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Phytochemicals Screening and Effectiveness of Free Radical Inhibitors of Garlic (*Allium sativum* L.) Ethanol Extract from Timor Island

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Abstract

The purpose of the study was to determine the content of secondary metabolites and the effectiveness of garlic (*Allium sativum* L.) ethanol extract from Timor island which has the potential to inhibit free radicals. The method used for testing secondary metabolites is phytochemical screening using color reagents. The effectiveness of free radical inhibitors from the ethanol extract of garlic (*Allium sativum* L.) from Timor island was carried out in two steps are: 1.) Determination of DPPH wavelength (λ) maximum and 2.) Measurement of antioxidant activity using the DPPH method. The results showed the ethanol extract of garlic (*Allium sativum* L.) from Timor Island contained secondary metabolites of flavonoids, phenols, and terpenoids. The ethanol extract of garlic (*Allium sativum* L.) from Timor Island also has strongest effectiveness in inhibiting free radicals, with the acquisition of IC_{50} values <50 ppm which is equal to 9,729 ppm.

Keywords: *Phytochemical, Free Radical, and Garlic* (*Allium sativum* L.)

INTRODUCTION

Medicinal plants have been known since ancient times by people in various parts of the world, especially in eastern Indonesia. Medicinal plants are used as an effort to overcome health problems that are often faced. Health is the key and the main condition in carrying out every event of human life. Knowledge of medicinal plants is a hereditary legacy long before formal health services with various modern chemical drugs touch the community. Even the use of garlic is currently experiencing rapid development not only limited to humans but also applied to plants and animals (Giofanny et al. 2014; Zuhri et al. 2017; Nanda et al. 2018).

Based on information obtained from various social media, both print and electronic media, the current state of the Indonesian economy is unstable. This resulted in an increase in the price of the dollar exchange rate and the weakening price of the rupiah from Rp. 13.000, to Rp. 14.000, -causing the price of various basic needs to increase to be affected also by the prices of synthetic drugs (Trihendrawan 2018). Most chemical raw materials for the manufacture of synthetic drugs in Indonesia are still imported from outside (Sidik 2018). In addition, the use of synthetic drugs can have harmful side effects on health. The use of synthetic drugs continuously and not in accordance with the recommended dosage can actually trigger damage to various other organs so that various diseases arise and can eventually cause death (Lawal et al. 2016). Based on this, it is necessary to increase the use of plants with natural medicinal properties in the community. Medicinal plants are considered relatively safe for consumption because they lack or even do not have harmful side effects when compared to synthetic drugs.

Medicinal plants have natural active chemical compounds called secondary metabolites. Secondary metabolites are

compounds that function as inhibitors of free radicals or antioxidants. Anti free radicals play a role in counteracting and inhibiting various negative effects caused by various free radicals. Free radicals come from inside and outside the body. Continuous exposure to free radicals can cause damage to cell death (Prasonto et al. 2017). Cells in the body exposed to free radicals will reduce adaptability so that it can cause interference known as disease. Various types of diseases caused by free radicals are chronic and degenerative diseases, such as diabetes, heart disease, allergies, strokes, and cancer (Tristantini et al. 2016).

Active chemical compounds that are free radical inhibitors in medicinal plants include flavonoids, phenols, and terpenoids. Allegedly these three compounds are also found in garlic. This is because garlic is not only used as a spice and flavoring food for thousands of years until now, but also has been used as a medicine such as maintaining stamina, clearing the respiratory tract from coughing and phlegm, maintaining healthy hair and skin, relieving nausea, and so on (Bisen & Emerald 2016). For this reason phytochemical screening is needed to determine secondary metabolites contained in garlic, and antioxidant activity test to determine the effectiveness of garlic in inhibiting free radicals.

MATERIALS AND METHODS

The research was conducted for 3 months at the Analytical Chemistry laboratory of the Faculty of Science and Engineering, Nusa Cendana University, Kupang. Testing of secondary metabolites and the effectiveness of free radical inhibitors from the ethanol extract of garlic (*Allium sativum* L.) from Timor island were carried out in several steps. The first step begins with collecting and preparing samples, followed by extraction using ethanol solvents. The next step is phytochemical screening secondary metabolite compounds (flavonoids, phenols, terpenoids, and alkaloids) using color reagents. The next step is the effectiveness of free radical inhibitors consisting of 1.) Determination of DPPH wavelength (λ) maximum; 2.) Measurement of antioxidant activity using the DPPH method.

Materials

The tools used in this study are glassware, non-glassware, and instruments. Glassware includes beakers, measuring cups, test tubes, funnels, vacuum funnels, drop pipettes, volume pipettes, volumetric flasks, erlenmeyer, watch glass, and mortals. Non glassware includes analytic, static, washing bottles, test tube racks, spatulas, incubators, evaporators and ovens. The instrument used was a UV-Vis spectrophotometer.

The materials used include garlic (*Allium sativum* L.), ethanol, HCl, $FeCl_3$ in ethanol, aquades, aquabides, DPPH solution, $CHCl_3$, concentrated H_2SO_4, $Bi(NO_3)_3$, KI, acetic acid anhydride, mercury (II) chloride, filter paper (Whatman).

Procedure

1. Sample Collection and Preparation

The sample used in the study was 3-4 months old garlic. Garlic is taken from a natural population that is maintained by communities from Timor island, exactly in Kapan village, Mollo Utara sub-district of NTT. Garlic is cleaned, then weighed 200 g and 200 g of onion sample is mashed.

2. Sample extraction

A total 200 g of garlic which has been mashed, macerated using ethanol solvent for 5 days in a light free condition. The part of ethanol extract formed is separated and supernatant was Precipitation and filtered. The filtrate is evaporated using a solvent in the evaporator.

3. Phytochemical screening

a. Flavonoid Test

A small amount of extract was added with Mg powder, then pressed with 5M HCl (Sibatha reagent), the presence of violet red indicates the presence of flavonoids (Markham 1998).

b. Phenol Test

One ml of extract was added with $FeCl_3$ in ethanol. If it creates a green, red, purple, blue or black color that is strong it means that it is positive to contain phenol compounds (Harborne 1996).

c. Terpenoid Test

A total of 0.5 mL $CHCl_3$ was added to 0.5 mL of extract, then 0.5 mL of anhydrous acetic acid was added and the last added concentrated H_2SO_4 (reagent Lieberman Buchard). If a brown

or reddish ring is formed it means that it contains terpenoids (Harborne 1996).

d. Alkaloid Test

A total of 1 mL of each sample was placed on 2 test tubes. Then 2 tubes were added with Dragendrof and Meyer reagents. If adding reagents Dragendrofs form orange deposits, which means they positively contain alkaloids. Whereas if added the Meyer reagent a white precipitate is formed, then it positively contains alkaloids (Harborne 1996).

4. Effectiveness of Free Radical Inhibitors

a. DPPH Absorbance Measurement

The maximum absorption wavelength is determined using a control solution, which is a DPPH solution dissolved in ethanol and measured at a wavelength of 450-600 nm.

b. Antioxidant Activity Measurement

The concentrated extract is made a solution by dissolving it in 100 mL of water. A total of 0.01 ppm; 0.5 ppm; 1 ppm; 2 ppm; 3 ppm; 4 ppm; 5 ppm and 6 ppm of solution are put into eight tubes. From each of these concentrations 1 mL was taken and added with a DPPH solution of 1 mL with a concentration 0.04 %. After that, it was incubated at 37^{o}C for 30 minutes. Then absorption is measured by a spectrophotometer at the maximum wavelength. This treatment was repeated 3 times.

5. Data Analysis

The percentage of inhibitor (%) is calculated based on {(absorption of blanks-sample)/blank absorption} x 100 %. The inhibitor value and concentration of extract are plotted on the x and y axes, and the line equation obtained is used to calculate IC_{50}.

RESULTS AND DISCUSSION

Based on the research that has been done, the results are:

1. Phytochemical screening

Phytochemical screening of garlic ethanol extract from Timor Island can be seen in table 1.

Table 1. Results of Phytochemical Screening

Extract	Secondary Metabolit	Reactor	Result	Changes Appear
Ethanol	Flavonoid	Sibatha	+	Purple
	Phenol	$FeCl_3$ in Ethanol	+	Purplish Brown
	Terpenoid	Lieberman Buchard	+	Reddish Brown Ring
	Alkaloid	Dragendrof	-	No Orange Sediment
		Meyer	-	No White Sediment

The results shown in table 1 that the samples of garlic from Timor island positively contain secondary metabolites, namely flavonoids, phenols, and terpenoids using ethanol as a solvent to extract active chemical compounds found in garlic samples. All components of the secondary metabolites to be dissolved in the solvent. Ethanol solvents are effectively used as solvents in the extraction process, both extraction of organic and inorganic chemicals because have universal nature. The universal nature of ethanol makes ethanol able to bind all chemical components contained in natural materials, both chemical compounds that are polar, semi-polar, and non-polar (Hanin & Pratiwi 2017).

2. Effectiveness of Free Radical Inhibitors

a. DPPH Absorbance

Based on the absorbance measurement results of the control solution measured at wavelengths of 450-600 nm, the maximum absorption wavelength is found at a wavelength of 515.5 nm with an absorbance of 0.665. The maximum wavelength of 515.5 nm is the optimum absorbance wavelength of the measured compound.

b. Antioxidant Activity

The antioxidant activity test was carried out to determine the effectiveness of inhibiting compounds found in garlic ethanol extract as a hydrogen donor to react with free radicals originating from DPPH. The optimum absorbance in measuring compounds causes high sensitivity and linearity. Small changes in concentration cause a large change in absorbance. It states that the change in the concentration of the compound is proportional to the change in absorbance of the compound. Data of changes the concentration of compounds in solution proportional to changes absorbance, and antioxidant activity of compounds

in garlic ethanol extract from Timor Island can be shown in table 2.

Table 2. Antioxidant Percentage of Garlic (*Allium sativum* L.) Ethanol Extract Sample

Absorbance of Control	Sample of Garlic (*Allium sativum* L.) Ethanol Extract					
	Concentration (ppm)	Absorbance of the Sample			Average Absorbance of Samples	Antioxid (%)
		1	2	3		
0.665	6	0.455	0.460	0.457	0.457	31.28
	5	0.482	0.481	0.448	0.484	27.22
	4	0.510	0.507	0.510	0.509	23.46
	3	0.531	0.538	0.535	0.535	19.55
	2	0.563	0.555	0.550	0.556	16.39
	1	0.595	0.595	0.597	0.596	10.38
	0.5	0.627	0.624	0.622	0.624	6.17
	0.01	0.655	0.651	0.650	0.652	1.95

The measurement results in table 2 show the variation of absorbance and variation of antioxidants (%) from each variation in concentration. The higher the concentration, the less absorption is produced, whereas, the less the concentration of the solution, the higher the absorption. This is related to the increasing number of antioxidant compounds as free radical inhibitors that become electron or hydrogen donors on DPPH free radicals so that DPPH color changes occur which cause the absorbance produced to be smaller (Elosta et al. 2017). Increasing the concentration with the percentage of antioxidants produced is a proportional relationship. If the concentration of the solution increases, the percent value of the antioxidant is also higher.

The data in table 2. were analyzed using a linear regression equation to obtain the correlation curve percent of antioxidant activity on the concentration of garlic ethanol extract samples in Figure 1, where the x axis is the variation in concentration and the y axis is antioxidant percent.

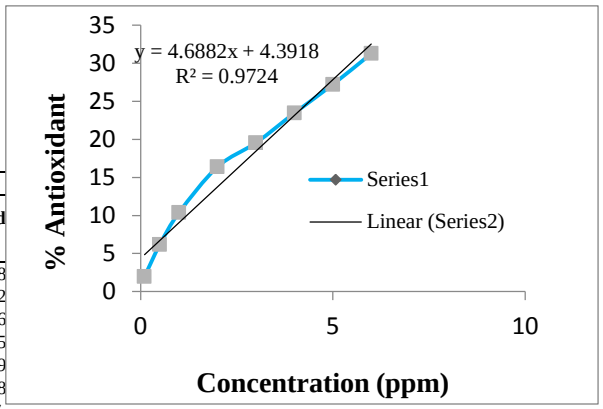

Figure 1. Correlation Curve Percentage of Antioxidant Activity to Concentration of Garlic Ethanol Extract from Timor Island

Based on Figure 1, a linear regression equation is obtained which is y = 4.391 + 4.688x. From this linear equation, the value of x as the effective concentration of garlic ethanol extract (IC_{50} value) can be determined. The IC_{50} value is the effective concentration of extract needed to inhibit 50 % of the total DPPH value, so the value of 50 is substituted as the y value in the linear regression equation above. In accordance with the parameters of the IC_{50} value in table 3, the calculation results show that garlic ethanol extract has very strong effectiveness in inhibiting DPPH free radicals, as evidenced by the IC_{50} value obtained is <50 ppm, which is equal to 9,729 ppm.

Table 3. Antioxidant Characteristics Based on IC_{50} Values (Molyneux 2004)

The IC_{50} Value	Antioxidant Characteristic
200 ppm - 150 ppm	Less
150 ppm - 100 ppm	Moderate
100 ppm - 50 ppm	Strong
<50 ppm	Very Strong

The very strong effectiveness possessed by the garlic sample from Timor Island as a free radical inhibitor is caused by the secondary metabolites of garlic. Based on the results of phytochemical screening, it was previously known that the sample contained secondary metabolites, namely flavonoids, phenols, and terpenoids.

Flavonoid and phenol compounds have -OH groups which are bound to carbon aromatic rings which function as inhibitors of free radicals, because it's have the ability to contribute hydrogen atoms so that free radicals can be reduced to a more stable form (Mohandas & Kumaraswamy 2018). The effectiveness of flavonoids and phenols in inhibiting DPPH free radicals is influenced by the number and position of phenolic hydrogen in molecules. Increasing the number of hydroxyl groups possessed by flavonoids and phenols, the greater the effectiveness produced in inhibiting free radicals (Wahdaningsih et al. 2011). In the structure of terpenoid compounds, the presence of a conjugated double bond functions as an inhibitor of free radicals, because of the ability to donate electrons so that it can stabilize the reactive charge of free radicals DPPH (Young & Lowe 2018). The content of secondary metabolites in prenatal garlic is influenced by the presence of genetic and environmental factors. In the postharvest phase, the storage time of garlic can affect the percentage of secondary metabolite content (Szychowski et al. 2018).

CONCLUSION

Based on the results of the study, it can be concluded that:

1. The ethanol extract of garlic (*Allium sativum* L.) from Timor Island contains secondary metabolites. There are flavonoids, phenols, terpenoids, and alkaloids.
2. The ethanol extract of garlic (*Allium sativum* L.) from Timor Island is very effective in inhibiting free radicals with the acquisition of IC_{50} values obtained at 9,729 ppm.

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