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Comparison of Pegagan (*Centella asiatica* (L.)) Extraction with Ultrasound-Assisted Extraction and Microwave-Assisted Extraction Methods Using Response Surface Methodology

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Abstract. Pegagan (*Centella asiatica* (L.)) is an herbaceous plant that grows and flowers throughout the year. Pegagan has been used for hundreds of years, especially in the dermatology and cosmetology industries. Pegagan (*Centella asiatica* (L.)) has distinctive bioactive components, namely triterpene ester glycoside compounds in the form of asiaticoside and madecoside, as well as triterpene group compounds in the form of asiatic acid and madecasic acid. There are various extraction methods to produce the following compounds, namely, conventional extraction methods and modern extraction methods. The two methods have differences in the results obtained. Therefore, the difference in the extraction method will determine the outcome of the difference in the levels of a compound. So that this research was carried out on the effect of Pegagan (*Centella asiatica* (L.)) extraction using a comparison of the Microwave-Assisted Extraction (MAE), and Ultrasound-Assisted Extraction (UAE) methods on the total phenol content in the Pegagan (*Centella asiatica* (L.)) extracted. The extracted bioactive content was analyzed by analyzing the total phenol content using the Folin-Ciocalteau reagent method and analyzing the data through the Analysis of Response Surface Design expert 11 to see the significance of the research variables. The results obtained from the study compare the optimal conditions for the total phenol content, namely, the comparison between the UAE and MAE methods for *Centella asiatica* (L.). Extracted with ethanol solvent, which is more optimal using the MAE method, because it can produce 1130,7882 mg quickly.

Keywords: *Ultrasound-Assisted Extraction, Microwave-Assisted Extraction, Centella asiatica* (L.)*, Phenolic Test.*

1. Introduction

Many new modern medicines are on the market; there is a global tendency to return to nature (back to nature). Some of the factors that encourage people to utilize natural medicines, among others, are the high prices of modern/synthetic medication and the many side effects

associated with these drugs. Therefore, natural medicines are now increasingly popular among the public, and their use is increasing in developing countries such as Indonesia and developed countries such as Germany and the United States. One of the plants that has medicinal properties is Pegagan (*Centella asiatica* (L.)) [1].

Pegagan (*Centella asiatica* (L.)) is an herbaceous plant that grows and flowers throughout the year. Pegagan is often found in rice fields, between grasses, on slightly moist soil, and in the low and highlands. This plant grows in tropical Asia and various countries such as the Philippines, China, India, Sri Lanka, Madagascar, and Indonesia [2]. Pegagan is a plant that people in Asian countries widely use. It contains various beneficial compounds that can be used as medicines, natural fungicides, and antimicrobials [3]. The Pegagan herb treats abdominal pain, cough, bloody cough, wound healing, dysentery, inflammation, aches, pains, asthma, hemorrhoids, tuberculosis, leprosy, fever, diabetes, and appetite enhancer [1]. Several researchers have tested the content of Pegagan compounds, such as Pegagan extract, which contains compounds that are antimicrobial and anti-fungal [4], as an antioxidant [5] and anticancer [6].

The components of the compounds contained in Pegagan are triterpenoids, including pentacyclic triterpenic acids, and glycosides, which consist of: asiatic acid, asiaticoside, mandecassic acid, m, andecassoside, brahmoside, brahmic acid, brahminoside, thankuniside, isothankuniside, centalloside, madasiatic acid, centic acid. Pegagan also contains flavonoids such as quercetin, kaempferol, and astragalin. Hydrocotylin alkaloids, as well as phytosterols, stigmasterol, and sitosterol. Other compounds are tannins, amino acids, B vitamins, and resins [3]. There are various extraction methods to produce the following compounds, namely, conventional extraction methods and modern extraction methods.

Conventional extraction methods include maceration and reflux. Meanwhile, modern extraction methods include Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) according to [7]. Extraction using the MAE method on onion skin with a power of 800 watts for 6 minutes produces a flavonoid level of 17.18%. Extraction using the maceration method for 3 days at room temperature produces a flavonoid level of 14.92%. Another study on white tea leaves showed that the optimum time and temperature for the UAE method was 40 °C for 19.5152 minutes with a flavonoid content of 0.39% [7]. In addition, research on corn cobs showed that extraction using the reflux method with a temperature of 50

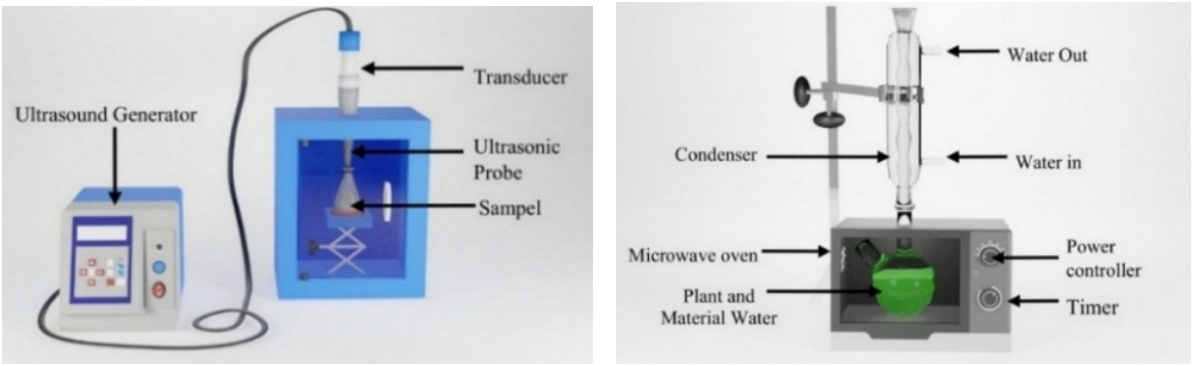
°C for 2 hours produced a phenolic content of 0.03% [8]. Therefore, the difference in the extraction method will determine the result of the difference in the levels of a compound.

Based on the problems and facts above, a study was conducted on the effect of Pegagan (*Centella asiatica* (L.)) extraction by using a comparison of the Microwave-Assisted Extraction (MAE) and Ultrasound-Assisted Extraction (UAE) methods on the total phenol content of the Pegagan (*Centella asiatica* (L.)) extracted. This research is expected to find the most optimum method to achieve optimal conditions by producing the highest total phenol content in the Pegagan (*Centella asiatica* (L.)) extract.

2. Materials and Methods

2.1 Materials

Pegagan leaves (*Centella asiatica* (L.)) were obtained from the Sumberarum area, Songgon District, Banyuwangi. The Pegagan leaves are dried in the sun for 2 days, the dried Pegagan leaves are crushed using a blender until they become powder, the Pegagan powder is sifted using a 40-mesh sieve, and data processing is done using expert design.



(a) (b)

Figure 1. Tool Schematic (a) Ultrasound-Assisted Extraction (b)

In Figure 1 (a), this *Ultrasound-Assisted Extraction* method uses a probe-type Sonicator. In contrast, Figure 1 (b) shows *Microwave-Assisted Extraction, which* uses a microwave oven type Electrolux model EMM2308X, 23 liters, max temperature 250, dimensions 292.5 x 485 x 370 mm. Uses 800 W of power.

The research procedure was carried out through 3 stages: (1) sample preparation, extraction using the method *Ultrasound-Assisted Extraction* and *Microwave-Assisted Extraction*, (3) Analysis of research results. Pegagan leaves were dried in the sun for 2 days with the determination of physical drying, then mashed with a size of 40 mesh with a mass of 1 gram. Furthermore, the extraction was carried out with several variables, including the UAE

concentration method (25%, 50%, 75%, time variables of 20 minutes, 40 minutes, and 60 minutes, and power variables of 30 watts, 150 watts, and 270 watts. As for the MAE method, the variables are concentration variables of 25%, 50%, and 75%, time variables are 5 minutes, 10 minutes, and 15 minutes, and variable power of 150 watts, 300 watts, and 450 watts. The extraction results were stored in an 8 ml vial at a temperature of 4 ℃.

2.2 Preparation of a Standard Solution of Gallic Acid at 100 Ppm

Weighed 0.01 grams of gallic acid, then added 1 ml of ethanol and one distilled water until the volume became 100 ml.

2.3 Determination of the Maximum Wavelength of Gallic Acid

Take 1 ml of 100 ppm gallic acid mother liquor, put it in a test tube, and add 1 ml of Folin's reagent. Then the solution mixture was shaken until homogeneous and allowed to stand at room temperature for 4-8 minutes. 4 ml of 10% Na_2CO_3 solution was added to a test tube, shaken until homogeneous, and allowed to stand for 15 minutes at room temperature. Analyzed using a UV-vis spectrophotometer with a wavelength range of 700 – 800 nm [9].

2.4 Preparation of Gallic Acid Calibration Curve with Folin-Ciocalteu Reagent

Take 100 ppm gallic acid mother liquor, 1 ml each, 3 ml, 5 ml, and 7 ml. Then diluted with distilled water to a final volume of 10 ml, a solution with a concentration of 10 ppm, 30 ppm, 50 ppm, and 70 ppm will be obtained. 0.2 ml of each solution was taken, put into a test tube, and 1 ml of Folin Ciocalteu reagent was added, shaken until homogeneous, and allowed to stand for 8 minutes. Then 3 ml of 10% Na_2CO_3 was added, shaken until homogeneous, and allowed to stand for 30 minutes at room temperature. Measure the absorption using the maximum wavelength that has been obtained previously. Then a calibration curve is made with the regression equation $y = ax + b$ [9].

2.5 Determination of Total Phenol Content using The Folin-Ciocalteu Method

Take 0.1 ml of the extract, add 9.9 ml of distilled water (dilution 100 times), add 1 ml of Folin-Ciocalteu reagent, then shake until homogeneous, and let stand for 8 minutes. Then add 3 ml of 10% Na_2CO_3 to the mixture, shake until homogeneous, and leave the solution for 1 hour at room temperature. Measure the absorption with a UV-vis spectrophotometer at its maximum wavelength. The content analysis was repeated 3 times so that the phenol content obtained was expressed as mg gallic acid equivalent/gram of fresh sample. Repeat 3 times so

that the total phenol level is described as equivalent gallic acid (Acid Equivalent Gallic/AGE) [9].

3. Result and Discussion

3.1 The Gallic Acid Standard Curve

Determination of total phenol content begins with determining the standard gallic acid curve. The aim is to choose a linear regression equation, which is then used to determine the sample's total phenol content. The standard gallic acid curve was obtained by measuring the absorbance of a series of standard solutions of gallic acid with several concentrations of gallic acid, namely 10 ppm, 30 ppm, 50 ppm, and 70 ppm, and the results from the measurement of the maximum wavelength of 765 nm. Based on the results of making a standard curve using a UV-vis spectrophotometer, the absorbance measurement of the standard solution of gallic acid from several concentrations was measured based on the maximum wavelength obtained, namely 765 nm. The following are the results of the standard gallic acid curve, and the straight-line equation that will be used to determine the total phenol concentration.

Based on the curve in Figure 2, the equation of a straight line is obtained, namely, y = 0.0078x + 0.2144. With the value of the correlation coefficient (R^2) = 0.9949. The correlation coefficient value shows the strength of the relationship between two variables. The strength and weakness of the relationship between the two variables, measured on an interval scale of 0-1. If the correlation coefficient value is close to 1, then the two variables have a firm relationship [10].

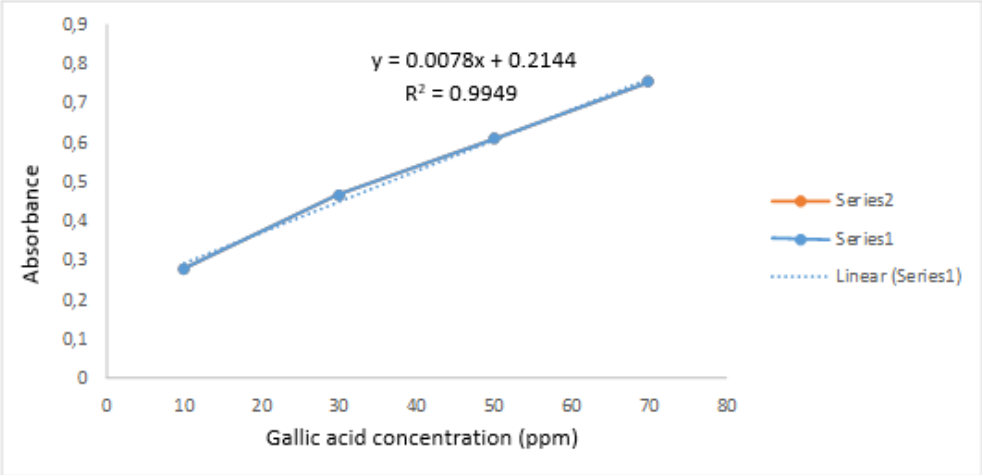


Figure 2. Gallic acid calibration curve *at 765nm wavelength.*

3.2 Extraction Results of Pegagan (*Centella asiatica* (L.)) using the UAE Method

The results of the absorbance and total phenol results from the research can be seen in Table 1 below.

Table 1. Total Phenol Content in Pegagan Plant Extract (*Centella asiatica* (L.)) in *the* UAE

No	Solvent Concentration (%)	Time (Min)	Power (Watts)	Absorbance (Average)	Total Phenol (mg.AGE/g)
1	50	40	150	0.675	589.87
2	50	40	150	0.704	627.05
3	50	40	150	0.656	566.15
4	75	20	150	0.507	357.13
5	75	40	270	0.621	521.28
6	50	40	150	0.682	598.85
7	75	40	30	0.432	278.97
8	25	40	30	0.442	291.79
9	25	60	150	0.531	405.90
10	50	20	270	0.732	662.95
11	50	60	270	0.867	836.67
12	25	40	270	0.662	573.21
13	50	40	150	0.707	631.54
14	50	60	30	0.561	443.72
15	75	60	150	0.563	446.92
16	25	20	150	0.544	422.56
17	50	20	30	0.603	498.21

It can be seen from the Table above that the highest total phenol content was 836.67 mg. The AGE/g of the sample in the UAE variable has a power of 270 watts, with a concentration of 50% for 60 minutes.

3.3 Analysis of Response Surface Design with ANOVA (*Analysis of Variance*)

Table 2 shows that the significance of the variables used is determined by the p-value, which is said to be significant if the p-value <0.05. The F-value of the model obtained from the calculation is 62.19. In the F distribution Table, the value of the F Table with the df model is 9, and the df error value is four at a probability of 0.05, the percentage point is 6.00, which means that the F-value is greater than the F Table. The hypothesis used in the F-value, namely, H_0, is the absence of a relationship between the independent and dependent variables. At the same time, H_1 is a relationship between the independent and dependent variables. H_1 is accepted if the F-value is greater than the F-Table, while H_0 is accepted if the F-value is smaller. So, based on the existing F-value, it shows that F-value > F-Table, which indicates a rejection of H_0, which means that H_1 is accepted. H_1 is a hypothesis that states a significant effect between the dependent and independent variables that has been tested and analyzed.

Table 2. Results of ANOVA Analysis on Pegagan Plant Extracted UAE Method

Source	Sum of Square	df	Mean Square	F-Value	P-Value	
Model	3.217E+05	9	35742.48	62.19	<0.0001	Significant
A-Concentration	632.97	1	632.97	1.10	0.3289	
B-Time	3800.18	1	3800.18	6.61	0.0369	
C-Power	1.426E+05	1	1.426E+05	254.35	<0.0001	
AB	1955.85	1	1955.85	3.40	0.1076	
air conditioning	382.40	1	382.40	0.6653	0.4415	
BC	13019.95	1	13019.95	22.65	0.0021	
A2	1.553E+05	1	1.553E+05	270.26	<0.0001	
B2	16.93	1	16.93	0.0295	0.8686	
C2	136.33	1	136.33	0.2372	0.6411	
Residual	4023.21	7	574.74			
Lack of Fit	1083.21	3	361.07	0.4912	0.7072	Not Significant
Pure Error	2940.00	4	735.00			
Total Cast	3.257E+05	16				

In addition, the F-value is inversely proportional to the value of the p-value, as shown in Table 2. The P-value is said to be significant if the P-value <0.05. The P's value is 0.0001, which means the meaning is smaller than the set probability of 5% or 0.05. *Centella asiatica* (L.) research model significantly affects the extract's total phenol content.

The analysis results in Table 2 also show that the UAE pore variable and extraction time significantly affect the total phenol content, with p-values of 0.0001 and 0.03695, respectively. For the interaction between variables on the response, the interaction of time on power has a real or significant effect on the total phenol content, with a p-value of 0.0021. However, the concentration variable has a p-value of 0.3289, which means that the p-value is greater than 0.005, so the concentration variable in the Pegagan extracted with the UAE method does not significantly affect the total phenol content. This is because the extraction power is too high, which can damage the material's compounds. This is by following per under research conducted by [11], the use of sonication in the extraction process can cause vibrations that have the potential to cause heat so that it can damage the extracted content which causes the acquisition of the total phenol content to below so that it can affect the significance of the model which means it has no real or insignificant effect on the total phenol content. The inaccuracy of the test or the lack of fit value means the deviation or inaccuracy of the model. Lack of Fit testing is required if there are repeated observations. The hypotheses used in the Lack of Fit test are:

H_0 = There is no Lack of Fit in the research model

H_1 = There is a lack of fit in the research model

The Lack of Fit test is carried out to determine whether the research model is appropriate [12]. In Table 2, based on the ANOVA Table, it is found that Lack of fit has a P-value of 0.7072 or more than 0.05, so that H_0 is acceptable, meaning there is no lack of fit in the research model. So, it can be interpreted that the research model used is suitable for predicting the total phenol content in the extraction process.

From the ANOVA analysis, the R-squared value of 0.9876 or 98.76% is more than 75%, indicating that the model fits the research results well. An adjusted R^2 value of 0.9718 indicates a significant relationship between ethanol concentration, extraction time, and UAE power on the response. The value of R-squared is declared according to the model if it is more than 75% [13]. So, the equation of the model can be used to predict the study's actual results. Based on the regression equation obtained, it can be concluded that the effect of concentration, time, and power variables can affect the total phenol concentration. The regression equation is as follows:

$$\text{Total Fenol} = 602.692 - 8.895\text{A} + 21.795\text{B} + 135.178\text{C} + 22.1125\text{AB} - 9.7775\text{AC} + 57.0525\text{BC} - 192.07\text{A}^2 + 2.00525\text{B}^2 + 5.69025\text{C}^2$$

The equation shows that the extraction time response will increase directly proportional to power, the interaction between concentration and time, the interaction between time and power, the interaction between time and power, and the interaction between power and power. A positive constant indicates this. The response of total phenol concentration will decrease with increasing concentration, as well as the interaction between concentration and power, and the interaction between concentrations. A negative constant indicates this.

3.4 Optimization of Total Phenol Content Using Response Surface Methodology with the UAE Method

The results of the extraction and testing of Pegagan (*Centella asiatica* (L.)) at various conditions of time, power, and concentration are presented in Table 4.1. The value of total phenol content of Pegagan extracted was the highest (836.67 mg.AGE/g sample). The results of the measurement data were analyzed statistically using the help of Design Expert ver.11 software (response of the contour plot, and surface response in each variable as well as the optimum condition of total phenol content of Pegagan (*Centella asiatica* (L.)). In this study, the variables used, among others, were solvent concentration, time, and power.

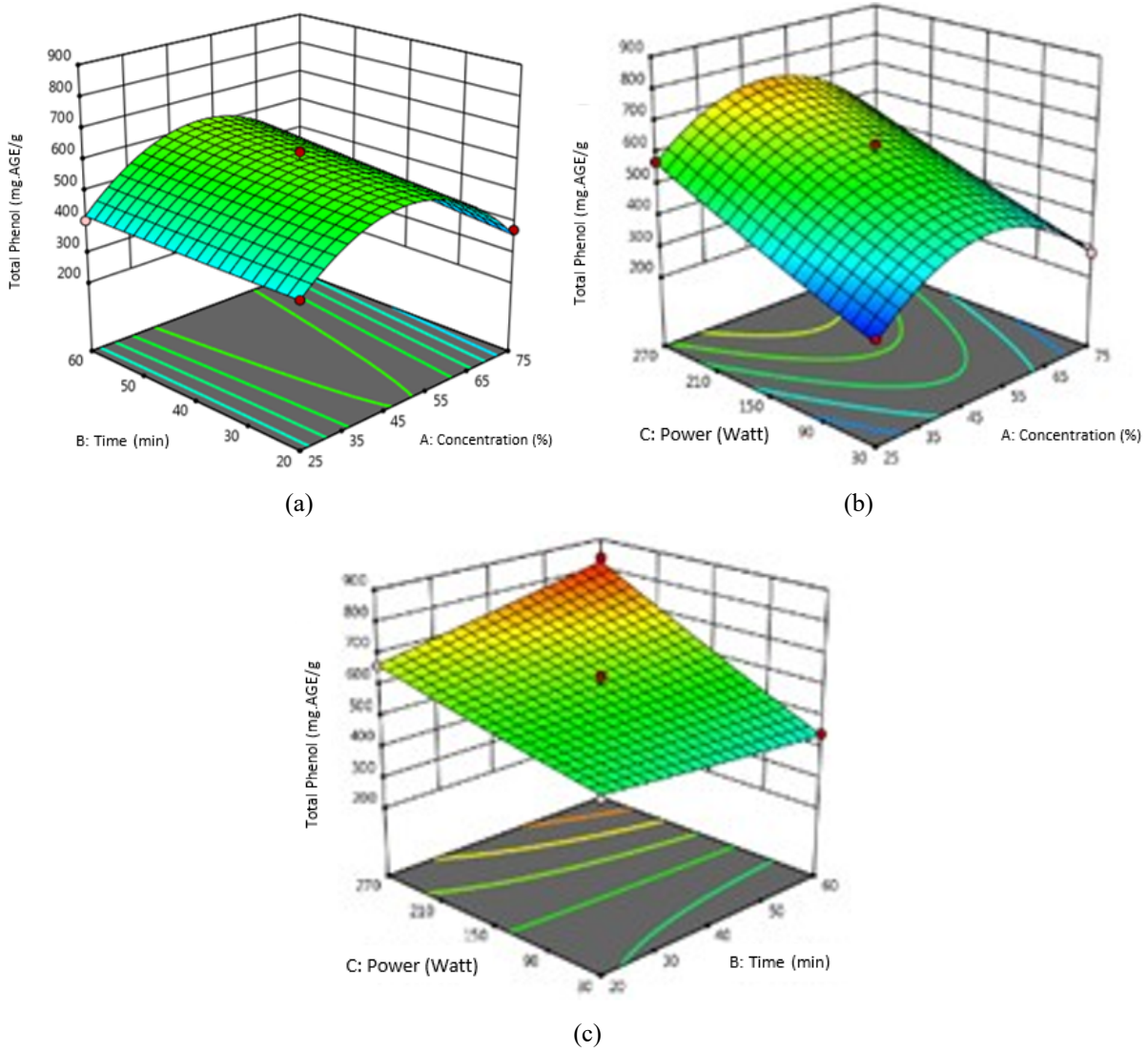


Figure 3. (a) Effect of concentration and time, (b) Concentration, and I, (c) Time, and power on total phenol bioactive compounds using the UAE method

Graph in Figure 3. Point (a) The graph shows the effect of the variable concentration of solvent and extraction time on the levels of total phenol bioactive compounds. The graph shows that at the time of the extraction, for 20 minutes, an ethanol concentration of 25% and 150 watts of power produced a total phenol content of 422.56 mg.AGE/g, the total phenol content produced increased with increasing time. The increase in extraction time can result in more prolonged contact between the material and the solvent. The longer the extraction time, the higher the levels of compounds extracted. This follows the research conducted by [14], which states that the longer the time, the more bioactive compounds are obtained. The longer the sonication time, the longer the contact of the mixture with the microbubble, so that more compounds contained in the sample cells are diffused with the solvent [15].

However, at a certain point, when the ethanol concentration is 50%, and the power is 150 watts for 40 minutes, the optimum total phenol content is 631.54 mg.AGE/g after the optimum point, the total phenol content produced or obtained decreases. The decrease in phenol levels occurred at the highest point or optimum point due to the high temperature, which was possible due to the increase in extraction time, so it was possible to experience the degradation process of bioactive compounds. Degradation is the termination or breaking of bonds in bioactive compounds, decreasing the number of compounds produced. Steam distillation is one example of factors that can affect the chemical composition of bioactive compounds because heat and water vapor can damage the molecular structure and hydrolyze double bonds [16].

The effect of ethanol concentration showed that at the time of extraction, using a solvent concentration of 25% for 20 minutes with a power of 150 watts resulted in a total phenol content of 422.56 mg.AGE/g, the higher the ethanol concentration, the higher the total phenol content obtained. This is because the solvent diffusion process into natural materials is getting better. Extraction of polyphenolic components from natural ingredients using solvents consists of two stages, namely the initiation and diffusion stages. At the initiation stage, the natural material particles will absorb the solvent so that the particles experience bubbles. The diffusion stage is characterized by the diffusion of the solvent to a deeper part, and the polyphenol component will also be extracted. This is based on the research conducted by [17], which indicates that the higher the ethanol concentration, the more metabolites extracted, either patterned or semipolar. The high concentration of ethanol gives a higher total phenol value.

However, at a certain point, the ethanol concentration is 50% and the power is 150 watts for 40 minutes with the optimum total phenol content of 631.54 mg.AGE/g after being at the optimum point, the total phenol content produced or obtained begins to decrease. This indicates a saturation point limit for the variable solvent concentration at the specified operating conditions. The decrease in phenol content occurs at the highest point or optimum point due to differences in the concentration of ethanol that can affect the solubility of phenolic compounds in the solvent. The higher the concentration of ethanol, the lower the polarity of the solvent. This follows the research [18], in *Centella asiatica* (L.), which showed a decrease in total phenol with treatment concentrations above 50%.

The effect of the power used by extraction using the UAE method can be seen in Figure 3, point (b), that the optimum conditions at the time of the extraction for 40 minutes with an ethanol concentration of 50%, and a power of 150 watts resulted in a total phenol content of

627.05 mg.AGE/g. The greater the power used, the higher the phenol content obtained. The results of this study are as follows, as shown by the research results, [19] which show that the addition of power in the UAE can increase the total phenol content. The greater the power given, the greater the ultrasonic waves used in the sample. The magnitude of the vibration of the ultrasonic wave will make it easier for the solvent to diffuse on the material's surface. The high power provided will accelerate the damage to the material's surface so that the extracted compounds will be more easily obtained, and the total phenol content will be greater. However, it should be noted that a power that is too high can potentially damage the content of compounds present in the extract. An increase in power will result in a decrease in the total phenol content. This is because the operating conditions with a power of 270 watts have exceeded the optimum point. This study's optimum point is operating conditions with a power of 150 watts. This is by following [under research [20]. The operating conditions that have passed the saturation point will not increase the extraction yield even though the solvent continues to be added.

Figure 3 point (c) shows the relationship between extraction power and extraction time to the total phenol produced. In the graph, the optimum condition for operating power is 150 watts with a concentration of 50% for 40 minutes to get a total phenol of 631.54 mg—AGE/g sample. The greater the power in the UAE, and the time it takes, the greater the total phenol obtained. This is because the longer the extraction time, the more compounds are extracted, and the power required is also greater, so that when the power is significant, the ultrasonic wave used for the sample is also greater because the magnitude of the vibration of the ultrasonic wave will facilitate the solvent diffusion on the surface of the material. This is by following the research [21], high power because the power acts as a driving force to break the structure of plant cell membranes so that the oil can diffuse out, and dissolve in the solvent. Thus, adding power will generally increase the phenol content and speed up the extraction time.

3.5 The Results of the Optimization of Pegagan Extraction Conditions with the UAE Method

Optimization of total phenol extraction from the Pegagan plant (*Centella asiatica* (L.)) using the UAE method can be seen in Figure 5. shows that the optimal value for the total phenol concentration-response was obtained at 270 watts of power, 50.2525% concentration, and within 60 minutes with a value of 824.422 mg.AGE/g sample obtained when the desirability value reaches 0.97804.

Figure 4. Graph of optimization plot with the UAE method

3.6 Extraction results of Pegagan (Centella asiatica (L.)) using the MAE method

The measurement results of the total phenol content in the Pegagan extract can be seen in Table 3.

Table 3. Total phenol content in the pegagan plant extracted by the MAE method

No.	Power (Watts)	Solvent Concentration (%)	Time (minutes)	Average Absorbance	Total Phenol (mg.AGE/g sample)
1	150	75	10	0.681	597.56
2	450	25	10	0.673	587.31
3	450	50	5	0.64765	553.97
4	300	75	5	0.571	456.54
5	150	50	15	0.687	605.90
6	300	50	10	0.715	641.79
7	450	50	15	0.857	823.85
8	300	75	15	0.951	943.72
9	300	25	15	0.687	605.90
10	300	50	10	0.83	789.23
11	450	75	10	1.191	1251.41
12	150	50	5	0.558	440.51
13	150	25	10	0.584	473.21
14	300	25	5	0.575	462.31
15	300	50	10	0.689	607.82
16	300	50	10	0.697	618.08
17	300	50	10	0.686	604.62

It can be seen from Table 3 that the highest total phenol content is 1251.41 mg—AGE/g of sample at 450Watt MAE power variable with 75% concentration for 10 minutes. The analysis results in this study can be seen in Table 5.

3.7 Analysis of Response Surface Design with ANOVA (*Analysis of Variance*)

Statistical analysis was carried out to prove whether the variables used for the *Centella asiatica* (L.) extraction process affect the resulting product, and the variable can be said to be significant if the p-value of the Analysis of Variance (ANOVA) method has a value of less than (5%) or < 5%. The Analysis of Variance (ANOVA) method can test the difference from the average of more than two independent groups. This is related to the research variables, which amounted to more than two: concentration, power, and extraction time. Each variant will be compared to determine the presence of significance in the data.

Table 4. Results of ANOVA analysis on the pegagan plant extracted using the MAE method

Source	Sum of Square	df	Mean Square	F-Value	P-Value	
Model	5.912E+05	9	65683.91	5.83	0.0150	Significant
There is	1.511E+05	1	1.511E+05	13.40	0.0081	
B-Concentration	1.569E+05	1	1.569E+05	13.92	0.0073	
C-Time	1.421E+05	1	1.421E+05	12.60	0.0093	
AB	72832.52	1	72832.52	2.46	0.0386	
air conditioning	2729.54	1	2729.54	0.2421	0.6378	
BC	29513.52	1	29513.52	2.62	0.1497	
A2	4312.19	1	4312.19	0.3825	0.5559	
B2	7807.82	1	7807.82	0.6925	0.4328	
C2	25783.13	1	25783.13	2.29	0.1742	
Residual	78919.19	7	11274.17			
Lack of Fit	54636.05	3	18212.02	3.00	0.1581	Not Significant
Pure Error	2483.15	4	6070.79			
Total Cast	6.701E+05	16				

From Table 4, the significance of the variables is determined by the p-value, which is said to be significant if the p-value < 0.05.

The F-value of the model obtained from the calculation is 5.83. In the F distribution Table, the value of the F Table with a model of 9, and a df error of 4 at a probability of 0.05, then the percentage point is 6.00, which means that the F-value is greater than the F Table.

In addition, the value of the F-value is inversely proportional to the value of the p-value, as shown in Table 4.2. If the F-value has no significant effect on the variable, then the P-value has a considerable or significant effect. The P-value can be seen as 0.0150, meaning the value is smaller than the set probability of 5% or 0.05. Therefore, the *Centella asiatica* (L.) analysis model significantly affects the extract's total phenol content.

The analysis results in Table 4 also show that the MAE power, extraction time, and solvent concentration significantly affect the total phenol content, with p-values of 0.0081, 0.0093, and 0.0073. Related to the inaccuracy of the test or the value of Lack of Fit, which means that the deviation or imprecision of the model.

In Table 4, the P-value on the Lack of Fit is 0.1581, and the F-value on the lack of fit is 3.00. This value is greater than the probability of 0.05, so that H_0 is accepted and declared insignificant, meaning that the model is suitable, or there is a discrepancy in the research model. The model is considered appropriate if the p-value of the lack of fit is inversely proportional to the e model, so based on the data from Table 4, it can be interpreted that the model is appropriate.

From the ANOVA analysis, the R-squared value was 0.8822 or 88.22%, indicating the model was a good fit under the research results. An adjusted R^2 value of 0.7308 indicates a close relationship between ethanol concentration, extraction time, and MAE power on the response. The value of R-squared is declared according to the model if it is more than 75% [13]. So, the equation of the model can be used to predict the study's actual results. Based on the regression equation obtained, it can be concluded that the effect of concentration, time, and power variables can affect the total phenol concentration. The regression equation is as follows:

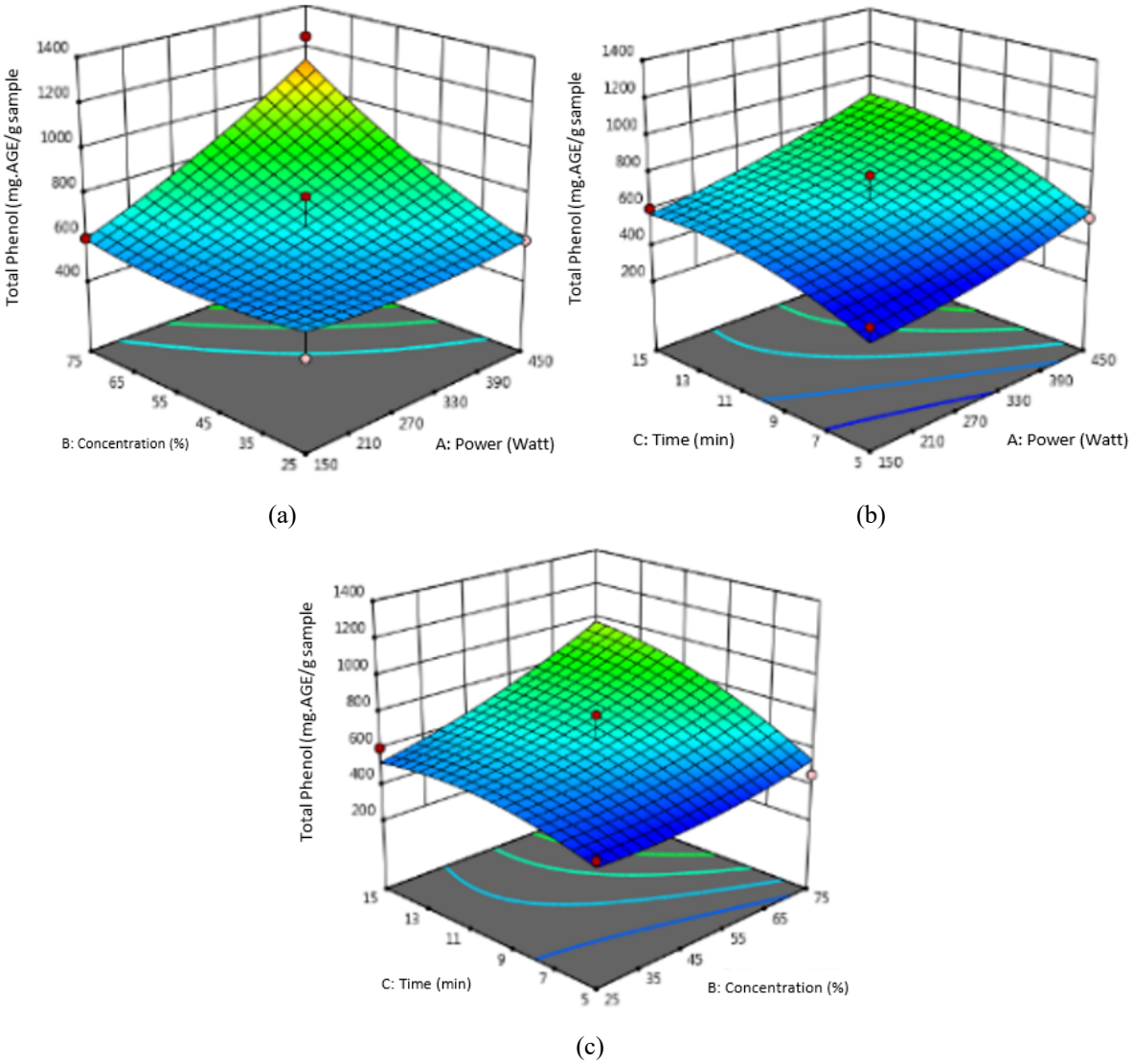
$$\text{Total Fenol} = 652.308 + 137.42A + 140.063B + 133.255C + 134.937AB + 26.1225AC + 85.8975BC + 32.0022A^2 + 43.0622B^2 - 78.2527C^2$$

The total phenol concentration response will decrease with increasing time. A negative constant indicates this.

3.8 Optimization of Total Phenol Content using Response Surface Methodology (RSM) with MAE Method.

Graph in Figure 5, point (a), which shows the effect of variable concentration of solvent and operating power on MAE on total phenol bioactive compounds. From the graph, it can be observed that at the time of the lowest extraction, with operating conditions for 10 minutes in a microwave of 150 watts, and a solvent concentration of 25% ethanol, a total phenol content of 473.21 mg AGE/g sample was produced. The total phenol content also increases with the increase in power and concentration. Thus, it will increase directly proportional to the rise in power and concentration. This is because, following the research conducted by [22], the higher the ethanol concentration, the higher the total phenol obtained. The increase in ethanol concentration is directly proportional to the total phenol obtained [23]. Similarly, the effect of

power, according to research conducted by [24], the higher the power on the MAE used, the hotter the resulting temperature, so that the energy produced in the MAE is radiation and rotation; therefore, the presence of microwave radiation and rotating vibrations will cause the pressure on the cell wall to increase. The cell swells and more bioactive compounds are released or obtained.



(a) (b)

(c)

Figure 5. (a) Effect of concentration and power, (b) Effect of power and time, (c) concentration and time on total phenolic bioactive compounds using the MAE method

On the graph in Figure 5, point (b) shows the effect of variable operating conditions of microwave power and extraction time on total phenol bioactive compounds. The graph shows that at the time of the lowest extraction, with operating conditions of 150 watts of power for 5 minutes, a solvent concentration of 50% ethanol, and a low total phenol content of 440.51 mg AGE/g sample. However, when the time and operating conditions increase, the total phenol content obtained is greater with the optimum condition for the total phenol content of 789.23 at

operating conditions of 300 watts of power, and extraction for 10 minutes with a concentration of 50%. Thus, it will increase directly proportional to the increase in power and time. This is because, according to research conducted by [25], the increase in the extraction time used will increase the penetration of the solvent into the material, making it easier for the solvent to pull chemicals out of the material.

In contrast, the shorter the extraction time, the more difficult it will be for the solvent to penetrate the walls of the material, so the increase in time will be directly proportional. Likewise, the effect of power, as shown in Figure 5 point (b), is that the higher the power, the higher the total phenol content obtained. This is because, according to research [21], the higher the microwave power produced, the greater he tendency to increase the total phenol obtained. This is because the greater the power, the higher the operating temperature, the greater the distillation rate (evaporation). The greater the power, the greater the energy the material receives to be converted into heat, so the total phenol produced is greater.

Graph in Figure 5, point (c), which shows the effect of the variable length of extraction, and the concentration of ethanol solvent on total phenol bioactive compounds. The graph shows that at the time of the lowest extraction, with a solvent concentration of 25% ethanol, for 5 minutes, and 300 watts of power, there was a low total phenol content of 462.31 mg.AGE/g sample. At the operating conditions of 300 watts of power, and extraction for 10 minutes with a concentration of 50%. Thus, it will increase directly proportional to the increase in concentration and time. This follows the research conducted [26], which states that the total phenol will increase, followed by an increase in extraction time until it reaches the optimum limit. If it exceeds the optimum limit, the total phenol will decrease because the compounds contained are degraded over a long time, which can cause the temperature to increase. Microwave heating will cause the extraction temperature to continue to increase, along with the increase in extraction time, which will cause the degradation of phenol compounds. Microwaves can also damage enzymatic compounds that can reduce the activity of the phenolase enzyme. Likewise, the increasing concentration of total phenol increases with increasing solvent concentration. The more that is used, the more the target compound will dissolve in the ethanol [27].

3.9 Result of Optimizing Conditions for The Extraction of Pegagan with the MAE Method

Figure 6 shows that the desirability (D) value reaches its maximum when the factor value is on the red line. The graph in Figure 9 shows that the optimal value for the response to the total phenol concentration was obtained at 450 watts of power, 75% concentration, and within 10 minutes, resulting in a value of 1130.7882 mg.AGE/g sample obtained when the desirability value reached 0.8513.

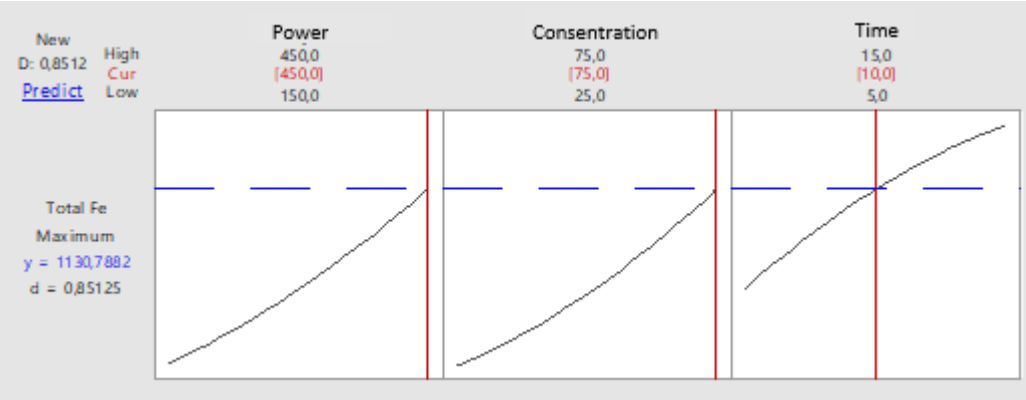


Figure 6. Graph of optimization plot with the MAE method

3.10 Comparison of the results of Pegagan (Centella asiatica (L.)) using the UAE method with the MAE method extraction results.

The analysis of the total phenol content represents the extraction process results for the content of bioactive compounds. From the UAE method using ethanol solvent, the optimal value for the total phenol concentration response was obtained at 270 watts, 50.2525% concentration, and within 60 minutes, resulting in a value of 824.422 mg.AGE/g sample. Obtained when the desirability value reaches 0.97804. While the extraction process results using the MAE method with ethanol solvent, the optimal value in response to the total phenol concentration was obtained at 450 watts of power, 75% concentration, and within 10 minutes, resulting in a value of 1130.7882 mg.AGE/g sample obtained when the desirability value reached 0.8513. So, it can be concluded that the extraction of Pegagan (*Centella asiatica* (L.)) using the microwave method gives the maximum results.

However, extraction using the UAE method also gives significant results, and the difference is not far from the MAE extraction method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing for total phenol in the extraction of Pegagan. It can produce a high total phenol content of 1130.7882 mg quickly.AGE/g. This study is quite high in the value of total phenol content compared to similar

studies by comparing the UAE and MAE methods because of the difference in the extraction method used in this study. Extraction using the UAE method also gives significant results, and the difference is not far from the extraction of the MAE method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing of total phenol in the Pegagan extract. It can produce a high total phenol content of 1130.7882 mg quickly.AGE/g. This study is quite high in the value of total phenol content compared to similar studies by comparing the UAE and MAE methods because of the difference in the extraction method used in this study. Extraction using the UAE method also gives significant results, and the difference is not far from the extraction of the MAE method, so it can be said that the MAE method is the most optimal for producing bioactive compounds through testing of total phenol in the Pegagan extract. It can produce a high total phenol content of 1130.7882 mg quickly.AGE/g. This study is quite high in the value of total phenol content compared to similar studies by comparing the UAE and MAE methods because of the difference in the extraction method used in this study. It can produce a high total phenol content of 1130.7882 mg quickly.AGE/g. This study is quite high in the value of total phenol content compared to similar studies by comparing the UAE and MAE methods because of the difference in the extraction method used in this study.

A short time can produce a high total phenol content of 1130.7882 mg.AGE/g. This study is quite high for the value of total phenol content compared to similar studies by comparing the UAE, and MAE methods, because of the difference in which extraction method is in this study [28] this study used the maceration method for *Centella asiatica* (L.) extracted with a maximum wavelength of 765 nm to determine the total phenol content, with a sample weight of 150 g in powder form resulting in a total phenol content of only 3.67 mg.AGE/g sample for 30 minutes with a concentration of 3.67 mg.AGE/g sample for 30 minutes with a concentration of 70% ethanol solvent. This differs greatly from the total phenol results obtained in the UAE method, with a lower ethanol concentration of 52.77%. The MAE method has a shorter time of 10 minutes, and both only use 1 gram of sample, but the MAE method has a higher total phenol content, 1130.7882 mg AGE/g, while the UAE method has a total phenol content of 824.422 mg AGE/g.

4. Conclusion

In the ANOVA analysis using the UAE method, variables significantly responded to the total phenol content: power, time, and ethanol concentration, with an R-squared value of 0.9876

or 98.76%. The optimum condition of the total phenol content using the UAE method is the optimal value for the response to the total phenol concentration obtained at 270 watts of power, 50.2525% concentration, and within 60 minutes by obtaining a value of 824.422 mg AGE/g sample. In the ANOVA analysis using the MAE method, variables significantly responded to the total phenol content: power, time, and ethanol concentration, with an R-squared value of 0.8822 or 88.22%.

So that the comparison between the UAE and MAE methods for *Centella asiatica* (L.) extracted with ethanol solvent is more optimal, namely using the MAE method, because in a short time of only 10 minutes, it can produce 1130.7882 mg AGE/g of total phenol sample, and the concentration of ethanol. Which is getting purer at 75%, so the MAE method is more efficient.

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