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

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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 a country with abundant natural resources, almost all types of plants can grow in this country. [1] Indonesia's wealth of biological resources ranks third in the world after Brazil and Zaire so that it is grouped in the mega country of biodiversity. [2] Most of it has been used by our ancestors to treat various diseases.

The active chemical compounds found in plants are generally in the form of secondary metabolites such as alkaloids, flavonoids, terpenoids, steroids, coumarin, and others. Compounds produced by secondary metabolites from plants have various activities, including having effects as anti-cancer, anti-inflammatory, antioxidant, anti-hepatoxic, and anti-diabetic. Therefore, research is needed on the effects of secondary metabolite compounds produced by plants. [17]

One of the many types of plants found in Indonesia is the Cucurbitaceae family. This family includes approximately 960 species divided into 125 genera. The Cucurbitaceae family is a flowering plant and is generally a creeping plant. The Cucurbitaceae family has been known as a source of secondary metabolites. The compounds of cucurbitacins, a tetracyclic triterpenoid that impart a bitter taste to many species of the cucurbitaceae, have been studied as beetle attractants (Whitaker and Davis, 1962). Alkaloids have been reported in Momordica, and saponins have been found in Cucurbita, Citrullus, Lagenaria and Momordica (Schultes, 1990). *6*

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 Cohinchina region in northern Vietnam. In Indonesia, Orange Halmahera or Tepurang or Pupia (*Momordica cochinchinensis* (Lour.) Spreng), is found in North Halmahera Regency, although its existence is still difficult to find. [7]

Today, non-traditional crops grown in European conditions are considered a promising source of raw materials for the food and pharmaceutical industries. In addition, almost all parts of the plant are used: leaves, fruit, caules and seeds (Taylor, 2002). The severe hypoglycemic effects of the momordica plant are caused by the presence of three groups of compounds known as harantin, insulin-like peptides and alkaloids. (Akhtar et al, 1981; Day et al, 1990). High content of fatty acids, vitamin E and carotenoids in the pulp. In addition, most carotenoids contain beta-carotene compounds. (Vuong, King, 2003; Kuhnlein, 2004; Ishida et al, 2004). The lycopene content in aryl (arillus) momordica reaches 308 mg / g, which is almost 10 times higher than other fruits, rich in lycopene content in fruits and vegetables (Aoki et al., 2002; Vuong et al., 2003). [12]

Momordica cochinchinensis (Lour.) Spreng. (Cucurbitaceae) is a dioecious plant group, forming seeds with a very strong woody seed coat.[12] Based on the research results, the seeds of orange halmahera (*Momordica cochinchinensis* (Lour.) Spreng) have strong antioxidant activity because they contain lycopene and alpha tocopherol. Consuming orange halmaher fruit seeds can increase beta carotene, lycopene, and essential fatty acids and is a good source of vitamin E (Vien, 1995; Vuong, 2003). [7]

In another study, the seeds of the orange halamahera fruit (*Momordica cochinchinensis* (Lour.) Spreng) have been used in traditional Chinese medicine for more than 1200 years. It has traditionally been used to treat internal and external disorders such as inflammation, tinea, glandular diseases, and skin infections such as wounds and ulcers.8 In Vietnam, orange seeds have also been used traditionally to promote longevity, making a tonic for children.

children and pregnant or nursing women, and can also treat dry eye. [15]

In Vietnam, Thailand, Cambodia, India, Malaysia, China and the Philippines, Momordica cochinchinensis Spreng is very popular, it is also known as Gac. This plant has rather large fruit, which until recently was only grown for use as food. Recently in Vietnam began production of a valuable oil from the seeds of this plant, rich in α-eleostearic acid. [12]

Given the lack of utilization of Halmahera oranges in the community, it still needs to be supported by scientific information regarding the properties and potential of the Halmahera orange fruit plant, so researchers are interested in conducting research on the identification and benefits of diltiazem compounds in Halmahera oranges (*Momordica cochinchinensis* (Lour.) Spreng) .

RESEARCH METHOD

Types of research

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

Population and Sample

1) 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 the village of Kacak, West Tobelo District, North Halmahera Regency.

2) Samples

The sample used in this study was the simplicia powder of the orange Halmahera fruit (*Momordica cochinchinensis* (Lour.) Spreng) as much as 200 grams.

Figure 1. Morphology of leaves, flowers (male, female), fruits (immature, mature) and seeds of Momordica cochinchinensis. A: Leaves, B: Male flowers with broad reinform bracts (arrows), C: Female flowers with scabrous ovaries (arrows). D: Immature fruit, E: mature fruit, F: Cross-cut fruit, G: Seed (arrow)

Work Procedures

1) Material Collection

The plants used are orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) taken as much as ± 2000 grams, after which it is washed with running water until it is clean of dirt or other foreign materials. Clean samples were chopped. After chopping, dry in the direct sun for 3-4 days to dry, then in the oven for 3 hours at 50^{o}C. Halmahera orange fruit samples were sorted dry then blended and sieved with 80 mesh sieve.

2) Preparation of Orange Fruit Ethanol Extract

A total of 200 grams of orange fruit simplicia is macerated by immersing the simplicia in 400 mL of 70% ethanol solvent (ratio 1: 2 w / v). 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 residue and macerate I. 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 aerated at room temperature until the total ethanol extract of Halmahera orange fruit was obtained.

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 Diltiazem Compounds

a) Fractionation

The ethanol extract of Halmahera orange fruit as much (3 g) was separated by column chromatography using silica gel. 264 (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 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 310ºC, detector 310ºC, injector 305ºC, program temperature (initial 50ºC, increase of 3.50ºC 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.

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 orange fruit (*Momordica cochichinensis* (Lour.) Spreng) is wet sorted. 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. Then, the fruit (fructus) orange Halmahera (*Momordica cochichinensis* (Lour.) Spreng) is chopped to expand the surface of the fruit used so that during the drying process it can dry evenly and quickly. After that, the drying is done in the traditional way, using heating under direct sunlight. Furthermore, the simplicia is milled into 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 the simplicia of Halmahera orange (*Momordica cochichinensis* (Lour.) Spreng) as in table 1:

No.	Halmahera orange Simplicia	Information
1.	Shape	Oval
2.	Color	Fruit skin: Orange / red Oval Fruit skin: Orange / red Flesh: Yellow

Source: Primary Data, 2020

C. Extraction and Extract Yield

The extraction method for the simplicia of Halmahera orange (*Momordica cochichinensis* (Lour.) Spreng) uses maceration. A total of 200 grams of simplicia powder of Halmahera orange fruit were macerated with 400 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 and then evaporated the solvent at room temperature to produce a thick ethanol extract of Halmahera orange fruit (*Momordica cochichinensis* (Lour.) Spreng).

The results of visual observation of the ethanol extract of Halmahera orange fruit (*Momordica cochichinensis* (Lour.) Spreng) in the form of light yellow maserate are then evaporated by being aerated until a thick, pale yellow ethanol extract is obtained like fat with an extract weight of 21 grams with extract yield of 10 , 5% as shown in the table 2:

Sample	**Simplicia Sample (gram)**	**Extract (gram)**	**Rendement (%)**
Halmahera Red Fruit	200	21	10,5

Source: Primary Data, 2020

D. Identification of Diltiazem Compounds in Orange Halmahera

The following is the identification result of the chemical compound of 70% ethanol extract of Halmahera orange (*Momordica cochichinensis* (Lour.) Spreng). as in the table 3 :

Table 3. Identification Results of Diltiazem Compound Orange Halmahera Fruit

Type / Sample Code	RT	Q	Name of Compound	Content (%)
Orange Halmahera Fruit	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**

Source: Primary Data, 2020

DISCUSSION

Based on the results of laboratory examinations of samples of Halamahera orange fruit extract (*Momordica cochichinensis* (Lour.) Spreng) which are presented in table 4, GC-MS chromatogram data shows that Halmahera orange pulp contains Diltiazem compounds in the percent area, namely 2.04% at the peak of absorption with retention time 31,227 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 orange fruit (*Momordica cochichinensis* (Lour.) Spreng) contains Diltiazem compounds in the percent area of 2.04% at the peak absorption with a retention time of 31,227 minutes.

Suggestions

The suggestion in this research is that it is necessary to do further research on the pharmacological effects of the orange Halmahera (Momordica cochichinensis (Lour.) Spreng) on trial and error.

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