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Antioxidant Activity Combination of Shallot (Allium Ascalonicum L.) and Black Garlic Extracts

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Abstract*—Shallots and garlic are spices in every home that are used as spices in the kitchen. Garlic is fermented at a certain temperature and humidity to produce black garlic. The purpose of this study was to determine the antioxidant activity of the combination of shallot and black garlic extracts in the ratio (4:1), (3:2), and (1:4) compared to vitamin C as a positive control using the DPPH method (1, 1-diphenyl-2-phicrylhydrasyl). The test results of the combination of shallot and black garlic extracts with a comparison (4:1) IC50 value of 81.14 ppm, (3:2) of 110.98 ppm, and (1:4) of 102.26 ppm . The three comparisons that have the highest activity are the ratio (4:1) with an IC50 value of 81.14 ppm with strong activity as an antioxidant, and when compared with the ratio (3:2) and (1:4) have the highest activity.*

***Keywords*—** *Antioxidant, allium ascalonicum L., black garlic, DPPH.*

I. INTRODUCTION

Indonesia is a country rich in plants and spices, and with the rapid advancement of scientific knowledge, it has become evident that there is a growing interest in natural medicine. However, there is still a lack of knowledge about the sorts of plants used as natural treatments [1], such as shallots and garlic, which are generally acknowledged to have health advantages and are commonly used as cooking spices in households [2],[3].Shallots are known to be effective in the treatment of ailments such as fever, diabetes, and cough, and they contain quercetin, a potent antioxidant that inhibits cancer cells growth. Shallots are also known to activate carcinogens and tumor initiators due to their high flavonoid content. Garlic, also known as Allium sativum, is absorbed by glucose in its original form. It is used to prevent and treat many diseases [4], inhibit brain degeneration and the immune system, aid in the aging process, inhibit cancer cell growth, reduce cholesterol levels, function as an antioxidant, and address heart disease, high blood pressure, and obesity [5]. Allicin and alliin are the active ingredients in garlic, which remain relatively stable when dried and have the potential to produce allicin compounds [6]. Black garlic is the result of the natural fermentation of garlic at specific temperature and humidity levels over an extended period [2].

Antioxidants are compounds capable of eliminating, preventing the formation of, or neutralizing the effects of reactive oxygen species. Antioxidant properties are exhibited when a compound donates one or more electrons to prooxidants, subsequently transforming oxidants compounds into more stable ones [7, 8]. Antioxidants biologically counteract the negative effects of oxidants in the body, such as cellular damage. Antioxidants are naturally produced by the human body to counteract the generation of free radicals resulting from stress factors[9]. Free radicals attack the nearest stable molecules and take electrons, the molecules that lose electrons become free radicals, leading to a chain reaction that ultimately damages cells. Antioxidants have the ability to stabilize free radicals before they harm cells [10, 11].

II. RESEARCH METHODOLOGY

A. Materials and Tools

An Ohaus digital scale, rotary evaporator, a Centrion centrifuge, a Shimadzu UV-Vis spectrophotometer, a Scientific vortex mixer, and various glassware are among the tools required. The ingredients include shallot and black garlic extracts, Libermann-Bouchardat reagent, Mayer's reagent, HCl (hydrochloric acid), FeCl3 (iron chloride), H2SO4 (sulfuric acid), ethanol, and DPPH (2,2-diphenyl-1-picrylhydrazyl).

B. Plant Determination

Determination was conducted to ensure that the plant's identity matches the condition of the plants to be studied, in order to avoid errors in selecting the plants for research. Determination was carried out at the Indonesian Institute of Sciences (LIPI), Jl. Ir. H. Juanda No. 13, PO.BOX 309, Bogor 16003, Indonesia [12].

C. The Preparation of Shallot and Black Garlic Extracts

Shallots were finely blended, then macerated with 98% ethanol and left for 24 hours with occasional stirring. The macerated mixture was filtered, and the filtrate was concentrated using a rotary evaporator to obtain a concentrated extract. Black garlic was prepared by fermenting garlic in a rice cooker by pressing the warm button for 10 days. The black garlic was blended, macerated with 98% ethanol, and allowed to stand for 24 hours with occasional stirring. Subsequently, it underwent filtration, and the filtrate was concentrated using a rotary evaporator to obtain a concentrated extract [13].

D. Phytochemical Screening of Shallot and Black Garlic Extracts

Phytochemical screening was conducted to determine the presence of secondary metabolites in the extracts. Phytochemical screening tests were performed to identify the groups of compounds, including alkaloids, triterpenoids, glycosides, flavonoids, saponins, phenols, and tannins [12].

E. Examination of the Quality of Raw Materials for Shallot and Black Garlic Extracts

1. Total Plate Count Test

The total plate count test (TPC) involved pipetting 1 mL from each dilution into sterilized petri dishes (in duplicate). Then, 5 mL of nutrient agar medium was poured into each dish and liquefied at approximately 45°C. The dishes were gently swirled to solidify the medium and then placed in an incubator at 35°C for 24 hours.

2. Mold and Yeast Test

The mold and yeast test included the addition of 5 mL of potato dextrose agar medium, previously liquefied at 45°C, to sterilized petri dishes (in duplicate). Then, 0.5 mL from each dilution was pipetted onto each dish (the seeding method). The dishes were gently swirled to ensure even distribution and incubated at room temperature for 7 days.

3. Aflatoxin Contamination Test

The aflatoxin contamination test was conducted using thin-layer chromatography.

4. Pesticide Residue Test

The pesticide residue test involved dissolving the extract in 95% ethanol, adding petroleum ether, and centrifuging the mixture three times with petroleum ether. The petroleum ether fraction was collected, evaporated, and subjected to column chromatography with florisil as the stationary phase (organochlorine), and gas chromatography (organophosphate).

5. Heavy Metal Contamination Test

The heavy metal contamination test consisted of adding 1 gram of the extract to concentrated HNO3, heating until dry, cooling, and then adding 10 mL of distilled water and acid percolate. The mixture was then measured using AAS [14], [15].

F. Antioxidant Activity

A blank solution was prepared by pipetting 2 mL of a DPPH solution (0.1 mM) into a reaction tube, homogenizing it, and then incubating it in a dark room for 30 minutes. The absorbance was measured using a UV-Vis spectrophotometer at a wavelength range of 400-800 nm, and the maximum wavelength was determined.

The standard curve of the DPPH solution was prepared by weighing 10 mg of DPPH (1,1-diphenyl-2-picrylhydrazyl), dissolving it in analytical grade ethanol in a 25.0 mL volumetric flask, and then diluting it to several concentrations, namely 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm. The absorbance of these solutions was measured at a wavelength of 517 nm.

A vitamin C solution was prepared as a positive control by weighing 10 mg of vitamin C, dissolving it in analytical grade ethanol in a 10.0 mL volumetric flask, and then diluting it to concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm. Each concentration was pipetted with 2 mL and mixed with 2 mL of a 0.1 mM DPPH solution, followed by incubation for 30 minutes and measurement using a spectrophotometer.

Antioxidant activity testing was carried out by preparing extracts of shallots and black garlic in three different ratios: (4:1), (3:2), and (1:4). Dilutions for antioxidant activity testing were made at concentrations of 20 ppm, 40 ppm, 80 ppm, 100 ppm, and 120 ppm from both extracts.

Determination of Percentage Inhibition and IC50 Value

Percentage inhibition represents the activity of the DPPH radical. The percentage inhibition against the DPPH radical for each concentration of the test solution is calculated using the following formula: [16], [17]

% = (Absorbance Blank - Absorbance Test) / Absorbance Blank × 100% [18]

Once the percentage inhibition is obtained, it can be plotted on both the x-axis and y-axis using the linear regression equation y = a - bx. This equation is used to calculate the IC50 values for each test [18]. The antioxidant strength level with the DPPH method is considered very strong when the IC50 value is less than 50 µg/mL, strong when the IC50 value is between 50 and 100 µg/mL, moderate when the IC50 value is between 101 and 150 µg/mL, and weak when the IC50 value is greater than 150 µg/mL [19].

III. Result and Discussion

Fresh samples of shallots and garlic were identified at the Indonesian Institute of Sciences (LIPI) as Allium ascalonicum L. for shallots and Allium sativum L. for garlic. Black garlic was prepared by fermenting garlic in a rice cooker using the warm button.

A. Phytochemical Screeening

Phytochemical screening was performed on the extracts of both shallots and black garlic to determine the presence of various chemical compounds such as alkaloids, steroids, triterpenoids, glycosides, flavonoids, phenols, and tannins. No saponin compounds were detected as there was no foaming observed when heated and vigorously shaken. The results are presented in Table 1.

TABLE 1. Results of Phytochemical Screening for Shallot and Black Garlic Extracts

Phytochemical Screening Test	Reagent	Shallot Extract	Black Garlic Extract	Results
Alkaloid	Wagner Mayer Dragendorf	Red Yellow Yellowish-brown	Red Yellow Yellowish-brown	+ + +
Triterpenoid	Chloroform and sulfuric acid	Brownish-yellow	Greenish brown	+
Glycoside	Glacial acetic acid and $FeCl_3$ + H_2SO_4	Brown ring	Brown ring	+
Flavonoid	NaOH	Brownish-yellow	Brownish-yellow	+
Saponin	HCl 2 N	No foam formed	No foam formed	-
Phenol	$FeCl_3$	Dark Green	Dark green	+
Tannin	$FeCl_3$	Green	Green	+

B. Results of Quality Control Testing for Raw Material of Shallot and Black Garlic Extracts include:

1. Ash Content

The determination of total ash content was carried out

using the gravimetric method, with results of 0.1262±0.04 for shallot and 0.2063±0.01 for black garlic extracts. The purpose of determining the ash content is to provide an overview of the internal and external mineral content originating from the initial process to the formation of the extract and to control the level of contamination by inorganic substances. The requirement is that the ash content in the extract should not exceed 0.9% [20].

2. Microbial Contamination

Microbial contamination testing is one of the purity tests for the extract. The microbial contamination test includes the total plate count and yeast and mold count. This test refers to the determination of the allowable number of microbes to indicate the presence or absence of specific microbes in the extract, ensuring that the extract does not contain harmful pathogenic and non-pathogenic microbes. The test results showed negative findings [21].

3. Aflatoxin Contamination

The results of aflatoxin contamination testing using high-performance liquid chromatography (HPLC) showed negative results. Aflatoxins are known to be toxic to humans, so testing is conducted to prevent potential contamination with secondary metabolites such as aflatoxins during the drying process, typically associated with Aspergillus sp. contamination [22]. Consequently, the results meet the requirements for being free from aflatoxin contamination.

4. Pesticide Residue

Pesticide residues are compounds that are carcinogenic, mutagenic, and teratogenic to humans. Therefore, it is essential to conduct testing on the extract used as a raw material for product manufacturing to ensure it does not pose risks to consumers. Pesticide residue testing is carried out using gas chromatography, and the results of the testing showed negative, indicating the absence of pesticide residues and compliance with the requirements [23].

5. Heavy Metal Contamination

The testing of lead content aims to provide assurance that the extract does not contain heavy metals exceeding the established limits, as they are hazardous to human health. These metals can directly or indirectly impact human health, causing fatal systemic disorders and can enter the body through skin contact [21]. Heavy metal contamination testing for Pb, Cd, Hg, and As showed results that meet the criteria, and the results are below the detection limits, confirming that both extracts are free from heavy metal contamination.

C. Antioxidant Activity Test Results

The determination of antioxidant activity values was conducted using the DPPH method. This method is chosen for its simplicity, ease of use, speed, sensitivity, and the requirement for only a small sample volume. It is widely used to evaluate the antioxidant activity of natural compounds, assessing their ability to act as electron donors. The principle of this method involves the quantitative measurement of antioxidant activity by measuring the DPPH radical scavenging capacity of compounds using UV-Vis spectrophotometry instrumentation. This allows the determination of the IC50 value (Inhibitory Concentration), defined as the concentration of the test compound needed to scavenge 50% of free radicals. A lower IC50 value indicates higher radical scavenging activity [24]. Antioxidant testing of the combination of shallot and black garlic extracts was performed at different ratios, namely (4:1), (3:2), and (1:4), using various concentrations of 20 ppm, 40 ppm, 80 ppm, 100 ppm, and 120 ppm. The testing process involved the following steps:

Measurement of a 0.1 mM DPPH solution, considered as a blank, to determine the absorption spectrum using UV-Vis spectrophotometry at a wavelength range of 400-800 nm and to identify its maximum absorption wavelength. The measurement of DPPH showed a maximum absorption wavelength at 516.5 nm with an absorbance of 0.779.

Preparation of a DPPH standard curve with concentrations of 5 ppm, 10 ppm, 15 ppm, 20 ppm, and 25 ppm to determine the maximum absorption wavelength for measurements in the visible region, resulting in a maximum absorption wavelength of 516.6 nm [24]. The results showed a linear relationship with a equation Y=0.0188x-0.0044 and an r-value of 0.9999, as depicted in Figure 1.

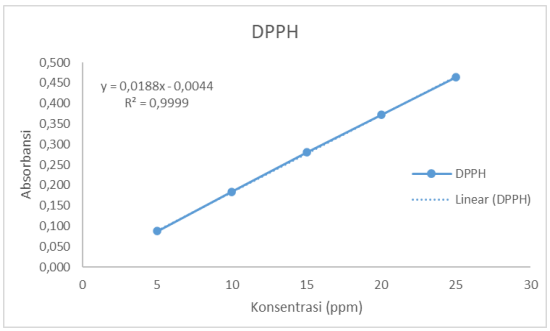

Figure 1. Linearity Graph of DPPH

Vitamin C was measured as a positive control due to its easy availability, widespread consumption, high and potent antioxidant activity. Vitamin C was used as a positive control and as a reference to determine whether the test substance had a similar effect to the standard antioxidant used as a positive control at concentrations of 1 ppm, 2 ppm, 3 ppm, 4 ppm, and 5 ppm. These lower concentrations were chosen because vitamin C is a pure compound. A linear regression was performed to calculate the IC50 value for vitamin C. The calculation resulted in a Y value of 9.115x + 48.909 and an r value of 0.9134. The IC50 value for vitamin C was determined to be 11.97 ppm, as shown in Figure 2.

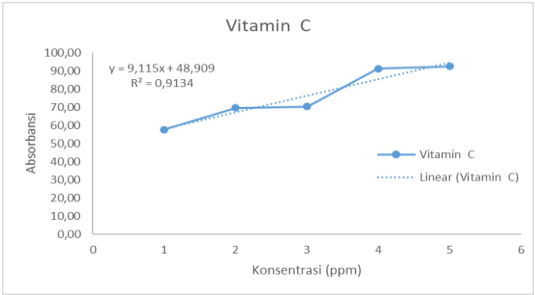

Figure 2. Linearity Graph of Vitamin C

Antioxidant activity testing was conducted for combinations of shallot and black garlic extracts at ratios of 4:1, 3:2, and 1:4, with concentrations of 20 ppm, 40 ppm, 80 ppm, 100 ppm, and 120 ppm. In the case of the 4:1 ratio, the calculation yielded a value of y=0.3451x + 49.72 with an r value of 0.8841, and the IC50 value was determined to be 81.1359 ppm, as depicted in Figure 3.

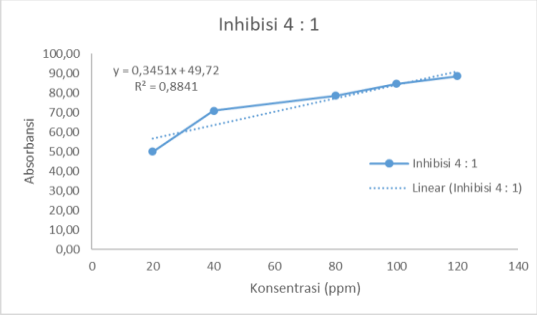

Figure 3. Linearity Graph of Ratio 4:1

For the 3:2 ratio, the calculation resulted in a value of y = 0.3451x + 49.617 and an r value of 0.7627. The IC50 value was determined to be 110.9823 ppm, as shown in Figure 4.

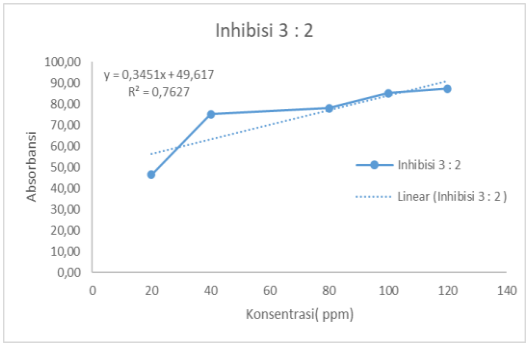

Figure 4. Linearity Graph of Ratio 3:2

In the case of the 1:4 ratio, the calculation yielded a value of y = 0.3237x + 49.669 and an r value of 0.7175. The IC50 value was determined to be 102.2552 ppm, as depicted in Figure 5.

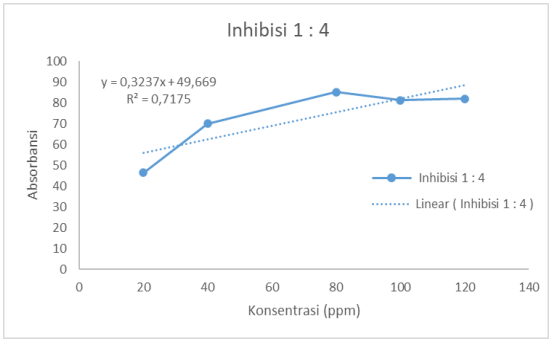

Figure 5. Linearity Graph of Ratio 1:4

IV. Conclusion

The combination of shallot and black garlic extracts at a ratio of 4:1 exhibited strong antioxidant activity, while the ratios of 2:3 and 1:4 demonstrated moderate antioxidant activity.

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Conflict of Interest

There are no conflicts of interest, and there will be none in the future.

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