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Formulation and Evaluation of Black Garlic (*Allium Sativum* L.) Lozenges As An Antioxidant Supplement

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Abstract

Free radicals are species of reactive chemical compounds that can cause degenerative diseases. Antioxidants work to deactivate free radicals by binding to these radicals to stabilize the radicals. One of the plants that contain antioxidants is black garlic. This study aimed to obtain the best formula from the preparation of lozenges of black garlic extract using the wet granulation method as an antioxidant supplement. The formula optimization was carried out using a statistical approach with a two-level factorial method using expert design software. The lozenges formula was made using wet granulation method. The granules and tablets were evaluated for its characteristic. Besides, the levels of polyphenols and antioxidant activity of the extracts and lozenges were also determined. The best formula is F2 obtained from a ratio of gum arabic and starch pregelatin of 3: 5 with a desirability value of 0.922. The Evaluation of black garlic extract granules is eligible, including the moisture content of 2.04%, an excellent flow rate is 18.3 g/s, and good compressibility of 9%. The uniformity of lozenge weight obtained is eligible, which is 3%. The hardness and disintegration time of 9.1 kg and 11.28 minutes, respectively. Meanwhile, the antioxidant activity of the extract and lozenges was 263 µg/mL and 323.9 µg/mL, respectively. The best formula obtained is with 3% gum arabic and 5% starch pregelatin.

Keywords: Antioxidant, Black Garlic, Design expert, Lozenges

1. Introduction

Free radicals are species of reactive chemical compounds, which have an unpaired free electron in their outermost orbital [1]. Excessive exposure of free radicals can cause degenerative diseases, such as cancer, and cardiovascular [2]. So it is needed antioxidants that work to

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deactivate free radicals by binding to these radicals, through electron donation so that radical species become more stable. It is will be neutral and unreactive [1]. One of the plants that contain antioxidants is black garlic [3].

Figure 1. Black Garlic

Black garlic is a product of garlic that undergoes an aging process (heating), at 65-80°C for several days. The heating process uses an electric rice cooker so the color changes due to the Millard reaction. This change also increases its bioactive compounds. These compounds are s-allyl cysteine, amino acids, polyphenols, and flavonoids [4]. In another study, it was proven that black garlic fermented for 15 days had a strong antioxidant, which is 2.41 g/ml [5].

Black garlic also has activity as antibacterial [6], antifungal [7], hepatoprotective [8], antidiabetic [9]. Black garlic has great potential to be developed into supplements and pharmaceutical preparations. However, black garlic has an unattractive shape, black color, sweet and sour taste, and a short shelf life [6]. To improve the taste and make it easier to use, lozenges can be made. In addition, by making lozenges, it can increase shelf life compared to the extract and can be made with the right dose. So in this study, lozenges were made from black garlic.

2. Methods

2.1 Materials

The materials to be used were aquadest, gallic acid, black garlic thick extract, 70% ethanol (Merck), folin-ciocalteau, methanol (Merck), NaOH, black garlic simplicia, DPPH reagent (2,2-Diphenyl-1-pikrilhidrazi). For the formulation materials, Avicel (CV. Quadrant), Gum Arabic (CV. Quadrant), starch pregelatin (CV. Quadrant), sucralos (CV. Quadrant) and talcum (CV. Quadrant) were used.

1.1 Extraction of Black Garlic

Garlic was heated using an electric rice cooker with several variations of time, which are 7, 14 and 21 days so that black garlic was obtained. Then black garlic simplicia was chopped and extracted by maceration method using 70% ethanol. It was carried out for 4x24 h while stirring occasionally. The extract obtained was filtered, then the filtrate was evaporated using a rotary evaporator at 30 rpm and a temperature of 50-60°C to obtain a concentrated extract.

1.2 Total Polyphenol Determination of extract and tablets of black garlic

The standard solution (gallic acid) was prepared with various concentrations of 20, 30, 40, 50, and 60 g/mL. Meanwhile, the test solution was prepared by weighing 0.2 g of extract and adding 25 ml of methanol and then stirring for 30 min with a magnetic stirrer. Then it was filtered and put into a 25 ml volumetric flask and it was added methanol up to the mark. Each 1 ml of the test solution and the comparison solution were put in a test tube and 5 ml of folin-ciocalteu (7.5% in water) was added. It was allowed to stand for 8 minutes, then added 4 ml of 1% NaOH and incubated for 1 hour. The polyphenol content of the extract was measured using a spectrophotometer at 765 nm [14].

1.3 Antioxidant activity test of extract and tablets of black garlic

Antioxidant activity test of black garlic extract and tablets is using DPPH. The antioxidant activity was quantitatively measured using a UV-Vis spectrophotometer. The concentration variations of the samples used were 100, 200, 300, 400, 500 ppm. Samples were added with DPPH reagent and incubated for 30 minutes in a dark room and then measure the absorbance at the optimal wavelength. Determination of the optimum wavelength by measuring the stock solution DPPH (2,2-diphenyl-1-picrylhydrazyl) at a wavelength of 400-800 nm with uv vis spectrophotometry [16].

2.2 Optimization of formulas and formulations of lozenges

Optimization using design expert software, with two factorial methods, to get the best formula from several variations. Optimization was carried out to get the best formula composition to be used. The formulation was carried out using the wet granulation method by mixing the internal phase and adding binders to form lumps and granulation. Oven-dried at 40^0C and printed using a tablet printer.

2.3 Evaluation of granules and lozenges

2.3.1 Granule flow time test

This was carried out by flowing 10 grams of granules in a diverter with a time of not more than 1 second [10].

2.3.2 Granule compressibility test

Determination was using a Tapped density tester. Compressibility was calculated from the density of granules by entering 100 ml of granules into a measuring cup. initial volume was recorded and the device was turned on. The final volume was observed and calculate the compressibility used this equation.

$$\%Compressibility = \frac{Initial\ Volume - Volume\ After\ Tapped}{Initial\ Volume} \times 100\%$$

2.3.3 Tablet visual test

It was carried out by observing the general appearance of the tablet such as: size, shape, color, presence or absence of odor, taste, surface shape and physical defects [10].

2.3.4 Tablet weight uniformity test

It was carried out by weighing 20 tablets and calculating the average weight of each tablet. The uniform requirement is that no more than 2 tablets deviate by more than 5%, and not one tablet deviates more than 10% [10].

2.3.5 Tablet size uniformity test

It used a caliper, by taking 20 or 10 tablets, then measuring the diameter and thickness of the tablets one by one. The requirements for a good tablet are to have a diameter of not more than 3 times and not less than one-third of the tablet [10].

2.3.6 Tablet hardness test

It used a hardness tester, by applying pressure to the tablet until the tablet cracks or breaks, the minimum tablet strength is 4 kg/cm^3 [10].

2.3.7 Tablet friability test

The examination was carried out by weighing the weight of 20 large tablets. It was inserted into the friability instrument and the instrument was operated for 4 minutes at a speed of 25 rpm, about 100 rotation. Then the tablets were cleaned and weighed again and the percent friability was calculated using the formula below. The requirement for a good tablet is the losses of weight less than 1% [10].

$$F = \frac{Wo - W1}{Wo} \times 100\% *$$

*F = Percent of friability ; Wo = initial weight ; W1 = final weight

2.3.8 Disintegration time test of tablets

It used a disintegration testertester, the disintegration time test was carried out on 6 tablets with the condition that all tablets disintegrated in no more than 30 minutes [11].

Data analysis

Determination of the best formula was using the two-level factorial method using the Design Expert software, by entering the responses generated from the evaluation of the tablet preparations in it. Furthermore, the optimal value will be obtained by setting goals and limits for each response. The Design Experts software perform analysis of variance (ANOVA) on the responses used.

3. Results

3.1 Black Garlic Extract

The heated black garlic was oval in shape and soft in texture, sweet and sour in taste, and the odor produced was not as strong as garlic. The longer heating will result in a darker black garlic color. This occurs because of the Millard reaction [15]. During the heating process, the substances contained in garlic will not be damaged because during the process the onions are wrapped in aluminum foil [20]. The results of the extraction of black garlic simplicia from each heating time obtained black garlic extract which was concentrated like caramel, brownish-black in color and has a distinctive smell.

3.2 Total Polyphenol Determination of extract and tablets of black garlic

3.2.1 Extract polyphenol testing

The principle of this method is that the phenolic compounds contained in the extract will be oxidized by Folin Ciocalteu reagent to form a blue complex solution. The highest total polyphenol value was found in the 14-day heating period, which was 15.18 mg GAE/g. x

Table 1. Polyphenol's total of black garlic extract

Heating time (days)	Polyphenol's total (mg GAE/g)
7	4.77 ± 0.0321
14	15.18 ± 0.0215
21	14.59 ± 0,057

3.2.2 Tablet polyphenol testing

From the results of the total polyphenol test, the extract which has the largest polyphenol was carried out for tablet formulations. The total polyphenol values obtained from black garlic extract tablets by heating for 14 days is shown in Table 2.

Table 2. Polyphenol content of tablets

Absorbance	Average	Polyphenol content (mg GAE/g) n = 6
0.4495		
0.4452	0.4453± 0.65	11.998 ± 0.00415
0.4412		

The total polyphenol content of the extract decreased after it was made into tablets, the decrease was not significant. This happens because polyphenols are compounds that are less stable to the effects of light, oxygen, temperature, and chemical changes [21].

3.3 Antioxidant activity test of extract and tablets of black garlic

3.3.1 Antioxidant activity of black garlic extract

The test was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. This method is a simple and fast method [11]. The initial determination of the maximum wavelength of the DPPH was carried out so that the measurements obtained have high sensitivity to changes that occur. The maximum wavelength of DPPH in this test was 516 nm. The measurement results obtained that the highest antioxidant activity was on heating for 14 days, which can be seen from the IC50 value. The smaller the IC50 value, the antioxidant activity is the greater.

Table 3. Antioxidant Activity of Black Garlic Extract

Heating time (days)	IC_{50} (µg/mL) n = 6
7	720.5 ± 0.843
14	263 ± 0.432
21	32.8 ± 0.581

3.3.2 Tablet antioxidant activity test

The principle of reaction change used is colorimetry, namely the change from purple color (DPPH radical) to yellow in the presence of antioxidants (polyphenols). The color change is proportional to the added antioxidant content [12]. The result of the antioxidant activity of black garlic extract tablet with heating for 14 days was 323.9 µg/mL. This is in accordance with the decrease in the levels of polyphenols in the preparation so that the antioxidant activity also decreases. This is because compounds that have a role as antioxidants are polyphenols contained in the extract.

Table 4. Antioxidant activity of black garlic tablet

Repetition	IC_{50}	Antioxidant activity (µg/mL) n = 6
1	325.5	
2	319.3	323.9 ± 4.04
3	326.9	

3.4 Optimization and formulation of lozenges

In the design expert optimization mode, the factors used were Arabic gum as a binder with a value of 1% (low) - 3% (High) and starch pregelatin as a disintegrant with a value of 5% (low) - 10% (High). The responses used were flow rate (g/s) and disintegration time (s). It was obtained 4 formula designs with varying concentrations of Gum Arab and Starch pregelatin.

Table 5. Optimization of lozenges preparation formula

Materials	F1 (%)	F2 (%)	F3 (%)	F4 (%)	Function
Black garlic extract	46.86%	46.86%	46.86%	46,86%	Bioactive component
Pregelatin strach	5%	5%	10%	10%	Disintegrant
Gummi arabicum	1%	3%	1%	3%	Binder

Talcum	2%	2%	2%	2%	Lubricants
Sucralose	0.1%	0.1%	0.1%	0.1%	Sweetener
Avicel	Ad.100%	Ad.100%	Ad.100%	Ad.100%	Diluent

The tablet formulation was carried out using the wet granulation method. The black garlic extract to be formulated has a poor flow rate, so this method is used to improve the flow rate and compressibility in order to form tablets that are homogeneous in content.

3.5 Evaluation of granules and tablets of black garlic extract

3.5.1 Granule flow rate evaluation

From the results of the tests carried out, the flow rate of formula 1 (F1) to formula 3 (F3) had a "very good" flow rate because they had a value of >10 g/s. While the formula 4 (F4) had a "good" flow rate [10].

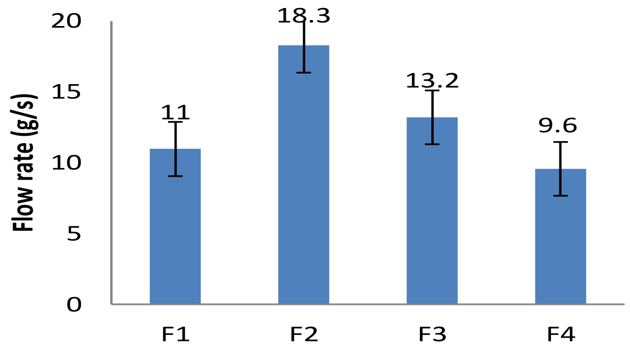

Figure 2. Granule flow rate

2.5.2 Moisture evaluation

Black garlic extract granules have good moisture content except for formula 1, this may be due to the content of excipients added in formulas is different, such as gum arabic and starch pregelatin which have water absorption.

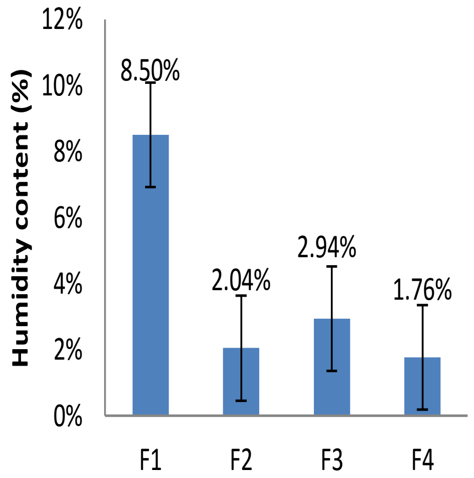

Figure 3. Humidity content of granule

3.5.3 Evaluation of granule compressibility

This test is carried out to determine the nature of the material if given pressure it will form a stable and compact mass [13]. In this work, the granules of F1, F2, and F4 had "very good" % compressibility and F3 had "good" compressibility.

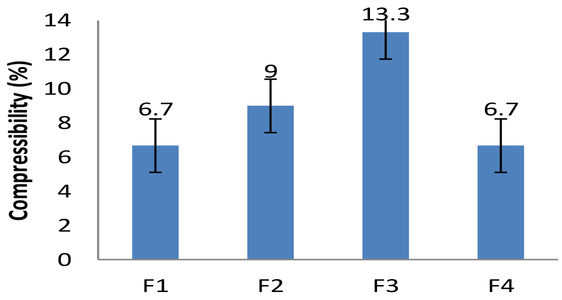

Figure 4. Compressibility of granule

3.5.4 Tablet visual evaluation

The visual form of each formula (F1, F2, F3 and F4) is not much different. Where the tablet was round flat, dark brown in color, sweet and sour in taste and characteristic in odor.

3.5.5 Evaluation of weight uniformity

All formulas (F1, F2, F3, and F4) met the requirements of good tablet. That was, no more than 2 tablets had a storage percentage of more than 5% and there was not a single tablet that deviates more than 10%.

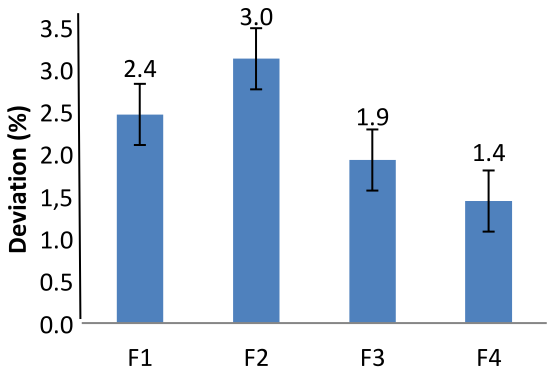

Figure 5. Tablet weight uniformity

3.5.6 Evaluate tablet hardness

The hardness of the tablet is strongly influenced by the compression pressure provided by the tool. The higher the pressure applied, the hardness will increase. In this study, all formulas (F1, F2, F3, and F4) met the requirements of good tablet which was more than 4 kg/cm^3.

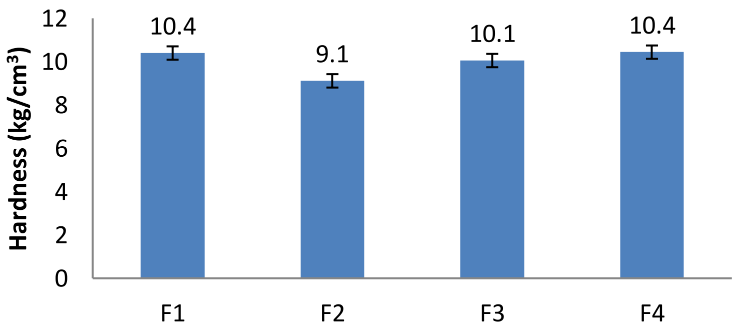

Figure 6. Hardness of tablet

3.5.7 Evaluation of tablet friability

The formulas (F1, F2 and F4) meet the requirements as good tablet. While F3 was not eligible. The eligible conditions were the tablet friability of less than 1%. This may be due to the influence of the binder and disintegrant used. F3 used a binder with a low concentration of 1% and a high concentration of disintegrant, which was 10%, so that the tablet formed had a high friability.

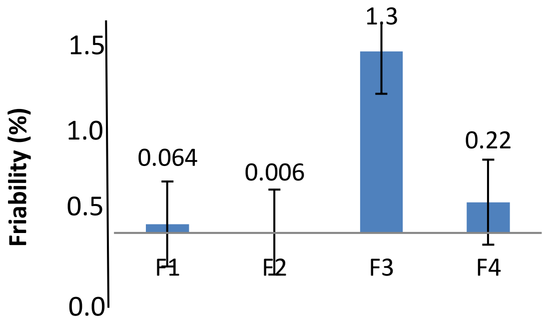

Figure 7. Friability of tablet

3.5.8 Evaluation of tablet disintegration time

Disintegration time testing was carried out to determine the tablet's ability to dissolve in the body. Where a good disintegration time requirement for lozenges is less than 30 minutes. The test results show that all formulas (F1, F2, F3, and F4) were eligible which were less than 30 minutes of disintegration time.

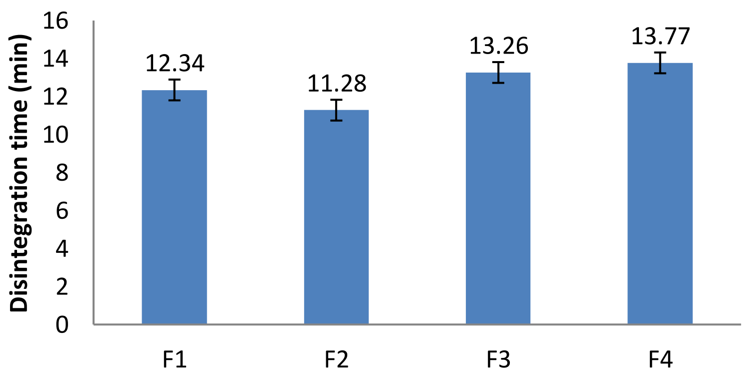

Figure 8. disintegration time of tablet

3.6 Data analysis

Response data included in the design expert were flow rate and disintegration time. Where the flow rate category used is "maximize", because a large flow rate value is directly proportional to good flowability. And the disintegration time category entered is “minimize” because the disintegration time of lozenges must be under 30 minutes. So the smaller of disintergartion time, the tablet is better.

The P-value of gum arabic and starch pregelatin on the response to flow rate and disintegration time showed a value of <0.05. So there was a significant difference between gum arabic and starch pregelatin on flow rate and disintegration time. So it shows that H0 is rejected and H1 is accepted. However, the value of gum arabic in response to disintegration had a value of more than 0.05 so that the effect of disintegration time on gum arabic was not significantly different. To determine the best formula, it can be seen from the desirability value of each formula.

Table 6. The desirability value of each formula

Formula	% Composition (Arabic gum : primojel)	Flow rate response (g/s)	Disintegration time response (s)	Desirability value
1	1:5	10.31	721	0.345
		10.53	735	
		12.05	766	
2	3:5	18.8	691	0.922
		18.5	664	
		17.5	676	
3	1:10	13.3	774	0.104
		13.5	789	
		12.8	825	
4	3:10	9.3	807	0.376
		9.4	810	
		10	861	

The desirability value is the value of the program's ability to meet the good characteristic, the best desirability value is close to 1. In this work the best desirability value from the above formula was formula 2 with a gum arabic value of 3% and a starch gelatin value of 5%, with a desirability value of 0.922.

4. Discussion

Antioxidants work to deactivate free radicals by binding to these radicals, through electron donation so that radical species become more stable [1]. The use of antioxidants is widely used from several plants such as turmeric, green tea, and black garlic [17,18]. Black garlic is a product of garlic that undergoes an aging process (heating), at a temperature of 65-80^{o}C for several days using an electric ricecooker so that changes occur due to the Millard reaction, this change also increases the bioactive compounds it contains. These compounds are s-allyl cysteine, amino acids, polyphenols and flavonoids [19]. So that black garlic is one of the plants that has high antioxidant activity and contains active compounds, namely phenols, flavonoids, pyruvate, thiosulfate, s-alilcysteine, and s-allymercaotocysteine.[3]

From some of these activities, according to Zhafira's research (2018), black garlic has a high antioxidant activity of 3,475 µg/g [18], and in Agustina et al's study (2020), proved that black onions fermented for 15 days have strong antioxidants, namely 2, 41 µg/ml.[5] In this study, it was found that the antioxidant activity of the extract was 263 µg/mL, and the antioxidant activity of lozenges was 323.9 ± 4.04 µg/mL. The result can be seen in Table 4. This shows that the antioxidant activity of black garlic in this study is smaller than that of other studies. This can occur due to several factors such as the heating process in the manufacture of black garlic which in this study showed that too long the heating process can reduce antioxidant activity but lack of heating also causes low antioxidant activity. This result can be seen in Table 3. Therefore, sufficient heating time is required to produce large antioxidant activity. In addition, the storage process can affect because storage for too long can reduce antioxidant activity. Then, the addition of excipients in the manufacture of these lozenges can also affect the content and antioxidant activity in them. In addition, the antioxidant activity is influenced by its

polyphenol content. The antioxidant activity of extract decreases, along with the decreasing levels of polyphenols in the preparation. We can conclude that it can be seen from tables 1,2, and 4 in result.
Then, to facilitate the consumption of antioxidants, lozenges can be made. In this manufacture of lozenges from this study, the best formula was obtained, namely F2 with a ratio of gum arabic and starch pregelatin (3:5) with a desirability value of 0.922. The evaluation of the black garlic extract granules met the requirements, including the moisture content of 2.04%. The flow rate is very good, namely 18.3 g/s. Good compressibility is 9%. The evaluation results of lozenges for the size uniformity test met the requirements, the weight uniformity test met the requirements, namely 3%. hardness of 9.1 kg. The disintegration time was 11.28 minutes. This shows that lozenges are suitable and good for making preparations from black garlic.

5. Conclusion

The results show that the best formula is F2 with a ratio of gum arabic and starch pregelatin (3:5) with a desirability value of 0.922. The antioxidant activity of the extract and tablet of black garlic is 263 µg/mL and 323.9 ± 4,04 µg/mL, respectively.

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