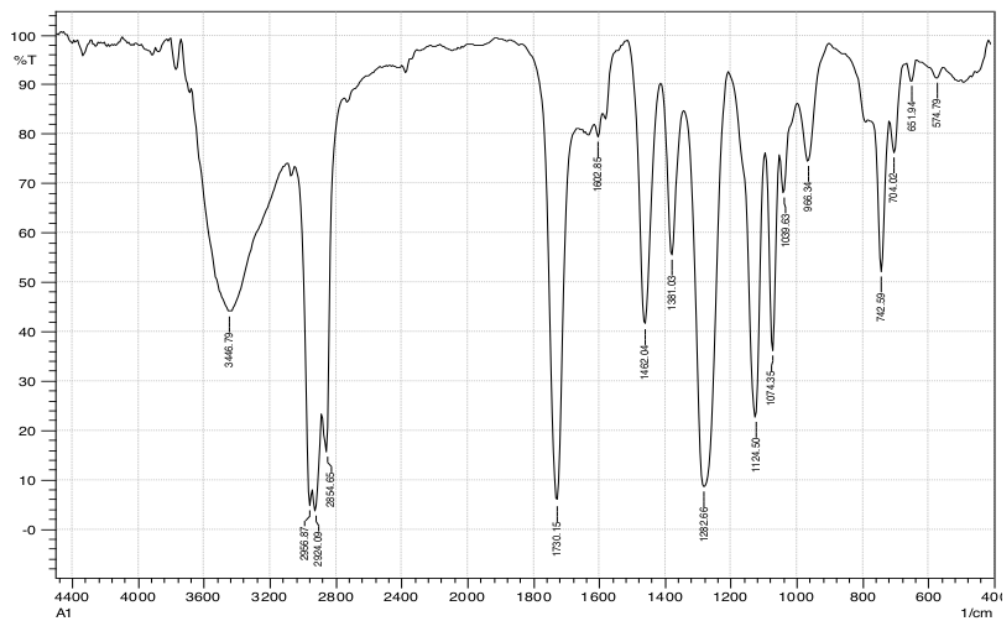
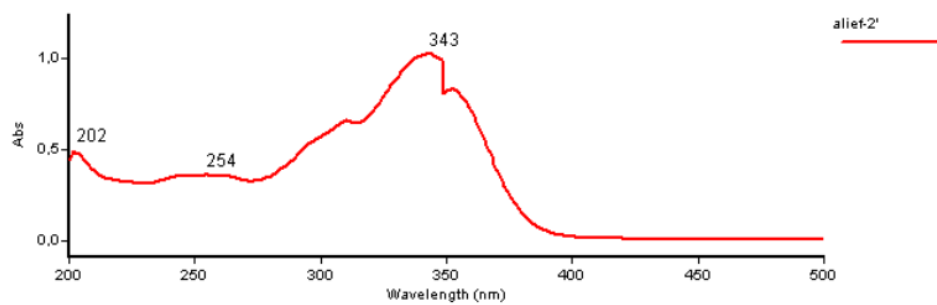


Figure 5. <sup>13</sup>C-NMR spectra of piperin

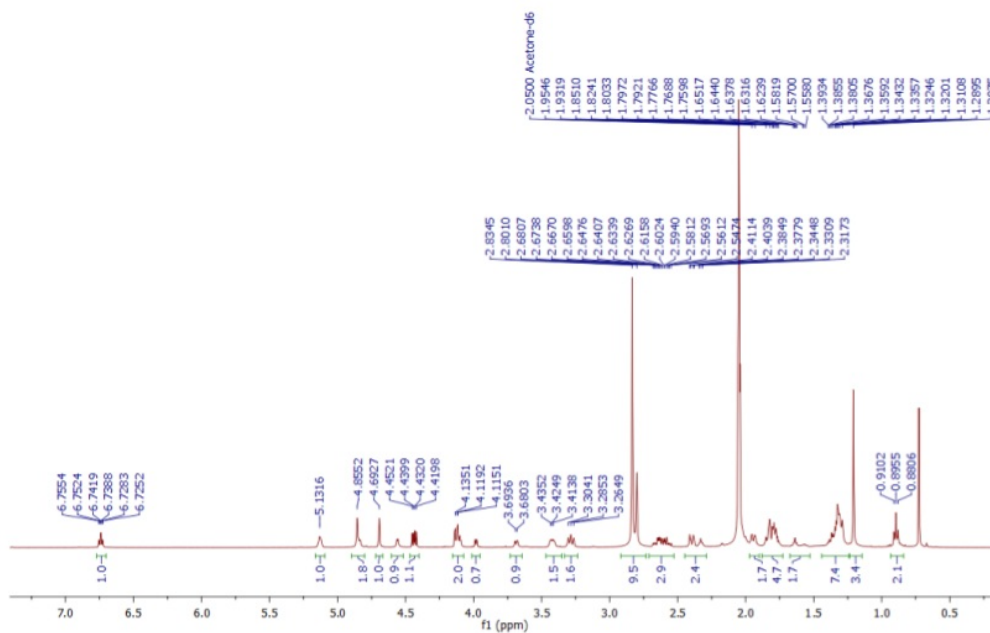


**Figure 6. IR spectra of piperin**

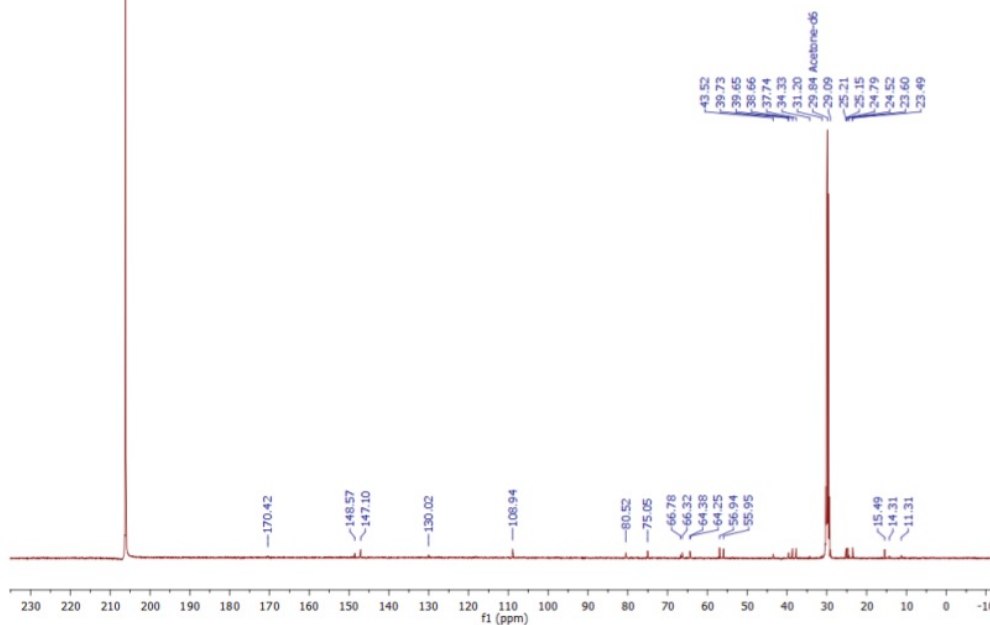


**Figure 7. IR spectra of piperin**

Alefman-mumi3\_1H

Figure 8. <sup>1</sup>H-NMR spectra of andrographolide

Alefman-mumi3\_13C

Figure 7. <sup>13</sup>C-NMR spectra of andrographolide

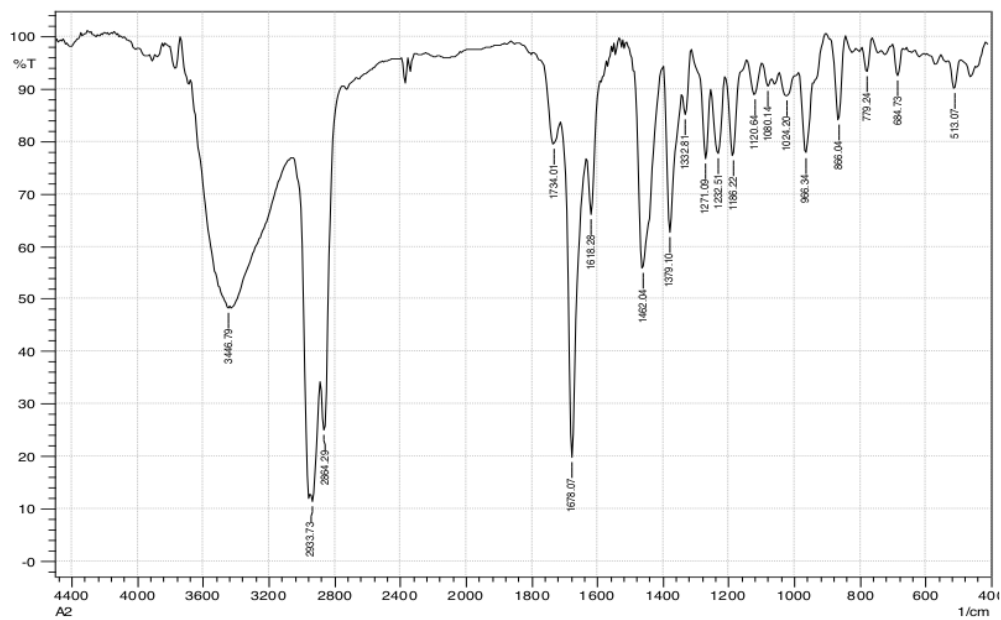


Figure 9. IR spectra of andrographolide

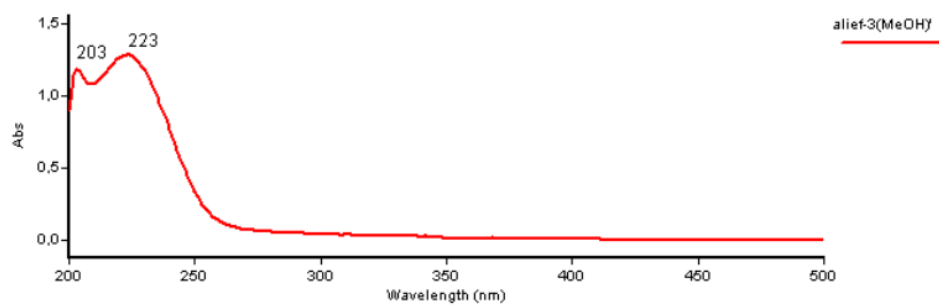


Figure 10. UV spectra of andrographolide

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