Formula Optimization and Antioxidant Activity Test of Combination Drink of Ginger (Zingiber Officinale var. rosc) and Lemongrass (Cymbopogon citratus): DPPH Method

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Abstract. Ginger and lemongrass are herbal plants that have antioxidant properties. Ginger contains phenolic compounds such as gingerol, shogaol, and paradol which function as antioxidants. Lemongrass has secondary metabolite compounds such as saponins, tannins, alkaloids, steroids, phenols, and flavonoids, which have the potential to be antioxidants. This study aims to formulate drinks made from ginger powder and lemongrass powder with brown sugar as a sweetener and identify each formula's antioxidant activity. This study is an experimental study consisting of 6 drink formulations, namely F1, F2, F3, F4, F5 and F6. The ginger and lemongrass drink formulas were tested for antioxidant activity using the DPPH method by calculating the IC50 value of each formula, and a hedonic test was carried out. Analysis of hedonic test data using the Global linear model test then continued with the Duncan test. The results of the hedonic test showed that F6 was the most preferred by panelists, with the highest score range of 3.76 for the taste parameter and 3.75 for the aroma parameter. The results of the antioxidant activity test showed that F6 had the highest antioxidant activity with an IC50 value of 92.51 ppm.

Keywords: Ginger, Lemongrass, Antioxidants, Antioxidant Activity, DPPH

1 INTRODUCTION

Free radicals are unstable and highly reactive molecules because they have one or more unpaired electrons [1]. Free radicals can arise from various complex chemical processes in the body, environmental pollutants, cigarette smoke, radiation from chemicals, toxins, high-intensity exercise, fast food, and foods with high-temperature frying [2]. Free radicals will react with surrounding molecules to obtain electron pairs to achieve molecular stability [3]. Free radicals can damage cells by damaging lipid membranes through lipid peroxidation. Lipid peroxidation occurs because cell membranes contain

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Polyunsaturated Fatty Acids (PUFA), which are very susceptible to free radicals [4]. High free radicals in the body cause continuous lipid peroxidation in the body and if not stopped will result in diseases such as cancer, premature aging, heart disease, and other degenerative diseases [3]. Antioxidants are needed to neutralize and prevent damage caused by free radicals. Antioxidants can complement the lack of electrons needed by free radicals and inhibit the occurrence of chain reactions from the formation of free radicals [5].

Ginger (Zingiber officinale var roscoe) and lemongrass (Cymbopogon citratus) are plants that contain bioactive compounds that have antioxidant properties [6,7]. Ginger contains bioactive compounds such as phenolics and terpenes. Phenolic compounds such as gingerol, shogaol and paradol function as antioxidants [8]. The potential mechanism of antioxidant action in ginger is that the 6-shogaol compound activates the nuclear factor erythroid 2-related factor 2 (NrF2) signaling pathway and increases the expression of the NrF2 target gene (endogenous antioxidant gene). Ginger shogaol prevents NrF2 from proteasomal degradation so that glutathione (GSH) levels increase and Reactive Oxygen Species (ROS) levels decrease [6]. Based on the results of the study, the antioxidant activity of ginger has an IC50 value of 57.14 ppm, which means it has potent antioxidant activity [9]. Lemongrass contains citronellal which can reduce the concentration of oxygen species reactions, lipid peroxidation, and 2,2-diphenyl-1picrylhydrazyl (DPPH) [10]. Lemongrass can also support the endogenous antioxidant defense system in alveolar macrophage cells by increasing superoxidase dismutase (SOD) activity and the formation of glutathione peroxidase (GPX) [11]. The results of the antioxidant activity test study on lemongrass drinks showed an IC50 value of 50.68 ppm, meaning that lemongrass drinks have potent antioxidant activity [12].

2 METHOD

The type of research conducted is an experimental study aimed at examining the antioxidant activity in samples of ginger and lemongrass combination drinks. The stages of this research include the process of selecting fresh ingredients, cleaning ginger and lemongrass from dirt, peeling, cutting into small sizes, drying, grinding, mixing ingredients and brewing, antioxidant activity testing and preference testing (Hedonic).

The research was conducted in the nutrient analysis laboratory of the University of Muhammadiyah Semarang. The materials used were fresh ginger, fresh lemongrass, brown sugar obtained from the Pedurungan market in Semarang City, DPPH solution, methanol PA, and distilled water. The tools used were knives, basins, kitchen scales, food dehydrators, blenders, 60 mesh sieves, analytical scales, measuring cups, Erlenmeyer flasks, test tubes, micropipettes, and UV Visible spectrometers.

2.1 Preparation of ginger powder

The process of making ginger powder begins with washing the ginger until it is clean from dirt, peeling the ginger, then thinly slicing it, then drying the ginger in a food

dehydrator at a temperature of 60°C for 24 hours. After the ginger is dry, it is blended until smooth and sieved with a 60 mesh sieve [13].

2.2 Preparation of lemongrass powder

The making of lemongrass powder starts from the lemongrass stalks which are washed clean then cut into small pieces, then dried in a food dehydrator at a temperature of 60°C for 24 hours. After that, the dried lemongrass stalks are blended until smooth and sieved using a 60 mesh sieve [14].

2.3 Making a combination drink of ginger and lemongrass

The making of the drink starts from mixing ginger powder and lemongrass powder by brewing it using 150ml of warm water at a temperature of 60°C and adding brown sugar as a sweetener. The ginger lemongrass drink formulation table can be seen in table 1.

Table 1. Formulation of a ginger and lemongrass combination drink

Formula	Comparison ginger : lemongrass	Ingredients Composition
F1	2 : 1	2g ginger : 1g lemongrass : 15g brown sugar
F2	1 : 1	1g ginger: 1g lemongrass : 15g brown sugar
F3	1 : 2	1g ginger: 2g lemongrass: 15g brown sugar
F4	2 : 1	2g ginger : 1g lemongrass : 20g brown sugar
F5	1 : 1	1g ginger : 1g lemongrass : 20g brown sugar
F6	1 : 2	1g ginger : 2g lemongrass : 20g brown sugar

2.4 Antioxidant Activity Test Using the DPPH Method

Antioxidant activity testing was carried out on all formulations of lemongrass ginger drinks to obtain the best formulation. Antioxidant activity testing used the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method. Measurement of antioxidant activity using a UV Visible spectrophotometer at a wavelength of 517 nm.

2.5 Preparation of DPPH Solution

DPPH powder was weighed as much as 0.006g then dissolved in 100mL of PA methanol then homogenized and covered with aluminum foil.

2.6 Preparation of Sample Solution

3g of material / 150 ml was diluted to 1000 ml to obtain a concentration of 1000 ppm. Then the solution was vortexed until homogeneous. Then dilution was carried out with different concentrations, namely 500 ppm, 250 ppm, 125 ppm, 62.5 ppm and 31.25 ppm.

2.7 Antioxidant Activity Test

Each concentration of ginger lemongrass drink sample solution F1, F2, F3, F4, F5 and F6 was pipetted 0.2 ml with a micro pipette, put into a reaction tube and added 3.9 ml of 0.16 mM DPPH solution. Shake with a vortex for 1 minute and incubate for 30 minutes at room temperature in a dark place. Perform absorbance on a spectrophotometer with a wavelength of 517 nm for each solution, observe and record the absorbance results.

2.8 IC50 Measurement

Antioxidant activity is expressed in percent inhibition. Based on the percent inhibition sample, each is plotted on the x and y axes in the linear regression equation $y = ax + b$. This equation is used in determining the IC50 value. Each sample can be expressed with a y value of 50 and an x of the IC50 value [15].

2.9 Hedonic Test

The Hedonic Test is expressed in two measurement scales, the hedonic and numeric scales. The hedonic scale shows respondents liking or disliking of the ginger lemongrass drink. The numeric scale shows the quantitative numbers of the hedonic scale assessment that can be used as a basis for statistical data analysis. The hedonic test of ginger lemongrass drinks includes taste, color, aroma, and texture. Five levels of hedonic scales and numeric scales were used in this study [16].

Table 2. Hedonic scale and numerical scale [17]

Hedonic scale	Numerical scale
Really like	5
Like	4
Quite Like	3
Dislike	2
Very Dislike	1

2.10 Data Analysis

The Excel program was used to analyze %inhibition and IC50, producing a concentration curve (ppm) against %inhibition. Then, the regression equation $y = ax + b$ was obtained. The hedonic test (preference) of the ginger lemongrass drink sample used a global linear model test and continued with the Duncan test to determine the best formulation.

3 RESULTS AND DISCUSSION

3.1 Antioxidant Activity

The antioxidant activity of ginger and lemongrass combination drinks was determined using the 2,2-diphenyl-2-picrylhydrazyl (DPPH) method by selecting the IC50 value. Antioxidant activity was analyzed on all formulations of ginger and lemongrass combination drinks. The results of the antioxidant activity test of ginger and lemongrass drinks from each sample F1, F2, F3, F4, F5, and F6 were obtained from DPPH absorbance measurements at a wavelength of 517 nm. The IC50 results and the antioxidant activity measurement curve of each formula can be seen in Table 3 to 8.

Table 3. Table 3. Results of antioxidant activity test of Formula 1

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.487	66.87	125.46
500	6.21	0.513	65.10	
250	5.52	0.683	53.54	
125	4.83	0.797	45.78	
62.5	4.13	0.821	44.15	
31.25	3.44	0.862	41.36	

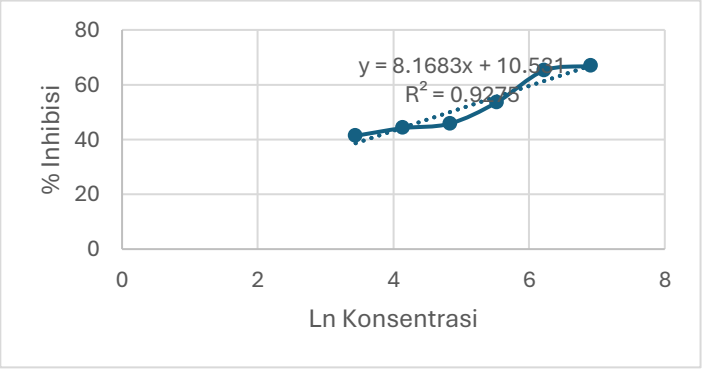

Fig. 1. Result curve of antioxidant activity test of formula 1

Table 4. Antioxidant activity test results for formula 2

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.582	60.41	142.28
500	6.21	0.614	58.23	
250	5.52	0.689	53.13	

125	4.83	0.734	50.06
62.5	4.13	0.825	43.88
31.25	3.44	0.856	41.77

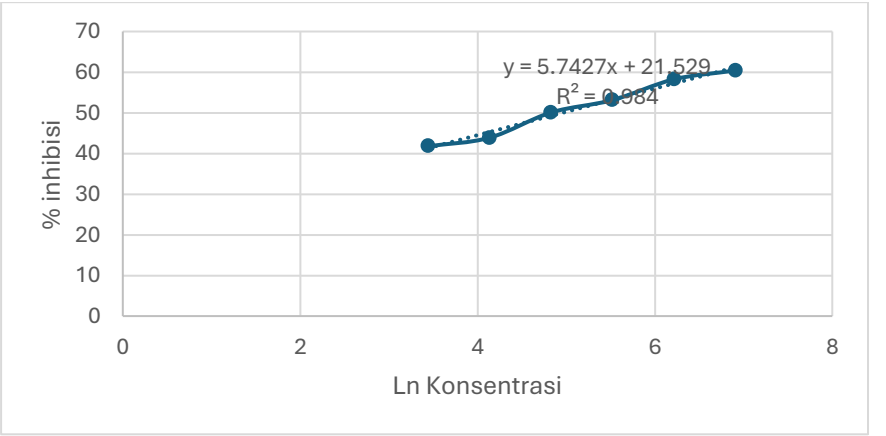

Fig. 2. Antioxidant activity test result curve of formula 2

Table 5. Results of antioxidant activity test of formula 3

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.478	67.48	119.15
500	6.21	0.583	60.34	
250	5.52	0.632	57.01	
125	4.83	0.756	48.57	
62.5	4.13	0.821	44.14	
31.25	3.44	0.868	40.95	

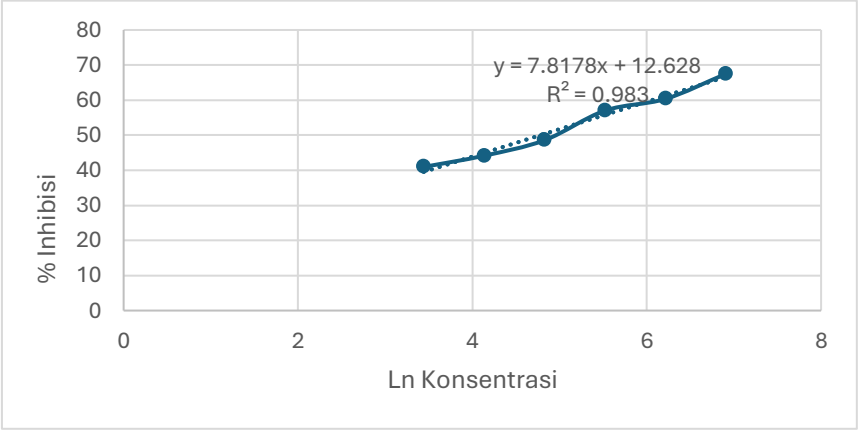

Fig. 3. Test Result Curve of Antioxidant Activity of Formula 3

Table 6. Results of antioxidant activity test of formula 4

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.489	66.73	108.53
500	6.21	0.534	63.67	
250	5.52	0.645	56.12	
125	4.83	0.715	51.36	
62.5	4.14	0.787	46.46	
31.25	3.44	0.895	39.11	

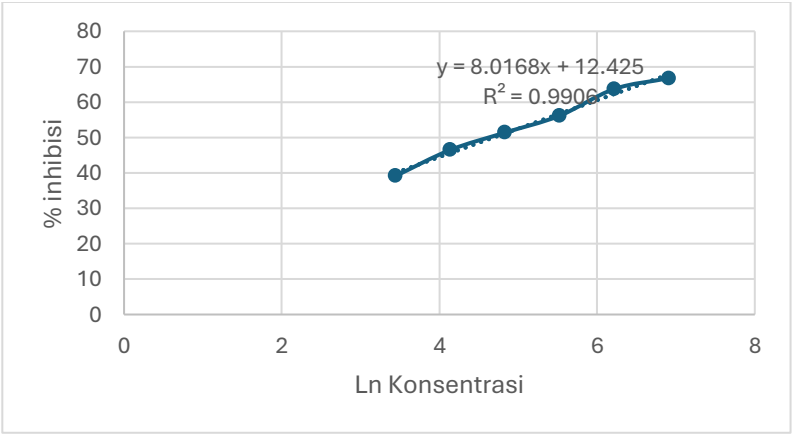

Fig. 4. Result curve of antioxidant activity test of formula 4

Table 7. Antioxidant activity test results for formula 5

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.531	63.88	123.46
500	6.21	0.597	59.39	
250	5.52	0.647	55.99	
125	4.83	0.714	51.43	
62.5	4.13	0.816	44.49	
31.25	3.44	0.884	39.86	

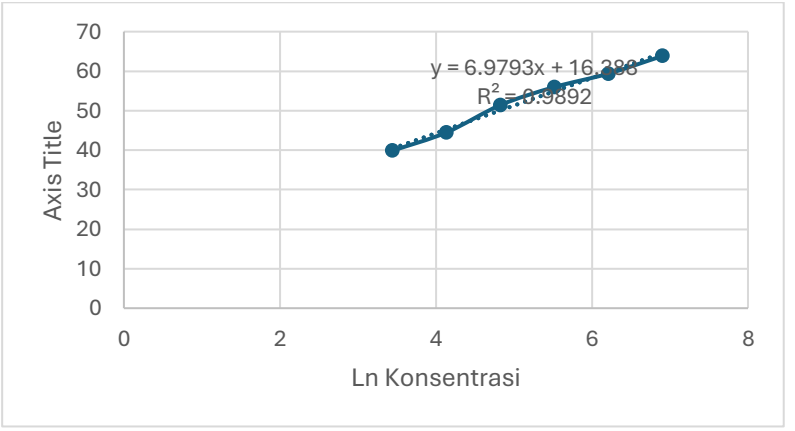

Fig. 5. Result curve of antioxidant activity test of formula 5

Table 8. Results of antioxidant activity test of formula 6

Concentration (ppm)	Ln Concentration	abs sample	%Inhibition	IC50
1000	6.91	0.453	69.18	92.51
500	6.21	0.522	64.49	
250	5.52	0.597	59.39	
125	4.83	0.654	55.51	
62.5	4.13	0.734	50.06	
31.25	3.44	0.945	35.71	

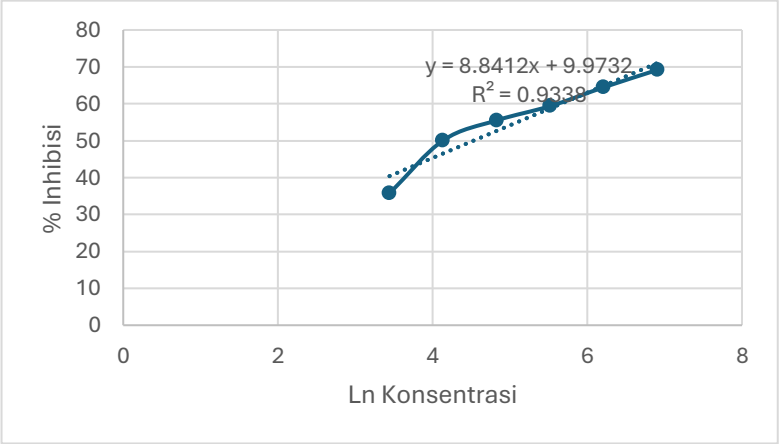

Fig. 6. Result curve of antioxidant activity test of formula 6

The results of % inhibition and IC50 of each formulation can be seen in Table 9.

Table 9. Results of Antioxidant Activity Tests of Various Formulas

Sample	%inhibition	IC50 (ppm)	Antioxidant
F1	66.87	125.46	Medium
F2	60.41	142.28	Medium
F3	67.48	119.15	Medium
F4	66.73	108.53	Medium
F5	63.87	123.46	Medium
F6	69.18	92.51	Strong

Based on the results of the antioxidant activity test above show that formulation 6 (F6) has a higher % inhibition than other formulations. Percent inhibition (% antioxidant activity) is one of the parameters that indicate the ability of an antioxidant to inhibit free radicals. The parameter used to determine the extent of the compound's ability as an antioxidant is the IC50 value. The IC50 value is the concentration of the antioxidant compound needed to capture 50% DPPH free radicals. The IC50 value can be obtained from the linear regression equation that states the relationship between solution concentration (x) and % inhibition (y) [16]. Based on the table 9, F6 has a vigorous antioxidant activity of 92.51 ppm. A compound is said to be a powerful antioxidant if the IC50 value is less than 50 ppm, strong if the IC50 value is between 50 - 100 ppm, moderate if the IC50 value ranges from 100 - 150 ppm, and weak if the IC50 value ranges from 150 - 200 ppm. If a substance has an IC50 of more than 500 ppm, then the substance is less active or weak [16]. The working principle of the DPPH method is the presence of hydrogen atoms from antioxidant compounds that bind to free electrons in radical compounds, causing a change from free radicals (diphenylpicrylhydrazyl) to non-radical compounds (diphenylpicrylhydrazine) indicated by a color change from purple to yellow (free radical compounds are reduced by the presence of antioxidants) [19].

3.2 Hedonic Test

The hedonic test was conducted by 55 panelists, including 30 students and 25 PS Undip football athletes. The ginger and lemongrass drink formulation consists of 6 formulas, as shown in Table 10.

Table 10. Hedonic test combination drink of ginger and lemongrass

Parameter	F1	F2	F3	F4	F5	F6	*P value*
Taste	2.56	3.53	3.13	2.75	3.60	**3.76**	0.001*
Aroma	3.75	3.49	3.31	3.44	3.36	**3.75**	0.003*
Color	3.09	**3.73**	3.33	3.16	3.47	3.15	0.001*
Texture	2.75	**3.82**	3.20	3.33	3.15	3.22	0.001*

Statistic Test **general linear model, Duncan*

The average assessment of the panelists on the organoleptic properties of taste was on a scale of 1-5, and the highest assessment was in F6 with a value of 3.76. The results of statistical tests using the global linear univariate model of taste parameters showed a significant value of <0.001, so it can be concluded that there is a difference in the results of the organoleptic taste test in the ginger and lemongrass drink formulas in the F1–F6 treatments. Furthermore, the Duncan post hoc test showed that F6 had the highest value of 3.76, which means that the panelists preferred formula six. Elephant ginger has a lower oleoresin content (2.8%) than red ginger (3.9%) and emprit ginger (3.5%). Oleoresin is an active compound in ginger that consists of gingerol, shogaol, and zingerone, giving ginger a spicy taste. The low oleoresin content makes the drink formula not too spicy and can be accepted by the panelists. Lemongrass is usually used to give a distinctive flavor to food or drinks so that it can increase the acceptability of ginger and lemongrass combination drinks [19].

Based on the aroma parameters, the highest average hedonic test results were at F6, which was 3.75. Ginger has a less sharp aroma, so ginger is suitable for making drinks because it has the opportunity to have an acceptable level of aroma [20]. Fresh dried lemongrass will cause the fresh aroma of lemongrass to come out. The aroma of the ingredients that are heated/dried will cause the substances in the ingredients to evaporate, which will result in a distinctive aroma [21].

The highest scoring color parameter for the hedonic test was at F2, which was 3.73. All ginger and lemongrass combination drink formulas had almost the same color. The brown color of the drink comes from the added brown sugar and the drying process of the ginger and lemongrass. The drying process of the ingredients can cause a browning reaction, causing a change in color and making it less attractive [22].

The drink's color can be combined with other ingredients to make it more attractive, such as adding sappanwood or talent leaves. The ginger drink with the highest texture score was F2, with a score of 3.78. Generally, the drink's texture is not much different for each formulation. The ingredients or the volume of water added can influence the drink's texture.

4 CONCLUSION

The antioxidant activity test of a combination of elephant ginger and lemongrass drinks with various variations obtained the best formulation, namely F6, with a composition of 1g ginger, 2g lemongrass, and 20g brown sugar and an IC50 value of 92.51 ppm. The results of the hedonic test of various formulations of ginger and lemongrass drinks on the enormous scale were F6 for taste parameters (3.76) and aroma (3.75) and F2 for color parameters (3.73) and texture (3.82).

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