

Analysis of Antibacterial and Antioxidant Activities of a Single Bulb of Garlic Fermented into Black Garlic

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Abstract—A single bulb of black garlic is the result of a single bulb of garlic fermentation which produces black colour as a result of the fermentation process at 80°C for 15 days. As it is known that garlic has pharmacological properties, but the properties possessed by black garlic cannot be separated from the result of heating during the fermentation process. This study aims to determine the differences in antibacterial and antioxidant activity in a single bulb of garlic and a single bulb of black garlic. The design of this study was a pure laboratory experimental study with a post-test only control group research design. In this study, the focus was to determine the antioxidant and antibacterial activity against *Streptococcus mutans*, *Streptococcus pyogenes* and *Enterococcus faecalis* on a single bulb of garlic and a single bulb of black garlic. Testing for antibacterial activity using the Kirby-Bauer diffusion method, while antioxidant testing was carried out by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The results showed that there was a significant increase in the antibacterial activity of black garlic against *Streptococcus mutans* and *Enterococcus faecalis* ($P < 0.05$). Meanwhile, the increase in antibacterial activity against *Streptococcus pyogenes* showed insignificant differences ($p > 0.05$). Likewise, the increase in antioxidant activity of single black garlic DPPH was significantly different from single bulb garlic ($P < 0.05$). Overall, the results of this study indicate that the garlic fermentation process by heating at 80°C for 15 days can cause changes in colour, texture, taste, and significantly increase the antibacterial and antioxidant activities.

Keywords—garlic, black garlic, fermentation, antibacterial activity, antioxidant activity

I. INTRODUCTION

Black garlic is a fermented garlic product that produces a black colour in the final product of the fermentation process. The health benefits of black garlic have long been developed in Japan and Korea. Black garlic or also known as Aged Black Garlic is made by storing fresh garlic at a certain room temperature and humidity for a period of several days to several months without treatment and certain additives until the colour of the garlic turns black. The result of the process of

making black garlic is aimed at increasing the activity of the active substances contained in garlic, eliminating the distinctive taste and aroma of garlic, and producing a sweet-sour taste in black garlic [1,2].

Previous studies have shown that the longer the fermentation process at temperatures of 40, 55, 70, and 85°C, the higher the levels of S-allyl cysteine (SAC) in black onions. On the other hand, compounds derived from SAC, namely allyl sulfide, diallyl disulfide (DADS), and diallyl trisulfide (DATS) have been shown to work as bactericides. The compounds in garlic are active against bacteria that are resistant to antibiotics, such as *Shigella dysenteriae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Eshceria coli*, *Streptococcus spp*, *Salmonella spp*, and *Proteus mirabilis*. Meanwhile, in an experiment conducted by Setyawati, garlic tuber extracts fermented for 15, 30, and 45 days at a temperature of 70°C had an antibacterial effect against *Staphylococcus aureus* [3].

Another benefit of a single bulb of garlic and a single bulb of black garlic also has a strong antioxidant effect, although it is a "physiological antioxidant" [4]. This is evidenced from several studies such as the results of research conducted by Prasonto, et al. In 2017 the results of the analysis of antioxidant activity were obtained with the antioxidant test of the DPPH method (1,1-diphenyl-2-picrylhydrazyl) which was carried out on single bulb garlic which had higher antioxidant power than other varieties of garlic [5]. In the research conducted by Xiong, F et al. in 2018 showed that black garlic at a temperature of 90°C for 4 days with 95% humidity have strong antioxidant activity, consuming black onions can significantly reduce levels of malondialdehyde (MDA) and increase superoxide dismutase (SOD) and glutathione peroxidase (GSH) activity -Px) [6].

II. METHODS

The design of this study was a laboratory experimental study with a post-test-only control group research design. In this study, the focus was to determine the antioxidant and

antibacterial activity against *Streptococcus mutans*, *Streptococcus pyogenes* and *Enterococcus faecalis* on a single bulb of garlic and a single bulb of black garlic. The method used to test for antibacterial is the Kirby-Bauer modified agar diffusion method, while the method used to test the antioxidant power uses the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl).

Ferment the garlic in the oven with a temperature setting of 80°C for 15 days. Then every single bulb garlic and black garlic extracted was carried out using the cold extraction method, namely by maceration. The characteristics of the active substances contained in black garlic are thermolabile and insoluble in water (hydrophobic) so that organic solvents are needed, one of which is ethanol, to attract these active substances from the black garlic cells as well as low temperatures to maintain stability.

Antibacterial activity testing was carried out using the Kirby-Bauer Paper Disk method with Muller Hinton Agar (MHA) media. The media plate was divided into 4 parts, parts 1 and 2 filled with dish samples of garlic or black garlic extract, part 3 positive control (antibiotic ciprofloxacin), and part 4 negative control Dimethyl sulfoxide (DMSO). Furthermore, the agar medium was incubated for 24-48 hours at 37°C. Results can be measured using a calliper or a ruler to measure the diameter of the clear zone of inhibition that forms around the well.

Antioxidant activity testing was carried out using the DPPH method. Each single bulb of garlic extract and single bulb of black garlic extract were prepared as much as 50 µg. Then the extract was dissolved with pro-analysis methanol to 50 ml to obtain a concentration of 1000 ppm. Then measure the absorption of the DPPH solution using a Pharma spec UV-Vis spectrophotometer at the maximum wavelength of the reagent solution. Antioxidant activity is determined by the amount of DPPH radical absorption inhibition by calculating the percentage of DPPH absorption inhibition. The results of the free radical reduction value were used for the IC50 value. The IC50 value is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50% [7].

III. RESULTS AND DISCUSSIONS

The result of a single bulb of garlic fermentation at a temperature of 80°C for 15 days with a humidity of 60-70% produces a single bulb of black garlic which has brownish black colour, sweet taste, chewy texture, and a distinctive aroma. Single black garlic has a sweet taste compared to single bulb garlic due to the reduction of sugar, hydrolysis and changes in pH that occur during the fermentation process. In addition, the brownish brown colour is due to the formation of brownish polymer groups that were formed during the fermentation process according to the Maillard reaction [8].

The results of single bulb garlic fermentation at a temperature of 80°C for 15 days can be seen in Figure 1.



Fig. 1. A. Single bulb garlic B. Black single bulb garlic.

The result of the garlic fermentation process by heating at 80°C for 15 days causes changes in colour, texture, and taste. The colour becomes black, the texture is softer, and the taste is sweet.

The results of the Kirby Bauer test on antibacterial activity against *S.pyogenes*, *E.faecalis*, and *S. mutans* can be seen in Table 1, Figure 2,3 and 4.

TABLE I. THE RESULTS OF MEASUREMENTS OF THE AVERAGE INHIBITION ZONE POWER OF ETHANOL EXTRACT OF SINGLE BULB GARLIC AND SINGLE BLACK GARLIC AGAINST S.PYOGENES, E.FAECALIS AND S. MUTANS

Bacteria	Average Zone of Inhibition (mm)					
	Single Garlic	(+)	(-)	Single Black Garlic	(+)	(-)
<i>S.pyogenes</i>	14,66	22	-	15,33	25,3	-
<i>E.faecalis</i>	8	21,33	-	13	24,6	-
<i>S.mutans</i>	7,67	24,67	-	11,33	24	-

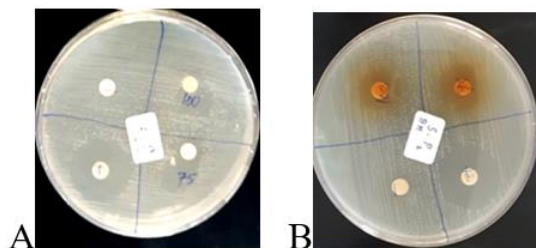


Fig. 2. The Result of Kirby Bauer test of Single bulb garlic (A) and Black Single bulb garlic against *S. pyogenes*.

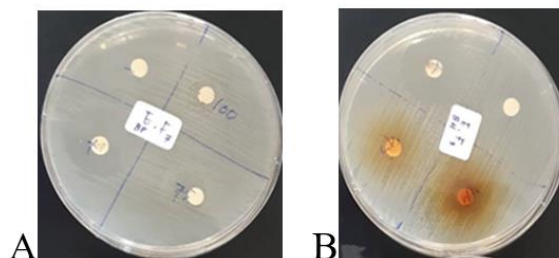


Fig. 3. The Result of Kirby Bauer test of Single bulb garlic (A) and Black Single bulb garlic against *E. faecalis*.

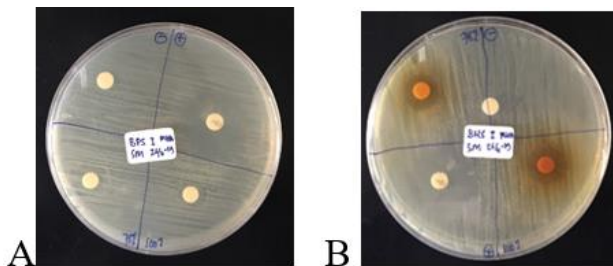


Fig. 4. The Result of Kirby Bauer test of Single bulb garlic (A) and Black Single bulb garlic against *S. mutans*.

According to Susanto Sudarajat and Ruga, the zone of inhibition observed on the media in order to be categorized as follows, for diameters > 20 mm are categorized as very strong, 11-20 mm are categorized as strong, 6-10 mm are categorized as moderate and <5 mm are categorized as weak [9].

Antibacterial test results showed an inhibition zone in the growth of *S. mutans*, *S. pyogenes*, and *E. faecalis* which was marked by the presence of clear areas in all which were given a single bulb garlic ethanol extract paper and a single black garlic ethanol extract. The inhibition zone was also formed in the positive control, namely chloramphenicol antibiotic dose of 30 µg, but the inhibition zone was not formed in the negative control, namely dimethyl sulfoxide (DMSO) [10].

TABLE II. T-INDEPENDENT PARAMETRIC TEST RESULTS

Bacteria	Average		P-Value
	Single Garlic	Single Black Garlic	
<i>S. mutans</i>	7,67	11,33	0,001*
<i>S. pyogenes</i>	14,66	15,33	0,75
<i>E. faecalis</i>	8	10,33	0,03*

Note *: p-value <0.05 indicates a significant difference

The difference in the antibacterial activity of single bulb of garlic and single bulb of black garlic ethanol extract on the growth of *Streptococcus mutans* and *Enterococcus faecalis* had P values of 0.001 and 0.03 (P <0.05) which mean the difference was statistically significant.

The difference in antibacterial activity between ethanol extract of single bulb of garlic and single bulb of black garlic against *Streptococcus pyogenes* has a P value of 0.75 (p > 0.05) which means that the P-value is not statistically significant. Based on these results, it can be concluded that there is an increase in antibacterial activity in single black garlic against *Streptococcus pyogenes* although the difference is not significant.

Fermentation is the process of changing sugar by the activity of microorganisms in the form of yeast, yeast, or yeast which can release very complex enzymes and are capable of converting sugars into ethanol and carbon dioxide. The fermentation carried out on this single black garlic uses the Maillard reaction. The Maillard reaction is a chemical reaction that occurs between amino acids and reducing sugars which results in a different brown taste and colour. This reaction is divided into several stages, namely in the first stage of sugars

and amino acids and followed by condensation of Amadori rearrangement and 1-amino-1-deoxy-2-ketose [11,12]. The second stage occurs dehydration and fragmentation occurs in sugar molecules and the formation of hydroxymethylfurfural products such as pyruvaldehyde and diacetyl occurs. The final stage of aldol condensation occurs and finally, a heterocyclic nitrogen compound is formed, melanoidin, which is very colourful. Melanoidin has health benefits as an antioxidant and antibiotic [8].

Alliin is the first and main organosulfur compound of the substance garlic. From one 10g garlic clove, 5 grams of Alliin will be produced. You can imagine how much alliin is contained in a clove of single bulb garlic which has more consistency than ordinary garlic, even up to 5-6 times [13]. Alliin comes from the damaged garlic that is excreted, from the vacuole to the cytoplasm of the cell and is assisted by the enzyme allinase. Alliin has S-S and S-O bonds, which will react with an enzyme that has a thiol group, namely the cysteine thiol residue to form a disulfide mixture by S-thioallylation.

A single bulb of black garlic has a higher content than regular garlic. This is due to changes in Alliin compounds into S-allylcysteine compounds, which are allin-forming components. Then allin will be converted by the allinase enzyme into alliin as a compound that has antibacterial activity. This proves that the antibacterial content is higher in single black garlic [14].

Measurement of the antioxidant test of a single bulb of garlic and a single bulb of black garlic ethanol extract was measured using the DPPH method. The following are the average results of measuring the antioxidant activity of a single bulb of garlic ethanol extract which can be seen in Table 3, figure 4 and 5.

A vitamin C solution with a concentration equal to the concentration of the test extract solution was used as the standard. This test was carried out 2 times.

TABLE III. MEASUREMENT RESULTS OF ANTIOXIDANT ACTIVITY OF SINGLE BULB OF GARLIC ETHANOL EXTRACT

Repetition	Concentration (ppm)	Absorbance	%Inhibition	IC50 (ppm)	Average IC50 (ppm)
1	0	0,911	0	1192,0	1182,9
	500	0,704	22,72		
	1000	0,561	38,42		
	1500	0,324	64,43		
	2000	0,144	84,19		
2	0	0,924	0	1173,7	1182,9
	500	0,700	24,24		
	1000	0,554	40,04		
	1500	0,324	64,94		
	2000	0,244	84,42		

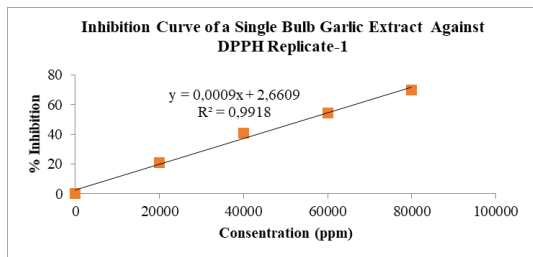


Fig. 5. Inhibition curve of a single bulb of garlic against DPPH replicate-1.

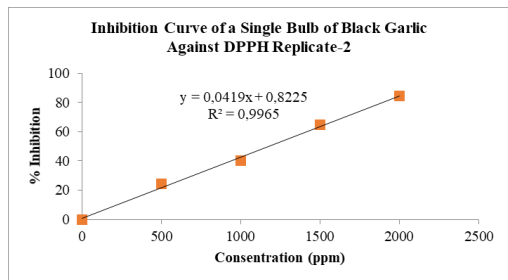


Fig. 8. Inhibition curve of a single bulb of black garlic against DPPH replicate-2.

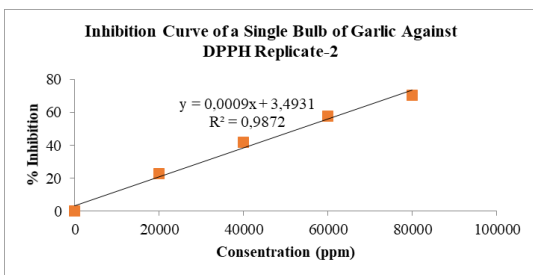


Fig. 6. Inhibition curve of a single bulb of garlic against DPPH replicate-2.

Based on the calculation of the IC50 value of single bulb garlic ethanol extract, the average IC50 value of single bulb garlic ethanol extract is 52136.7 ppm, which means that a single bulb of garlic ethanol extract solution of 52136.7 ppm is needed to capture 50% DPPH free radicals.

Furthermore, the results of measuring the antioxidant activity of a single black garlic ethanol extract can be seen in Table 4, figure 7 and 8.

TABLE IV. MEASUREMENT RESULTS OF THE ANTIOXIDANT ACTIVITY OF SINGLE BULB OF BLACK GARLIC ETHANOL EXTRACT

Repetition	Concentration (ppm)	Absorbance	%Inhibition	IC50 (ppm)	Average IC50 (ppm)
1	0	0,911	0	1192,0	1182,9
	500	0,704	22,72		
	1000	0,561	38,42		
	1500	0,324	64,43		
	2000	0,144	84,19		
2	0	0,924	0	1173,7	
	500	0,700	24,24		
	1000	0,554	40,04		
	1500	0,324	64,94		
	2000	0,244	84,42		

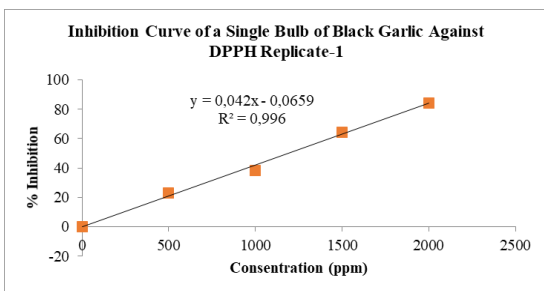


Fig. 7. Inhibition curve of a single bulb of black garlic against DPPH replicate-1.

Based on the results of the calculation of the IC50 value of the single black garlic extract, the average value of IC50 in the single black garlic ethanol extract is 1182.9 ppm, which means that 1182.9 ppm of single black garlic ethanol extract is needed to capture DPPH free radicals of 50 %.

The results of testing the antioxidant activity of single bulb of garlic and a single bulb black garlic ethanol extract can be concluded that the IC50 value in the ethanol extract of single bulb of garlic is smaller than the ethanol extract of single bulb of garlic. The smaller the IC50 value, the greater the ability of a product to capture free radicals, which can be seen in table 5.

TABLE V. ANTIOXIDANT ACTIVITY OF A SINGLE BULB OF GARLIC AND A SINGLE BULB OF BLACK GARLIC ETHANOL EXTRACT

	A Single bulb of garlic	A Single bulb of Black garlic	Difference
Average IC50 (ppm)	52136,7	1182,9	50953,8

The results of the antioxidant activity showed that the ethanol extract of single black garlic was 51 times greater than that of the single bulb of garlic extract.

The results showed that the antioxidant activity of a single bulb of garlic ethanol extract was greater than that of a single bulb of black garlic ethanol extract. Because the data obtained is only small and the data is too extreme, the normality test is not carried out and the data is considered normal.

Furthermore, a parametric statistical test was carried out using the T-independent test which can be seen in Table 6. The difference in the antioxidant activity of a single bulb of black garlic ethanol extract and a single bulb of garlic ethanol extract showed that the IC50 (% Inhibition) value of the single bulb of black garlic ethanol extract was smaller, namely 1182.85 ppm than the single bulb of garlic ethanol extract antioxidant value, namely 52136.65 ppm. Based on the P-value of 0.000 (P < 0.05), which means that the P-value is significantly different in the IC50 value (% inhibition) which is statistically significant between the ethanol extract of a single bulb of black garlic and the ethanol extract of single bulb of garlic.

TABLE VI. T-INDEPENDENT PARAMETRIC TEST RESULTS

Variable	Average		P-Value
	A Single bulb of garlic	A Single bulb of Black garlic	
IC50 (ppm)	52136,65	1182,85	0,000*

Note *: p-value <0.05 indicates a significant difference

The greater the IC50 value, the smaller the ability of a product to scavenge free radicals. Based on the results of the above research, there was an increase in antioxidant activity which was indicated by a decrease in the IC50 value after the fermentation process was carried out for 15 days at 80 ° C. The result of antioxidant activity of a single bulb of black garlic ethanol extract was 51x greater than that of a single bulb of garlic extract or significantly different P <0.05 statistically in neutralizing free radicals.

Previous research has shown that black garlic fermented at 90°C for 4 days with 95% humidity has strong antioxidant activity, consuming black garlic can significantly reduce MDA levels and increase Superoxide Dismutase (SOD) and Glutathione Peroxidase (GSH-Px) activity. 17 Garlic fermented at 70°C with 90% humidity has higher levels of antioxidants than fresh garlic. In black garlic, there is an increase in antibacterial and antioxidant properties. This increase was due to the increased allicin content due to the fermentation process [6].

The bioactive compounds contained in black garlic include SAC, polyphenols, and flavonoids. The three compounds are formed through a heating process. The heating time used is responsible for the increased content of antioxidant compounds in black garlic.

Antioxidants are compounds that are capable of removing, cleaning, and resisting the formation of the effects of reactive oxygen species. An antioxidant is a donor compound (electron donor) or reductant. This compound has a small molecular weight but is able to inactivate the development of oxidation reactions by preventing the formation of radicals. Antioxidants have the ability to work as an inhibitor (inhibitor) of oxidation reactions by reactive free radicals which are one of the triggers for various diseases such as cancer, heart disease, and symptoms of aging. Antioxidants are divided into two, namely the enzyme antioxidants SOD, catalase, and GSH.Px and antioxidants vitamins alpha-tocopherol or vitamin E, beta carotene, and ascorbic acid or vitamin C which are widely found in plants and animals [15].

IV. CONCLUSION

The results showed that there was a significant increase in the antibacterial activity of a single bulb of black garlics against *Streptococcus mutans* and *Enterococcus faecalis* (P <0.05). Meanwhile, the increase in antibacterial activity against *Streptococcus pyogenes* showed insignificant differences (p > 0.05). Likewise, the increase in antioxidant activity of a single bulb of black garlic DPPH was significantly different from single bulb of garlic (P <0.05).

Overall, the results of this study indicate that the garlic fermentation process by heating at 80°C for 15 days can cause changes in colour, texture, taste, and significantly increase the antibacterial activity and antioxidant activity.

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