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Phytochemicals Screening and Antioxidant Effectiveness of Garlic (*Allium sativum*) from Timor Island

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Abstract

The people of Timor Island only know garlic as a kitchen spice. This research provides new knowledge of the benefits of garlic in the health sector, especially as an inhibitor of free radical that can trigger various degenerative diseases. The aims of this research were to identify secondary metabolites contained in the ethanolic extract of garlic (*Allium sativum* L.) from Timor Island and to determine its effectiveness in inhibiting free radicals. The method used to test secondary metabolites was phytochemical screening using color reagents. Testing the effectiveness of free radical inhibitors from garlic ethanol extract from Timor Island was carried out in 2 stages: 1.) Determination of DPPH maximum wavelength (λ) and 2.) Measurement of antioxidant activity using DPPH method. The results showed that the ethanolic extract of garlic from Timor Island contained secondary metabolites of flavonoids, phenols and terpenoids. It was also very effective in inhibiting free radicals, with the acquisition of IC50 values <50 ppm which was equal to 9.729 ppm. This research gives some information that can be used for the pharmacological ingredients, i.e. as a natural medicine that safe for the body to be consumed by the people of Timor Island. Moreover that also can impact on demand of garlic in the market. This has a very positive impact on improving the economy of garlic farmers on Timor Island.

How to Cite

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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. 13,000.00 to Rp. 14,000.00, 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 the damage to various other organs, so that, various diseases arise and can eventually cause death (Lawal et al., 2016). Based on these information, 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. Antioxidants play a role in counteracting and inhibiting various negative effects caused by various free radicals (Ni'mah et al., 2017). Free radicals come from inside and outside the body. Continuous exposure to free radicals can cause damage to cell (Prasonto et al., 2017). When the cells in the body exposed to free radicals, they will reduce their adaptability that leads to interference known as disease (Tillah et al., 2017). Various types of diseases caused by free radicals are chronic and degenerative diseases.

Degenerative diseases were chronic diseases caused by damage to the body's tissue system (attacking the immune system) due to unhealthy lifestyles, such as smoking, radiation, lack of exercise, obesity, hormones, viruses, and carcinogenic chemicals (Gebreyohannes & Gebreyohannes, 2013 ; Nasr, 2014; Arreola et al., 2015; Gao & Huang, 2019). Degenerative diseases were often suffered by humans in general, namely osteoporosis, heart disease, diabetes, high blood pressure, allegory, stroke, and cholesterol. Uncontrolled growth of cells in the human body tissue caused damage that can lead to cancer (Tristantini et al., 2016; Divya et al., 2017).

Active chemical compounds that act as 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 since thousands of years ago until now, but has been used as a medicine such as in maintaining stamina, clearing the respiratory tract from coughing and phlegm, maintaining healthy hair and skin, relieving nausea, treating toothache, treat from insect and snake bites, prevent infection, etc. (Bayan et al., 2014; Bisen & Emerald, 2016). Based on the explanation above phytochemical screening is needed to determine secondary metabolites contained in garlic as well as, antioxidant activity test to determine the effectiveness of garlic in inhibiting free radicals.

The objectives of this research were to identify secondary metabolites contained in garlic ethanol extract and to determine the effectiveness of ethanolic extract of garlic from Timor Island in inhibiting free radicals. This research was expected to give information that can be used for pharmacological ingredients, i.e. as a natural medicine that safe for the body to be consumed by the people of Timor Island. Moreover, it is also expected that to have an impact on demand of garlic in the market. This has a very positive impact on improving the economy of garlic farmers on Timor Island.

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 (*A. sativum*) from Timor Island were carried out in several steps. The first step began with collecting and preparing samples, followed by extraction using ethanol solvents. The next step was phytochemi-

cal screening secondary metabolite compounds (flavonoids, phenols, terpenoids, and alkaloids) using color reagents. The next step was testing the effectiveness of free radical inhibitors consisting of 1.) Determination of DPPH wavelength (λ) maximum; 2.) Measurement of antioxidant activity using the DPPH method.

The tools used in this research were glassware, non-glassware, and instruments. Glassware apparatus included beakers, measuring cups, test tubes, funnels, vacuum funnels, drop pipettes, volume pipettes, volumetric flasks, Erlenmeyer, watch glass, and mortals. Non-glassware apparatus included 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 (*A. sativum*), ethanol 98%, HCl, $FeCl_3$ 1%, distilled water, double-distilled water, DPPH solution, $CHCl_3$, concentrated H_2SO_4, acetic acid anhydride, chloroform, NH_3, Mg powder and filter paper (Whatman).

Sample Collection and Preparation

The sample used in the research was 3-4 months old garlic. Garlic was taken from a natural population maintained by communities from Timor Island, precisely in Kapan Village, Mollo Utara Sub-district of NTT. Garlic was cleaned, then weighed of 200 g then the sample was mashed.

The garlic which has been mashed was macerated using 98% ethanol solvent as much as 400 mL for 5 days in a light free condition. The part of ethanol extract formed was separated and supernatant was filtered. The filtrate is evaporated using a solvent in the evaporator.

Phytochemical screening

Flavonoid Test. A small amount of extract was added with 0.5 mg Mg powder, and was pressed with 0.5 mL 5M HCl (Sibatha reagent), the presence of violet red indicates the presence of flavonoids.

Phenol Test. 1 ml of extract was added with 1% $FeCl_3$ solution. Formation of strong green, red, purple, blue or black color gives an indication of the presence of phenol compounds.

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 slowly, finally, 1 drop of concentrated H_2SO_4 solution (reagent Lieberman Buchard) was added. If a brown or reddish ring is formed, it means that it contains terpenoids.

Alkaloid Test. A total of 1 mL of each sample was placed on 2 test tubes. The two tubes were then added with 1 mL of 2N H_2SO_4 and were shaken until two layers were formed. The top layer was pipetted, then the chloroform and ammonia were added. Then each one put in each test tubes. The first test tube was added with two drops of Dragendorf reagent. The second test tube was added with two drops of Meyer reagent. If the addition of Dragendorf forms an orange precipitate, it positively contains alkaloids, and if the addition of Meyer reagent forms white precipitate, then it positively contains alkaloids.

Effectiveness of Free Radical Inhibitors

DPPH Absorbance Measurement. The maximum absorption wavelength was determined using a DPPH solution (control solution) dissolved in ethanol and measured at a wavelength of 450-600 nm using a UV-Vis spectrophotometer.

Antioxidant Activity Measurement. Various extract concentration were made by dissolving the solution 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 were put into eight tubes. From each of these concentrations, 1 mL of solution was taken and added with 1 mL DPPH solution with a concentration 0.04 %. After that, it was incubated at 37°C for 30 minutes. Then absorption was measured at the maximum wavelength using a UV-Vis spectrophotometer. This treatment was repeated 3 times.

Data Analysis

The percentage of inhibitor (%) was calculated based on (absorption of blanks-sample / blank absorption) x 100 %. The inhibitor value and concentration of extract were plotted on the x and y axes, and the line equation obtained was used to calculate IC50.

RESULTS AND DISCUSSION

Phytochemical screening was a qualitative preliminary test to find out the secondary metabolite compounds contained in the plants would be research using the dyestuff testing method by looking at the changes that might occur in the test solution during the reaction process. Changes in reactions that occur are: changes in color, sediment, and formation of rings (Simaremare, 2014; Pratita, 2017).

Phytochemical screening

The picture of garlic (*A. sativum*) from Ti-

mor Island can be seen in Figure 1 and the results of phytochemical screening of garlic ethanol extract from Timor Island are shown in Table 1.

Figure 1. Garlic (*A. sativum*) from Timor Island

The test results shown in Table 1 shows that the sample of garlic from Timor Island is positively containing various types of secondary metabolites, i.e. flavonoids, phenols, terpenoids, and alkaloids that can function as a pharmacological ingredients. The amount of secondary metabolite content had not been tested further because this study only conducted qualitative testing, namely phytochemical tests (Radam & Purnamasari, 2016).

Ethanol in this research was used as a solvent to extract secondary metabolites contained in garlic samples. This caused all of the components that were in these secondary metabolites compound dissolved in the solvent. Ethanol solvents are effectively used as solvents in the extraction process, both in extraction of organic and inorganic chemicals due to their universal nature. The universal nature of ethanol makes it is able to bind all chemical components (polar, semi-polar, to non-polar) contained in natural materials (Hanin & Pratiwi, 2017).

Effectiveness of Free Radical Inhibitors DPPH Absorbance

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

Antioxidant Activity

The antioxidant activity test was carried out to determine the effectiveness of antioxidant 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. The change in the concentration of the compounds is proportional to the change in their absorbance. Data of antioxidant activity in ethanolic extract of garlic from Timor Island is presented in Table 2.

The measurement results in Table 2 shows the variation of absorbance that leads to variation

Table 1. Results of Phytochemical Screening

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

Table 2. Antioxidant Percentage of Garlic (*A. sativum*) Ethanol Extract Samples

Absorbance of Control	Sample of Garlic (*A. sativum*) Ethanol Extract					
	Concentration (ppm)	Absorbance of the Sample			Average Absorbance of Samples	Antioxidant (%)
		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

of antioxidants (%) in each concentration. The higher concentration shows lower absorbance. 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 extract concentration is proportional to the percentage of antioxidants produced. If the concentration of the solution increases, the percent value of the antioxidant also increases.

The data in Table 2. were analyzed using a linear regression equation to obtain the correlation curve of antioxidant activity (%) and the concentration of ethanolic extract of garlic (ppm) (Figure 2).

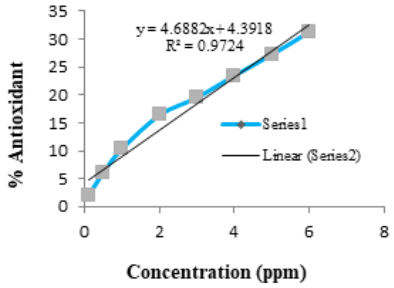

Figure 2. Correlation Curve of the Percentage of Antioxidant Activity and Concentration of Ethanolic Extract of Garlic from Timor Island

Based on Figure 2, 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 ethanolic extract of garlic (IC50 value) can be determined. The IC50 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 IC50 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 IC50 value obtained that is <50 ppm (9.729 ppm).

Table 3. Antioxidant Characteristics Based on IC50 Values (Molyneux, 2004)

The IC50 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 samples of ethanolic extract of garlic from Timor Island as a free radical inhibitor is caused by the secondary metabolites contained in garlic. Based on the results of phytochemical screening, it was previously known that the sample contained secondary metabolites, i.e. flavonoids, phenols, and terpenoids. Flavonoid and phenol compounds have an -OH group bounds to the aromatic carbon ring namely 5,7,4'-trihydroxyl group which functions as a free radical inhibitor, because it has the ability to donate hydrogen atoms so that the free radicals can be reduced to a more stable form (Santoso et al., 2016; 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. An increase in the number of hydroxyl groups (indicated by flavonoids and phenols) will produce the greater effectiveness in inhibiting free radicals (Wahdaningsih et al., 2011; Sulistyaningtyas & Wilson, 2018). In the structure of terpenoid compounds, the presence of a conjugated double bond functions as an inhibitor of free radicals, because of its ability to donate electrons so that it can stabilize the reactive charge of DPPH free radicals (Young & Lowe, 2018). The content of secondary metabolites in garlic before harvest 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).

The people of Timor Island basically only know garlic as a kitchen spice. This research provides new knowledge on the benefits of garlic from Timor Island in the health sector, as a safe natural medicine the inhibitor of free radical that can trigger various degenerative diseases in the body. Moreover that also can impact on demand of garlic in the market. This has a very positive impact on improving the economy of garlic farmers on Timor Island.

CONCLUSION

Secondary metabolites contained in the ethanolic extract of garlic (*A. sativum*) from Timor Island are flavonoids, phenols, terpenoids, and alkaloids. These compounds are very effective in inhibiting free radicals with the acquisition of IC50 values obtained at 9.729 ppm.

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