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Antioxidant Activity Of Various Preparations Of Garlic Extract (*Allium Sativum*) By 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Method

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----------***ABSTRACT***----------
This study investigated the antioxidant activity of various preparations of garlic (Allium sativum) extract using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Different preparation of garlic, which is fresh garlic, black garlic, laba garlic, and powdered dried garlic were employed to evaluate their impact on the antioxidant capacity of the resulting extracts compared to ascorbic acid and quercetin. The antioxidant activity was assessed by measuring the ability of the extracts to scavenge DPPH radicals. Various preparations of garlic exhibit different antioxidant activity strength. From our result, black garlic showed the best one (IC_{50} = 165.09, but still weaker in strength compared to ascorbic acid, IC_{50} = 32.35, and quercetin, IC_{50} = 22.76), followed by powdered dried garlic, laba garlic and fresh garlic. The research contributes to the understanding of garlic's antioxidant properties and its potential as a natural antioxidant source.

Keywords: *garlic, black garlic, laba garlic, DPPH, antioxidant*

I. INTRODUCTION

Free radicals are molecules with unpaired electrons that easily react with other molecules. The imbalance between free radicals and antioxidants can interfere with organ function and can cause disease (Irianti et al., 2021). In fighting free radicals, antioxidants contribute electrons to free radicals to maintain their stability, thereby reducing free radical reactivity and the effects of oxidative stress. Antioxidants produced in the liver, such as glutathione, are very important in preventing cell damage and aging, and preventing the development of degenerative diseases (Huang et al., 2021). The mechanism of action of antioxidants is very complex and involves various strategies to protect body cells from oxidative damage. One of the main mechanisms is the ability of antioxidants to donate electrons to free radicals. Free radicals, including superoxide (O2-) and hydroxyl (OH-) ions, are highly reactive molecules that steal electrons from other molecules, leading to cellular damage. When antioxidants donate electrons to free radicals, they neutralize them and stop the chain reaction that can damage cells (Johnson & Brown, 2018).

Antioxidants are categorized into two types: endogenous and exogenous. Endogenous antioxidants are produced naturally by the human body and help maintain cellular redox balance. Exogenous antioxidants, sourced from external factors, are abundant in various foods and supplements. Vitamin C, also known as ascorbic acid, is a prominent exogenous antioxidant capable of neutralizing free radicals and restoring oxidized vitamin E (Johnson & Brown, 2022).

Garlic (Allium sativum) is a rich source of antioxidants. Its bioactive compounds, including allicin, diallyl disulfide (DADS), S-allyl cysteine (SAC), ajoene, vinyldithiins, and flavonoids, contribute to its diverse health benefits. These compounds have been linked to improved cardiovascular health, antimicrobial, and anticancer effects (Smith et al., 2023).

This study suggests that consuming fresh garlic will increase the levels of antioxidant enzymes in the body such as glutathione peroxidase and superoxide dismutase, which may help reduce oxidative stress (Banerjee & Maulik, 2016). Fresh garlic may also have cardioprotective effects, which help to lower blood pressure and improve blood lipid profiles, making it useful in preventing cardiovascular disease (Jeong et al., 2016). In addition to fresh garlic, there are also several processed garlic that will be compared to the antioxidant content in this study including black garlic, laba garlic and powdered garlic. However, storage and processing can affect the effectiveness of fresh garlic. Some studies have shown that the active compounds in fresh garlic can degrade over time if not stored properly.

Black garlic is the result of fermenting fresh garlic under certain conditions, where the garlic is placed at controlled temperature and humidity for 14-20 days. This process transforms the garlic through the Maillard reaction resulting in changes in color, texture and active ingredient content (Clark & Macready, 2021).

Laba garlic are also called fermented garlic with the addition of vinegar marinated for 4 days. This is another variation of garlic processing. This process will produce garlic with a greenish color and a sourer taste than fresh garlic. Laba garlic are also often used in Asian cuisine and have unique antioxidant properties.

Powdered sun-dried garlic is garlic that has been oven-dried and is ready for extraction. The process begins with the collection of ingredients, where fresh garlic is cleaned of dirt and the outer skin is cut or chopped into small pieces (Kim et al., 2019).

The test method in this study is DPPH (2,2-diphenyl-1-picrylhydrazyl) test method, which is commonly used in measuring antioxidant activity. The basic principle of this method is the ability of antioxidant compounds to donate electrons or hydrogen to reduce free radicals from DPPH into a more stable and colorless form. This color change is then measured using a spectrophotometer, where the degree of color change indicates the level of antioxidant activity in the sample (Shahidi, F., & Zhong, Y. 2015).

II. METHODOLOGY

The method used in this research is a quantitative method. This research method refers to strategies, techniques and data collection that can be measured numerically. In data collection, measurements must be made with objective and standardized tools and with numerical calculations and data quantity.

Preparation of Processed Garlic

Black garlic is created through a slow-cooking process. Begin by selecting firm, unpeeled garlic bulbs. Place them in a slow cooker or rice cooker set to the "warm" setting. The garlic will gradually transform into a soft, black delicacy with a sweet and savory flavor. It's essential to maintain a consistent warm temperature and avoid opening the appliance frequently. Once the cloves are soft and black, the garlic is store in an airtight container.

Laba garlic, is a pickled garlic that turns a vibrant green. To make it, start by peeling garlic cloves and removing any damaged parts. Soak the garlic in salted water to remove impurities. Then, pack the garlic tightly into clean glass jars. Prepare a pickling solution with white vinegar, sugar, and salt. Bring the solution to a boil, let it cool slightly, then pour it over the garlic. Seal the jars tightly and store them in a cool, dark place. The garlic will gradually turn green over a few days. The resulting laba garlic has a tangy and slightly sweet flavor.

To create powdered dried garlic, begin by separating garlic cloves from the bulb and peeling them. Slice the cloves thinly and dehydrate the garlic slices in dehydrator at 50 °C for 2 hours. Ensure the garlic is completely dry before proceeding. Once dried, grind the garlic into a fine powder using a spice grinder and filtered with 200 mesh sieves. The powdered garlic is stored in an airtight container in a cool and dark place.

Extraction Process

Extraction of simplisia is done using various equipment such as shaker rotator, vacuum rotatory evaporator, hot plate, glassware, and other. The extraction carried out with cold method (maceration), where simplisia is soaked in a 96% ethanol solvent to extract soluble components, with a ratio of 1:10 between simplisia and solvent volume, and shaken in a shaker rotator for 24 hours. The macerate is filtered through filter paper and further concentrated using a vacuum rotary evaporator. At the end of extraction process, the yield is calculated.

Assessment of Antioxidant Activity

To assess antioxidant activity, a DPPH solution was prepared. 11.5 grams of DPPH powder was weighed and dissolved in 115 mL of ethanol. The mixture was vortexed until the DPPH was completely dissolved. Subsequently, 3 mL of this DPPH solution was pipetted into a new container and diluted to a final volume of 5 mL with additional ethanol. The solution was then allowed to stabilize in the dark for 30 minutes.

The maximum wavelength for DPPH absorption was determined using the method described by (Sulastri et al., 2022). A 3 mL aliquot of the prepared DPPH solution was transferred to a cuvette and diluted with 2 mL of ethanol. The mixture was kept in the dark for 30 minutes to ensure stability. Subsequently, the absorbance of the solution was measured at 517 nm using a UV-Vis spectrophotometer (Rigol U3660).

Each sample of garlic extract were diluted to have concentration of 12.5, 25, 50, 100, 200, 400, and 800 ppm using ethanol as dilution solvent. Thus, the same procedure with above 2 ml of ethanol applied for each concentration to be measured for its absorbance at 517 nm.

$$\text{DPPH radical scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{\text{Absorbance of control}} x100$$

Where:
Absorbance of control: The absorbance of the DPPH solution without the sample.
Absorbance of sample: The absorbance of the DPPH solution with the sample.

Furthermore, data is calculated to gain IC_{50} value, which is concentration of the sample that inhibits the DPPH radical by 50%, using AAT Bioquest IC_{50} calculator. It is often determined by plotting a graph of percentage inhibition against sample concentration and finding the corresponding concentration for 50% inhibition.

III. RESULTS AND DISCUSSION

Extraction Yield

The extraction yield is a critical parameter in evaluating the efficiency of a particular extraction process. It represents the amount of desired compounds recovered from the plant material relative to the initial sample weight. In this study, the extraction yield of processed garlic simplisia using maceration extraction method with 96% ethanol as solvent was determined. The results obtained provide valuable insights into the efficacy of the extraction process (Table 1).

Table 1. Extraction Yield of Simplisia

Sample	Yield (from 50 gr simplisia)
Fresh garlic	10.36% (5.18 gr)
Black garlic	37.48% (18.74 gr)
Laba garlic	23.56% (11.78 gr)
Sun-dried garlic	10.88% (5.44 gr)

Antioxidant Activity Test

The DPPH radical scavenging assay revealed that the processed garlic extract exhibited antioxidant activity. Data processing technique is done by comparing the concentration with the percent value of antioxidant activity of each sample. The results of antioxidant activity test are showed in the Table 2, Table 3, Table 4, Table 5, Table 6, and Table 7 for consequently antioxidant activity of fresh garlic, black garlic, laba garlic, powdered dried garlic, ascorbic acid, and quercetin, where the last two were act as the comparing compounds.

Table 2. Antioxidant Activity of Fresh Garlic Extract

Concentration (ppm)	*% inhibition*	***IC50***
12.5	3.47	
25	4.33	
50	4.66	
100	4.98	1390.10
200	6.01	
400	6.82	
800	8.50	

Table 3. Antioxidant Activity of Black Garlic Extract

Concentration (ppm)	*% inhibition*	***IC50***
12.5	0.01	
25	0.58	
50	1.03	
100	1.84	165.09
200	6.28	
400	10.30	
800	14.37	

Table 4. Antioxidant Activity of Laba Garlic Extract

Concentration (ppm)	*% inhibition*	*IC50*
12.5	0.56	
25	0.65	
50	1.10	
100	1.48	250.19
200	2.38	
400	2.87	
800	3.37	

Table 5. Antioxidant Activity of Powdered Dried Garlic Extract

Concentration (ppm)	*% inhibition*	*IC50*
12.5	2.50	
25	2.82	
50	3.25	
100	3.92	180.39
200	5.11	
400	8.46	
800	12.07	

Table 6. Antioxidant Activity of Ascorbic Acid

Concentration (ppm)	*% inhibition*	*IC50*
12.5	18.84	
25	37.2	
50	85.36	
100	96.21	32.35
200	96.26	
400	96.07	
800	95.27	

Table 6. Antioxidant Activity of Quercetin

Concentration (ppm)	*% inhibition*	*IC50*
12.5	55.44	
25	78.88	
50	93.83	
100	94.21	22.76
200	94.69	
400	96.69	
800	94.42	

The test results showed that fresh garlic, black garlic, laba garlic, and powdered dried garlic extracts showed their DPPH free radical scavenging ability. From the IC_{50} value, black garlic showed the best processed garlic with relatively good value (IC_{50} = 165.09) which is weaker in strength to ascorbic acid (vitamin C) (IC_{50} = 32.35) and quercetin (IC_{50} = 22.76). The lower IC_{50} value, the stronger its antioxidant activity.

The observed potent antioxidant activity of the garlic extract in this study is consistent with previous research. Garlic is well-recognized for its rich phytochemical composition, including organosulfur compounds, flavonoids, and phenolic compounds, which are known for their potent antioxidant properties. These compounds effectively scavenge free radicals, thereby mitigating oxidative damage (Furdak et al., 2023). The high antioxidant capacity demonstrated by the garlic extract suggests its potential as a natural antioxidant source for various applications, including food preservation and nutraceutical development.

The results obtained in this study corroborate previous findings highlighting garlic's potential as a valuable source of natural antioxidants for various applications (El-Saber Batiha et al., 2020). Further investigations are warranted to isolate and characterize the specific compounds responsible for the observed antioxidant effects.

IV. CONCLUSION

In conclusion, the present study successfully demonstrated the significant antioxidant potential of garlic extract. The results obtained from the DPPH assay revealed a remarkable ability of the extract to scavenge free radicals. Based on the antioxidant activity test results, various preparations of garlic (*Alium sativum*) exhibit different antioxidant activity strength. From our result, black garlic showed the best one, followed by powdered dried garlic, laba garlic and fresh garlic. This finding underscores the importance of garlic as a rich source of natural antioxidants for its promising applications in the pharmaceutical, food, and cosmetic industries. Further research is encouraged to elucidate the underlying mechanisms of its antioxidant action and to explore its potential health benefits.

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