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Extraction of Bioactive Compounds from Coffee Husk with Acetone Using Microwave Assisted Extraction Method and Analysis of Phenolic Compounds

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Abstract. Nowadays, coffee husks are limited to animal feed and fertilizer. Therefore, a study used Robusta coffee husk waste as a raw material. The purpose of this study was to determine the effect of several variables and to determine the optimum conditions in the process of extracting bioactive compounds from coffee husk waste. The coffee husk contains bioactive compounds, including anthocyanins and polyphenol compounds, such as flavonols, flavan-3-ols, hydroxycinnamic acids, and caffeine. Coffee husk waste will be extracted using the Microwave-Assisted Extraction (MAE) method with acetone solvent, with several variables: the ratio of material to solvent, microwave power, and extraction time. Analysis of the identification of phenolic compound content was then also carried out using UV-Vis spectrophotometry. The variables of material ratio, extraction time, and microwave power were proven to be interrelated so that they could produce total phenol at optimum conditions. The optimum conditions for extracting bioactive compounds from coffee husk waste were obtained at a material ratio of 0.04 g/mL, 9 minutes, and power of 300 watts, with a total phenol yield of 8.65 GAE/g sample.

Keywords*: coffee husk, acetone, extraction, MAE, total phenol*

1. Introduction

Indonesia is one of the largest coffee-producing countries in the world, ranking 4th after Colombia, Vietnam, and Brazil, with coffee production reaching 762.38 thousand tons in 2020 [1]. Coffee is one of the plantation commodities with a relatively high economic value among other plantation plants, and it plays a vital role as a source of foreign exchange for the country and a source of income for coffee farmers in Indonesia. In coffee processing, several stages are carried out, from peeling the coffee husk until the coffee becomes the final product of ground coffee. Coffee husks are the most significant waste (40% - 45%) from coffee processing [2].

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Nowadays, coffee husks are limited to animal feed and fertilizer. Lack of public attention and minimal information obtained regarding the use of coffee husk waste are the causes of the lack of utilization and processing of coffee husk waste [3]. Coffee husk waste contains ferulic acid, caffeic acid, gallic acid, and p-coumaric acid. In addition, coffee husk also contains active secondary metabolite compounds, including anthocyanins and polyphenol compounds such as flavonols, flavan-3-ols, hydroxycinnamic acids, and caffeine [4].

Natural phenolic compounds are generally polyphenols that form ether, ester, or glycoside compounds, including flavonoids, tannins, tocopherols, coumarins, lignins, cinnamic acid derivatives, and polyfunctional organic acids. The total phenolic content determines an extract's potential for free radical scavengers [5]. A study reported that dry coffee husk contains phenolic compounds of around 1.8-8.56% of the total primary and secondary metabolite content [6].

These bioactive compounds can be obtained by extraction. Several developments have emerged from conventional methods of extracting a sample, one of them being the microwave-assisted extraction method. MAE is an extraction that utilizes microwave radiation to accelerate selective extraction through a rapid and efficient solvent heating [7].

Based on these potentials, a study utilized Robusta coffee husk waste as a raw material for research. Coffee husk waste will be extracted using the MAE method with acetone solvent. This study uses several variables to determine optimal results: the ratio of material to solvent, microwave power, and extraction time. An analysis was also carried out to identify the presence of phenolic compounds using UV-Vis spectrophotometry. The objectives of this study include determining the effect of variations in the ratio of material to solvent, microwave power, and time in the extraction process, as well as determining the optimum conditions in the extraction process of bioactive compounds from coffee husk waste.

2. Material and Methods

2.1 Equipment

The equipment used in this study includes a set of MAE method tools, namely a microwave (Samsung MS23K3515AS-SE), condenser, and measuring flask. Some glass equipment such as measuring cups, beakers, Erlenmeyer flasks, stirring rods, measuring pipettes, test tubes, cuvettes, funnels, and supporting tools such as analytical balance (Pioneer), hoses, clamps, stands, micropipettes, and UV-Vis spectrophotometers 752AP.

2.2 Materials

The materials used in this study were robusta coffee husk waste, raw materials obtained from coffee farmers in Tanah Wulan Village, Bondowoso Regency, East Java, Indonesia. There are also distilled water, acetone (technical), gallic acid (p.a. Merck), Folin-Ciocalteu reagent (p.a. Merck), Na_2CO_3 (p.a. Merck), aluminum foil, and filter paper.

2.3 Methods

2.3.1 Design Expert

This study used the Design Expert application (software) with CCD type (Central Composite Design) to obtain experimental design. The results of running through Design Expert used several variables such as extraction time (3 min, 6 min, 9 min), microwave power (100, 200, 300 Watt), and the ratio of material to solvent (0.2 (10 g: 0 mL), 0.12 (6 g:50 mL); 0.04 (2 g:50 mL)).

2.3.2 Preparation of Coffee Husk Waste Simplicia

Robusta coffee husk waste simplicia was taken from robusta coffee farmers in the Tanah Wulan, Bondowoso Regency. Simplicia's robusta coffee husk waste was dried in the sun until the coffee husk waste was dehydrated. The dried simplicia was ground using a grinding machine and then sieved with a sieve size of 80 mesh.

2.3.3 Coffee Husk Simplicia Water Content Test

The water content of coffee husk waste is determined using the oven method [8]. A total of 5 grams of sample is put into the oven for 2 hours at a temperature of 100 °C and then weighed. After that, the sample is put into the oven for 10 minutes and weighed again. The drying process is repeated 3 times until a constant weight is obtained, with a water content result of ± 10%. The water content can be calculated using Equation 1 [8].

$$Water\ Content = \frac{initial\ mass(g) - final\ mass(g)}{initial\ mass\ of\ coffee\ husk}\ x\ 100\% \quad (1)$$

2.3.4 Coffee Husk Extraction Using the MAE Method

The extraction process in this study used the MAE method because it was considered more effective. This method has the advantage that the process takes a short time, and the total phenol content produced is higher [9]. The extraction process begins by dissolving the sample in acetone solvent using a beaker glass. The ratio of materials and solvents used in this study is 0.2 (10 g:50 mL), 0.12 (6 g:50 mL), 0.04 (2 g:50 mL). The sample solution is then put into

an extraction container (measuring flask) and extracted using a microwave with time variations of 3 min, 6 min, and 9 min and power variations of 100, 200, and 300 watts. The extracted sample solution is filtered with filter paper to separate the filtrate.

2.3.5 Analysis of Total Phenol Content with UV-Vis Spectrophotometry

According to research by Ayuchecaria et al. (2020), the analysis of phenolic compound content using UV-Vis spectrophotometry has several stages, namely [10] :

2.3.5.1 Preparation of Gallic Acid Stock Solution (100 ppm)

A total of 0.01 grams of gallic acid is dissolved in 1 mL of acetone. Then, the solution is diluted with distilled water to 100 mL.

2.3.5.2 Determination of Maximum Wavelength of Gallic Acid

A total of 0.2 mL of gallic acid stock solution was put into a test tube, and 1 mL of Folin-Ciocalteu reagent was added and then shaken until homogeneous. The solution was left for 5 minutes at room temperature. After being left to stand, 2 mL of 10% Na_2CO_3 was added to the solution, and distilled water was added until the volume became 10 mL, then shaken until homogeneous and left for 8 minutes. The solution was then analyzed using UV-Vis spectrophotometry with a 600-800 nm wavelength interval.

2.3.5.3 Preparation of Gallic Acid Standard Curve

A 100 ppm gallic acid stock solution was taken, each 1, 3, 5, and 7 mL. The solution was then diluted with distilled water until the final volume was 10 mL, and a concentration of 10, 30, 50, and 70 ppm was obtained. Each solution was taken in 0.2 mL and put into a test tube. The next step was to add 1 mL of Folin-Ciocalteu reagent, shake until the mixture of the two solutions became homogeneous, and then leave for 5 minutes at room temperature. The maximum wavelength absorption that had been obtained previously was measured. Then, a calibration curve was made with the regression equation $y = ax+b$.

2.3.5.4 Determination of Total Phenolic Content

Take 0.2 mL of diluted extract (0.01 mL of sample diluted to a volume of 10 mL), add 6.8 mL of distilled water, and add 1 mL of Folin-Ciocalteu reagent then shake until the solution mixture becomes homogeneous and left for 5 minutes. Then, 2 mL of 10% Na_2CO_3 is added to the mixture, shaken again until the solution becomes homogeneous, and then the solution is

left for 8 minutes at room temperature. The absorbance is measured using a UV-Vis spectrophotometer at the maximum wavelength that has been obtained.

3. Results and Discussion

3.1 *Design Expert*

Based on the running results using CCD, 20 samples were obtained. The CCD type Design Expert was used because it has the advantage that the design basis is a factorial design, so it can be used for order one or order 2. The running results obtained 20 designs using variations in extraction time, the ratio of material to solvent, and the microwave power used.

3.2 Raw Material Preparation

The raw material in the form of coffee husk obtained from coffee farmers is first dried using sunlight for 3 days to remove the water content contained in the coffee husk. The dried coffee husk is then ground using a grinding machine to make it powder. The raw material in the form of powder aims to facilitate the extraction process so that it is easier to mix. The coffee husk powder is then sieved using an 80-mesh sieve. The finer the coffee husk powder used, the larger the surface area, and the easier it is to penetrate by microwaves, so the higher the solubility level is, the greater the yield produced [11]. The determination of the water content of the coffee husk powder is then carried out using the oven method. The raw material's high or low water content affects the process and extraction results [12]. This water content determination was repeated 3 times until the results were stable to obtain a valid water content percentage. The water content of the sample used was 7.15%; this shows that the percentage of the water content of the robusta coffee husk powder has met the simplex standard, where the water content should not be more than 10% [13].

Table 1. Water Content Test Results

Sample weight (g)	Sample weight after drying (g)	Water content (%)
5	4.65	6.96
5	4.64	7.12
5	4.63	7.38
Average	4.64	7.15

3.3 Coffee Husk Extraction Using MAE Method

The finely ground robusta coffee husk powder is then weighed to be extracted using the MAE method. The robusta coffee husk powder is then dissolved in acetone solvent according

to the ratio obtained through the Design Expert software. The selection of acetone solvent is based on its polarity, which can dissolve secondary metabolite compounds that are polar to non-polar, including phenolic compounds in robusta coffee husk. The extraction process produces sediment and filtrate, which are filtered using filter paper and a funnel. The filtration of the extraction results aims to obtain maximum separation results so that the filtrate is not mixed with the coffee husk powder [14].

3.4 Analysis of Total Phenol Content with UV-Vis Spectrophotometry

The determination of wavelength aims to determine the absorption area that can produce the absorbance value of the parent solution whose absorbance is measured. The wavelength used is the wavelength that has maximum absorbance so that it shows maximum sensitivity. The spectrophotometric tool is set to the wavelength range used, which is around 600-800. Based on research conducted Ayuchecaria et al. (2020), the maximum wavelength is 600-800 nm, and it states that this range obtains ideal wavelength results in determining the presence of bioactive compounds in plants [10]. Septiani et al. (2018) Said that the maximum wavelength is 765 nm [13]. The graph in Figure 1 shows the wavelength measurement with a range of 600-800 nm, and the maximum wavelength is obtained at 760 nm, which has the highest absorbance value. To be more specific, then the wavelength measurement was carried out again with a range of 762-768 nm. Table 2 shows the maximum wavelength is 766 nm.

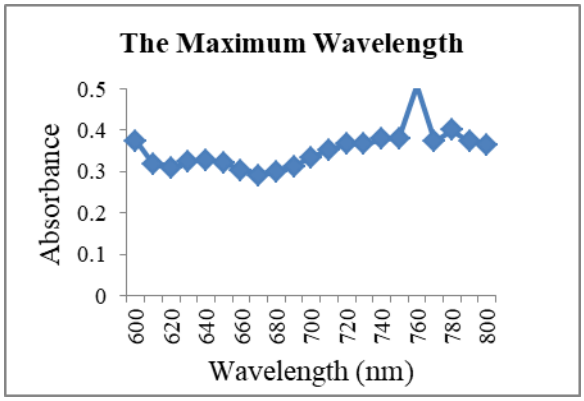

Figure 1. The Measurement of Maximum Wavelength

Table 2. The Measurement of Maximum Wavelength

Wavelength (nm)	Absorbance
762	0.477
764	0.488
766	**0.568**
768	0.422

3.4.1 Gallic Acid Standard Curve

The standard curve was made using a gallic acid solution that had previously been made with several concentrations, namely 10, 30, 50, and 70 ppm. The standard curve was created based on the determination of the absorbance values of several concentrations and measured based on the maximum wavelength that had been obtained. The calibration curve states the relationship between the concentration of gallic acid and the absorbance value expressed by a linear line. The standard curve results obtained a linear regression equation y = 0.0098x + 0.1222 with a correlation coefficient R^2 of 0.9914. According to Miller & Miller (2005), in the study Arikalang et al. (2018), based on the R^2 results obtained, the correlation coefficient gave linear results because it met the acceptable criteria, namely 0.99 [15]. These results meet the requirements, so they can be used to determine the total phenol content of coffee husk extract.

3.4.2 Analysis of Total Phenol Content

The extraction process used the MAE technique with influential variables in time, solvent ratio, and microwave power. The selection of variables is based on factors that affect the extraction process using the microwave technique: time, the ratio of material to solvent, and the amount of power [16]. The extraction results will be filtered using filter paper to obtain maximum separation results so the filtrate is not mixed with robusta coffee husk powder.

The extraction results were analyzed for total phenol content using visible spectrophotometry. Total phenol analysis was carried out with the help of the Folin reagent, which aims to show that the coffee husk extract contains phenolics, which is indicated by a change in the color of the solution to blue. The blue color indicates the presence of bioactive compounds in the coffee husk extract. The results of this analysis are expressed in gallic acid equivalents (mg GAE/g sample).

The results of the absorbance value and total phenol content of coffee husk extract based on the research that has been carried out are presented in Table 3. Table 3 below shows that the highest total phenol results were produced by sample 17 with an extraction time variable of 9 minutes, a material-to-solvent ratio of 0.04 g/mL, and a microwave power of 300 watts. The total phenol produced was 8.65 mg GAE/g sample with an absorbance value of 0.97. The results of this study are higher when compared to previous research conducted Rahayu et al. (2022),Simlipi wherewith the same raw materials and extraction methods but using ethanol solvent, the highest total phenol was obtained at a ratio of 1:30 and a time of 10 minutes, namely 8.55 mg GAE/g [17].

3.3 Effect of Microwave Power, Material Ratio, and Extraction Time on Total Phenol Content

Table 3. Total Phenolic Content in Coffee Husk Extract

Run	Time (minutes)	Ratio (g/mL)	Power (watt)	Absorbance	Total phenol (mg GAE/g sample)
1	6	0.12	200	0.62	1.69
2	6	0.2	200	0.89	1.58
3	6	0.2	200	0.89	1.58
4	3	0.04	100	0.59	4.82
5	9	0.12	200	0.75	2.14
6	9	0.2	100	0.91	1.62
7	6	0.12	300	0.70	1.97
8	9	0.2	300	0.93	1.65
9	3	0.04	300	0.60	4.89
10	3	0.2	300	0.92	1.63
11	3	0.12	200	0.59	1.59
12	9	0.04	100	0.89	7.86
13	6	0.2	100	0.97	1.73
14	3	0.2	100	0.86	1.51
15	6	0.2	200	0.89	1.58
16	6	0.2	200	0.89	1.58
17	**9**	**0.04**	**300**	**0.97**	**8.65**
18	6	0.04	200	0.59	4.84
19	6	0.2	200	0.89	1.58
20	6	0.2	200	0.89	1.58

Based on the results of the research that has been done, the effect of several variables used on the total phenol produced can be seen in Figures 2(a), (b), and (c). Figure 2(a) shows the effect of time and power variables on the total phenol produced. The highest total phenol results were produced at 9 minutes and 300 watts of microwave power of 8.65 GAE/g sample, while the lowest total phenol content was at 3 minutes and 100 watts of power, which was 1.51 GAE/g sample. It can be concluded that the longer the extraction time and the higher the microwave power used, the higher the total phenol produced.

Figure 2(b) shows the effect of the ratio of materials and time variables on the total phenol produced. The highest total phenol results were obtained at a ratio of 0.04 g/mL and an extraction time of 9 minutes, resulting in a total phenol of 8.65 GAE/g sample. It can be concluded that the smaller the ratio of materials to solvents but with a longer extraction time, the higher the total phenol produced. The use of a large ratio will result in a decreasing total phenol. This is because there is a decrease in microwave absorption in the material, so more energy needs to be absorbed by the solvent [17].

Figure 2(c) shows the effect of power variables and material ratio on the total phenol produced. The highest total phenol yield was obtained at a ratio of 0.04 g/mL and 300 watts of microwave power, which was 8.65 GAE/g sample. It can be concluded that the smaller the ratio of material to solvent but with higher microwave power, the higher the total phenol will be produced.

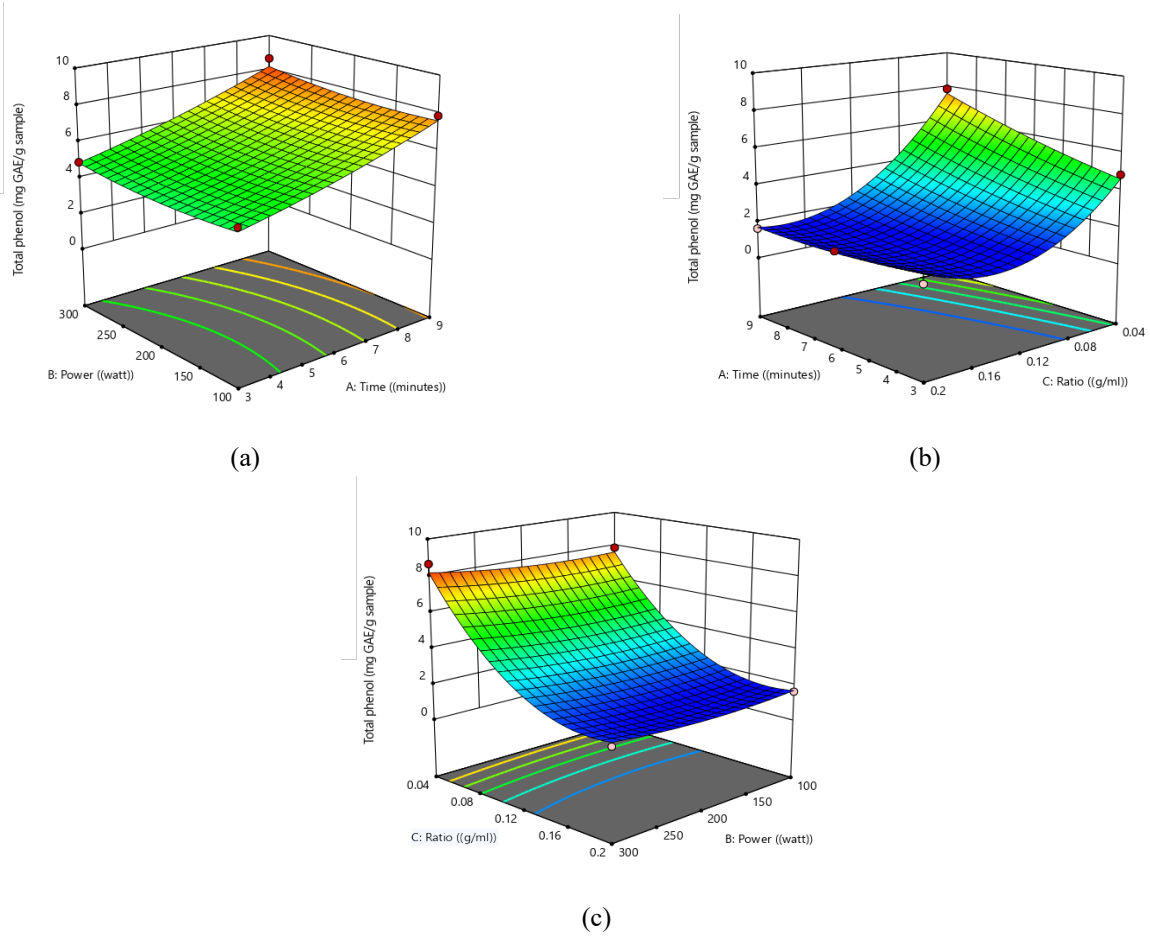

Figure 2. Response Surface Analysis on Total Phenol (a) Effect of Time vs Power Variable, (b) Effect of Ratio vs Time Variable, (c) Effect of Power vs Ratio Variable

The ratio of material to solvent is a reasonably necessary parameter because it affects the total phenol results of robusta coffee husk extract. A ratio of 0.04 (2 g: 50 mL) gives a significant average total phenol value because there is more effective contact between the material and the solvent when compared to other solvent ratios. The ratios of 0.2 and 0.12 are less effective because the weight of the material used is too much, so the material only partially dissolves into the solvent. The greater the ratio of material extracted to the solvent used, the more optimal the results obtained [18].

The time variable in the extraction process is also a parameter that significantly influences the total phenol produced. Extraction time is the contact time between microwaves and the material to be extracted. Extraction time that is too short causes the solubility of phenolic compounds to be less than optimal, so the material is not extracted perfectly and vice versa. The longer the extraction time, the more analytes are extracted because the contact between the solvent and the solute will be longer, so the dissolution of phenolic compounds will continue [19]. This research produced the highest total phenol at the optimum time, namely 9 minutes.

Microwave power variables also affect the total phenol yield, so selecting the power used is necessary. The use of power that is too high, namely above 300 Watt, produces temperatures above 60 °C so that it can cause degradation of the target compound structure and excess pressure in the extraction process and affect the quality of the resulting extract, so a power of 100-300 watt is selected [18]. At low power usage, the cell wall breaks down gradually so that the solvent can be selective to the target compound. In the principle of MAE extraction, the heat generated by particle friction due to microwave energy can cause the cell matrix to break down, and the target compound will come out. So, if the temperature is too low, the cell matrix will not break down completely, and the amount of dissolved target compound is small [18]. Microwave power and extraction time are closely related, where the combination of high power below 400 and longer time becomes the variable with the best results in the MAE method. This study obtained the highest total phenol results at 300 watts of power and 9 minutes, while the lowest were produced at 100 watts of power and 3 minutes.

3.4 ANOVA Analysis

Analysis of research results using the ANOVA method aims to show that the variables used in this study affect the resulting research product. The response value used in this study is total phenol. The results of the ANOVA analysis have several parameters shown in Table 4. The F-value is a specific value used to compare whether the test is significant or not, and the p-value is the magnitude of the observed probability from the statistical test. Several parameters observed include extraction time (A), ratio of material to solvent (B), and microwave power (C).

Table 4. The Results of ANOVA Analysis

Source	Sum of Squares	Df	Mean Square	F-value	p-value
Model	89.75	3	9.97	42.43	0.0001
A-Time	5.57	1	5.57	23.71	0.0007
B-Ratio	58.11	1	58.11	247.24	0.0001
C-Power	0.1140	1	0.1140	0.4853	0.5019
Pure Error	0.0062	5	0.0012		

Parameters with a P-value smaller than 5% (0.05) can be significant or have a real effect. Based on Table 4 above shows that the models and variables A, B, and C used are substantial. A value greater than 0.1 indicates an insignificant model. The F value of the model of 42.43 suggests that the model is significant.

Based on the ANOVA analysis, the R^2 value was obtained at 95.16%, as shown in Table 5. This value indicates that the model used is according to the research results. The R^2 value is stated to be in according to the model if the resulting percentage is more significant than 75% [20]. This can indicate that the equation used to predict the influential variables is appropriate and can be used to predict the study's actual results.

Table 5. The R^2 Value in ANOVA

R^2	0.9516
Adjusted R^2	0.9345
Predicted R^2	0.7934

Based on the ANOVA equation obtained, it can be concluded that the influence of process parameters, namely extraction time, microwave power, and the ratio of material to solvent, can affect the total phenol produced. The ANOVA equation is as follows:

$$\text{Total Phenol} = 7.10 + 0.33A + 78.62B + 0.0095C \quad (2)$$

Description:

A: extraction time

B: ratio of material to solvent

C: microwave power

The equation shows that extraction time, material-to-solvent ratio, and microwave power are directly proportional to the total phenol value. Total phenol increases with increasing extraction time, material-to-solvent ratio, and microwave power. A positive constant value indicates this.

Figure 3 shows a plot of actual data with model-predicted data, which is close to accurate; there is a strong correlation between actual and predicted data. The expected data line is the result of computer calculations using the Design Expert application based on the variables that have been inputted, and the points around the line are the actual data from the experiments that have been carried out. Based on Figure 3, the data plot touches a line that shows that the actual data is close to the predicted data, supported by an R^2 value of 95.16%.

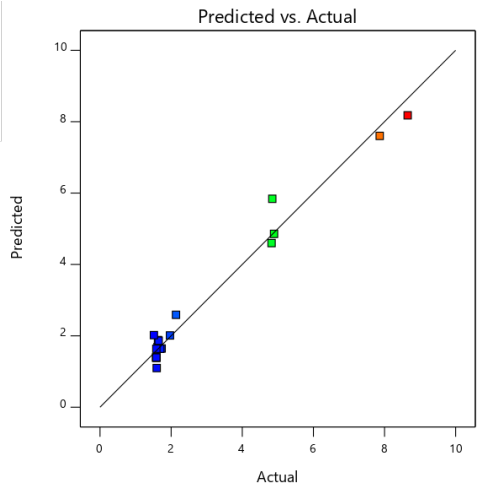

Figure 3. Predicted Data vs Actual Data Plot

4. Conclusion

The variables used in the study of the extraction of bioactive compounds from coffee husk waste using the MAE method affect the total phenol produced. The variables of the ratio of material to solvent, extraction time, and microwave power are related to specific combinations that can result in total phenol at optimum conditions. The optimum conditions in the process of extracting bioactive compounds from coffee husk waste using the MAE method were produced at a variable ratio of material to solvent of 0.04 g/mL, a time of 9 minutes, and a power of 300 Watt with a total phenol yield of 8.65 GAE/g sample.

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