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Optimization of Extraction of Bioactive Compound from Pegagan Leaves Using Ethanol Solvent With Microwave-Assisted Extraction Method (MAE)

Fira Ulvatur Romah [a], Atiqa Rahmawati [b,*], Meta Fitri Rizkiana [a], Ari Susanti [c]

[a]Department of Chemical Engineering, Universitas Jember, Indonesia 68121

[b]Department of Leather Engineering and Skin Preservation, Politeknik ATK Yogyakarta, Indonesia 55188

[c]Department of Chemical Engineering, Politeknik Negeri Malang, Indonesia 65141

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Abstract. Treatment using natural ingredients in Indonesia is the main choice that is growing in society. This is because treatment with natural ingredients has relatively milder side effects compared to synthetic treatment. Therefore, further research is needed on natural ingredients that can be used as natural medicines, one of which is pegagan (*Centella asiatica* (L.)). Several studies have found bioactive compounds in pegagan that can be used as medicine by various methods. The author wants to know the optimal conditions for extracting pegagan bioactive compounds using the microwave-assisted extraction (MAE) method. This study used pegagan leaf size 40 mesh that had been dried. pegagan leaves were extracted using ethanol as a solvent with microwave power, solvent concentration, and extraction time as variables. Variable power 150 watts, 300 watts, and 450 watts. Variable solvent concentration 25%, 50%, and 75%. Variable extraction time for 5, 10, and 15 minutes. Analysis of the results of the study was carried out using total phenol analysis using the Folin-Ciocalten method. The research data obtained optimum operating conditions at 75% solvent concentration, 450-watt microwave power, and extraction time of 10 minutes with a total phenol content of 1251.410225 mg AGE/g sample.

Keywords: *extraction, pegagan, ethanol, bioactive compounds, microwave assisted extraction, optimum conditions*

1. Introduction

Recently, in Indonesia, treatment using natural ingredients has become the main choice that has developed in the community. This is because treatments made with natural ingredients

*corresponding author: tiqa054@gmail.com

have relatively milder side effects when compared to synthetic treatments. Therefore, a more in-depth research is needed on the natural ingredients used as natural medicines. One of the natural ingredients that can be used as medicine is pegagan (*Centella asiatica* (L.)) [1].

Pegagan (*Centella asiatica* (L.)) is one of the wild plants that grow in large numbers in various places such as plantations, fields, and in the yard. Pegagan has good prospects as a medicinal plant. Pegagan has been designated as a traditional medicinal plant since 1884 [2]. Pegagan is commonly used as a food additive, herbal tea, and component in cosmetics. In addition, this plant can also be used in the pharmaceutical world, namely as a therapeutic application [3]. Pegagan has long been used as a traditional medicine in the form of dry ingredients, fresh, or in the form of ingredients. This pegagan plant has a pharmacological effect that has been proven from several studies, such as pegagan used as a medicine in healing wounds, rheumatism, inflammation, hemorrhoids, asthma, leprosy, tuberculosis, fever, dysentery, and can increase appetite in Australia [4].

The pegagan plant has been used as a traditional medicinal herb in Asia for hundreds of years, such as traditional Chinese medicine and Ayurvedic medicine [5]. Pegagan geographically comes from China, Indonesia, India, Madagascar, Sri Lanka, and Malaysia, and grows in humid places. Because pegagan can be used in the health sector, this plant can reach the borders of Turkey, the West Indies, and North America [6]. *Centella asiatica* (L.) has many bioactive ingredients, such as asiaticoside, Asiatic acid, betulinic acid, thankunic acid, madeccasic acid, and madecassoside [7]. Asiaticoside in pegagan was identified as the most active major compound [8], so it can be used as a characteristic of the pegagan plant. *Centella asiatica* (L.) contains asiaticoside as an active constituent which plays an important role in increasing the stimulation of antioxidant levels that can assist in the wound healing process by helping the proliferation of fibroblasts and extracular matrix which have an important role in the wound healing process [9].

The bioactive compounds contained in *Centella asiatica* (L.) can be extracted by several methods. Based on several studies that have been carried out, the extraction of these bioactive compounds was carried out using the Soxhlet extraction method, maceration [10], subcritical water [11], viscozyme [12], and microwave-assisted extraction [13]. In its use, the pegagan plant is usually produced in the form of an extract first. Pegagan extract can be produced by maceration, fluidization, continuous filtration, and percolation. In general, the solvent specified in the extraction process is ethanol, water, ether, or a mixture of water with ethanol [3].

In the research conducted by [3] namely pegagan extraction using the microwave-assisted extraction method to determine the optimal conditions. This research used dry pegagan and ethanol as solvents. The results obtained in this study are the optimal conditions obtained with a ratio of 10 ml/g: 58% ethanol (solid/liquid ratio) at 300 W microwave power and in 3.4 minutes. The MAE process accelerates mass transfer and produces higher yields when using less solvent. The proportion of ethanol in water has a major influence in extracting the desired product quantity. Other studies have shown that the MAE method in extracting bioactive compounds from pegagan using ethanol as a solvent has a yield that is twice as large as using the Soxhlet extraction method [10]. The advantages of the microwave as an extraction method are time efficiency, reducing the use of organic solvents, and as an environmentally friendly extraction method [14]

In this study, the extraction of bioactive components from the *Centella asiatica* (L.) plant using the microwave-assisted extraction method will be carried out to study the optimization of the extraction of bioactive compounds from pegagan extract using ethanol as a solvent. Optimization results will be obtained using the Box-Behnken design (BBD) model and to determine the bioactive content contained in pegagan plants, a total phenolic analysis will be carried out.

2. Materials and Methods

2.1. Materials

The materials used in this study included 40 mesh size dried pegagan leaves, 96% technical ethanol, aquadest, Na_2CO_3, Folin-Ciocalteu reagent, and gallic acid.

2.2. Methods

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. Extraction was carried out with several variables, including at a solvent concentration of 25%; 50%; and 75%, 150 watts of microwave power; 300 watts; and 450 watts, and variable extraction time for 5 minutes; 10 minutes; and 15 minutes. The extraction results were stored in an 8 ml vial at a temperature of 4^oC.

2.3. Preparation of gallic acid solution 100 ppm

Weigh 0.01 grams of gallic acid, then add 1 ml of ethanol and add distilled water to a volume of 100 ml.

2.4. 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, shake the two liquid mixtures until they are homogeneous, and allow to stand at room temperature for 4-8 minutes. Add 4 ml of 10% Na_2CO_3 solution into a test tube, shake, until homogeneous and allowed to stand for 15 minutes at room temperature. Then the solution was analyzed with a UV-vis spectrophotometer with a wavelength range of 700-800 nm.

2.5. Preparation of the gallic acid calibration curve for the folin-ciocalteu reagent

100 ppm gallic acid mother liquor, taken 1 ml each; 3 ml; 5 ml; and 7 ml. Then diluted with distilled water, to a final volume of 10 ml, so that a solution with a concentration of 10 ppm will be obtained; 30 ppm; 50 ppm; and 70 ppm. Each of these solutions was taken as much as 0.2 ml, put into a test tube, and added 1 ml of Folin-Ciocalteu reagent, shaken until homogeneous, 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 with the maximum wavelength that has been obtained previously. Then a calibration curve is made using the regression equation $y = ax + b$.

2.6. Determination of total phenolic content by the folin-ciocalteu method

Take 0.1 ml of the extract, add 9.9 ml of distilled water (dilution 100 times) and add 1 ml of Folin-Ciocalteu reagent then shaken until homogeneous and allowed to stand for 8 minutes. Then add 3 ml of 10% Na_2CO_3 to the mixture, shake until homogeneous and let the solution stand 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 as mg gallic acid equivalent/gram of fresh sample.

3. Result and Discussion

The research was carried out in November 2020 s.d. December 2020 at the Basic Chemistry Laboratory and Bioprocess Laboratory, Chemical Engineering Study Program, Department of Mechanical Engineering, Faculty of Engineering, University of Jember. This study uses pegagan plants extracted to be taken bioactive compounds or components with the Microwave-Assisted Extraction method. The results of measuring the total phenol content in the pegagan plant extract can be seen in Table 1.

Tabel 1. Total phenol content in pegagan plant extract

No.	Power (Watt)	Concentration Pelarut (%)	Time (minute)	Absorbance Rata-Rata	Total Phenol (mgAGE/g sampel)
1	150	75	10	0.680	597.564102
2	450	25	10	0.672	587.307692
3	450	50	5	0.646	553.974359
4	300	75	5	0.570	456.538461
5	150	50	15	0.687	605.897435
6	300	50	10	0.715	641.794871
7	450	50	15	0.857	823.846153
8	300	75	15	0.950	943.717948
9	300	25	15	0.687	605.897435
10	300	50	15	0.830	789.230769
11	450	75	10	1.190	1251.41025
12	150	50	5	0.558	440.512820
13	150	25	10	0.583	473.205128
14	300	25	5	0.575	462.307692
15	300	50	10	0.688	607.820512
16	300	50	10	0.696	618.076923
17	300	50	10	0.686	604.615384

3.1. Gallic acid standard curve

Gallic acid standard curves were made using several concentrations of gallic acid, namely 10 ppm; 30 ppm; 50 ppm; and 70 ppm. The absorbance was measured with a maximum wavelength of 765 nm that had been previously obtained. The standard curve for gallic acid and the straight-line equation that will be used in determining the concentration of gallic acid can be seen in Figure 1.

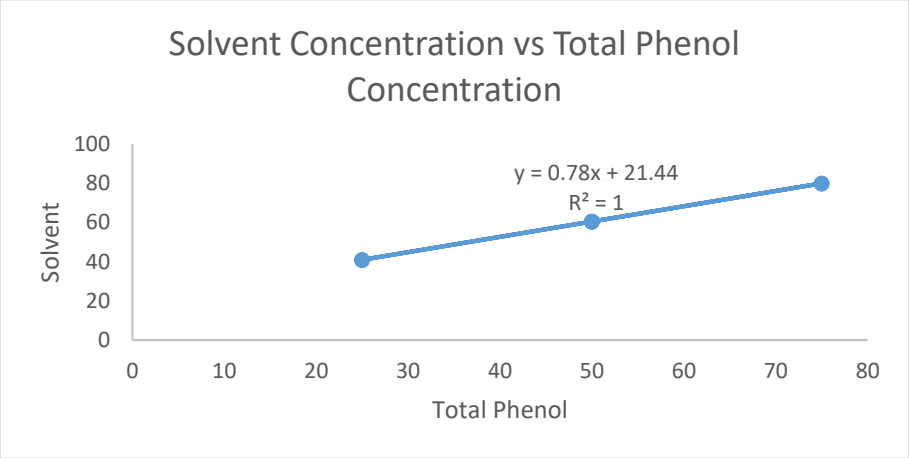


Figure 1. Gallic acid standard curve with various concentrations of 10, 30, 50, 70 ppm

From the curve, a straight line equation is obtained, namely y = 0.78x + 21.44, with R^2 = 1 which shows that the straight-line equation can be used to determine the total phenol content.

3.2. Analysis of total phenol by Response Surface Method (RSM)

Analysis of total phenol using the response surface method (RSM) was carried out to prove whether the variables used in the pegagan extraction process could affect the resulting product. The variable can be said to be significant if the p-value of the RSM analysis method has an alpha value (5%). P-value <0.05 indicates that the antioxidant activity produced is a response to the treatment variables which include solvent concentration, power, and extraction time. The F-value is inversely proportional to the F-table value, seen in Table 2.

Table 2. Results of Analysis of Variety (ANOVA) response of antioxidant activity

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	607455	67495	7.54	0.007
Linear	3	486103	162034	18.11	0.001
Power	1	151074	151074	16.89	0.005
Concentration	1	156944	156944	17.54	0.004
Time	1	178086	178086	19.91	0.003
Square	3	32114	10705	1.20	0.378
Power*Power	1	6743	6743	0.75	0.414
concentration * concentration	1	10973	10973	1.23	0.305
Time*Time	1	11390	11390	1.27	0.296
2-Way Interaction	3	105074	35025	3.92	0.062
Power* Concentration	1	72831	72831	8.14	0.025
Power*Time	1	2729	2729	0.31	0.598
Concentration *Time	1	29513	29513	3.30	0.112
Error	7	62620	8946		
Lack-of-Fit	4	61771	15443	54.57	0.004
Pure Error	3	849	283		
Total	16	670075			

The F-value is 7.54, the df value is 9 with the number of samples 17 at alpha 0.05, the F-table value is 2.49. So that the F-value is greater than the F-table which indicates that the model used has a significant effect on the response. The value of the P-value can be seen as 0.007 which means that the value is smaller than the set probability of 0.05. So that the analysis model of Pegagan (*Centella asiatica* (L.)) plant extract has a significant or significant effect on the total phenol content of the extract.

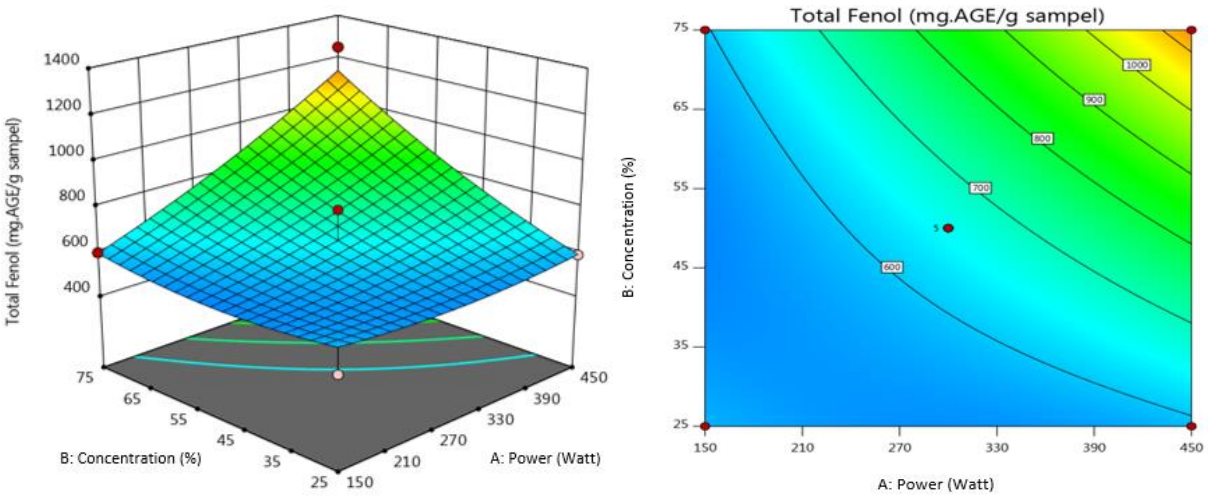
Tabel 3. Model Summery

S	R-sq	R-sq(adj)	R-sq(pred)
94.5819	90.65%	78.64%	0.00%

This analysis also obtained an R square value of 90.65% which indicates that the model used is by the results of the study. The value of R square can be stated according to the model if the value is more than 75% (Yingngam et al., 2020). The adjusted R-sq value of 78.64% indicates that there is a strong relationship between ethanol concentration, extraction time, and microwave power on the response.

3.3. Effect of variables (power, ethanol concentration, and radiation time) on the total phenol content

Figures 2, 3, and 4 are graphs showing the effect of each variable (concentration; power, time; power, concentration; time) on the total concentration of phenol. The figure shows that there are combinations of parameters that influence the response value through the presence of different colors. The lines that consist of the dots on the counterplot graph are a combination of 3 factors. The combination is formed from differences in the proportion of factors and produces the same response value for the total phenol content.



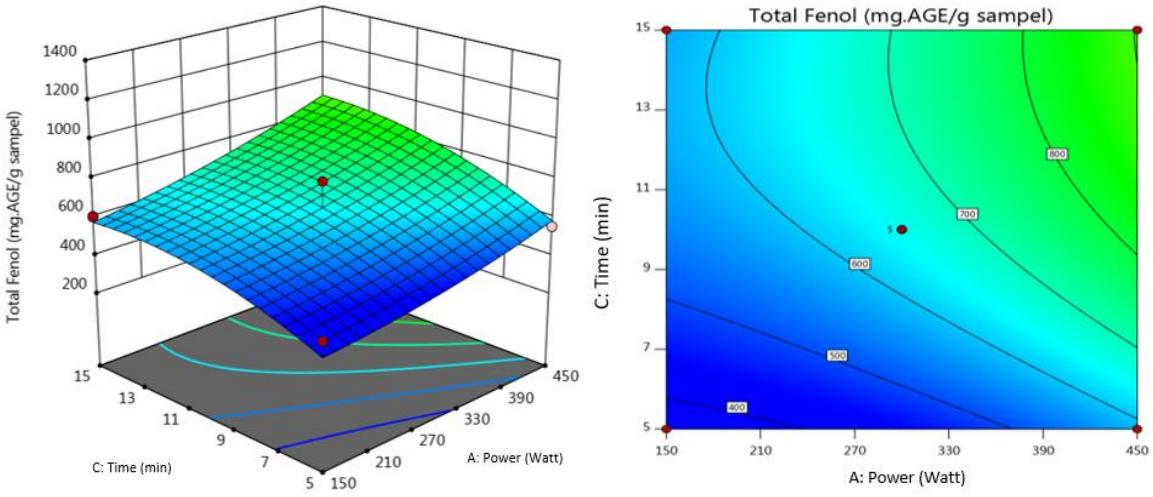
(a) (b)

Figure 2. Effect of Concentration and Power on Total Phenol of Pegagan Bioactive Compounds

The graph in Figure 2 point (a) can be observed that the extraction that produces the lowest yield at the operating conditions of the microwave power is 150 watts and the solvent concentration of ethanol is 25%. Thus, it can be seen that the total phenol content will increase with the increase in microwave power and solvent concentration. The effect of microwave

power in this study is following the research conducted by [15] which shows the higher the microwave power used will produce an increasing temperature by causing the energy generated in the microwave (radiation and rotation), so that there is microwave radiation and rotating vibrations which cause pressure on the cell wall to increase and the cell will swell, so that more and more bioactive compounds are produced.

figure 2 point (b), the X-axis shows the extraction power used, while the Y-axis shows the concentration of ethanol solvent used (%), and the lines in the contour indicate the response. The figure shows that with increasing power and solvent concentration, the total phenol concentration will increase. This can be seen from the change in the color of the area starting from the lowest in the blue area and rising to the orange area, namely the area with a higher total phenol concentration.



(a) (b)

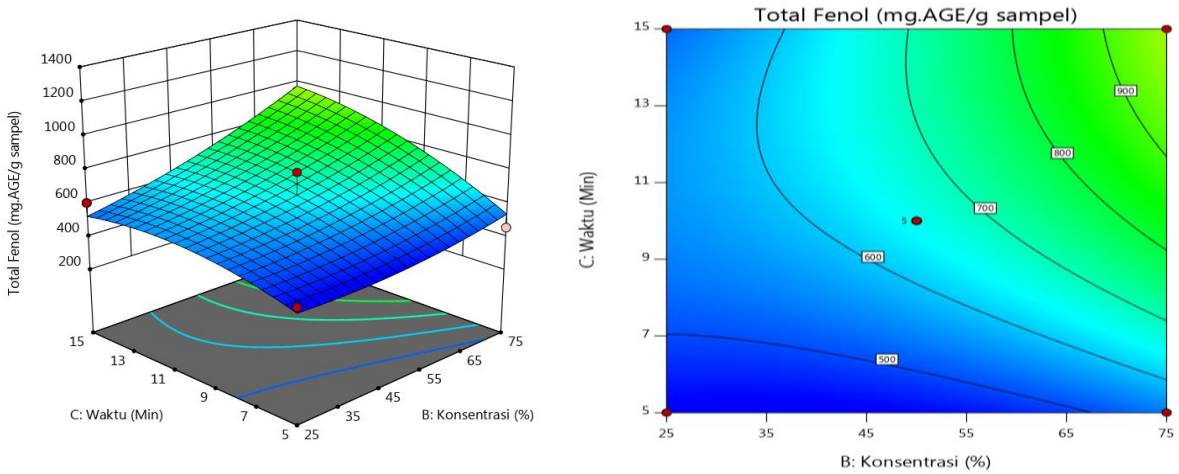
Figure 3. Effect of time and power interaction on total phenol of pegagan bioactive compounds

Figure 3 point (a) shows the response surface graph for the yield response, namely the relationship between extraction time and power to the total phenol produced. The graph in the figure shows that the extraction that produces the lowest yield is at the operating conditions of the 150-watt microwave power for 5 minutes. When the extraction time and microwave power are increased, the total phenol content produced will increase by producing the optimum total phenol content. This shows that the increase in total phenol content is directly proportional to the increase in power and extraction time.

The results obtained are by the results of research by [16] in extracting soursop leaves using the microwave-assisted extraction method, which results in an increased response of antioxidant activity with increasing extraction time. So that more and more target compounds can be extracted with ethanol solvent and MAE method, but the extraction time which increases

beyond the optimal extraction time will result in a decrease in the total phenol content. Microwave heating will cause the extraction temperature to increase with the increase in extraction time which causes the degradation of phenol compounds. In addition, microwaves can also reduce enzymatic activity which is the result of damage to the extracted compounds, the result of microwave heat will be an inhibitor of phenolase enzyme activity. And in [17] regarding extracts of phenolic compounds from rosella flower petals with microwaves which stated that an increase in the total phenol produced was in line with an increase in microwave power. This increase is due to the direct influence of microwave energy on biomolecules by ionic conduction and dipole rotation resulting in molecular motion and heating.

Figure 3 point (b) shows the response lines, where the outer line shows the lowest response value and the deeper line shows the higher response value. The figure shows that the total phenol concentration will increase with increasing extraction time and power. This can also be seen from the color change in the contour graph. This is also by the research conducted by [18] in determining the optimization of the total flavonoid content of brown algae by producing a significant effect of the interaction between power and extraction time, namely the flavonoid content increased with an increase in power of 300-450 watts and at 7-9 minutes.



(a) (b)

Figure 4. Effect of time and concentration interaction on total phenol of pegagan bioactive compounds

In figure 4 point (a) it is known that when the operating conditions of time and power increase, the total phenol content obtained increases. This shows that the increase in total phenol content is directly proportional to the increase in solvent concentration and extraction time. This is following the research conducted by [19], namely extraction with the MAE method on the antioxidant activity of corn silk extract by obtaining results showing that the higher the

concentration of ethanol solvent and the longer extraction time, the yield of corn silk extract will also increase.

Figure 4 point (b) also shows the response to the total phenol concentration which increases with increasing time and solvent concentration as evidenced by a change in color from the outermost (low) region to the deepest region, which shows a higher response value.

3.4. Regression Equation

Total phenol concentration = 932 – 2.30 power – 20.25 concentration + 25.24 time + 0.00178 power*power + 0.0819 concentration * concentration – 2.09 time*time+ 0.0360 power* concentration + 0.0348 power*time + 0.678 concentration *time

The regression equation above can be used to determine the response value of the total phenol concentration obtained if the solvent concentration, power, and extraction time are different. The coefficient of power, concentration, time shows the amount of increase or decrease in the value of the total phenol concentration. If the coefficient of power, concentration, and time are negative it will decrease the value of the total phenol concentration, whereas if it is positive it will increase the value of the total phenol concentration. In this equation, the value of the interaction coefficient between time and time has a negative value. This indicates that there is a maximum stationary point of the response surface [20].

The equation shows the interaction coefficient between power and time, power and concentration, and the interaction between concentration and time is positive, which means that the interaction between these variables can affect the response. The extraction time in the equation shows a positive value, where increasing the extraction time will increase the total phenol response until it reaches the optimal value. This is by [21], namely the longer the extraction time used, the longer the exposure time to microwaves in the sample, resulting in a high value of the antioxidant activity.

3.5. Optimization of total phenol

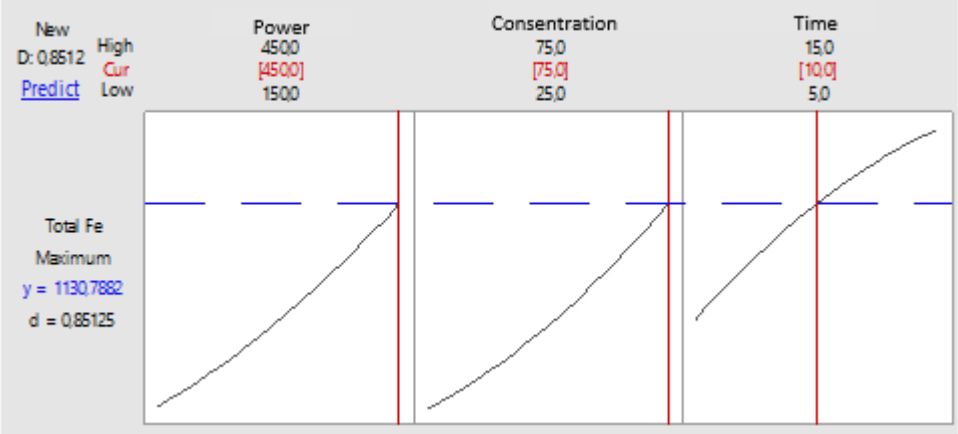


Figure 5. Graph of Optimization Value

Figure 5 shows that the desirability value reaches its maximum value when the factor value is in the red line. The desirability value is used to determine the accuracy of the optimal solution results, where on a scale of 1.00 - 0.80 it shows a very good number [22]. Judging from Figure 5, the desirability value obtained is 0.85125 which indicates that the variables used have a very good effect on the response. In the figure, the black line shows the desirability value for each response and the blue dotted line shows the response value at a certain desirability value. The graph in Figure 5 shows that the optimal value for the total phenol concentration-response was obtained at 450 watts of power, 75% concentration, and within 10 minutes by obtaining a value of 1130.7882 mgAGE/g samples obtained when the desirability value reached 0.85125.

4. Conclusion

The maximum total phenol content in the process of extracting gotu kola bioactive compounds using the MAE method resulted in a total phenol of 1251.410225 mg AGE/g sample. The operating conditions resulted in optimum total phenol at 75% solvent concentration, 450 watt microwave power, and within 10 minutes with an R-square value of 90.65%.

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