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Original Research Article

Antioxidant Activity Ethanol Extract of Garlic (*Allium sativum* L.) and Black Garlic

Zikra Azizah, Putri Yani, Rina Desni Yetti

College of Pharmacy (STIFARM), Jl. Raya Siteba Kurao Pagang, Padang, West Sumatera, Indonesia

Corresponding Author: Rina Desni Yetti

ABSTRACT

A research on the antioxidant activity ethanol extract of garlic (*Allium sativum* L.) and black garlic has been done. This study determined the compound and content of secondary metabolites and antioxidant activity from the ethanol extract of garlic (*Allium sativum* L.) and black garlic. Extraction using the maceration method with ethanol solvent, then analyze qualitative, quantitative and antioxidant testing with 1.1-diphenyl-2-picrylhydrazyl (DPPH) method. The results of qualitative analysis showed that the ethanol extract of garlic and black garlic contained flavonoids, alkaloids, and saponins compounds. Ethanol extract of garlic in the flavonoid content was 2.5836% ± 0.0982, the alkaloid content was 5,413% ± 0.2357, saponin content was 1.1007% ±0.1797. In ethanol extract of black garlic the flavonoid content was 3.0117% ± 0.1783, the alkaloid content was 6.9658% ± 1.5362, saponin content was 1.7835% ± 0.2017. The result of the calculation IC50 ethanol extract of garlic was 670.0333 mg / mL ± 1.8609 (very weak antioxidant), in ethanol extract of black garlic was 637.7955 mg / mL ± 1.7879 (very weak antioxidant). The results of statistical tests in the ethanol extract of garlic and black garlic on flavonoids, saponins, and IC50 there is a significant difference, but the alkaloid content there is no significant difference.

Keywords: *Antioxidant, DPPH, Garlic, Black Garlic*

INTRODUCTION

The use of plants as traditional medicines have existed since long ago, even the last 20 years of world attention to traditional medicines are increasing, both in the developing country and developed countries. The World Health Organization mentions that up to 65% of developed countries use traditional medicine and medicines from natural ingredients. [1]

Garlic (*Allium sativum* L.) is a high-production horticultural commodity in Indonesia. According to the Ministry of Agriculture of the Republic of Indonesia (2017) that in 2016 there was about 2,407 Ha of garlic harvests with a total production of 21,150 tons. In West Sumatra, the area of garlic harvested in 2016 amounted to 90 Ha with a total production of 590 tons. [2]

According to Hernawan & Setyawan (2003), [3] Garlic has biological activity of antidiabetic, antihypertensive, antioxidant, Anticholesterol and Antimikroba. Also besides, garlic is usually also consumed directly in the form of fresh because it has various efficacy. Some of the efficacy of garlic can increase appetite, lower blood pressure, decrease cholesterol levels, asthma, accelerate the eyes of swollen abscesses or ulcers, diarrhea and so forth. [4]

One alternative way of processing garlic to increase antioxidant activity is by fermentation. Processing garlic by fermentation will produce black garlic or black garlic that can be consumed directly. Black garlic is a fermented product of garlic that is heated at a temperature of 70-90°C for 12-40 days without any additional treatment. [5]

Lately, the use of antioxidant compounds develops rapidly both for food and treatment. Antioxidants are known to inhibit free radical work. [6] Antioxidants are

compounds that can inhibit oxidation by capturing free radicals. Natural antioxidants contained in plants are usually flavonoid compounds, phenolic, and tannins because these three compounds are phenol compounds, i.e. compounds with the OH-cluster that are bound to the carbon aromatic rings. This phenol compound can donate hydrogen atoms so that DPPH radicals can be reduced to a more stable form. [7]

Based on the explanation above, researchers want to do research that is the antioxidant activity test of garlic ethanol extract (Allium sativum L.) and black garlic.

RESEARCH METHODS

Tools

The tools used: Double beam UV-Vis spectrophotometer (Shimadzu UV-1800), Analytical scales (Precisa), Grinder (Miyako), Desiccator, beaker glass (Pyrex), Spatel, suction balls, macerator, Rotary evaporator (Ika), Erlenmeyer (Iwaki), volumetric flask (Iwaki), stirring rod, infrared moisture balance, measuring pipette (Iwaki), pipette drops, test tubes, test tube racks, oven, furnace (Carbolite), silica gel plates 60 F254 and plate drops.

Materials

The ingredients used are fresh garlic bulbs (*Allium sativum* L.) as much as 6 kg. The chemicals used are ethanol (C_2H_5OH) 70% (PT. Bratachem), Ethanol (C_2H_5OH) 95% (PT. Bratachem), Hydrochloric Acid (HCl) (Merck), Iron (III) Chloride ($FeCl_3$) (Merck), Mercury (II) Chloride ($HgCl_2$) (Merck), Potassium Iodide (KI) (Merck), Iodide (I_2) (Merck), Chloroform ($CHCl_3$) (Merck), Aquadest (PT Bratachem), Methanol (CH_3OH) p. a (Merck), Toluene ($C_6H_5CH_3$) (Merck), Ethyl Acetate ($CH_3CH_2OC(O)CH_3$) (Merck), DPPH (1.1-Diphenyl-2-Picrilhydrazil) p.a (SIGMA), Gallic Acid ($C_7H_6O_5$) (PT Bratachem), Zinc Powder (Zn) (Merck), Sulfuric Acid (H_2SO_4) (PT Bratachem), Ammonia (NH_3) (PT Bratachem), Quercetin ($C_{15}H_{10}O_7$) (PT Bratachem), Petroleum Ether (Merck), Butanol (C_4H_9OH) (PT Bratachem).

Sampling

The sample of garlic tuber (*Allium sativum* L.) is 6 kg derived from Paimban in Pariangan Village, Pariangan Sub District, Tanah Datar Regency, West Sumatera.

Plant Identification

Identification of plants conducted at Herbarium Andalas (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA), Andalas University (UNAND) Padang.

Preparation of Simplisia

In general, the manufacture of Simplisia includes, collecting samples, wet sorting, washing, drying, sorting dry, preparation of powder simplisia, storage, and inspection of quality. [8]

Preparation of Black Garlic

Garlic as much as 3 kg was chosen that is large, not rotten, and still intact blends with the other cloves instead of the rupture. Garlic is left without being peeled in a dry and unmoist state. Garlic is inserted into the rice cooker and is styled not overlapping to prevent damage to the black garlic form. The Rice cooker is closed and adjusted in warm keep mode (temperature ± 70-90°C) and left for 12 days, checked the changes that occur every 2 days. [9]

Characterization Simplisia

Simplisia characterization test includes drying shrinkage, total ash content, insoluble ash content, water-soluble sari content, soluble juice of ethanol. [1]

Preparation of Extract

200 grams of simplisia powder garlic and black garlic is macerated by soaking the simplisia into the ethanol solvent 70% as much as 2000 mL (ratio 1:10 W/V). Marinated for the first 6 hours while occasionally stirring, then let stand for 18 hours. Maserate is separated by filtration (filtering), the screening process is repeated 2 times, using the same type and number of solvents. All the maserat are collected, and then in the evaporator with a rotary steam device at a temperature below ± 50°C so that a condensed extract is obtained. The obtained yield is weighed and recorded.

Characterization Extract

The characterization of extracts includes organoleptic, identity, moisture determination, total ash content, and acid insoluble ash content. [10]

Qualitative Analysis

1. Phenol Test

Extracts (50 mg) dissolved in 5 mL of distilled water. Added 3-4 drops of iron (III) chloride 5%. The phenol compound will give green to black blue with the addition of iron salt (III) chloride solution. [11]

2. Flavonoids Test

Experimental solution

The 0.5 g extract was added with 10 mL methanol P, using a back cooling for 10 minutes. Filter heat through a small, folded filter paper. Dilute filtrate with 10 mL of water. After cold add 5 mL ether kerosene P, shake carefully, let stand. Take the methanol layer, darken at a temperature of 40ºC under pressure. Time dissolved in 5 mL ethyl acetate p, strain.

wipe to dry 1 mL of the test solution, the remaining dissolved in 1 mL to 2 mL ethanol (95%) P, add 0.5 g of zinc powder P and 2 mL 2 N hydrochloric acids, let stand for 1 minute. Add 10 drops of concentrated hydrochloric acid, if within 2 to 5 minutes occurs intensive red color, indicating the presence of flavonoids (glycosides-3-Flovanol).

To dry 1 mL of the test solution, the remaining dissolved in 1 mL ethanol (95%) P, add 0.1 g of magnesium P powder and 10 drops of P hydrochloric acid, in case of red-orange to red-violet, indicating the presence of flavonoids. If there is an orange-yellow color indicates the presence of Flavon, Kalkon, and Auron. [12]

3. Alkaloids Test

Extract 0.5 g add 1 mL 2 N hydrochloric acid and 9 mL water, heat over a water bath for 2 minutes, chill and strain. Move the 3 drops of filtrate on the watch glass, add 2 drops of Bouchardat LP to the form of precipitate colored brown to black, then there is a possibility of alkaloids. If with Mayer LP formed an agglomerate white or yellow precipitate. [12]

4. Saponin Test

Method of bribe

Put 0.5 g of the extract inspected into the test tube, add 10 ml of hot water, chill and then shake strong-strong for 10 seconds. (If the checked substance is a liquid dosage, the 1mL of the dosage is examined with 10 mL of water and beaten firmly for 10 minutes, forming a steady froth for no less than 10 minutes, as high as 1 cm to 10 cm. Add 1 drop of 2 N hydrochloric acids , Froth is not lost. [12]

Extract 1 mL added 3 drops reagent FeCl3 5% produce green or blue to black color. [12]

6. Steroid Test

1 mL extract is added chloroform and sees the formed layer, then the chloroform layer is dried. Then add 3 drops H2SO4 P. It will form a blue color. The formation of blue can be observed at the edges of the plate drops. [13]

Quantitative Analysis

1. Flavonoids

Determination of total flavonoids levels using spectrophotometers with the reagent of aluminium chloride solution. Comparative solution is made by careful weighing 10 mg quercetin dissolve in 100 mL ethanol 80% (100 μg/mL), to 30, 40, 50, 60, 70 μg/mL by take 3, 4, 5, 6, 7 mL of the solution 100 μg/mL, each of which is included in a 10 mL measuring flask and in a short until the boundary mark with ethanol 80%. Create a test solution with a careful weigh 50 mg of extract, dissolved into ethanol 80%, volume was chopped down to a flask limit of 50 mL (1000 μg/mL).

To obtain maximum absorption is done by testing the absorption of a comparative solution concentrations quercetin 50 μg/mL. After the maximum absorption is obtained, then measure the solution that has been gradually diluted at the maximum wavelength to be obtained calibration curve. Pipettes 0.5 mL of quercetin Comparator solution, add 1.5 mL ethanol P, aluminum chloride P 10% 0.1 mL, sodium acetate 1 M 0.1 mL and 2.8 mL distilled water. Shake and let stand for 30 minutes at room temperature. Measure

absorption at maximum wavelengths. After that determine the absorption of test solution utilizing 0.5 mL test solution add 1.5 mL ethanol P, aluminum chloride P 10% 0.1 mL, sodium acetate 1 M and 2.8 mL distilled water. Shake and let stand for 30 minutes at room temperature. Measure absorption at the maximum wavelength. [1]

2. Alkaloid

Weigh approximately 2 grams of extract add 100 mL methanol P and 10 mL Ammoniac P, preheat above the water bath for 30 minutes, strain. Repeat extraction 2 times using the same type and number of solvents. Add 50 mL of 1 N hydrochloric acid and collect filtrate, darken it to a volume of approximately 25 mL, strain it into a separating funnel. Base filtrate with Ammoniac P to pH ± 10 using pH indicator, extract 3 times with 25 mL chloroform P. Collect and darken the chloroform phase at a temperature of 50ºC, then dry at a temperature of 100ºC to a fixed weight. Calculate residual drying as total alkaloids. [1]

3. Saponin

The determination of saponins levels is done by the gravimetric method. An extract of 1.25 G was reflux with 50 mL of petroleum ether at 60-80°C for 30 minutes. After cold, the petroleum ether solution is discarded and the residue left is dissolved in 50 mL of ethyl acetate. The solution is transferred to the separating funnel and then separated ethyl acetate solution. The residual residue is dissolved with N-butanol by 3 repetitions each 50 mL, then evaporated, the remaining evaporation is dissolved with methanol 10 mL and then this solution is placed into 50 mL of diethyl ether while stirring. Precipitate formed in the mixture is poured on a filter paper that has been known to the weight. Precipitates are then rinsed with 10 mL of diethyl ether. The filter paper is dried at 105°C to a fixed weight. The difference in the weight of the filter paper before and after the screening is set as the weight of saponins. [14]

Determination of Antioxidant Activity

Preparation of DPPH Solution 25 µg/mL

Weighed more or less 10 mg of DPPH dissolved with methanol p.a up to 100 mL, then placed in a volumetric flask coated with aluminum foil. Dip the dissolution until the boundary mark then shakes until homogeneous and obtained DPPH solutions with a concentration of 100 µg/mL. Then diluted 12.5 mL solution DPPH concentration 100 µg/mL insert in a flask 50 mL dipped methanol p.a until the boundary mark then shake to homogeneous and obtained DPPH solution with a concentration of 25 µg/mL.

Preparation of Blanko Solution and Maximum Wavelength Optimization DPPH

Pipette 3.8 mL of DPPH solution (25 µg/mL) into the reaction tube. Then added methanol p.a as much as 0.2 mL and homogenized and the vial was closed with aluminum foil. It was then incubated in a dark room for 30 minutes. Determine the absorbance spectrum using the UV-Visible spectrophotometer at 400-800 nm wavelengths and determine the maximum wavelength.

Preparation of Gallic Acid Comparative Solution

Weighed gallic Acid as much as 10 mg. dissolved with methanol p.a, included in the measuring flask and then added water up to 100 mL (100 µg/mL). Furthermore, the concentration series is 40, 50, 60, 70, 80 µg/mL with a way pipette 4, 5, 6, 7 and 8 mL from a solution of 100 µg/mL each included in a 10 mL measuring flask and added methanol p.a to the boundary mark. Each concentration was pipette as much as 0.2 ml of the acid Gallic solution and input into the vial, then add 3.8 ml of DPPH solution 25 µg/mL. The mixture is homogenized and left for 30 minutes in the dark, determine the absorbance spectrum using the UV-Visible spectrophotometer at the maximum wavelength of DPPH. [15]

Antioxidant Activity Ethanol Extract of Garlic and Black Garlic

Weighed extract of 100 mg, then dissolved with methanol p.a in a flask of 100 mL, then obtained a concentration of 1000 μg/mL. Then dilute by adding methanol p.a that obtained samples with concentrations of 100, 300, 500, 700, 900 μg/ml through pipette 1, 3, 5, 7, 9 mL of a solution of 1000 μg/mL, each of which is included in the 10 mL flask and added methanol p.a to the limit. For the determination of the antioxidant activity of each concentration pipette as much as 0.2 ml of the sample solution and input into the vial, then add 3.8 ml of DPPH solution 25 μg/mL. The mixture is homogenized and left for 30 minutes in the dark, absorbance is measured by the UV-Vis spectrophotometer at the maximum wavelength of DPPH. The sample antioxidant activity is determined by the magnitude of the DPPH radical absorption barrier through the calculation of DPPH absorption inhibition. [15]

Analyze Data

Analyze data uses linear regression equations, calculation of inhibition and IC50 percent with regression equations.

Calculation formula of percent inhibition:

$$\%\text{Inhibition} = \frac{abs\ control - abs\ sample}{abs\ control} \times 100\%$$

Data obtained is processed using statistic test One Way ANOVA.

RESULTS AND DISCUSSION

The samples used are garlic bulbs derived from Paimban in Pariangan Village, Pariangan Sub District, Tanah Datar Regency, West Sumatera. Plant identification has been conducted in the Herbarium Laboratory of Biology Department of FMIPA, Andalas University (ANDA) Campus of Limau Manis, Padang, West Sumatera. The purpose of identification is to know the identity of the sample to be used. Based on the results of such identification can be noted that the sample used in this study is garlic (*Allium sativum* L.) with the Amaryllidaceae family.

Result of the characterization of garlic simplisia, namely the determination of the drying drill which aims to give minimal or range limitation on the size of the compounds that are lost in the drying process, the shrinkage of the tuber bulbs of garlic, amount about 9.816% ± 0.0240. Determination of the total ash content that aims to determine the mineral content contained in the symptoms, total ash content of 2.5508% ± 0.0701. The acid insoluble ash content of 0.377% ± 0.0328. Water-soluble content of 12.6556% ± 0.3730. Ethanol soluble pollen rate of 4.7077% ± 0.0982. Of all of the Simplisia characterization test is done stating that the tuber powder simplisia of garlic meets the requirements because the determination of the shrinkage drying of not more than 10%, total ash content of not more than 3.0%, the ash content of insoluble acids no more From 1.0%, water-soluble content of not less than 5.0% and soluble pollen content of ethanol is not less than 4.0%. [1]

Fresh garlic bulbs are processed into black garlic by inserted garlic bulbs into the rice cooker and arranged no overlap to prevent the damage. The Rice cooker is closed and set in warm keep mode and left for 12 days. During the process of warming, garlic occurs color change, texture, flavor and aroma to black garlic. The color change that occurs is from white to yellow, then light brown, dark brown until finally to black. The texture of garlic changes from hard, then flaccid and juicy to dry, and into black garlic. The garlic flavor changes from spicy to sweet when it becomes black garlic. The scent of garlic changes from the pungent smell until it doesn't sting when it becomes black garlic.

During the process of heating an unstable component of garlic including allysine transformed into a stable component like S-Allyl Cysteine (SAC) (Lee et al., 2009). The increase of S-allyl Cysteine (SAC) occurs because of the enzymatic hydrolysis of γ-glutamyl-S-allyl Cysteine by γ-glutamyl transpeptidase to S-allyl Cysteine (SAC). S-allyl Cysteine (SAC) is one of the components that serve as an antioxidant. [16]

Garlic and black garlic were extracted by the method of maceration using

the 70% ethanol solvent and the yield obtained a garlic ethanol extract 20.4835% and a black garlic ethanol extract. 13.3153%.

The specific characterization results of extracts on the identity test of garlic ethanol extract obtained the name of the extract of Allii sativi bulbii extractum spissum from the plant of garlic (Allium sativum L.), the part used is the tuber. The identity test of the black garlic ethanol extract obtained the name of the extract of Allii sativi bulbii extractum spissum made from black garlic, the part used is the tuber. The results of the organoleptic test of garlic ethanol extract and black garlic ethanol extract form a viscous extract, brown colored, typical smell of aromatic sting, with a spicy flavor, but on the black garlic extract taste sweet.

Non-specific characterization of extracts on the determination of total ash content of garlic ethanol extract obtained yield 1.9555% ± 0.1299. The total ash content of black garlic ethanol extract is 1.3052% ± 0.3098. The insoluble ash content of garlic ethanol extract is 0.5935% ± 0.1261. The insoluble ash content of the black garlic ethanol extract is 0.5801% ± 0.3106. The moisture content of garlic ethanol extract is 7.6667% ± 0.5186. The water content of black garlic ethanol extract is 6.74% ± 0.4563. Of all the characterization of extracts made stating that garlic ethanol extract and black garlic Ethanol extract meets the requirements because the total ash content is no more than 2.7%, the ash content of insoluble acids is not more than 0.7% and moisture content not more than 12%. [17]

Qualitative analysis of garlic ethanol extract and black garlic Ethanol extract was carried out six Tests namely phenol test, flavonoid test, alkaloid test, saponin test, tannin test, and steroid test. The positive phytochemical screening test contains a flavonoids compound characterized by an orange-red color reaction, the extract reacts when added ethanol, Mg and concentrated hydrochloric acid. The test of the positive phenol compound is characterized by a blackish-blue color or blackish-green when reacted with iron (III) chloride.

The result of the saponin test is positive the reacted with 10 mL water and after shaken formed fixed foam for 10 minutes as high as 1 cm in addition of HCl 2 N foam is not lost. The test of tannins is reacted with iron (III) 10% chloride, positive results are characterized by dark blackish-blue or blackish-green, steroids that show positive results provide a green color when added chloroform and sulfuric acid.

The results obtained on garlic ethanol extract and black garlic Ethanol extract showed positive results in the flavonoid compounds, alkaloids, and saponins. The results can be seen in Table 1.

Table I. Qualitative Analysis Result

No.	Pengujian	Ekstrak Etanol Bawang Putih	Esktrak Etanol Bawang Putih Hitam
1.	Fenol a. With $FeCl_3$	- (Does not form blackish green color)	- (Does not form blackish green color)
2.	Flavonoid a. Glikosida-3-flovanol (wth zink powder)	- (Does not form intensive red color)	- (Does not form intensive red color)
	b. Flavon, kalkon, auron (with Mg powder)	+ (Orange)	+ (Orange)
3.	Alkaloid a. With reagen Bouchardat LP	+ (Brown to black precipitate)	+ (Brown to black precipitate)
	b. With reagen Mayer LP	+ (White precipitate)	+ (White precipitate)
4.	Saponin a. With hot water + HCl	+ (Formed Froth)	+ (Formed Froth)
5.	Steroid a. With kloroform	- (Not formed in blue at the edges of the plate drops)	- (Not formed in blue at the edges of the plate drops)
6.	Tannin a. With $FeCl_3$	- (Does not form blackish green color)	- (t Does not form blackish green color)

Keterangan : (+) = Contains chemical compounds
(-) = No chemical compound content

Determination of flavonoids levels as quercetin is done using the standard solution quercetin, first do determination of the maximum wavelength quercetin at concentrations 50 µg/mL obtained wavelength 438.5 nm. Then made the calibration curve with some concentrations of quercetin namely 30, 40, 50, 60 and 70 µg/mL obtained absorbance 0.281, 0.386, 0.484, 0.588, 0.690 and acquired a linear regression y = 0, 0102x-0.0242. Can be seen in Figure 1.

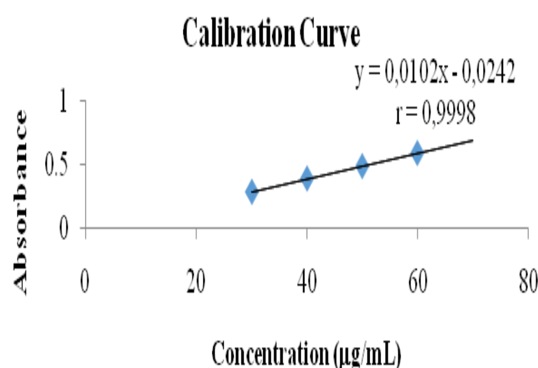

Figure 1. Calibration Curve

Then determined the flavonoids levels on garlic ethanol extract and black garlic ethanol extract by measuring the absorption of the sample at concentrations of 1000 µg/mL performed 3 times the repetition. In garlic ethanol extract obtained absorbance 0.243, 0.247, 0.228 and obtained a rate of 2.6196%, 2.6588%, 2.4725% obtained average levels of flavonoids 2.5836% ± 0.0982. In black garlic ethanol extract obtained absorbance 0.304, 0.273, 0.272 and obtained a rate of 3.2176%, 2.9137%, 2.9039% obtained average flavonoids levels 3.0117% ± 0.1783. Once conducted a statistical test it is known that the flavonoids extracts of garlic ethanol extract and black garlic ethanol extract have a significant difference (P-value < 0.05).

The flavonoids content of black garlic ethanol extract is greater than that of black garlic. According to Choi *et al* (2014), [5] this can be caused by warming that is done on garlic so that it affects the availability of flavonoids in black garlic, depending on the magnitude of temperature and length of heating, sensitivity to heat and physicochemical environments. Due to the heating process, phenolic components will increase the free fraction of phenolic acid where there will be decreased esters, glycosides, and ester bonds. [18]

Determination of alkaloid levels using the gravimetric method, determination of alkaloid levels is calculated from the dried result at a fixed temperature. In ethanol extract of garlic obtained an alkaloids rate of 5.4130% ± 0.2357. In ethanol extract of black garlic obtained an alkaloid rate of 6.9658% ± 1.5362. Once the statistical test is conducted, it can be noted that the alkaloid levels in garlic and garlic ethanol extracts are not significantly different (P-value > 0.05).

Determination of saponins levels using the Gravimetric method, the weight difference of the filter paper before and after filtration is set as the weight of saponins. In garlic ethanol extract obtained saponins rate of 1.1007% ± 0.1797. In black garlic ethanol extract obtained saponins rate of 1.7835% ± 0.2017. Once a statistical test is obtained the results of the saponins rate on garlic and black garlic Ethanol extract have a significant difference (P-value of < 0.05).

Determination of the power of antioxidant activity using the reagent of DPPH determined by the UV-Vis double beam spectrophotometry. Antioxidants are defined as compounds that are capable of protecting cells from the dangers of reactive oxygen free radicals. [19] DPPH will donate a hydrogen atom when reacting with the compound will then be a reduced form marked with a loss of violet color. The radical method 1.1-Diphenyl, 2-Pikril Hydrazyl (DPPH) is a measurement of antioxidant activity that uses only small quantities of samples and short amounts of time. The antioxidant activity of a compound is demonstrated by the absorption barrier of DPPH at a wavelength of 515-517 nm. Where DPPH has a strong absorption at 515-517 nm wavelengths with

a dark violet color. DPPH has a silent electron that causes the DPPH to be highly reactive to capture electrons or other hydrogen radicals to become stable molecules. This method was first proposed by Marsden Blois in 1958. [20]

The maximum wavelength of DPPH obtained is 515.5 nm with Absorbance 0.681. The magnitude of antioxidant activity is characterized by the value of IC_{50}, which is the concentration of sample solution needed to inhibit 50% of DPPH free radicals. In addition, antioxidant activity is also dependent on its chemical content.

At the test of antioxidant activity of the Gallic Acid, the IC_{50} value or the activity of free radical antidote of 50% of Gallic acid obtained at 66.0801 μg/mL, 66.0197 μg/mL, and 66.4301 μg/mL, obtained an average IC_{50} value of 66.1766 μg/mL ± 0.2215 (strong antioxidant 50-100 mg/mL). Then at testing the antioxidant activity of garlic ethanol extract, the value of IC_{50} or the activity of free radical amounting to 50% of garlic ethanol extract obtained at 671.7395 μg/mL, 670.3115 μg/mL, and 668.0490 μg/mL, obtained an average IC_{50} value of 670.0333 μg/mL ± 1.8609 (very weak antioxidant 200-1000 mg/mL). Then at testing the antioxidant activity of black garlic ethanol extract, the value of IC_{50} or the activity of free radical amounting to 50% of black garlic ethanol extract obtained at 635.9285 μg/mL, 639.4923 μg/mL, and 637.9658 μg/mL, obtained an average IC_{50} value of 637.7955 μg/mL ± 1.7879 (very weak antioxidant 200-1000 mg/mL).

Then the IC_{50} value obtained is a statistical test using SPSS to see if the IC_{50} value differs significantly or not. Testing conducted is a test of normality, homogeneity, and One Way ANOVA. When the normality test is obtained the value of P-Value Gallic acid $0.261 > 0.05 = H_0$ received = Normal distributed data, value p-value of garlic ethanol extract $0.752 > 0.05 = H_0$ received = Normal distributed data, and the P-value value of onion ethanol extract Black white $0.842 > 0.05 = H_0$ received = Normal distributed data. Followed by Test homogeneity, obtained P-value value $0.194 > 0.05 = H_0$ received = Data spread homogeneous. After that, it is followed by a test of One Way ANOVA, obtained P-value value of $0.000 < 0.05 = H_0$ rejected = There is a significant difference. Based on statistical test results, it is known that the value of IC_{50} Gallic acid, garlic ethanol extract and black garlic ethanol extract have a significant difference.

The factors that affect the small antioxidant activity are physical factors, where the high oxygen pressure, the breadth of contact with oxygen and heating that causes decreased antioxidant activity of the sample. It can also be caused by a less precise manner of workmanship such as choosing the manner of drying that is hardened, where the pressure and extent of contact with oxygen is high and the heating temperature is not adjustable so that decreased activity Antioxidant. Improper drying process can decrease antioxidant activity. [21]

Decreased antioxidant activity can also be caused in the process of thickening extract is at the time of the vacuum rotary evaporator, because it uses a temperature of 70°C so that the compounds that serve as antioxidants are damaged in the process of thickening The. It is recommended that the vacuum rotary evaporator is performed at a temperature of no more than 60°C, so that the compounds that do not hold heating are not damaged. In the process of storage can also cause decreased antioxidant activity, because the storage temperature is not set, the storage temperature should be set and also the place is not directly exposed to the sun. Direct exposure to the sun on the extracts can cause the compounds in the extract to be oxidized, resulting in decreased oxidative activity. [22]

CONCLUSION

Based on research that has been conducted on the antioxidant activity test of garlic ethanol extract (Allium sativum L.) and black garlic, it can be concluded that:

1. The chemical compounds contained in garlic ethanol extract and black garlic Ethanol extract showed positive results in the flavonoid compounds, alkaloids and saponins.
2. Quantitative analysis results of garlic ethanol extract flavonoids compounds was 2.5836% ± 0.0982, alkaloids was 5.413% ± 0.2357 and saponins amounting to 1.1007% ± 0.1797. On the black garlic ethanol extract flavonoids compounds was 3.0117% ± 0.1783, alkaloids was 6.9658% ± 1.5362 and saponins amounted to 1.7835% ± 0.2017. Based on a statistical test it is known that the levels of flavonoids and saponins in garlic and black garlic have significant difference, while alkaloid levels do not have significant differences.
3. Antioxidant activity of Gallic acid obtained IC_{50} at concentrations 66.1766 µg/mL ± 0.2215 (strong antioxidant 50-100 µg/mL), in garlic ethanol extract obtained the value of IC_{50} at concentrations 670.0333 µg/mL ± 1.8609 (very weak antioxidant 200-1000 µg/mL) and in black garlic ethanol extract obtained IC_{50} value at a concentration 637.7955 µg/mL ± 1.7879 (very weak antioxidant 200-1000 µg/mL). Based on a statistical test it is known that the value of IC_{50} on the Gallic acid, garlic ethanol extract and black garlic ethanol extract have significant difference.

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