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Formulation and Antioxidant Effectivity Test of Single Bulb Black Garlic Lotion With DPPH Method (1,1-diphenyl-2-picrylhydrazyl)

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ABSTRACT
Single bulb black garlic is a fermented product of single-bulb garlic for 30 days with a certain temperature and humidity. It experiences a Maillard reaction and has an increased content of active compounds. Single bulb black garlic contains secondary metabolites in the form of flavonoids, tannins, and saponins which have the potential as antioxidants so that they can inhibit free radicals and can be used as a substitute for synthetic antioxidants. Single bulb black garlic is formulated as an antioxidant lotion to facilitate its use. The objective of this study is to determine the optimal antioxidant activity in a lotion preparation containing single bulb black garlic on DPPH, to find out the concentration of single bulb black garlic which is effective as an antioxidant in a lotion preparation and to find out the best physical properties as a lotion preparation. This type of research is experimental research. Single bulb black garlic is formulated in four lotion formulas with concentrations of FI (0%), FII (1%), FIII (5%), and FIV (10%). Then, the antioxidant activity was tested on DPPH and IC50 values were calculated on the formula of 250.59 ppm, 118.77 ppm, 96 ppm, and 70.17 ppm, and physical evaluation was tested to discover results that met the physical requirements of lotion preparations (adhesion, organoleptic, homogeneity, lotion type, spread ability, viscosity, and pH). Statistical test results showed a significant difference in each group on antioxidant activity ($p<0.05$). The conclusion of this study found that a single bulb black garlic concentration of 10% had optimal antioxidant effectivity; the four formulas produced showed optimal antioxidant activity and the FII formula showed the best physical properties of the lotion.

Keywords: *Single bulb black garlic, lotion, antioxidant, DPPH*

1. INTRODUCTION

Garlic (*Allium sativum L.*) is one of crops with high yields in Indonesia. Approximately, 5,013 hectares of harvested land for garlic (*Allium sativum L.*) produced 39,301 tons in 2018 [1]. Generally, garlic consists of many cloves, but may also consist of only one clove which is known as single garlic. The difference between single garlic and ordinary garlic lies in its active compound content which is equivalent to 5-6 ordinary garlic cloves [2].

Garlic is commonly consumed directly in its raw form. However, it is rarely preferred due to its spicy taste and sharp aroma. Hence, one alternative that can be administered is to increase the sensory quality using fermentation. Processing by fermentation is conducted at a temperature of 65º-80ºC, 90% humidity for 30-40 days [3] produces black garlic products, light, which have aroma and taste that is not as strong as fresh garlic due to a Maillard reaction. During this process, S-allyl cysteine and flavonoids are formed in increasing numbers [2]. Black garlic is useful as anti-inflammatory, anti-diabetic, anti-carcinogenic, hypocholesterolemia, antioxidant, and allergenic [3]. Environment is one of factors which affect the skin, causing damage to the skin. It is characterized by the appearance of dry, rough, wrinkled, dull skin, and black spots [4].

Synthetic antioxidant ingredients such as BHT, BHA, and TBHQ are limited in use because of their carcinogenic effects. It is an encouraging reason to find new sources of antioxidants derived from natural ingredients expected to replace synthetic antioxidant materials [5]. One of

antioxidant testing is the DPPH method. DPPH is a free radical reagent [6]. The mechanism of this method is to react antioxidants in the sample with DPPH. The antioxidants work by donating their hydrogen atoms to inhibit free radical activity.

2. METHODS

This research is a quantitative laboratory experimental study, to determine the antioxidant activity of single bulb black garlic and single bulb black garlic lotion preparations. The tools used in this study consisted of a freeze-drying device, climatic chamber, Lamy rheology viscometer, blender, rotary evaporator, Whatman No.1 filter paper, oven, test tube, porcelain plate, analytical scales, glassware (Pyrex), tools-measuring instruments (Pyrex), aluminum foil, mortar, pestle, stationery, UV-Vis spectrophotometer (Shimadzu UV-1601), watch glass, water bath, and pH meter. The ingredients used were single black garlic (*Allium sativum L.*) which had been fermented for 30 days, DPPH powder, 70% ethanol, methanol pa, aquades, vitamin C, stearic acid, liquid paraffin, methylparaben, TEA, cetyl alcohol, and glycerin.

2.1. Plant Determination

The determination was conducted at the Herbarium Bogoriense, Botany Division of the Biology Research and Development Center-LIPI, Cibinong to discover the truth which will be used in the research.

2.2. Making a single black garlic

1 kg of fresh single garlic was fermented by placing it in the Climatic Chamber with a temperature of 65°-80°C, 90% humidity for 30-40 days [3].

2.3. Single bulb black garlic drying

As much as 200 grams of single bulb black garlic was cleaned from the skin and mashed using a blender, then dissolved using 70% ethanol and filtered, filtrate rotary evaporator until thick. Then,frozen at -80°C and vacuum freeze-drying.

2.4. Phytochemical Screening

Phytochemical screening includedtesting for alkaloids, saponins, tannins, flavonoids, triterpenoids/steroids, in order to determine the bioactive components contained in single black garlic.

2.5. Lotion Preparation Formulation

Table 1. Single Bulb Black Garlic Lotion Formula

Material	Composition (%)					Function
	F1	F2	F3	F4	Vit C	
Black garlic	0	1	5	10	-	Active Substance
AscorbicAcid	-	-	-	-	0,02	Comparator
Liquid paraffin	7	7	7	7	7	Moisturizer
Stearic Acid	2,5	2,5	2,5	2,5	2,5	Emulsifier
Triethanolamine	1	1	1	1	1	*Alkalizing Agent*
Glycerin	5	5	5	5	5	Humectants
Cetyl alcohol	1	1	1	1	1	Thickener
Methyl Paraben	0,1	0,1	0,1	0,1	0,1	Preservative
Patchouli Oil	5 drops	5 drops	5 drops	5 drops	5 drops	Perfume
Aquades	ad 100	ad 100	ad 100	ad 100	ad 100	Solvent

2.6. Formula

2.6.1. Making Lotion Formulas

Materials including the oil phase such as stearic acid, cetyl alcohol, and liquid paraffin were placed in evaporator cup A, and those which include the water phase such as triethanolamine, glycerin, and aquades were placed in the evaporating cup B. The oil phase and the water phase were heated and stirred at temperature 70-75°C separately until melted. The liquid preparations were mixed in a hot mortar. The mixing of the two different phases was administered at a temperature of 70°C. The stirring process was performed until the mixture of the two phases was homogeneous and reached a temperature of 40°C (preparation 1). The preservative (nipagin) and the active substance, which wasthe single dissolved black garlic, were added to preparation 1 at 35°C and then stirred for about one minute. Then, it was added patchouli oil to the mixture until it was homogeneous and put it in the container.

2.6.2. Physical evaluation of lotion preparations

Physical evaluation of lotion preparations was administered every 1 week for 4 weeks (0, 7, 14, 21 days).

2.6.3. Organoleptic

Organoleptic tests were conducted by directly observing the shape, color, and smell of the lotion.

2.6.4. pH test

This test was conducted by entering the pH meter into the diluted lotion preparation, then measuring it with a pH meter.

2.6.5. Spread ability

A total of 0.5 grams was placed on a transparent glass coated with graph paper and covered with transparent glass. Then, it was given a load of 150g and leave for 60 seconds, andmeasured the diameter of the preparation [7].

2.6.7. Homogeneity

The sample was smeared on a slide, the preparation must show a homogeneous arrangement and do not show any coarse particles.

2.6.8. Adhesiveness

A total of 0.2g was placed on a glass plate. Then, it was put another glass plate on top of the lotion and stick it. It was given a load of 1kg for 5 minutes, after which the load was taken. Finally, the two glasses were removed and the time was recorded until the two glass plates were released [7].

2.6.9. Lotion Type

A few drops of methylene blue were mixed into the lotion formula. If all the lotions were uniform in color, the tested lotion has M/A type.

2.6.10. Viscosity

The viscosity test used a Lamy rheology viscometer with an L-4 spindle, a speed of 30 rpm, and a time of 30 seconds.The lotion was based on the Non-Newton flow system.

2.7. Testing Antioxidant Activity with the DPPH Method

1. Preparation of 0.05 mM DPPH Solution
   Weigh 2 mg DPPH (BM 394.32) dissolved with methanol p.a and put into a 100 ml volumetric flask. The volume was sufficient with methanol p.a up to the limit mark and store in a dark bottle [8].

2. Determination of the Maximum Wavelength of 0.05 mM DPPH
   The maximum wavelength measurement was conductedby measuring the absorbance of the DPPH solution 0.05 mM on a UV-Vis spectrophotometer with a wavelength of 400-800 nm. The maximum wavelength was obtained from the maximum absorbance value [9]. The absorbance value obtained was the absorbance blank.

3. Preparation of Test Solution (1000 ppm)
   Weigh 10 mg of extract dissolved in 10 mL of methanol p.a until homogeneous. The test solution (1000 ppm) made a concentration series of 12.5: 25; 50; 100; and 200 ppm, then the volume was sufficient with methanol p.a up to 10 mL.

4. Determination of Operating Time
   Determination of operating time was administered by taking 50 µL of test solution plus 4.0 ml of 0.05 mM DPPH solution then vortexed and measured at minutes 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 at the maximum wavelengths obtained. The time which produced the most stable DPPH absorbance was the operating time [9].

5. Preparation of Vitamin C Solution
   Weigh 10 mg of vitamin C powder dissolved with 100 mL of methanol p.a in a 100 mL volumetric flask to obtain a concentration of 100 ppm. A series of concentrations of 2.5, 5, 7.5, 10, and 12.5 ppm were made in a volumetric flask, and the volume was sufficient with methanol p.a up to 5 mL. Each concentration of 2 mL of vitamin C comparison solution was inserted into the test tube. 2 mL of 0.05 mM DPPH solution was added, homogenized, and incubated in a dark room for 30 minutes [8]. The absorption was measured at the maximum wavelength of the measurements that had been made.

6. Single Bulb Black Garlic Antioxidant Activity Testing
   Each 2 mL concentration of the test solution was inserted into the test tube. 2 mL of 0.05 mM DPPH solution was administered, homogenized, and incubated in a dark room for 50 minutes. Then, the absorption was measured at the maximum wavelength of the measurements that had been made.

7. Testing the Antioxidant Activity of Single Bulb Black GarlicLotion
   Weigh 10 mg of a Single bulb black garlic lotion then dissolved it in 10 mL of methanol p.a until homogeny,

to obtain a concentration of 1000 ppm. Then, a series of concentrations of 10, 20, 30, 40, and 50 ppm were made into a volumetric flask, and the volume was sufficient with methanol p.a up to 10 mL. Each concentration series solution was taken 1 mL input into the test tube added 1 mL of 0.05 mM DPPH solution and 2 mL of methanol [10] which was homogenized with the vortex. Furthermore, it was incubated in a dark room for 50 minutes and the absorption was measured at the maximum wavelength of the measurements which had been made. Vitamin C lotion and lotion X as positive control were applied with the same treatment.

8. Determination of Percent Inhibition and IC50
   The parameter that shows antioxidant activity with the DPPH method was Inhibitory Concentration (IC50). To calculate the IC50 value, data on percent inhibition of the test were needed.

3. RESULT

3.1. Phytochemical Screening

Phytochemical screening is a qualitative test as an important step to obtain initial information from plants regarding the class of secondary metabolite compounds contained therein. The results of a single phytochemical screening on black garlic are provided in table 2.

Table 2. Results of Phytochemical Screening Test

Secondary Metabolites	Results
Alkaloids	-
Flavonoids	+
Saponins	+
Tannins	+
Triterpenoids/Steroids	-

(+): There are Secondary Metabolites
(-): No Secondary Metabolites

Based on the results of phytochemical screening, single bulb black garlic was positive for flavonoids, saponins, and tannins.

1. Physical Evaluation of Lotion Preparations
   The organoleptic test
   The organoleptic test was administered to determine the physical properties of the lotion which experienced changes in color, smell, and shape during storage. The results of the observation show that the lotion has a relatively stable color, shape, and odor stability. During storage, there was no change in the lotion preparation, it is because the active substance and lotion base were evenly mixed.

Table 3. Organoleptic Test

Formula	Checking Type			
	Odor	Color	Shape	RAL
FI	Typical patchouli oil	RAL 9016 *Traffic White*	Semi solid	
FII	Typical patchouli oil	RAL 1015 *Light Ivory*	Semi solid	
FIII	Typical*black garlic*	RAL 1001 *Beige*	Semi solid	
FIV	Typical*black garlic*	RAL 1011 *Brown Beige*	Semi solid	
FV	Typical patchouli oil	RAL 9002 *Grey White*	Semi solid	
Lotion X	Typical lilies	RAL 5024 *Pastel Blue*	Semi solid	

The difference in color and odor occurred due to variations in the concentration of a single bulb black garlic extract. The most visible changes were in the third and fourth formulas. Color changes could occur because single bulb black garlic undergoes a non-enzymatic browning reaction and contains melanoidin.

3.2. pH testing

Table 4. pH Test

Formula	pH test during storage day			
	0	7	14	21
F I	8,0	8,0	7,9	7,9
F II	7,7	7,6	7,8	7,5
F III	7,2	7,3	7,2	7,1
F IV	6,9	6,8	6,8	6,8
F V	7,7	7,7	7,7	7,6
Lotion X	7,1	7,2	7,1	7,1

The objective of pH testing is to determine the comfort and safety of lotion preparations at the time of use, hence it will not cause irritation to the skin [11]. The pH measurement results on all preparations ranged from 6.8 to 8.0. It fulfilled the quality requirements of skin moisturizers, which was 4.5-8.0. The pH of the lotion preparation should not be too acidic because it irritates the skin and should not be too alkaline because it can cause the skin to become dry [12].

3.3. The spread ability test

Table 5. The spread ability test

Formula	Diameter of Spread ability During Storage Day (cm)			
	0	7	14	21
F I	5,135	5,425	5,481	5,760
F II	6,052	6,205	6,211	6,254
F III	6,220	6,266	6,415	6,727
F IV	6,375	6,476	6,576	6,913
F V	5,275	5,345	5,492	5,776
Lotion X	5,210	5,538	5,759	5,889

The spread ability test was conducted to determine the amount of force required for the lotion to spread over the skin surface when used. The spread ability of all preparations was in the range of 5.13-6.91cm. It is in accordance with the requirements for the spread ability of the lotion, which is 5-7 cm. Good spread ability will make it easier to use, without strong emphasis [13].

The homogeneity test was administered to determine that all the raw materials were mixed evenly in the lotion preparation. The results of the homogeneity test for each formula showed homogeneous results and did not displayany coarse grains and no separation of components or uniformity in shape. It is because the ingredients used in making lotions were well mixed.

3.4. The homogeneity test

Table 6. The homogeneity test

Formula	**Observations During the Storage of the Day**			
	0	**7**	**14**	**21**
F I	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F II	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F III	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F IV	Homogeneous	Homogeneous	Homogeneous	Homogeneous
F V	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Lotion X	Homogeneous	Homogeneous	Homogeneous	Homogeneous

3.5. Adhesiveness

The adhesion test was conducted to determine the ability of the lotion to adhere to the skin when it was used. The adhesion test results of all formulas have adhesion time of 05.03-07.97 seconds, it fulfills the requirements for good adhesion of lotions, which is not less than fourseconds [14]. The longer the stickiness of the preparation, the stronger the ability to adhere to the skin, andthe longer and more optimal the absorption in the skin [15].

Table 7. Adhesion Test

Formula	Adhesion Time During Storage Day (Second)			
	0	7	14	21
F I	07.01	06.65	05.89	05.35
F II	06.86	06.26	06.10	05.68
F III	06.71	05.97	05.80	05.07
F IV	06.01	05.64	05.32	05.03
F V	07.97	07.83	07.09	06.47
Lotion X	07.80	06.64	06.17	05.19

3.6. The Lotion Type

The lotion type test was conducted to determine the type of lotion on the preparation whether it was oil in water or water in oil. The test results of the lotion type in each formula showed that it was included in the oil in water type, marked by the mixing of the lotion preparation with methylene blue (water-soluble dye) evenly after stirring.

3.7. Viscosity Test

Viscosity testing was administered to determine the thickness of preparation, thus, it can be seen whether the preparation was made easy to pour or not when used. The viscosity in each formula has a viscosity ranging from 5588-8987 cPs. It isin accordance with the viscosity requirements for skin moisturizers, which was 2000-50000 cPs [16]. A viscosity which is too high will reduce the comfort level of use, while a viscosity that is too low will drip when applied to the skin [17].

Table 8. Viscosity Test

Formula	ViscosityTime During Storage Day (cps)			
	0	7	14	21
F I	7930	7562	7091	6458
F II	7032	6699	6041	5588
F III	6826	6391	6022	5680
F IV	6468	6353	6004	5873
F V	8987	7270	7250	6573
Lotion X	8055	7848	7522	7320

3.8. Testing Antioxidant Activity with the DPPH Method

Quantitative antioxidant activity testing was performed using the DPPH (1,1-diphenyl-2-pycryhydrazyl) method.

This method is simple, fast and easy for screening the radical scavenger activity of several other compounds and only requires a small sample for testing the antioxidant activity of natural ingredients [18]. The principle of this method is that there is a change in the purple color intensity of DPPH. DPPH free radicals possessing unpaired electrons produce purple color, it will turn yellow when the electrons are paired [19].

The parameter used WasIC50, which was the effective concentration of the compound in the sample which can inhibit 50% of the absorbance of the DPPH control. The smaller the IC50 value, the stronger the antioxidant power [19].

a. Determination of Maximum Wavelength (λmax).The objective of the maximum wavelength determination is to determine the wavelength at which the compound to be measured provides the most optimum absorbance, thus,it has a high sensitivity measurement [20]. The λmax of 0.05 mM DPPH obtained was517 nm. These results were included in the λ DPPH range which rangedfrom 515-520 nm [19].
b. Determination of Operating Time (OT)
The objective of the operating time determination isto determine the time needed to properly react, so as to minimize errors in measurement [20]. OT results at 50 minutes showed a relatively stable absorbance of DPPH so that a single bulb black garlic antioxidant activity test was administered in the 50th minute, while vitamin C took 30 minutes [19]. The reaction time or OT of various compounds varied. Hence, the determination of OT of the most appropriate test compound was performedto determine the perfect time of the reaction, which was indicated by the absence of a decrease in absorbance.

c. Testing of Sample Antioxidant Activity
The samples used in testing antioxidant activity using the DPPH method were single bulb black garlic extract, vitamin C, and lotion. It comprised of vitamin C as a comparison, single black garlic, single bulb black garlic lotion as a test sample, base lotion as a negative control, and vitamin C and market X lotion as a positive control. Vitamin C has the strongest antioxidant activity compared to vitamin A and vitamin E [21]. If the IC50 value of the sample is the same or close to the IC50 value of the positive control for vitamin C, the sample has the potential to be one of very strong alternative antioxidants [9]. The IC50 sample value can be seen in Table 3.

Table 9. IC50

Sample	IC50 (µg/ml)
Black garlic	14,957
Formula I	250,59
Formula II	118,77
Formula III	96,00
Formula IV	70,47
Formula Vit. C	52,80
Lotion X	95,21

Single bulb black garlic has two times higher antioxidant activity than fresh garlic because it contains flavonoid compounds in the form of flavanols, flavones,flavanones, and S-allyl cysteine [22].

Flavonoids are one of the compounds which function as antioxidants because these compounds have an–OH group attached to the aromatic carbon ring.The free radical products of this compound are resonantly stabilized and are not reactive compared to most other free radicals. Thus,they can function as effective antioxidants. The most effective formula as an antioxidant lotion from single bulb black garlic is F IV, which contains 10% single bulb black garlic with an IC50 value of 70.47 ppm. It was proven after conducting the same study using the DPPH method with F. Vit. C as a positive control containing vitamin C 0.02% with an IC50 value of 52.8 ppm which was classified as a strong antioxidant. Hence, the IC50 value for formula F IV with a single bulb black garlic concentration of 10% is close to the IC50 value for positive control for vitamin C lotion.

3.9. Data Analysis

Based on the analysis of the Anova test data, it was obtained that the data were normally distributed and not homogeneous because the two conditions of Anova were not fulfilled. Meanwhile, the data were analyzed using the Kruskal-Wallis test. The Kruskal-Wallis results showed that there were significant differences in the antioxidant activity data ($p \leq 0.05$). The Mann-Whitney analysis was performed to identify the differences in each lotion formula. The results of the analysis showed that in each FI, FII, FIII, FIV, and F Vit.C,lotion X had a significant difference ($p \leq 0.05$), but in FIII, lotion X showed no significant difference ($p > 0,05$). FIII is the best formula because it has the largest sig value. and approaching lotion X as a comparison preparation according to statistical tests.

4. CONCLUSION

Single bulb black garlic has an IC50 value of 14,957 ppm. A single bulb black garlic lotion preparation has antioxidant activity with an IC50 value in each formula, which was FI of 250.59 ppm, FII of 118.77 ppm, FIII of 96 ppm, FIV of 70.47 ppm, F. Vit. C which was52.8 ppm and lotion X which was 95.21 ppm. The single bulb black garlic lotion preparation in FIII showed the best physical properties of the lotion preparation.

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