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Optimization of Ultrasonic Assisted Extraction Process on Antioxidant Activity of Honje Fruit Extract (*Etlingera elatior*) using Surface Response Method

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Abstract

Honje fruit (*Etlingera elatior*) contains bioactive compounds as antioxidants. An antioxidant can be obtained by extraction. This research aimed to determine the best combination of solvent amount and extraction time to produce the optimal yield and antioxidant activity. The extraction method was UAE (*Ultrasound Assisted Extraction*) using ethanol 96% solvent amount 125 mL to 250 mL and time level of 30 to 60 minutes. The RSM (Response Surface Methodology) in the Design Expert 11 application was used to arrange the extraction combination treatment, which resulted in 13 runs. Parameters analyzed were total yield, antioxidant activity, pH, specific gravity, and color. The results showed that total yield was revealed quadratic, $Y_1 = 19.05 - 1.76A + 0.32B - 0.002AB + 0.023A^2 - 0.0005B^2$ and the antioxidant activity was revealed linear, $Y_2 = 408.147 - 6.424A + 0.326B$. The optimum treatment was achieved in the amount of solvent 174.815 ml and extraction time of 60 minutes resulted in a total yield of 17.125% and antioxidant activity of 77.55 ppm that could be classified as strong.

Keywords: antioxidant, honje fruit extract, *Response Surface Methodology, Ultrasound Assisted Extraction*

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1. INTRODUCTION

One source of antioxidant compounds is plants with a high content of polyphenolic compounds. One kind of plants has potential as a source of natural antioxidants is honje (*Etlingera elatior*). According to Naufalin *et al.* (2010), the phytochemical content of flowers, stems, rhizomes, fruits and leaves of kecombrang include alkaloids, saponins, tannins, phenolics, flavonoids, triterpenoids, steroids, and glycosides that act as antioxidants. Antioxidants are compounds that are able to inhibit the rate of oxidation of other molecules or neutralize free radicals (Tristantini *et al*, 2016). According to the research of Jabbar *et al*. (2019) the antioxidant activity of the ethanol extract of honje fruit using the maceration extraction method was 72.518 ppm with a mass of honje fruit powder used of 1870 grams. The ethanol extract of honje fruit belongs to the category of strong antioxidant activity.

Extraction is a very important process in the isolation and identification of phenolic compounds. The content of flavonoid compounds and antioxidant capacity of plant extracts is highly dependent on the extraction conditions and the composition of chemical compounds in plant cells (Lapornik *et al.*, 2005; Ksouri *et al.*, 2008). The extraction process used in this study is Ultrasonic Assisted Extraction or commonly referred to as UAE. Ultrasound Assisted Extraction (UAE) is an extraction technique by applying ultrasonic waves to the material to be extracted (Bermúdez-Aguirre *et al.*, 2011). The advantages of using ultrasonic technology according to Radi *et al.* (2014) is that this technology can extract faster, consumes less energy and allows solvent reduction, unit operation, emits less CO_2 and can produce pure products with higher yields.

In the UAE extraction process, ultrasonic waves move through the solvent and produce bubbles that cause a pressure drop or also known as cavitation. This cavitation process will cause damage to plant cells which cell membranes in honje fruit is being damaged and broken so that the extract content will be easily taken up and dissolved in solvents (Wardiyati, 2004). According to Zivkovic *et al.* (2018), the extraction of antioxidant content using UAE is depended by the type of solvent, temperature, time, and the ratio amount of materials and solvents. In addition, the sonication intensity or amplitude also affects the extraction process with the UAE (Wardiyati, 2004). According to Chemat *et al.* (2011), extraction with the UAE has advantages which is this extraction does not cause high temperatures, efficient in mass and energy transfer, and produces high yields. Determination of the optimum conditions for this extraction treatment is known by using RSM. According to Montgomery (1991) in Oramahi (2008) RSM is some of mathematical and statistical techniques used for modeling and problem analysis in a response that is influenced by several variables and the goal is to optimize the response. RSM makes it possible to find the optimum conditions for a response that is influenced by complex variables (Oramahi, 2008).

In this study, UAE was carried out with a combination of variations in the amount of solvent and variations in extraction time. The method used to test the antioxidant activity of this research is the *1,1-diphenyl-2-picrylhydrazyl* (DPPH) method. The principle of spectrophotometry at visible light wave lengths around 515-517 nm is used to measure antioxidant activity by the DPPH method, which changed from the dark purple color due to the reaction of DPPH compounds in ethanol (Molyneux, 2004). The yield is usually expressed as the effective concentration (IC50), which corresponds to the number of samples required to reduce the initial concentration of the DPPH radical by 50%, this unit of expression allows comparison of results independent of the sample concentration (Moharram, 2014).

The purpose of this study was to examine the effect of the amount of solvent and extraction time in the UAE on the yield and antioxidant activity of the honje fruit extract and to determine the best combination of treatments that produced the highest yield and the strongest antioxidant activity. The expected benefit from this research is to provide information about the extraction conditions at an exact combination of time and solvent amount in the extraction of honje fruit using UAE with the strongest antioxidant activity value so that it can be useful as a reference in academic and industrial fields.

2. MATERIALS AND METHODS

Tools

The tools used are 30 mesh sieve, glass beaker, dark amber bottle, cup, glass funnel, colorflex, desiccator, erlenmeyer, measuring cup, grinder, incubator, infrared thermometer, Whatman No. filter paper. 42, refrigerator, 25 ml volumetric flask, magnetic stirrer, oven, pycnometer, pipette, Q-Sonica Ultrasonic Processor (Qsonica – Q500, 500 W, 20 kHz), Rotary Vacuum Evaporatorm (Heidolph p/n 562 – 01300–00), UV-Vis spectrophotometer (Reyleigh UV-9200), digital and analytical balance, and vortex (Hwashin 250VM).

Materials

The material used is fresh honje fruit (Etlingera elatior) from Pangandaran, West Java, dark red-purple in color, 10-20 cm in diameter per 1 fruit bulb, each 2-2.5 cm in size of 1-1.5 kg which is then dried and powdered. The chemicals needed to carry out the extraction are 96% ethanol and aquadest. Other chemicals used to perform the antioxidant test are DPPH (2,2-diphenyl-1-picrylhydrazyl) and methanol.

Method

This study used a laboratory experimental method where the number of treatments (running) is determined by the Design Expert version 11 application using RSM type CCD (Central Composite Design). This design combines 2 independent variables to produce 2 responses. The first independent variable is the extraction time with a minimum limit of 30 minutes and a maximum limit of 60 minutes. The second independent variable is the amount of solvent with a minimum amount 125 ml and a maximum amount 250 ml.

Raw Material Preparation

The process of preparing research raw materials was carried out by crushing the honje fruit from the hump then drying the honje fruit in an oven at 60℃ for 65 hours. Next, the dried honje fruit was reduced in size by using a mesh of 30 to become honje fruit powder.

UAE Extraction

The extraction process used 10 grams of honje fruit powder with 96% ethanol as solvent. The amount of solvent used is adjusted to the RSM application. Extraction using the UAE method with an amplitude of 65% and a time determined by the RSM application. The extraction process used a Q-Sonica Ultrasonic Processor with pulse intervals of 3 seconds on 2 seconds off. The extract was filtered using filter paper and evaporated at a temperature of 50℃, 80 rpm to obtain a concentrated extract.

Antioxidants Test

Quantitative test of antioxidant activity was carried out using the DPPH (*1,1-Diphenyl-2-picrylhydrazyl*) method. Determination of antioxidant activity (IC50) used the method from Molyneux (2004). The stock solution of *1,1-Diphenyl-2-picrylhydrazyl* was prepared in a 25 mL volumetric flask using methanol as a solvent at a concentration of 160 ppm. The extract was again diluted with methanol at concentrations of 200 ppm, 400 ppm, and 1000 ppm then homogenized and adjusted using a 25 ml volumetric flask. The test is done by preparing a blank solution and samples. The blank solution and sample were vortexed and incubated in the dark for 30 minutes. Absorbance measurements were carried out at wavelength 517 using a UV-Vis spectrophotometer.

Total Yield

The total yield is the ratio of the mass of the resulting honje fruit powder extract with the mass of the extracted raw materials. The total yield calculation uses the following equation.

$$\text{Total Yield} = \frac{\text{honje powder mass} - (\text{extract mass} \times \text{residual solvent level})}{\text{initial fruit mass}} \times 100\% \quad (1)$$

Residual Solvent Level

This analysis described the remaining solvent in the extract which is calculated based on the weight of the solvent evaporated from each unit weight of the evaporated material. Measurement of residual solvent content refers to Guenther (1987). Calculation of residual solvent content is as follows.

$$\text{Residual Solvent Level} = \frac{b-c}{b-a} \times 100\% \quad (2)$$

Where,a = mass of empty evaporator flask (g); b = initial mass of pumpkin + honje fruit extract (g); c = mass of flask + extract after evaporation (g)

Specific Gravity

Specific gravity can be used to describe the level of purity of the resulting extract. The method of measuring the density of maserate/honje fruit extract is based on the ratio of the mass of maserate/honje fruit extract to the mass of water (aquades) at the same volume and temperature. The measurement of the specific gravity of the extract refers to SNI-06-2385-2006. The calculation of the specific gravity of the extract is as follows.

$$\text{Specific gravity} = \frac{m_2 - m}{m_1 - m} \quad (3)$$

Where,
m = mass of empty picnometer (g); m1 = pycnometer mass + aquades (g); m2 = pycnometer mass + extract (g)

Color Analysis

Color analysis of the honje fruit extract was carried out using color flex with the principle of reading the notation on the color flex consisting of the values of L, a*, b*, C, and Hue. Total Color Difference (TCD) analysis was also carried out to see the effect of the ultrasonic-assisted extraction process on the color changes that occur in the material. TCD analysis was performed using the following equation.

$$TCD = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (4)$$

Where, $\Delta L = L_{extract} - L_{raw\ material}$; $\Delta a^* = a^*_{extract} - a^*_{raw\ material}$; $\Delta b^* = b^*_{extract} - b^*_{raw\ material}$

pH Analysis

The pH analysis is performed as an indicator of the acid-base of a solution. The pH test was carried out using a pH meter by inserting the pH meter into the extract solution which had been diluted with a dilution of 1 gram of extract with 10 ml of distilled water.

3. RESULTS AND DISCUSSION

Raw Material Characteristics

These characteristic tests include testing the moisture content of fresh honje fruit, testing the moisture content of honje fruit after drying and testing the moisture content of honje fruit powder. The table below shows the moisture content of fresh honje fruit, moisture content of dried honje fruit, and moisture content of honje fruit powder.

Table 1. Moisture Content of Dried Honje and Honje Powder Sample (%)

Sample	Moisture Content (%)
Fresh Honje	59.10
Dried Honje	2.30
Honje Powder	4.29

The average moisture content of honje fruit powder is 4.2 9%. The water content of the powder increased when compared to dried honje fruit. This is due to the fruit powder which is hygroscopic or easily absorbs water from the environment (Wingate, 1989). In addition, the grinding treatment is also the cause of the increase in the moisture content of the honje fruit powder. The frictional force generated during the powdering process causes heat in the grinder. The heat in the closed grinder causes steam so that there is an increase in the moisture content of the honje fruit powder when the grinding process is carried out.

UAE Condition

The extraction process was carried out using ultrasonic waves, ultrasonic waves were channeled through an ultrasound probe which will then hit the material to be extracted. Several factors that can affect the UAE process, including the type of solvent, the amount of solvent used, the length of the extraction time, and the magnitude of the amplitude used during the extraction process. Extraction was carried out following the combination of treatments shown in the design expert 11 application, which amounted to 13 treatments, where there were 5 repetitions of the midpoint. The combination of extraction treatments given by RSM can be seen in Table 2. below.

Tabel 1. RSM combination

Treatment	Time (minutes)	Solvent Amount (mL)
1	60	125
2	45	99.11
3	45	187.50
4	66.21	187.50
5	45	187.50
6	45	275.88
7	45	187.50
8	45	187.50
9	60	250
10	23.78	187.50
11	30	250
12	45	187.50
13	30	125

The data in Table 1 showed the lowest extraction time treatment was 23.78 minutes and the highest was 66.21 minutes with the lowest amount of solvent used was 99.11 ml and the highest was 275.88 ml.

Total Yield Extraction

The total yield is the ratio of the mass of honje fruit extract obtained with the mass of the raw materials used. The total yield is an important parameter that is observed and analyzed in this study because it is one of the responses to the variables used in RSM. The extraction process includes extraction with the UAE. filtration to separate the dregs and filtrate. and evaporation with temperature conditions of 50°C and 80 rpm. The evaporation process produces a more concentrated extract. This extract was then tested for residual solvent content to obtain the total yield value. The residual solvent level needs to be tested because it shows the residual ethanol solvent content in the extract. The residual solvent content in an extract represents the amount of solvent remaining in the extract. The value of the residual solvent content is obtained from the percentage by weight of the solvent that is evaporated from the unit weight of the tested material. The results of the calculation of the total yield of honje fruit extract can be seen in Table 3. below.

The data in Table 3. shows the highest percentage of total yield was in treatment 11 with a 30 minute extraction time treatment with 250 ml of solvent. This is presumably because the extraction time of 30 minutes is sufficient to extract the honje fruit powder with 250 ml of solvent. The higher the ultrasonic amplitude that passes through a medium, the breakdown of cavitation bubbles can occur around or on the surface of the sample membrane, causing larger microfactures. Yields tend to be high at high amplitudes even though the excitation time is shorter (Vinatoru, 2001). Therefore, in this study, the total yield produced was quite high because it used an amplitude of 65%. The long extraction time will support the active compounds in the ingredients to be more active.

Table 3. Total Yield of Honje Fruit Extract

Treatment	Residual Solvent (%)	Total Yield (%)
1	80.58	15.06
2	89.00	5.26
3	79.00	10.09
4	80.19	9.28
5	85.29	13.70
6	89.32	8.85
7	88.35	11.30
8	86.54	13.29
9	90.38	16.62
10	84.76	19.62
11	83.49	23.18
12	91.35	11.71
13	82.69	13.15

Antioxidants Test

Testing of antioxidant activity is one of the responses to the RSM application so that it becomes an important parameter to do. Antioxidant activity test was carried out using the DPPH method (*2.2 diphenyl 1 picrylhydrazil*). Antioxidant activity is usually expressed as a concentration that causes a loss of 50% of DPPH activity (Molyneux. 2004) or known as the IC50 value. The antioxidant value or IC50 value indicates a very strong antioxidant activity. strong. currently. weak. and inactive are shown in the table below.

Table 4. IC_{50} value

IC50	Description
<50	Very strong
50 – 100	Strong
100 – 250	Medium
250 – 500	Weak
>500	Inactive

Sumber: Molyneux (2004)

The antioxidant test of honje fruit extract was carried out on each combination of honje fruit extract treatments. Table 5. below is the IC50 value of each honje fruit extract.

Table 5. IC_{50} value of honje fruit extract

Treatment	IC_{50} (ppm)	Description
1	100.28	Strong
2	75.37	Strong
3	49.38	Strong
4	100.29	Strong
5	153.88	Medium
6	220.29	Medium
7	152.40	Medium
8	270.03	Weak
9	68.71	Strong
10	328.09	Weak
11	303.72	Weak
12	206.07	Medium
13	314.04	Weak

Based on the data from the analysis of antioxidant activity that has been carried out, it shows that the highest IC50 value is in treatment 3, which is 49.38 ppm with the amount of solvent being 99.97 ml and extraction time of 45 minutes, while the lowest IC50 value is in treatment 10, which is 328.09 ppm with the amount of solvent 187.5 ml and treatment. extraction time 23.78 minutes. The high and low IC50 values are thought to be influenced by the suitability of the extraction time with the amount of solvent used which affects the temperature increase of the extraction process. Based on the results of this study. It is suspected that a large number of solvents should be extracted for a long time so that there is a significant temperature increase in the extraction process because the antioxidant activity increases when the extraction temperature is in the range 45-85°C.

Optimization Using RSM Application

The optimization carried out in this research is the total yield and antioxidant activity so that the application of RSM will produce 2 analytical responses, namely total yield analysis and antioxidant activity analysis. The analysis of the optimization results of the total yield of honje fruit extract using the RSM application can be seen in the table showing the ANOVA analysis and Figure 1. which shows the following optimization graph.

Tabel 2. ANOVA Analysis on Total Yield

Source	Sum of Squares	df	Mean Square	F-value	p-value	Note
Model	308.87	5	61.77	9.73	0.0047	Significant
A-time	0.2609	1	0.2609	0.0411	0.8541	
B-solven	39.68	1	39.68	6.25	0.0410	
AB	16.15	1	16.15	2.54	0.1547	
A	201.89	1	2.54201.89	31.81	0.0008	
B	27.25	1	31.8127.25	4.29	0.0770	
Residual	44.43	7	4.296.35			
Lack of Fit	33.00	3	11.00	3.85	0.1128	Not significant
Pure Error	11.43	4	2.86			
Cor Total	353.30	12				

Tabel 3. ANOVA Analysis on Antioxidants Test

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	77614.70	2	38807.35	9.12	0.0056	Significant
A-time	74291.22	1	74291.22	17.46	0.0019	
B-solvent	3323.48	1	3323.48	0.7812	0.3975	
Residual	42544.60	10	4254.46			
Lack of Fit	16185.29	6	2697.55	0.4094	0.8426	Not significant
Pure Error	26359.31	4	6589.83			
Cor Total	1.202E+05	12				

$$Y1 = 19.05 - 1.76A + 0.32B - 0.002\ AB + 0.023A^2 - 0.0005\ B^2 \quad (5)$$

Where, Y1 = total yield (%); A = solvent volume (ml); B = extraction time (minutes)

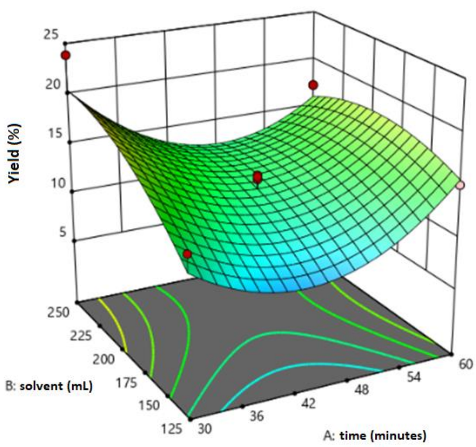

Figure 1. Total yield optimization graph by RSM

The results of the analysis conducted by RSM show that the F-value has a value of 9.73 which indicates that the model is significant. The p-value is less than 0.05 which means that the model is significant. The lack of fit value for the model is 3.85 which indicates that the lack of fit value is not significant. The lack of fit value is the error value that occurs so it is expected that the lack of fit value is insignificant, which means the model has a small error value. The relationship between the amount of solvent and the extraction time with the yield as a response forms a quadratic mathematical equation model as follows equation (5). Based on the following equation, it was obtained that the total extract yield was influenced by the amount of solvent. extraction time. multiplication of solvent amount and extraction time. the square of the amount of solvent. and the square of the extraction time.

The analysis of the antioxidant optimization results of honje fruit extract analyzed using RSM can be seen in Figure 2. and the table below.

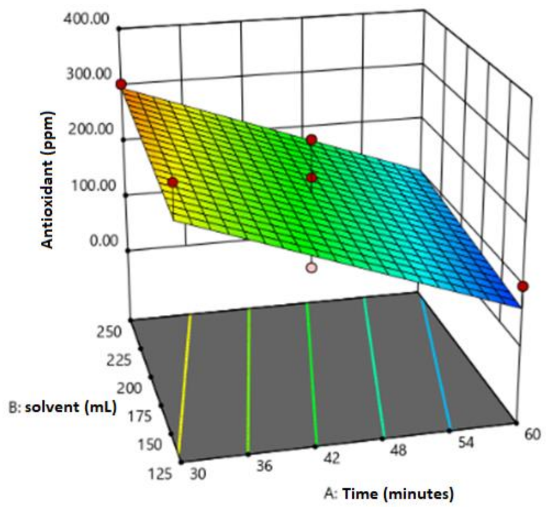

Figure 2. Antioxidants optimization graph by RSM

RSM analysis for optimization of antioxidant activity showsthat the F-value has a value of 9.12 with a p-value of less than 0.05 indicating that the model is significant. The lack of fit value is 0.4094 which indicates the lack of fit is not significant, which means the model has a small error value. The relationship between the amount of solvent and the extraction time with the yield as a response forms a linear mathematical equation model as follows. The equation below is influenced by the amount of solvent and extraction time.

$$Y2 = 408.147 – 6.424A + 0.362B \quad (6)$$

Where, Y2 = Antioxidants (ppm); A = Solvent amount (ml); B = Extraction time (minutes)

The best combination of treatments can be known by optimizing so that the response that is expected can be known. In the RSM application using 2 responses. each response has an expected goal. In this study, the expected goal of the total yield value is maximization and the expected goal of the antioxidant activity value is minimization because the smaller the antioxidant value, the stronger the antioxidant activity.

The best treatment combination solution is shown by the high desirability target value. namely the desirability value that is close to 1. The combination of treatments in this study that has the highest desirability value is the solution with a desirability value of 0.772 with an extraction time of 60 minutes and the amount of solvent as much as 174.815 mL. The combination of these treatments is expected to produce honje fruit extract which has characteristics according to the optimization target of total yield of 17.69% and antioxidant activity of 79.718 ppm.

Optimization Validation Results Analysis

The combination of treatments obtained from the RSM analysis resulted in the optimum point which is the best combination to produce the maximum total yield and the minimum IC50 value. The optimum point obtained from this RSM analysis is a combination of 174.815 ml of solvent with an extraction time of 60 minutes. The optimum conditions were tested with two repetitions with the average results as shown in the following table.

The validation carried out resulted in a total yield of 17.125%. When compared with the predicted value of RSM, which is 17.689%, the validation value obtained is 96.81%. These data indicate that the extraction process carried out to obtain the optimum yield of honje fruit extract is quite consistent. This is because the prediction interval value used in RSM is 95%. This means that the maximum tolerance for the difference between the actual result and the predicted result is 5%. The comparison results show that the difference between the prediction value and validation is less than 5%, meaning that the validation value is in accordance with the predicted value (Mariana et al. 2017).

Table 8. Validation results of honje extract optimization

Extract	Total Yield (%)	IC_{50} Value (ppm)	Residual Solvent (%)	Specific Gravity (g/ml)	pH	Color (*Hue*)
Validation	17.125	77.55	79.50	0.855 ± 0.003	2.65	42.18 (*Red*)

Validation of the antioxidant activity response of the optimization validation results using a sample concentration of 1000 ppm. Predicted antioxidant value by RSM is 79,693 ppm. The resulting validation is 77.55 ppm. The IC50 value of the validated extract is classified as a strong antioxidant activity and when compared with the predicted result of RSM which is 79.693 ppm, the validation value obtained is 97.31%. The antioxidant response validation test data also showed that the antioxidant testing process for the optimum honje fruit extract was quite consistent. The following is an image that shows a graph of the antioxidant test results from the optimum point validation.

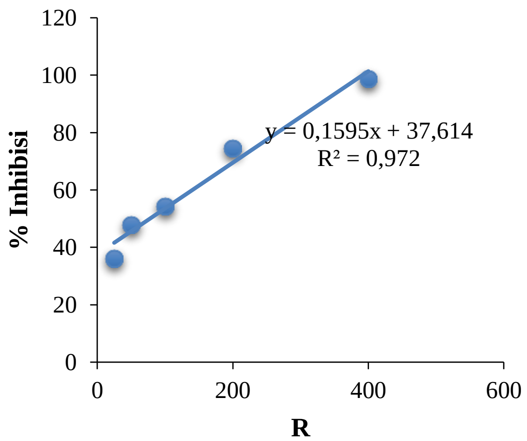

Figure 3. Validation of antioxidants value's graph

When compared with the research of Jabbar et. al (2019) found that 1870 grams of honje fruit powder was extracted using the maceration method for 3x24 hours to produce 96,214 grams of thick extract with antioxidant activity of 72,518 ppm. This shows that the UAE treatment can reduce the use of raw materials, especially for raw materials that are difficult to find. This is due to the cavitation effect which will damage plant cells so that the compounds in the material will be extracted. So that the compounds in honje fruit powder that act as antioxidants are easily extracted in a short time.

Characteristic tests were carried out on the validated extracts including the residual solvent content test, color test, specific gravity, and pH value. In the test of residual solvent content, the optimum point validation process has an average Residual Solvent value of 79.50%. The specific gravity value of the treated honje fruit extract had an average of 0.8556 ± 0.0033 g/ml. The pH test results of the optimum validation extract were 2.65 on average and the extract was classified as acidic. The color of the treated extract and the color of the extract from the validation process showed red or red chromaticity.

Characteristic Test

Color Analysis

The color test results using the ColorFlex EZ Hunter Lab tool produce an L* value. a*. b*. chroma. and Hue directly. In this study, the color test of each sample of honje fruit extract was carried out. Color test results (L*. a*. b*). Chroma and Hue values for the extraction process are shown in the table 9.

Specific Gravity

Specific gravity is the number of components contained in a substance which shows the weight fraction of the components in it. Specific gravity measurements were carried out for each combination of extracts and were repeated three times. Based on the specific gravity data that has been obtained, it shows that the specific gravity of the extract is greater than the specific gravity of the solvent, which is 0.798 g/ml. This is due to the 96% ethanol solvent binding to the compounds contained in the honje fruit extract. The table 10 shows the specific gravity of each extract.

pH Value

The pH value is a value that states the degree of acidity used to express the level of acidity or alkalinity possessed by a solution. The pH measurement range changes from 0 to 14 with a value of 7 being neutral. A pH value of less than 7 indicates the acidity of the solution. while a pH of more than 7 indicates the basicity of the solution. The pH value of each extract is shown in the table 11. Based on these data, it is known that the average pH value of the extract obtained in each treatment combination is 2.80 and it can be concluded that all extracts are acidic.

Table 4. Honje Extract Color Test

Run	**L***	**a***	**b***	***Chroma***	***Hue***	**TCD**	**Chromaticity**	**Figure**
Raw material	55.06	15.89	7.77	17.69	26.07			-
1	5.76	14.19	6.68	25.1	15.44	49.34	Red (R)	
2	3.28	11.04	2.27	11.27	11.63	52.29	Red (R)	
3	12.95	30.3	10.42	32.04	18.98	44.58	Red (R)	
4	14.07	34.49	14.5	37.41	22.8	45.51	Red (R)	
5	24.62	26.36	4.94	26.82	10.62	32.31	Red (R)	
6	19.47	40.36	19.84	44.97	26.18	44.85	Red (R)	
7	12.83	36.24	17.39	40.19	25.63	47.85	Red (R)	
8	13.65	32.8	14.46	35.84	23.79	45.23	Red (R)	
9	14.4	30.83	11.74	32.99	20.85	43.49	Red (R)	
10	10.7	30.25	11.67	32.42	21.09	46.79	Red (R)	
11	16.27	27.78	7.74	28.84	15.56	40.57	Red (R)	
12	11.48	27.54	9.32	29.07	18.69	45.13	Red (R)	
13	13.41	27.06	11.63	29.46	23.25	43.2942	Red (R)	

Table 5. Specific gravity value on honje extract

Treatment	Specific Gravity (g/ml)
1	0.8512 ± 0.0002
2	0.8764 ± 0.0002
3	0.8647 ± 0.0002
4	0.8693 ± 0.0001
5	0.8194 ± 0.0002
6	0.8309 ± 0.0001
7	0.8195 ± 0.0002
8	0.8125 ± 0.0001
9	0.8406 ± 0.0001
10	0.8376 ± 0.0002
11	0.8021 ± 0.0002
12	0.8090 ± 0.0001
13	0.9112 ± 0.0001
Average	0.8419 ± 0.0002

Table 6. pH value

Treatment	pH value
1	2.66
2	2.83
3	2.85
4	2.80
5	2.86
6	2.81
7	2.87
8	2.76
9	2.76
10	2.65
11	2.95
12	2.80
13	2.85
Average	2.80

4. CONCLUSION

The combination of the amount of solvent and the length of extraction time affects the total extraction yield and antioxidant activity contained in the material. The optimum extraction conditions were the amount of solvent 174.815 ml and the extraction time of 60 minutes resulting in a total yield of 17.125%; antioxidant activity 77.55 ppm; Residual Solvent value of 79.5%; the specific gravity of the extract is 0.8556; extract pH value 2.66; and the chromaticity color of the extract is red.

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