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IDENTIFICATION OF CHEMICAL COMPOUNDS EXTRACT OF ORANGE HALMAHERA FRUIT (*Momordica cochinchinensis* (Lour.) Spreng) USING CHROMATOGRAPHY-MASS SPECTROSCOPY GAS METHOD (GC-MS)

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ABSTRACT

Background: Indonesia is a country with abundant natural resources, almost all types of plants can grow in this country. Research Objectives: To identify diltiazem compounds in orange fruit (*Momordica cochinchinensis* (Lour.) Spreng) with Gas Chromatography-Mass Spectrophotometry (GC-MS).

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 orange fruit (*Momordica cochichinensis* (Lour.) Spreng) contains Diltiazem compound in the percent area of 2.04% at the peak absorption with a retention time of 31,227 minutes. Diltiazem is a drug to lower blood pressure in hypertension and prevent chest pain (angina).

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INTRODUCTION

Indonesia is one of the countries whose biodiversity is abundant with adequate natural resources. The availability of adequate natural resources can indirectly be said to have contributed a million potential which is useful for human life. Especially for the health sector. Indonesia is very rich in plant species that can be used for medical research, pharmacy, prevention and treatment of various diseases.¹

There are many plants and herbs around us that have potential as herbal remedies that are easy to find. Even parents in our families often use plants and herbs as medicine. In addition, the growth of the pharmaceutical industry in Indonesia ranges from 10-14% annually, while Indonesia itself still lacks medicinal raw materials, so the raw materials must be imported from outside. Thus, Indonesia still depends on raw materials which have the potential to make Indonesia a consumptive country.² Drugs that are currently being developed are well developed from natural materials and structures, with a long period of time of 25 years, especially drugs to block the Corona virus, HIV / AIDS.³

The active chemical compounds found in plants are generally in the form of secondary metabolite compounds such as alkaloids, flavonoids, terpenoids, steroids, coumarin. Compounds produced by secondary metabolites from plants have various activities, including having effects as anti-cancer, anti-inflammatory, antioxidant, anti-hepatotoxic, and anti-diabetic. Therefore we need research on the effects of secondary metabolite compounds produced by plants.⁴

One of the medicinal plants that can be used as medicine is the fruit of Orange Halmahera or Tepurang or Pupia (*Momordica cochinchinensis* (Lour.) Spreng). This plant belongs to the Cucurbitaceae family in

Southeast Asia, the species of *cochinchinensis* originates from the Cochinchina region in northern Vietnam. In Indonesia, orange Halmahera or Tepurang is found in North Halmahera Regency, although its existence is still difficult to find.⁵

In previous studies, the seeds of the orange halmahera (*Momordica cochinchinensis* (Lour.) Spreng) have been used in traditional Chinese medicine for more than 1200 years. It has also traditionally been used to treat internal and external disorders such as inflammation, tinea, glandular diseases, and skin infections such as wounds and ulcers.⁷ In Vietnam, orange seeds have also been used traditionally to promote longevity, make a tonic for children and pregnant or nursing women, and can also treat dry eyes.⁸

Based on the above background, the researchers are interested in conducting research on the identification of the chemical compounds of the ethanol extract of Halmahera orange fruit (*Momordica cochinchinensis* (Lour.) Spreng) using the Gas Chromatography-Mass Spectroscopy method (GC-MS).

RESEARCH METHOD

Types of research

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

Population and Sample

Population

Population is the whole object of research. The population in this study was the orange Halmahera (*Momordica cochinchinensis* (Lour.) Spreng) which was taken from Simau Village, Kec. Galela, Kab. North Halmahera.

Sample

The sample used in the study was the simplicia powder of the orange Halmahera fruit (*Momordica cochichinensis* (Lour.) Spreng) as much as 500 g.

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

Work procedures

1) Sample Preparation

± 1000 g of fresh samples of Halmahera orange (*Momordica cochichinensis* (Lour.) Spreng) were taken, then washed with running water until they were clean of dirt or other foreign materials. The clean sample is chopped, then dried in direct sun for 3-4 days to dry, then in the oven for 3 hours at 50 ° C. Halmahera orange fruit samples were dry sorted.

2) Preparation of Ethanol Extract of Orange Halmahera Fruit

The samples extracted from Halmahera orange fruit simplicia (*Momordica cochichinensis* (Lour.) Spreng) using the maceration method with 70% ethanol solvent. A total of 500 grams of Halmahera orange fruit simplicia were soaked in 70% ethanol until all simplicia was wetted and ethanol added again until the solvent limit was approximately 2 cm above the simplicia. The maceration container is closed and stored for 5 × 24 hours in a protected place from the sun, stirring occasionally. Then filtered, separated between the pulp and the filtrate. After that, the remaceration was carried out for 3 × 24 hours with the same type and amount of solvent. Maserat I and II were collected and concentrated using a rotary evaporator at 60 ° C to obtain a total ethanol extract of Halmahera orange fruit.

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:

Identification of Chemical Compounds

a) Fractionation

The ethanol extract of Halmahera orange fruit as much (3 g) was separated by column chromatography using silica gel 60 (70-230 mesh) with a diameter of 2 cm. The process of separating the compounds is carried out with a gradient elution system using ethanol and n-hexane solvents, meaning that the polarity of the eluent used to elute the sample is increased gradually to produce various fractions. The results of the separation are accommodated in a numbered vial. After that it is calculated and combined based on the value of the Retardation factor (Rf) and purified to obtain a purer compound.

b) GC-MS (*Gas Chromatography Mass Spektrofotometry*)

The structure of the compounds from the fraction was determined by using a GS-MS spectrometer. Analyzes were 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 50oC, increase of 3.50°C per minute to maximum temperature 310°C), 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.

RESULTS

1. Sample Preparation

The collection of raw materials or research on Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) was taken from Simau Village, Galela, North Halmahera. This research was conducted at the Integrated Laboratory of the Pharmacy Study Program of the Makariwo Halmahera College of Health Sciences in October-November 2020. Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) is wet sorted / separated from foreign materials then washed in running water. The fruit (fructus) of Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) is chopped or made into small parts, which aims to expand the surface of the raw material because the wider the surface, the faster the raw material will dry, then wind it up. After drying in the sun then the sample was weighed again and the Simplicia weight of Orange Halmahera was obtained as much as 200 grams. Then the simplicia is extracted by maceration.

1. Preparation of Ethanol Extract of Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng)

Simplicia Orange Halmahera Fruit as much as 200 grams is macerated using ethanol solvent with a ratio of 1: 2 (1 gram of sample in 20 ml of ethanol) for 5 days, then the ethanol extract of Halmahera Orange Fruit is filtered, the filtrate is taken and the residue is removed. Maceration again for 2 days (remaseration), then evaporated using a rotary evaporator until a thick extract was obtained. The thick extract obtained is brownish yellow as much as 30 grams.

2. Description of Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) Thick Orange Fruit Extract

The thick extract of Orange Halmahera Fruit (*Momordica cochichinensis* (Lour.) Spreng) was obtained as much as 30 grams and has a brownish yellow color.

1. Extract yield calculation

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

$$\begin{aligned} \% \text{ Rendement} &= \frac{30 \text{ gram}}{200 \text{ gram}} = 100 \% \\ \text{Rendement} &= 15 \% \end{aligned}$$

2. Organoleptic Examination of Extract

Thick, brownish yellow color, fishy taste and smells rancid.

3. Identification of the Chemical Compounds of Orange Halmahera Fruit Extract (*Momordica cochichinensis* (Lour.) Spreng).

The following is the identification result of a chemical compound of 70% ethanol extract of Halmahera orange (*Momordica cochichinensis* (Lour.) Spreng). as in table 1.

Table 1. Identification Results of Orange Halmahera Fruit Chemical Compounds

Jenis/Kode Sampel	RT (R. Time)	Q	Nama Senyawa	Kandungan (%)
<i>Orange Halmahera Fruit</i>	27.648	53	1-Tertadecene	1,25
	28.275	99	Hexadecanoic acid, ethyl ester	1,59
	28.937	99	Hexadecanoic acid	15,28
	29.358	91	11-Hexadecenal, (Z)-	1,25
	29.441	99	9,12-Octadecadienoic Methyl Ester	2,32
	29.475	99	7-Octadecenoic acid, Methyl Ester	2,41
	30.020	99	9,12-Octadecadienoic acid (Z,Z)-	25,94
	30.151	91	Oleic acid	1,35
	30.234	99	Methyl 9 cis, 11 trans, 13 trans octadecatrienoate	1,33
	30.482	64	Fumaric acid, 2-dimethyl amino ethyl octadecyl ester	1,09
	30.558	90	2-Methyl-Z,Z-3,13-octadecadienol	1,31
	30.634	43	Ethyl 5,5-Dimethyl-4,5-Dihydro-1,3-Oxazole-4-Carboxylate	1,85
	30.896	96	Z,Z-10,12-Hexadecadien-1-ol acetat	1,20
31.227	72	Diltiazem	2,04	

DISCUSSION

The sample used in this study was Orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng). The solvent used in the ethanol extraction process, because ethanol solvent can dissolve in polar, semi-polar and non-polar substances. The tool used to identify the compounds contained in the orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng). namely by using (GC-MS) Gas Chromatography Mass Spectrometry because the advantage of this tool is that it is faster analysis, does not damage the sample, has high sensitivity so that it can separate various compounds that mix with each other and is able to analyze various compounds even at low levels / concentrations.

Results of Chemical Compound Identification Using GC-MS (*Gas Chromatography Mass Spectrometry*) : 1 – Tetradecene (1,25%), Hexadecanoic Acid, ethyl ester (1,59 %), Hexadecanoic acid (15,28 %), 11-Hexadecenal, (Z) -, 9,12-octadecadienoic methyl ester (2,32%), 7-Octadecenoic acid, methyl ester (2,41%). 9,12-octadecenoic acid (Z,Z) – (25,94%), Octadecanoic Acid (4,50%), Oleic Acid (1,35%), Methyl 9 cis, 11 trans t, 13 trans octadecatrienoate (1,33%), Fumaric acid, 2-dimethylamioethyloctadecyl ester (1,09%), 2-Methyl-Z,Z-3, 13-octadecadienol (1,31%), Ethyl 5,5-dimethyl-4,5-carboxylate (1,85%), Z,Z-10,12-Hexadecadien-1-ol acetat (1,20), Diltiazem (2,04%). Previous research results from Orange Fruit (*Momordica cochichinensis* (Lour.) Spreng). mentioned that orange fruit has benefits as an antioxidant; Anti cancer; and Vitamin A activity.¹⁵

Momordica cochinchinensis has been used in traditional Chinese medicine for over 1200 years as a preparation known as "Mubeizi". They have traditionally been used to treat internal and external disorders such as inflammation, tinea, scrofula and skin infections such as wounds, ulcers and furuncles. 16 Fresh arils and oil were traditionally used by indigenous people in Vietnam to

promote longevity and vitality. Burke Aril is also used to make a tonic for children and nursing or pregnant women and to treat "dry eye" (xerophthalmia) and night blindness.¹⁷

CONCLUSION

Based on the results of the study, it can be concluded that the Orange Halmahera *Momordica* contains chemical compounds such as: 1 – Tetradecene (1,25%), Hexadecanoic Acid, ethyl ester (1,59 %), Hexadecanoic acid (15,28 %), 11-Hexadecenal, (Z) -, 9,12-octadecadienoic methyl ester (2,32%), 7-Octadecenoic acid, methyl ester (2,41%). 9,12-octadecenoic acid (Z,Z) – (25,94%), Octadecanoic Acid (4,50%), Oleic Acid (1,35%), Methyl 9 cis, 11 trans t, 13 trans octadecatrienoate (1,33%), Fumaric acid, 2-dimethylamioethyloctadecyl ester (1,09%), 2-Methyl-Z,Z-3, 13-octadecadienol (1,31%), Ethyl 5,5-dimethyl-4,5-carboxylate (1,85%), Z,Z-10,12-Hexadecadien-1-ol acetat (1,20), Diltiazem (2,04%).

SUGGESTION

Based on the results of the study, the authors make several suggestions:

1. With this research, it is hoped that it can add information to the development of the pharmaceutical world regarding the chemical compounds of the orange fruit Halmahera *Momordica cochinchinensis* (Lour.) Spreng) so that it can be made in the form of a health supplement dosage form.
2. With this research the community can cultivate the orange fruit Halmahera *Momordica cochinchinensis* (Lour.) Spreng) in order to increase the economic value of the Halmahera orange fruit so that it can be used by the community as a medicinal ingredient.

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