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IDENTIFICATION OF ANTIOXIDANT ACTIVITY OF *GOLOBE HALMAHERA* (HORNSTEDTIASP, ZINGIBERACEAE) FRUIT EXTRACT

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ABSTRACT

Golobe halmahera (Zingiberaceae) fruit extracts prove to have a wide variety of pharmacological activities. Chemical content in their seed extracts provides pharmacological activities. Phytochemical screening aims to provide an overview of the class of antioxidant compounds contained in the plant being studied. Samples used are extracts of fresh and dried *Golobe halmahera* extracted with hot water and ethanol solvents for 24 hours. The DPPH method was used to determine the antioxidant activity. The content of free radical scavenging compound with a high DPPH was identified with a lower IC50 value, i.e. anti-oxidant with a concentration of IC50 = 6.54 ng / ml.

Key words: *Golobe halmahera* (Zingiberaceae), Antioxidants, Free radicals, Flavonoids, DPPH.

INTRODUCTION

Diseases in the body are caused by various factors, one of which is free radicals. Free radicals are oxygen or nitrogen based radicals with unpaired electrons that are normally produced in the body during metabolism. Excessive free radicals can lead to many issues in the body's metabolism and has the potential to become the originator of various degenerative diseases, diabetes, coronary heart disease, and cancer [1]. Free radicals found in the environment include metals such as iron and copper, cigarette smoke, drugs, packaged food, and educational materials.

Antioxidants are compounds that can bind to free radicals in the body. In protecting the body against free radicals, antioxidant substances function to restore the complete lack of free electrons in free radicals from molecules that inhibit the chain [2].

Windono found that antioxidant is a compound that can be used to protect food from damage, rancidity, or discoloration caused by oxidation. Antioxidants are able to act as a developer of hydrogen radical or can act as an acceptor of free radicals that can delay the initiation stage of free radicals formation.

Natural antioxidants, such as phenolic compounds, or synthetic, can inhibit lipid oxidation to prevent damage to the organic component change in foods so that it can extend the shelf life [3].

Various diseases in the body are caused by free radicals. Free radicals are atoms or groups which have one or more unpaired electrons. Free radicals are also found on metal (e.g., iron and copper), cigarette smoke and pollution from vehicles, drugs, packaged food, addictive substance, etc [4].

The principle of the antioxidant mechanism is through inhibition of the radical by stabilizing and preventing free radicals reactivity [5]. Consuming plants or foods that contain antioxidants can help the body reduce free radicals in the body [6].

Golobe halmahera (Zingiberaceae) is a plant that grows in tropical areas, including in Halmahera. This plant is used to supply energy while hunting in the jungle, cure wounds and infections, and be herbal remedies for indigestion. Its fruits and seeds are consumed by all ages continuously for more than four generations in Halmahera [7]. *Golobe halmahera* (Zingiberaceae) until now has not

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been studied in Indonesia for its antioxidants content as well as active substances. This is the first study conducted to do so and will be developed to determine the active chemicals that can be beneficial to human health [7].

The utilization of medicinal plants includes the prevention and treatment of a disease and the maintenance of health. One of the plants that is extracted is mangosteen fruit, which is used as an antioxidant, anti-cancer, anti-skin inflammatory, anti-allergic, anti-bacterial, anti-fungal anti-virus and anti-malaria [8].

Differences in environmental conditions where plants grow can lead to differences in the type and amount of secondary metabolites contained in plants [9]. Genetic factors, methods of cultivation, harvest time, as well as post-harvest management also contribute to the differences [10]. It is important to determine the chemical content responsible in causing activity and resulting in quality control of herbal production [11]. Phytochemical screening is a preliminary stage in a phytochemical research that aims to provide an overview of the classes of compounds contained in the plant being studied. An important thing in phytochemical screening is the recovery of solvents and extraction methods [12].

The determination of antioxidant activity by DPPH is a common and relatively quick method to assess the activity of free radicals catcher from various plant extracts. Further research is needed to know the reproductive power of *Golobe halmahera* (Zingiberaceae) with other methods. DPPH and FTC are normally used to test a good antioxidant, and they are reliable to identify antioxidants activity [13].

MATERIALS AND METHODS

Instruments

UV-VIS spectrophotometer (SHIMADZU), cuvettes, rotary evaporator, water heater, funnel buncher, and glass tools, and analytical scale.

Materials

Golobe halmahera fruits, DPPH (Sigma), absolute ethanol, pa (Merck), 95% ethanol, 2.5% oleic acid, 0.02 phosphate buffer pH 7, distilled water, 30% ammonium thiocyanate, iron (n) chloride (Merck), CO_2free water, Folin–Ciocalteu reagent (Merck), Na carbonate, Gallic acid (sigma), potassium chloride, potassium acetate, quercetin, Vitamin C, aquabidestilate, and aluminum foil.

Materials collection

Golobe halmahera fruits obtained from agricultural lands in Tobelo, Loloda North Halmahera were terminated at the UGM (Universitas Gadjah Mada) pharmaceutical laboratory to ensure that samples are reliable. Golobe seeds, which consist of seed sugar, were drawn, drained and then dried. The dried seeds are then crushed into powder and extracted with 90% ethanol solvent by maceration method.

Extraction

A total of 500gr Halmahera Golobe fruit powder is subsequently screened with 90% ethanol for 7 times, carried out for 3 days and repeated 3 times. Once evaporated, it is dissolved with a vacuum rotary evaporator to obtain concentrated extract. The ethanol extract is then used to create a series of concentration to be tested for its antioxidant power.

The yield

The yield is calculated from the weight of the extract obtained is divided for simplicia made is 100%.

Determination of flavonoids with DPPH

The principle of the antioxidant activity of a sample is shown by the magnitude of the radical DPPH absorption decline at a wavelength of 517 nm. DPPH (2.2-diphenyl-1-picrylhydrazyl) is monitoring antioxidants activity. Concentration extracts/fractions that give50% antioxidant activity are compared to the control via a linear regression equation. Control solution used was DPPH 0.4 mM solution in ethanol.

Test procedure

The fraction that gives 50% antioxidant activity was compared to the control via a linear regression equation added with 1.0 ml of DPPH 0.4 mM and 3,950 ml of ethanol mixture. This solution then was vortexed and left for 30 minutes. Solution absorption rate was then measured at a wavelength of 517 nm with the blank consisting of 50 ml of extract and 4,950 ml of ethanol. Control absorption, consisting of 1.0 ml of DPPH and 4.0 ml of ethanol, were also measured, and vitamin C with a range of 1-5 mg/ml was used as a comparison.

The study of antioxidant activity in Rezali Zadah *et al* used a modification, i.e. 1.0 ml of DPPH 0.4 mM added with *Golobe halmahera* extract with a 50 ml concentration added with 50 ml of ethanol and then left for 30 minutes in a dark condition. Solution uptake was measured using spectrophotometer at a wavelength of 517nm with an ethanol blank.

The determination of antioxidant activity was performed by calculating the concentration inhibitory (IC_{50}). IC_{50} is the sample concentration and vitamin C that showed 50% anti-radical activity compared to the control via a linear regression line between content and capture percentage [14].

$$Radical\ activity\ \% = \frac{control\ absorption - sample\ absorption}{sample\ absorption}\ x\ 100\%$$

RESULTS AND DISCUSSION

Golobe halmahera (Zingiberaceae) seed extracts were cleaned of impurities, washed with water, and dried at a temperature of 40^{o}C using an oven. Then, the extracts were crushed using a blender, and then soaked with ethanol at a ratio of 1:10 and then scraped using a magnetic Styron for 24 hours. After that, the extracts were filtered using fitration filter paper and evaporated with a water bath at a temperature of 45^{o}C and stirred until they became thick. The results of the *Golobe halmahera* fruit extracts are shown in Table 1.

The total flavonoid content was measured to determine the potential of *Golobe halmahera* extracts as

free-radical scavengers. The human body produces antioxidant compounds but in limited amount so it is often not enough to neutralize free radicals that enter the body. The chemical components that act as antioxidants are phenolic and flavonoid. Those compounds are widely available in nature, particularly in plants, and possess an ability to capture free radicals [15].

Compounds that have the ability to counteract free radicals are generally a donor or hydrogen (H), so that the H atom can be captured by DPPH radical to be converted into its neutral form. The determination of IC_{50} was done with the regression equation. Test to determine the value of $IC_{50}$was calculated using a linear regression formula, i.e. y = ax ± b, where the value of y is 50 and x is the IC_{50}. The calculation result of IC_{50} value of each extract can be seen in Table 3.

Based on data in Table 3, the level of antioxidant activity is characterized by the value of IC_{50}, namely the concentration of the sample solution needed to inhibit 50% of DPPH free radicals. The smaller the IC_{50} value, the greater the activity of DPPH free radical scavengers.

Based on this, the test of antioxidant activity using DPPH method to *Golobe halmahera* fruit extracts and water at concentrations of 20, 30, 40, 50, 60 mg/l for the determination of IC_{50}values showed that the extracts have greater potential as radical scavengers with a smaller concentration. With only 6.55 ug/ml, the extracts were able to ward off free radicals by 50%.

Table 1. Percentage of free radicals capture by *Golobe halmahera* extracts

Content Ug/ml	I	II	III	Capture of free radicals (%)			Free follicle (%)
				I	II	III	
Control	0,628	0,625	0,628				
4,0	0,507	0,505	0,502	18,880	19,200	19,680	19,25
6,0	0,416	0,395	0,398	33,440	36,800	36,320	35,52
8,1	0,327	0,325	0,326	47,680	48,000	47,840	47,84
10,1	0,258	0,254	0,257	58,720	59,360	58,880	58,99
12,1	0,180	0,179	0,118	71,200	71,360	81,200	74,59

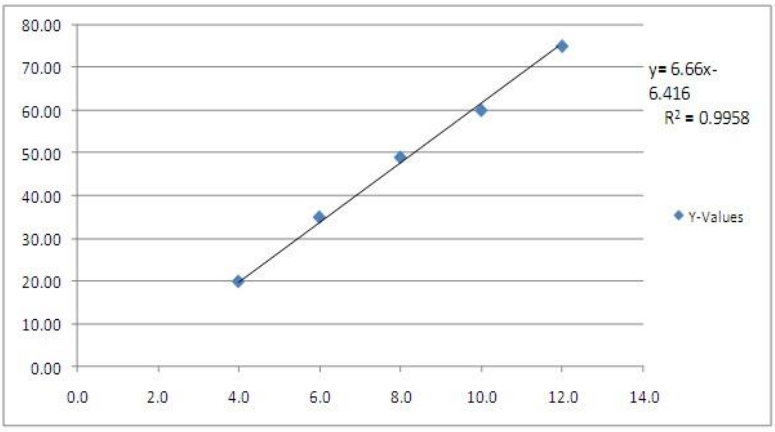
Table 2. Sample collection with several measures

Sample collection (ml)	Content (ug/ml)
20	4,0
30	6,0
40	8,1
50	10,1
60	12,1

Table 3. The regression equation and ic_{50} of *Golobe halmahera* fruit extracts

Extract	Equation	R_2	IC_{50}	Concentration ng/ml
Golobe halmahera	Y=6,66x-6.416	0,9958	6.544 ng/ml	0,0065 ng/ml

Fig 1. DPPH free-radical scavengers of *Golobe halmahera* extracts activity graph



CONCLUSION

Based on the results, it can be concluded that the *Golobe halmahera* fruit extract contains large antioxidant activity of flavonoids. With only 6.54 ng/ml, it can capture free radicals well, thus, is able to prevent many degenerative diseases, metabolic, and coronary such as heart disease, diabetes, stroke, kidney and other body disorders.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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