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IDENTIFICATION OF PYRIDINE COMPOUNDS IN GOLOBE HALMAHERA FRUIT (*Hornstedtia alliacea*) USING CHROMATOGRAPHY-MASS SPECTROMETRY (GC-MS) GAS METHOD

Adlien Rafenska Malige*, Arend L. Mapanawang, Silvester Wungow

¹Program Studi Farmasi, Sekolah Tinggi Ilmu Kesehatan Makariwo Halmahera (STIKMAH) - Tobelo

²Yayasan Medika Mandiri Halmahera - Tobelo

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***Correspondence to Author:
Adlien Rafenska Malige**

ABSTRACT

Background: One of the plant families that have been widely used as medicinal ingredients is Zingiberaceae or better known as the rhizome family. Zingiberaceae consists of approximately 52 genera and 1300 species of plant species. *Hornstedtia alliacea* with the local name Goloba, ethnic Makian-Kayoa call it Bahim. The people of the North Maluku islands, especially Maba, Tidore, Makian, and Bacan as a medicine for malaria, fever, and heartburn.

Research Objectives: Pyridine compound in the ethanol extract of Halmahera golobe fruit (*Hornstedtia alliacea*) using the Gas Chromatography-Mass Spectroscopy (GC-MS) method. **Type of Research:** This type of research is experimental research with a laboratory scale. Based on the results of GC-MS analysis that has been carried out, it can be concluded that the ethanol extract of Halmahera golobe fruit (*Hornstedtia alliacea*) contains pyridine compounds in the percent area, namely 5.88% at the peak absorption with a retention time of 37.14 minutes.

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INTRODUCTION

Indonesia is an archipelagic country located in the equatorial zone and is known for its diversity of plant species, but not all of this potential can be utilized as industrial materials, especially medicinal plants.⁴ Indonesia's biological resources are ranked third in the world after Brazil and Zaire so that they are grouped in mega biodiversity country. With the existing potential, Indonesia has a great opportunity to develop all of its natural wealth, but until now the utilization has not been maximized.¹

One plant family that has been widely used as a medicinal ingredient is Zingiberaceae or better known as the rhizome family. Zingiberaceae consists of approximately 52 genera and 1300 species of plant species. Plants from the Zingiberaceae family generally contain essential oils. Several phytochemical tests of this plant have been reported to have many interesting biological activities, including reports related to Zingiberaceae extract, namely antimicrobial, antioxidant, anticancer and stimulant effects on the immune system.²

Hornstedtia is a genus of the Zingiberaceae family (Ginger). The rhizomes of Zingiberaceae are strong aromatic and generally contain essential oils which have been reported to have antioxidant, anti-inflammatory and antimicrobial properties. Zingiberaceae can also be used in chronic diseases, such as osteoarthritis, rheumatoid arthritis and depression.

Hornstedtia alliacea with the local name Goloba, ethnic Makian-Kayoa call it Bahim. The people of the North Maluku islands, especially Maba, Tidore, Makian, and Bacan as a medicine for malaria, fever, and heartburn. Small children in the village usually take it to chew because it tastes sweet when the fruit is ripe. The shape of the ripe seeds is black while the unripe seeds are yellow. This plant from the Zingiberaceae family has a

habitat in tropical rain forests. In addition to utilizing its fruit, the community also takes its stems to be used as fuel for cooking meat. According to the ethnicity of Maba, the stem of the goloba plant can give a distinctive aroma to fish and deer meat.⁵

Golobe Halmahera (Zingiberaceae), is a plant that grows in tropical areas including Halmahera. Utilization of Golobe (Zingiberaceae) by the people of Halmahera as energy supply for hunting in the forest, treating wounds and infections, and herbal medicine for peptic diseases, the fruit and seeds are consumed by all ages, children and adults continuously for more than 4 generations in Halmahera.¹

Based on the above background, the researchers are interested in conducting research on the identification of pyridine compounds in Golobe Halmahera (*Hornstedtia alliacea*) using the Gas Chromatography-Mass Spectroscopy (GC-MS) method.

RESEARCH METHOD

Types of research

This type of research is experimental research with a laboratory scale.

Population and Sample

Population

The population is the whole object of the research. The population in this study is the fruit of the golobe Halmahera (*Hornstedtia alliacea*) taken from the village of Kacak, Kec. North Tobelo, Kab. North Halmahera

Samples

The sample used in the study was the simplicia powder of golobe Halmahera (*Hornstedtia alliacea*) fruit which was taken randomly and homogeneously as much as 400 g

Work Procedures

1) Sample Preparation

± 5000 g of fresh samples of globe Halmahera (*Hornstedtia alliacea*) were taken, then washed with running water until they were clean of dirt or other foreign materials. The peel of the sample that has been clean is peeled off. After that, it is dried in direct sun for 3-4 days to dry, then in the oven for 3 hours at 50 ° C. The dried globe fruit samples were sorted dry from the remaining impurities. The sorted simplicia is then mashed by means of a coarse ground until a small powder is obtained. After that, sieving with an 80 mesh sieve is intended for simplicia size uniformity so as to facilitate the extraction process.

2) Making Ethanol Extract of Golobe Halmahera Fruit

Golobe fruit extract was made by maceration. A total of 400 grams of Halmahera golobe fruit powder is put into a maceration vessel, then soaked in ethanol with a ratio of 1: 2 (w / v) covered with aluminum foil and left for 5 × 24 hours at room temperature and protected from direct sunlight and the occasional sauce. Stirring is carried out, the samper is filtered using filter paper to produce macerate I and residue I. After that, the residue I is then added with ethanol with a ratio of 1: 2 (w / v), covered with aluminum foil and left for 3 × 24 hours, the sample filtered using filter paper to produce macerate II and residue II. Maserate I and II are mixed together then filtered, then evaporated using a rotary evaporator until dry and then weighed using an analytical balance.

The extract yield was calculated by dividing the extract weight by the dry sample weight. The concentrated extract was weighed and the yield of the sample was obtained. Extract yield expressed in percent and calculated using the equation:

$$\text{Rendement (\%)} = \frac{\text{Extract weight obtained (grams)}}{\text{the extracted simplicia weight}} \times 100\%$$

3) Identification of Pyridine Compounds

a. Fractionation

The crude extract of Halmahera golobe fruit obtained was put into a separating funnel, then dissolved with 70% ethanol and added with 1: 1 (v / v) n-hexane solvent and then shaken in a separating funnel until homogeneous. Left to form an ethanol layer and an n-hexane layer. Each layer is accommodated in a different container. The ethanol and n-hexane layers are then evaporated using a rotary evaporator to dry, then weighed and the results are called ethanol and n-hexane fraction.

b. GC-MS (Gas Chromatography Mass Spectrophotometry)

The structure of the compounds from the fraction was determined by using a GS-MS spectrometer. The analysis was performed using GC-MS Agilent 6890 N 2006 with 5 MS DB column dimensions of 30m x 250µm x 0.25µm. System conditions are oven temperature 310°C, detector 310°C, injector 305°C, program temperature (initial 50°C, increase of 3.50°C per minute to maximum temperature 310oC), analysis time 100 minutes, column pressure 14.12 psi with a flow rate of 1.7 mL per minute and injection volume of 2 µL. Analysis using the Gas Chromatography-Mass Spectrophotometry (GC-MS) method is the key in the analysis of unknown plant chemical components. GC-MS analysis has been widely used to identify hundreds of compound components contained in plant cells that cannot be done by ordinary phytochemical screening. This is because phytochemical screening is limited to the identification of groups of compounds

RESULTS

A. Sample Preparation

The collection of raw materials for simplicia was carried out in Kursan Village, West Tobelo District, North Halmahera Regency. The raw material for Halmahera (*Hornstedtia alliacea*) golobe fruit is wet sort. The purpose of doing this wet sorting is to separate the foreign organic material that is carried away during the harvesting process such as sand, soil, stone, sand which can interfere with the next process. Even though it's called wet sorting, this process doesn't use water for the process. Furthermore, washing is carried out using running water so that the water that cleans the plants to be made for simplicia is always new. The purpose of washing is to better clean the remnants of foreign organic matter that are still attached to the wet sorting process. After that, the fruit is dried by drying it in direct sunlight. Furthermore, it is milled into a powder to make it easier during the maceration process. Powdered simplicia is sieved and packed properly in an airtight container made of glass.

B. Organoleptic Examination

Organoleptic examination of Halmahera golobe fruit simplicia (*Hornstedtia alliacea*) as presented in the following table 1:

| No. | Halmahera Golobe Fruit Simplicia | Information |
|-----|----------------------------------|--------------------|
| 1. | Shape | Oval |
| 2. | Color | Fruit skin: Red |
| 3. | Smell | Flesh: Black |
| 4. | Taste | Typical |

Source: Primary Data, 2020

C. Extraction and Extract Yield

The crude extraction method for Halmahera golobe fruit (*Hornstedtia alliacea*) uses maceration. A total of 400 grams of Halmahera golobe fruit simplicia powder was macerated with 800 mL of 70% ethanol in a ratio (1: 2). The extraction process lasts for 5 days at room

temperature and is protected from direct sunlight while stirring occasionally. After 5 days, the macerate I was filtered and the residue was squeezed out. Then the residue was remacerated with 400 ml of 70% ethanol for 3 days, after which, macerate II was filtered. Maserat I and II were combined then evaporated the solvent at room temperature to produce a thick ethanol extract of the Halmahera golobe fruit.

The results of visual observation of Halmahera golobe fruit ethanol extract in the form of blackish green maserate are then evaporated by aerating until a thick black ethanol extract is obtained with an extract weight of 27 grams with an extract yield of 6.75% as presented in the following table 2:

| Sample | Simplicia Sample (gram) | Extract (gram) | Renderment (%) |
|--------------|-------------------------|----------------|----------------|
| Golobe fruit | 400 | 27 | 6,75 |

Source: Primary Data, 2020

D. Identification of the Halmahera Golobe Fruit Pyridine Compound

The following is the identification result of the chemical compound identification of the 70% ethanol extract of Halmahera golobe fruit (*Hornstedtia alliacea*) as in the table 3 :

Table 3. Identification Results of Golobe Halmahera Fruit Pyridine Compounds

| Type / Sample Code | RT | Q | Name of Compound | Content (%) |
|---------------------|--------|-------------|------------------------------------|-------------|
| Golobe fruit | 12.08 | 76 | 2,3-Dihydro-3,5-Dihydroxy-6-Methyl | 5,31 |
| | 22.41 | 99 | Beta-Caryophyllene | 4,74 |
| | 23.95 | 98 | Alpha-Humulene | 12,46 |
| | 34.28 | 93 | Hexadecanoic acid, ethyl ester | 1,22 |
| | 34.50 | 98 | Palmitic acid | 7,29 |
| | 34.61 | 56 | 2-Formyl-5-isopropyl-8-Methylspiro | 2,22 |
| | 35.52 | 9 | 3'H-Cycloprop (1,2)-5-cholest-1-en | 1,40 |
| | 35.77. | 95 | 9,17-Octadecadienal | 14,19 |
| | 36.37 | 99 | Nonacosane | 2,81 |
| | 37.14 | 50 | 2,6-Diethylpyridine | 5,88 |
| | 37.44 | 74 | C6-D-Indolinocodeine | 2,94 |
| | 37.79 | 98 | Docosane | 6,13 |
| | 39.42 | 97 | 11-Tricosene | 1,38 |
| | 39.57 | 99 | Heptacosane | 1,96 |
| | 41.24 | 93 | Trans-Caryophyllene | 1,91 |
| | 41.81 | 91 | Cyclopentane | 1,57 |
| 41.98 | 64 | Cyclohexane | 3,98 | |

Source: Primary Data, 2020

DISCUSSION

Based on the results of laboratory examinations of Halmahera golobe fruit extract samples presented in table 4, GC-MS chromatogram data showed that Halmahera golobe fruit contained pyridine compounds in the percent area, namely 5.88% at the peak absorption with a retention time of 37.14 minutes.

Analysis using the Gas Chromatography-Mass Spectrophotometry (GC-MS) method is a key in the analysis of unknown plant chemical components. Analysis by GC-MS has been widely used in identifying hundreds of components of compounds present in plant cells that cannot be done by ordinary phytochemical screening. This is because

phytochemical screening is limited to the identification of compound groups. 14

CONCLUSION

Based on the results of the GC-MS analysis that has been carried out, it can be concluded that the ethanol extract of Halmahera golobe fruit (*Hornstedtia alliacea*) contains pyridine compounds in the percent area of 5.88% at the absorption peak with a retention time of 37.14 minutes.

SUGGESTIONS

The suggestion in this study is that further research is needed regarding pharmacological effects and toxicity tests in experimental animals.

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