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Black Garlic (*Allium sativum* L.) Ethanol Extract: Total Phenolic Content and Antioxidant Activity Test Determination Using the Soxhletation Method

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Abstract:
A plant that is commonly used in the food and health industries is garlic (Allium sativum L.). One powerful antioxidant found in garlic is called allicin. The garlic used in this study was heated to 70°C in order to produce black garlic. Determining the phenolic content overall and the antioxidant activity of the Black Garlic ethanol extract are the goals of this investigation. The Folin Ciocalteu method was used to calculate the total phenolic content, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was used to test for antioxidant activity, and the soxhletation method was used to extract black garlic. The findings indicated that the ethanol extract of black garlic had an overall phenolic content of 8.96% and an IC50 value of 4.7527 µg/mL (extremely strong antioxidant) for its antioxidant activity.

Keywords: Garlic, Black Garlic, Soxhletation, Total Phenolics, Antioxidant.

1. Introduction

One kind of plant that has been employed extensively in the culinary and medical industries is garlic (*Allium sativum* L.). In traditional medicine, garlic (*Allium sativum* L.) has been utilized extensively (Pramitha & Nikomang, 2020). Regarding its composition, garlic comprises roughly 63% water, 28% carbohydrates, 2.3% organosulfur component acids, 2% protein (alliinase), 1.2% free amino acids, and 1.5% fiber. When garlic is chopped or crushed, alliinase is released, which transforms alliin into allicin (Kimura *et al*., 2017).

Black garlic can be made by fermenting garlic. A fermented product of garlic, black garlic is fermented for about a month to 65–80 degrees Celsius and 70–80% relative humidity. According to Pramitha & Nikomang (2020), black garlic has a taste that is similar to that of garlic, a moderate scent, and a black color due to the low water content.

Garlic has several recognized pharmacological benefits, including antibacterial, antihypertensive, anti-cholesterol, and antioxidant properties (Azhar & Yuliawati, 2021). Antioxidants, also known as free radical trapping compounds, are chemicals that have the ability to neutralize free radicals or that work to shield the biological system of the body from harmful effects resulting from reactions or processes that produce excessive oxidation. According to Fessenden & Fessenden (1986), free radical compounds are produced by the body through a variety of intricate chemical processes. These processes include breathing, cell metabolism, excessive exercise, inflammation, and exposure to environmental pollutants like chemicals, smoke from cigarettes, motor vehicle fumes, and sunlight.

A lengthy heating duration of 7, 14, 21, 28, and 35 days is used to make black garlics, according to research by Choi *et al*. (2014). The results of this study indicate that roasting black garlic for 21 days increased its

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antioxidant activity to its maximum. With fresh garlic having an antioxidant activity of 4.65% and black garlic having a greater antioxidant activity of 74.48%, the test results were obtained using the DPPH method.

The antioxidant activity of black garlic extracted with ethanol was studied by Sukmawati (2022). Black garlic was heated for 21 days to obtain the extract by the maceration method, and 70% ethanol was used as the solvent. This resulted in an IC50 value of 6.6000 µg/mL (very strong antioxidant <50 µg/mL). Putri's research (2022) employed ethyl acetate and n-hexane fractions, heating black garlic for 21 days to obtain extracts using the maceration method. Ethanol 70% was used as the solvent, and the IC50 values for the n-hexane fraction were 194.5885 µg/mL (weak antioxidant >100 µg/mL) and the ethyl acetate fraction were 31.6818 µg/mL (very strong antioxidant <50µg/mL).

Soxhletation is a process that involves repeatedly filtering solid materials with certain solvents and a soxhlet instrument in order to separate their constituent parts. The extraction of chemical compounds from natural materials using volatile solvents that can dissolve such compounds through repeated filtering is the basis of the soxhletation process. When compared to other procedures, the soxhletation method has the benefit of requiring less solvent and sample, and it also uses less time because the process proceeds quickly (Marjoni, 2016).

The author's research interests are centered around determining the total phenolic content and evaluating the antioxidant activity of an ethanol extract of black garlic (*Allium sativum* L.) through the use of the soxhletation method. The folin ciocalteu principle is used to determine levels, and the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method is used to test for antioxidant activity.

2. Data Collection Methods

Tools and materials

A UV-visible spectrophotometer (Shimadzu UV 1800), oven (Memmert), furnace (carbolite), water bath (Memmert Basic Water Bath – WNB 14), rice cooker (Miyako), rotary evaporator (Heidolph), analytical balance (Biobase), and standard laboratory glassware were employed in this study.

The ingredients used are garlic bulbs (*Allium sativum L.*), ethanol (C_2H_5OH) 96% (Novalindo), distilled water (Novalindo), Gallic acid ($C_7H_6O_5$) (Sigma), 1,1- diphenyl-2-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$) (Himedia), Folin-Ciocalteu (Merck), methanol (CH_3OH) p.a (Merck), ammonia (NH_3) (Novalindo), chloroform ($CHCl_3$) (Merck), sulfuric acid (H_2SO_4) (Merck), acetic acid (CH_3COOH) (Merck), iron III Chloride ($FeCl_3$) (Merck), hydrochloric acid (HCl) (Merck), mercury II chloride ($HgCl_2$) (Merck), potassium iodide (KI) (Merck), iodide (I_2) (Merck), sodium hydroxide (NaOH) (Merck), bismuth nitrate $Bi(NO_3)_2$ (Merck).

3. Analytical Methods

Procedures

Garlic Sampling (*Allium sativum L.*)

Two kilograms of garlic bulbs (*Allium sativum* L.), bought at the Ibuh Payakumbuh market in the West Payakumbuh District of Payakumbuh City, West Sumatra, served as the sample.

Plant Identification

Identification of Garlic Bulbs (*Allium sativum L.*) was carried out at the Andalas University (UNAND) Herbarium, Biology Department, FMIPA, Andalas University.

How to Prepare Black Garlic

How to fermented Black Garlic

The first step is to choose 2 kg of intact garlic bulbs and place them in a rice cooker covered with aluminum foil at 70°C for 21 days to produce black garlic (Azizah 2020). Following formation, sorting is done to separate the black garlic from dirt or other unwanted materials. This is done by eliminating any unnecessary components and peeling the skin of the Black Garlic bulbs to obtain pure, usable black garlic. Next, slice into little pieces after sorting. A knife can be used to chop black garlic into thin slices or pieces the right size (Republic of Indonesia Ministry of Health, 1985).

Preparing Black Garlic Extract for Ethanol.

96% ethanol is used as a solvent in the soxhletation process to create black garlic ethanol extract. A sleeve or lead is used to hold 100 grams of finely chopped black garlic that has been wrapped in filter paper and secured with thread. Fill a round-bottom flask with two liters of solvent using a socket set (Marjoni, 2016). Until the

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cycle droplets lose their color, soxhletation is continued. After that, a thick extract of black garlic was produced by evaporating the liquid extract in a rotary evaporator set at 40°C (Puspitasari, 2017).

Examination of Extract Characterization Black Garlic Ethanol

1. Specific Characterization of the Extract

a. Extract Identity Check Description of the nomenclature includes: name of the extract, Latin name of the plant, part of the plant, part of the plant used, and the Indonesian name of the plant (Ministry of Health of the Republic of Indonesia, 2000).

b. Organoleptic Examination

The extract obtained was tested organoleptically using five sense observations to describe the shape, color, smell and taste of the extract (Ministry of Health of the Republic of Indonesia, 2000).

2. Non-Specific Characterization Extract

Determining non-specific characterization of extracts, namely determining chemical, microbiological and physiological aspects that will affect consumer safety and stability. Non-specific characterization of extracts includes:

a. Drying Shrinkage

The extract is weighed carefully in the amount of 1 gram to 2 grams and placed in a closed shallow weighing bottle which has previously been heated at 105°C for 30 minutes and has been tarred. Before weighing, the extract is spread evenly in a weighing bottle, by shaking the bottle, until it forms a layer approximately 5 mm to 10 mm thick. If the extract being tested is a thick extract, blend it with a stir stick. Then cooled in a desiccator for ± 30 minutes and then weighed (Department of Health of the Republic of Indonesia, 2000).

b. Determination of Total Ash Content

Weigh out two grams of the crushed extract, then carefully weigh it, transfer it into a ground-and-leveled silicate crucible. Gradually burn until the charcoal is consumed, then let it cool and weigh it. If the charcoal cannot be removed using this procedure, add hot water and filter through paper that is free of ash. In the same crucible, put the remaining filtration and filter paper. Put the filtrate in a crucible, let it evaporate, light it, and then weigh it to ensure it maintains its weight (Department of Health, Republic of Indonesia, 2000). Determine the amount of ash in materials that have been air dried.

c. Determination of Insoluble Ash Content Sour

Ash obtained from determining the ash content, boil with 25 mL of dilute sulfuric acid for 5 minutes, collect the part that does not dissolve in the acid, filter through ash-free filter paper, wash with hot water, ignite until the weight remains constant, weigh. Calculate the acid-insoluble ash content of the air-dried material.

Extract Phytochemical Screening Test

Phytochemical screening tests of ethanol extract from Black Garlic include:

1. Alkaloid test

Add 1 ml of 2N HCl and 9 mL of water to 0.5 grams of black garlic extract, heat in a water bath for 2 minutes, cool and strain. Divided into 3 tubes then added to each of Mayer's, Wagner's and reagents Dragendrof. The presence of alkaloids is indicated by the formation of a white or yellowish precipitate in Mayer's reagent, a brown precipitate in Wagner's reagent and a red precipitate in Dragendorf's reagent (Department of Health of the Republic of Indonesia, 1995).

2. Flavonoid test

A total of 0.5 grams of black garlic extract was added to 3 ml of 95% ethanol, added to 100 mg of magnesium powder and 10 drops of concentrated hydrochloric acid, if a red-orange to purple-red color occurs, this indicates the presence of flavonoids. (Ministry of Health of the Republic of Indonesia, 1995).

3. Saponin test

Put 0.5 grams of black garlic ethanol extract into a test tube, add 10 mL of hot water, cool, and then shake for a long time. 10 seconds. Positive results are indicated by the formation of stable foam for no less than 10 minutes, when 1 drop of 2 N hydrochloric acid is added, the foam does not disappear (Ministry of Health of the Republic of Indonesia, 1995).

ISSN: 2519-9889
Impact Factor: 5.9

4. Phenol test

A total of 0.5 g of black garlic ethanol extract was added to a solution of vanillin P 10% w/v in ethanol (96%) then added 2 drops of hydrochloric acid P, the part containing phenol had an intense red color (Hanani, 2017).

5. Tannins test

A total of 0.5 g of black garlic ethanol extract added 3 drops of 1% $FeCl_3$ reagent, positive results containing tannins were indicated by a blackish green color (Harborne, 1987).

6. Terpenoid Test

A total of 0.5 g of black garlic ethanol extract was added with 3 mL of chloroform or 3 mL of 96% ethanol and 2 mL of concentrated sulfuric acid (H_2SO_4) and 2 mL of anhydrous acetic acid were added. The formation of a brownish color between the surfaces indicates the presence of terpenoid compounds (Department of Health of the Republic of Indonesia, 1979).

Determination of Total Phenolic Content

Determination of total phenolic content using Folin-Ciocalteu solution reagent with UV-Vis spectrophotometry.

A. Determination of Wavelength Maximum Gallic Acid

Weigh 10 mg of gallic acid, then dissolve it with methanol p.a to 100 mL (100 µg/mL). Next, a gallic acid solution was made with a concentration of 60 µg/mL by pipetting 6 mL of the stock solution (100 µg/mL) into a 10 mL volumetric flask. Then pipette Put 1 mL of 50 µg/mL concentration solution into the vial, add 5 mL of Folin-Ciocalteu LP solution (7.5% in water). Let stand for 8 minutes, add 4 mL of 1% NaOH then incubate for 1 hour at room temperature. Determine the maximum wavelength using a UV-Vis spectrophotometer at a wavelength of 400-800 nm (Ministry of Health of the Republic of Indonesia, 2011).

B. Making a Calibration Curve Gallic Acid

A concentration solution of 30, 40, 50, 60, and 70 µg/mL by pipetting 3 mL, 4 mL, 5 mL, 6 mL, and 7 mL respectively from the 100 µg/mL gallic acid stock solution. then put into a 10 mL measuring flask, add methanol p.a to the mark. From each pipette concentration of 1 mL, put it into the vial, add 5 mL of Folin-Ciocalteu LP solution (7.5% in water). Leave for 8 minutes, add 4 mL of 1% NaOH, incubate for 1 hour at room temperature. Determine the absorbance at the maximum wavelength with a UV-Vis Spectrophotometer (Ministry of Health of the Republic of Indonesia, 2011).

C. Determination of Total Phenolic Content of Ethanol Extract of Black Garlic

Weigh out 10 mg of Black Garlic Ethanol Extract, transfer it to a 100 mL volumetric flask, and then gradually add methanol up to the limit (100 µg/mL). Next, 5 mL of the stock solution (100 µg/mL) were pipetted into a 10 mL volumetric flask, and methanol was added p.a. until the limit was reached. This resulted in a solution with a concentration of 50 µg/mL. 5 mL of the Folin-Ciocalteu LP solution (7.5% in water) should be added to a 1 mL pipette that has been filled with water. After 8 minutes, add 4 mL of 1% NaOH, and let it sit at room temperature for an hour. Using a UV-Vis Spectrophotometer, ascertain the absorbance at the maximum wavelength (Ministry of Health of the Republic of Indonesia, 2011).

Antioxidant Activity Test

A. Preparation of 30 µg/mL DPPH Solution

Weigh 10 mg DPPH (BM 394.33) then put it in a 100 mL measuring flask, add methanol p.a to the limit mark, line the measuring flask with aluminium foil, then shake until homogeneous and obtain a DPPH solution with a concentration of 100 µg/mL. Then dilute by pipetting 15 mL of DPPH solution with a concentration of 100 µg/mL, put it in a 50 mL measuring flask, add enough solvent to the limit mark, then shake until homogeneous and obtain a DPPH solution with this concentration 30 µg/mL (Molyneux, 2004).

B. Making Optimization Blank Solutions

DPPH Maximum Wavelength Pipette 3.8 mL of DPPH solution (30 µg/mL) into the vial. Then 0.2 mL of methanol was added, homogenized and the vial was covered with aluminium foil, then incubated in a dark room for 30 minutes. Determine the maximum wavelength using a UV-Vis spectrophotometer at a wavelength of 400-800 nm (Andayani *et al*., 2008).

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C. Making a comparison solution Gallic Acid

Gallic acid concentration 100 µg/mL. Next, a concentration series of 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL, and 5 µg/mL. By pipetting the stock solution (100 µg/mL) into 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL respectively. then put into a 10 mL flask until boundary sign. To determine the antioxidant activity of each concentration, pipet 0.2 mL of solution into a vial then add 3.8 mL of DPPH solution (30 µg/mL) then cover the vial with aluminium foil. The mixture was homogenized and incubated for 30 minutes in the dark. Absorbance of various concentrations was measured using UV-Vis spectrophotometry at the maximum DPPH wavelength (Andayani *et al*., 2008).

D. Testing of Antioxidant Activity of Ethanol Extract of Black Garlic

Extract solution concentration 100 µg/mL. Next, a series of concentrations of 1 µg/mL, 2 µg/mL, 3 µg/mL, 4 µg/mL and 5 µg/mL were made, by pipetting each 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, 0.5 mL then put into a 10 mL flask then add methanol p.a until the mark. To determine the antioxidant activity, the concentration of Black Garlic was pipetted at 0.2 mL and put into a vial, then added 3.8 mL of 30 µg/mL DPPH solution and covered with aluminium foil. The mixture was homogenized and incubated for 30 minutes in a dark place. Absorption was measured with a UV-Vis spectrophotometer at maximum wavelength. The antioxidant activity of the sample is determined by the amount of inhibition of DPPH radical absorption through the percentage of DPPH absorption inhibition. (Andayani *et al*., 2008).

IC50 determination

The percentage of inhibition of DPPH radicals from the sample solution can be calculated using the formula:

$$\%\ Inhibition = \frac{Control\ Absorbance - Sample\ Absorbance}{Control\ Absorbance}\ x\ 100\%$$

Information:

% Inhibition	: Percentage of antioxidant activity power
Control absorbance (A1)	: DPPH absorbance 30 µg/mL
Sample absorbance (A2)	: Sample absorbance test

After obtaining the percentage of inhibition from each concentration, then the percent of inhibition is plotted on the x and y axes respectively in the linear regression equation y = a + bx. This equation can be used to determine the IC50 value with the formula (Andayani *et al*., 2008).

$$IC50 = \frac{50 - a}{b}$$

RESULTS AND DISCUSSION

Garlic bulbs from the Ibuh Payakumbuh Market in the West Payakumbuh District of Payakumbuh City, West Sumatra, served as the study's samples. At the FMIPA Herbarium Laboratory of the Biology Department, Andalas University (ANDA), Limau Manis Campus, Padang, West Sumatra, plant identification was done. Finding out the identity of the sample that will be used is the goal of sample identification. The materials employed in this investigation were garlic bulbs (*Allium sativum* L.), which belong to the Amaryllidaceae family, according to the identification results. Black garlic is made from whole, fresh garlic bulbs that are fermented at 70°C while covered with aluminium foil. The bulbs are positioned carefully so as not to overlap and to preserve their shape. After that, the rice cooker was left in the "keep warm" setting for 21 days. The garlic undergoes color, texture, flavor, and scent changes while being heated until it turns black. The color shift of black garlic can also be influenced by heating. One way that garlic turns black is by a non-enzymatic browning process. The Maillard reaction is the reason behind the non-enzymatic browning that takes place when garlic is heated. The Maillard reaction is a reaction that occurs between reducing sugars and a number of amino acids.

Then, using a 96% ethanol solvent and the soxhletation process, the black garlic was extracted. After a duration of two and a half hours, the soxhletation process—which consists of six cycles—ends when the cycle droplets on the siphon tube are colorless. If the soxhletation device's siphon tube is full and the filter fluid flows back into the round-bottom flask, then one soxhletation cycle is recorded. A thick extract is obtained by

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Impact Factor: 5.9

separating the liquid extract from the filter liquid using a rotary evaporator, which is the outcome of the soxhletation process. The yield of black garlic extracted with ethanol is 40.13%. Testing was done on the black garlic ethanol extract to determine its specific and non-specific qualities. As well as non-specific extract characterization tests like total ash content, acid insoluble ash content, and drying shrinkage tests, extract specific tests include identification and organoleptic tests. The name Alii sativi bulbus extractum spissum extract from the garlic plant (*Allium sativum* L.) was obtained based on the findings of the specific extract characterization test in the identity test of the ethanol extract of black garlic. The part of the garlic employed was the bulb. The ethanol extract of black garlic yielded positive organoleptic test findings, appearing as a thick, brownish black liquid with a sharp, fragrant scent and a slightly sour and sweet flavor.

Non-specific characterization test of the extract. The first, namely the drying loss test, aims to find out how many compounds are lost or easily evaporate during drying. Drying loss is a parameter for an extract to maintain its quality prevent fungal growth. The results obtained from the drying loss of the ethanol extract of Black Garlic were 8.08%. The next test is to determine the total ash content which aims to determine the mineral content contained in the extract. The results obtained from determining the total ash content of the ethanol extract of Black Garlic are 2.01%. Then proceed with determining the acid insoluble ash content which aims to determine the amount of impurities from external factors originating from impurities. The ash used is ash obtained from determining the total ash content. The result is an acid insoluble ash content of 0.58%. Of all the extract standardizations carried out, it was stated that the ethanol extract of Black Garlic met the requirements because the drying loss was not more than 10%, the total ash content was not more than 2.7%, and the acid insoluble ash content was not more than 0.7% (Department Republic of Indonesia Health, 2011).

The next test is a phytochemical screening test to determine the content of secondary metabolite compounds contained in the ethanol extract of black garlic. The ethanol extract of Black Garlic showed positive results for alkaloids, flavonoids, phenols, tannins, saponins and terpenoids. Determination of the total phenolic content in the ethanol extract of Black Garlic was carried out using the Folin-Ciocalteu method using gallic acid as a standard solution because gallic acid is classified as a simple phenolic acid which is a derivative of hydroxybenzoic acid (Kupina., *et al* 2018). Determination of the maximum absorption wavelength of gallic acid at a concentration of 60 µg/mL as measured by a Double Beam Uv-Vis spectrophotometer. Maximum absorption was obtained at a wavelength of 731.50 nm with absorbance 0.517. It can be seen in Figure 1.

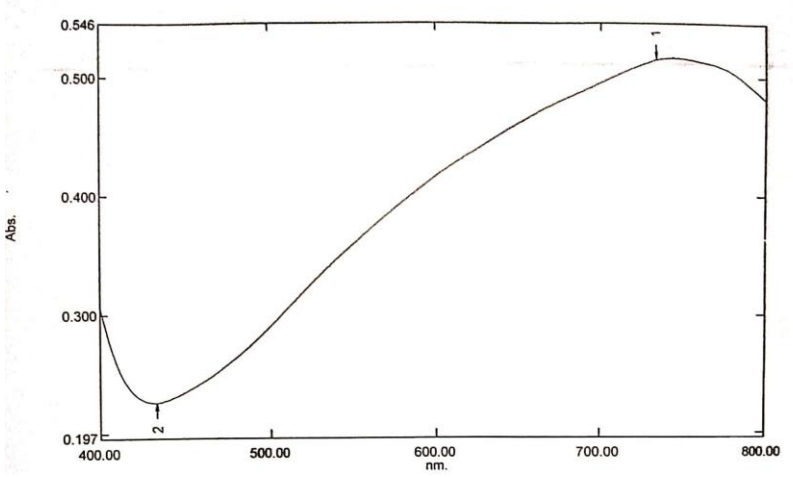

Figure 1. Maximum Absorption Wavelength of Gallic Acid Concentration 60 µg/mL.

The results of determining gallic acid phenolics as a standard for phenolic content at a maximum absorption of 731.50 nm with a concentration of 30, 40, 50, 60, and 70 µg/mL obtained solution absorbance of 0.241, 0.320, 0.402, 0.482, 0.556, we get the equation y = 0.00792x + 0.0042 with a regression coefficient of 0.99985. Can be seen in Figure. 2.

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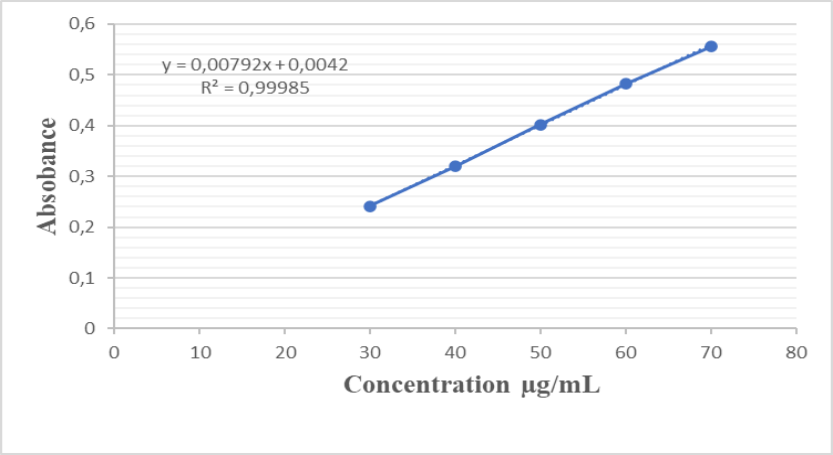

Figure 2. Calibration curve for variations in gallic acid concentration on absorbance.

The results of measuring the total phenolic content of ethanol extract of Black Garlic with a concentration of 50 µg/mL were measured using a UV-Vis spectrophotometer at a maximum gallic acid wavelength of 731.50 nm, and the absorbance with 3 repetitions was 0.364, 0.357, 0.357 obtained an average percent of total phenolic content of 8.96%. Phenolic compounds have oxidation reduction properties which act as antioxidants because phenolic compounds have hydroxyl groups on them aromatic rings which function as donors of hydrogen atoms when reacting with free radical compounds so that the oxidation process is inhibited and free radical compounds are reduced. So, if the phenolic compound has more hydroxyl groups, the antioxidant activity it has will also be higher (Lee *et al*., 2003).

Determination of antioxidant activity using DPPH reagent which was measured using a UV-Vis Double beam spectrophotometer. Antioxidants are compounds that can protect cells from the dangers of reactive oxygen free radicals (Hazima *et al*, 2013). DPPH is a free radical that is stable at room temperature in the form of a blackish violet colored powder, quickly oxidized by light, easily soluble in methanol, with a BM of 394.3 (Molyneux, 2004).

In a reaction with a molecule, DPPH will donate an atom of hydrogen and transform into a reduced form that loses its violet color. A quick and easy way to test antioxidant activity is with the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical technique, which only requires a tiny amount of sample. The absorption barrier of DPPH, which is measured at 515.50 nm, indicates a compound's activity. Dark violet in color, DPPH exhibits high absorption at 515.50 nm. Due to its quiet electrons, DPPH is extremely reactive and can absorb electrons or other hydrogen radicals to transform into stable molecules (Molyneux, 2004). The maximum DPPH wavelength obtained is 515.50 nm and the absorbance is 0.668. Can be seen in Figure 3.

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Impact Factor: 5.9

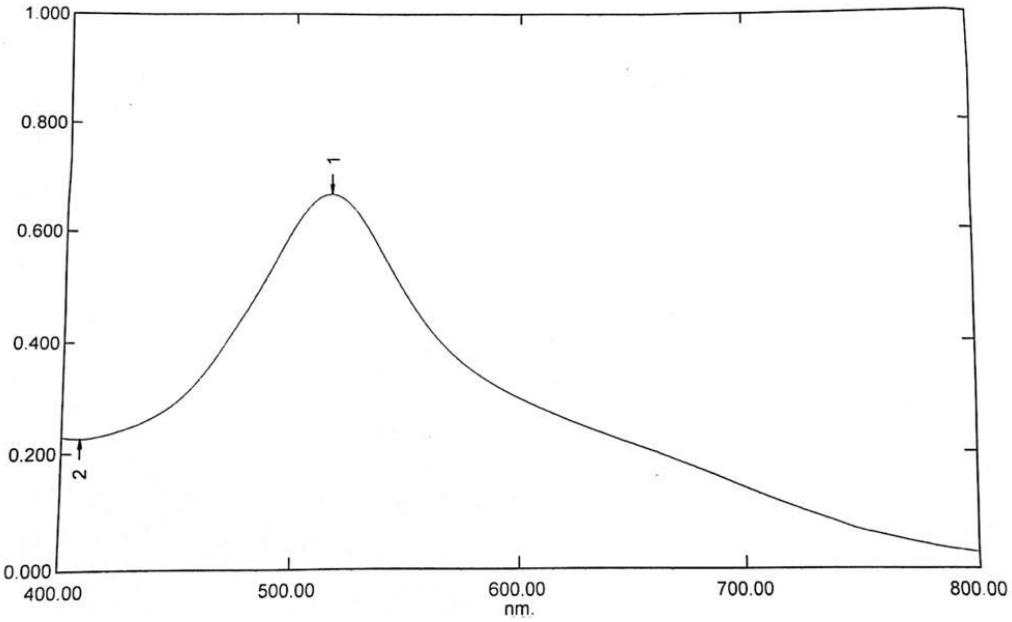

Figure 3. Absorption Wavelength Maximum DPPH Concentration 30 µg/mL.

As a reference, the absorbance of the solution in the test of gallic acid's antioxidant activity was 0.634, 0.558, 0.476, 0.392, and 0.307, while the percent radical scavenging activity was 5.0898%, 16.4670%, 28.7425%, 41.3173%, and 54.0419%. Based on the absorbance results, it can be observed that the absorbance value obtained decreases with increasing sample concentration. This is because the antioxidant compounds have a greater ability to reduce or ward off radicals in DPPH, resulting in a greater percentage of inhibition (Bahriul *et al*, 2014). The gallic acid comparison 4.7000 µg/mL yields the IC50 value, or free radical scavenger activity, which is 50% (extremely strong antioxidant <50 µg/mL).

Table 1. Results of measurement of the absorbance of DPPH + ethanol extract of Black Garlic at a wavelength of 515.50 nm and an absorbance of 0.668 with a double beam spectrophotometer.

No	Concentration(µg/mL)	Absorbance		% inhibition	IC_{50}
		A1	A2		
1.	1	0,668	0,539	19,3113%	4,7527 µg/mL
2.	2		0,482	27,8443%	
3.	3		0,428	35,9281%	
4.	4		0,374	44,0119%	
5.	5		0,322	51,7964%	

Information:
A1: Absorption of 30 µg/mL DPPH radical solution at a maximum wavelength of 515.50 nm
A2: Absorption of 30 µg/mL DPPH radical solution + ethanol extract of black garlic

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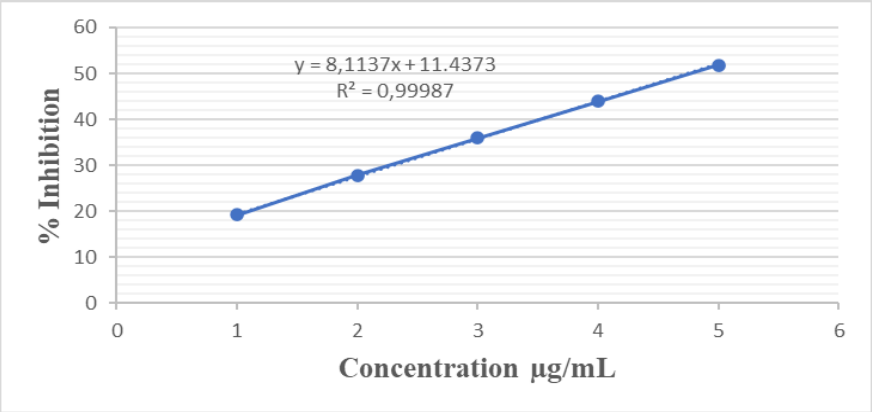

Figure 4. Correlation Curve Between Concentration and % Inhibition of Black Garlic Ethanol Extract.

When black garlic was tested for antioxidant activity, absorbance values of 0.539, 0.482, 0.428, 0.374, and 0.322 were obtained, and percentages of radical scavenging activity were 19.3113%, 27.8443%, 35.9281%, 44.0119%, and 51.7964% (Table 1, Figure 4). Based on the absorbance data, it is evident that lower absorbance values are obtained at higher sample concentrations. This is because higher concentrations of antioxidant compounds are more effective at reducing or inhibiting radicals in DPPH, resulting in higher percentages of inhibition (Bahriul *et al*, 2014). From extract 4.7527 µg/mL (extremely strong antioxidant <50 µg/mL), the IC50 value, or free radical scavenger activity, was obtained.

In previous research, Sukmawati, (2022) tested the antioxidant activity of ethanol extract of Black Garlic by heating the garlic to black garlic for 21 days, obtaining the extract using the maceration method and using 70% ethanol solvent to obtain an IC50 value 6.6000 µg/mL (very strong antioxidant <50 µg/mL. Research by Putri, (2022) tested the activity of the ethyl acetate fraction and the n-hexane fraction of garlic that was blackened with long heating garlic becomes black garlic, namely for 21 days, the extract is obtained using the maceration method and using 70% ethanol solvent, the antioxidant activity of the ethyl acetate fraction has an IC50 value 31.6818 µg/mL (very strong antioxidant <50 µg/mL), the antioxidant activity of the n-hexane fraction had an IC50 value of 194.5385 µg/mL (weak > 100 µg/mL). Based on this, it can be concluded that the method of obtaining the extract or method for extracting the sample and the solvent used can also influence the antioxidant activity obtained. Antioxidant activity in the ethanol extract of garlic that was blackened for 21 days using the soxhletation method obtained the strongest antioxidant activity compared to the maceration method. The advantages of using the soxhletation method are that the solvent and sample used are relatively small and the time used is more efficient because the process is fast (Marjoni, 2016).

CONCLUSION

1. The secondary metabolite compounds of the ethanol extract of Black Garlic showed positive results for alkaloids, flavonoids, tannins, phenols, saponins and terpenoids.
2. The ethanol extract of Black Garlic has a phenolic content of 8.96%.
3. The antioxidant activity of the ethanol extract of Black Garlic was obtained IC50 value 4.7527 µg/mL (very strong antioxidant <50 µg/mL).

SUGGESTION

Future researchers are advised to carry out antioxidant testing using other methods and using different solvents than this researcher, as well as further testing of Black Garlic such as tests in the fields of pharmacology and biology. With a basic heater and a relative humidity of about 70%, making black garlic is fairly straightforward. Antioxidant, antibacterial, anti-inflammatory, and many more properties are among the many advantages of black garlic. Black garlic's secondary metabolite levels will vary according on the extraction technique used.

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