



## Extraction of Phenolic Active Compounds from Coffee Leaves (*Coffea sp.*) Using the Ultrasound-Assisted Extraction Method and Total Phenol Analysis

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**Abstract.** *Coffea sp.* is one of the largest plantation commodities in Indonesia, especially coffee leaves containing phenolic compounds. This research was conducted to extract phenolic compounds using the Ultrasound-Assisted Extraction method with ethanol solvent which aims to determine the optimum operating conditions of extraction as well as the influence of variations in amplitude, time, and ratio of solvents produced. The experimental design was carried out using Design Expert 13 software with the response surface method box-Behnken design. The research variables used were amplitude variations (50%, 60%, and 70%), time (10, 20, and 30 minutes), and solvent ratios (0.1; 0.15; and 0.2 g/mL). Based on our study, these parameters affect the total phenolic content. The model equation for the total phenolic content of coffee leaves obtained is  $Y = 0.1349 - 0.0016 A - 0.0505 B + 0.0010 C + 0.0018 AB + 0.0043 AC - 0.0018 BC - 0.0004 A^2 + 0.0178 B^2 - 0.0014 C^2$  ( $R^2 = 0.9758$ ) with the optimum total phenolic content located in the 17th running of 0.209 mg GAE/g under conditions of 20 minutes, the ratio of material to solvent is 0.2 g/mL, and an amplitude of 50%.

**Keywords:** *Coffea sp.*, phenolic compound, ethanol, ultrasound-assisted extraction.

### 1. Introduction

Indonesia is known as an agricultural country with a tropical climate, most of the land in Indonesia is used for agricultural land, reaching 82.71% (Badan Pusat Statistik, 2015). One of the agricultural or plantation products is coffee. In 2016 the total production of coffee in Indonesia was 663,900 tons, then in 2018, the total production increased to 722,500 tons (Luh Gede *et al.*, 2021). Coffee exports rank fourth in 2018 of the largest commodities in Indonesia after palm oil, rubber, and coconut with a weight of 666,000 tons (Riska *et al.*, 2021). The most common types of coffee found on the market are Arabica coffee (*Coffea arabica*) and Robusta coffee (*Coffea canephora*).

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The utilization of coffee leaves is still not used optimally, so far the processing of coffee plants has only focused on processing coffee beans. The use that is often done is to make compost without processing it first or as a drink that is rich in nutrients (Setiawan *et al*, 2015). According to Campa, *et al* (2012), coffee leaves contain mangiferin, which functions to lower cholesterol which can protect neurons in the brain, and reduce the risk of diabetes, when made into tea it can relieve pain and boost the immune system. According to Baiq *et al* (2017), the use of coffee leaves can be used as something of economic value, coffee leaves contain alkaloids, caffeine, saponins, flavonoids, and polyphenols which can be used to remove free radicals in the body and carcinogenic drugs. Compounds contained in coffee leaves have the potential to inhibit inflammation, diabetes, oxidation, and bacterial growth (Khare *et al*, 2016).

Polyphenols are natural antioxidant, antitumor, and antibiotic compounds that are beneficial to human health. Phenol compounds have one or more hydroxyl groups attached to aromatic compounds, derivatives of phenolic compounds are called the most metabolites produced by plants (Vermerris *et al*, 2006). Extraction is a process of separating natural products contained in raw materials with the help of solvents (Saadatin *et al*, 2019). Compounds will be easy to extract when using the appropriate solvents, the solvents used are organic solvents or inorganic solvents such as ethanol, methanol, acetone, and ethyl acetate (Haslina *et al*, 2019). Factors that influence the extraction process include the solvent, the size of the material aims to increase the contact area of the material with the solvent, and the extraction temperature (Tambun *et al*, 2016). Research on coffee extraction using the ultrasonic method can be seen in table 1 below.

**Table 1.** Coffee extraction by ultrasonic method

Material	Condition	Results	Reference
Coffee Leaves	60% ethanol concentration, time variations of 10, 20, 30, and 40 minutes, 210 W ultrasound power	The highest total phenol amounted to 0,3 mg GAE/g	(Xiumin Chen <i>et al</i> , 2020)
Coffee Skin	Concentration of 80% ethanol, 60 mesh sieve, and time variations of 10, 20, 30, 40, and 60 minutes with 3 times each treatment	The yield and highest anthocyanin value were respectively 11% and 136,67 ppm	(Adinda <i>et al</i> , 2021)
Coffee Seed	Ethanol Concentration 70%, 40 mesh sieve, time variations of 15, 30, and 60 minutes	The optimum yield values for caffeine and chlorogenic acid are	(Adisya <i>et al</i> , 2019)

Material	Condition	Results	Reference
		respectively 4,85 mg/g and 13,06 mg/g	
Coffee Seed 2	Ethanol Concentration 50%, temperature variations 30, 40, 50, and 60°C, time variations 5, 10, 20, 30, 40, and 60 minutes	The highest antioxidant value of 68,9%	(Patrick <i>et al</i> , 2016)
Coffee Seed 3	Ethanol Concentration 60%, frequency 40 kHz, power 300 W	The highest caffeine value of 14,24 g/kg dw	(Guglielmetti <i>et al</i> , 2017)

The extraction process can be carried out in various methods, some use conventional and non-conventional methods. Extraction methods that are often used are maceration, percolation, soxhlet, reflux or steam distillation, Microwave Assisted Extraction, Enzyme Assisted Extraction, Pulsed-electric Field Extraction, Ultrasonic Assisted Extraction. Phenolic extraction is generally carried out using the maceration and soxhlet methods with the consideration that this method is easier and also cheaper, but the weakness of this method is that it takes longer. So the Ultrasonic Assisted Extraction method was used (Mansano *et al*, 2019). Ultrasonic Assisted Extraction (UAE) is an extraction method that produces high yields of bioactive compounds in a relatively short time (Djaeni *et al*, 2019).

The advantage of using ultrasonic waves in extraction is that it is safer, shorter, and increases the amount of crude yield. The type of solvent and the difference in solvent concentration affect the extraction rate so that the solvent used in the extraction process must have a polarity level similar to the compound identified (Intan *et al*, 2021). The use of ethanol solvents is often used in the extraction process because it has a low price, is easy to obtain and has the same polarity level as bioactive compounds or substances, so it is very appropriate to be used to extract phenolic compounds (Noviyanti, 2016)

## 2. Materials and Methods

### 2.1 Materials

The materials used in this study included robusta coffee leaves obtained from Badean Village, Jember Regency, East Java. Ethanol with a concentration of 96% (Technical), aquadest (Technical), gallic acid (p.a, E. Merck), Na<sub>2</sub>CO<sub>3</sub> (p.a, E. Merck), Folin-cicalteu reagent (p.a, Planet Kimia).

## 2.2 Equipment

The tools used in this study included: a blender (Philips HR-2115), oven (Maspion MOT-600), measuring cup (Herma), stirring rod, test tube (Pyrex), Erlenmeyer (Pyrex), filter paper, analytical balance (Ohaus), UV-VIS spectrophotometer (752AP), ultrasonic tool (ultrasonic probe), 60 mesh sieve.

## 2.3 Methods

### 2.3.1 Sample Preparation

Coffee leaves are dried using conventional methods with the help of sunlight until the samples are dry. Coffee leaves drying is carried out at room temperature of 25°C for 1 week until the water content is <10% (Devi *et al*, 2017). The dry coffee leaves are then crushed using a blender until smooth. The fine coffee leaves are then sieved using a sieve with a size of 60 mesh (Andriani *et al*, 2019). Furthermore, coffee leaves are weighed according to the ratio determined by the design expert.

### 2.3.2 Water Content Analysis

Determination of coffee leaves water content was carried out using the oven method (Fahmi Arwangga *et al*, 2016). The determination of the water content of the simplicia was carried out by means of gravimetry (Wijaya *et al*, 2022). A total of 5 grams of sample was put in the oven for 2 hours at 100°C, then the sample was weighed. Calculation of % water content using the following equation:

$$\text{Water Content} = \frac{\text{initial mass(g)} - \text{final mass(g)}}{\text{initial mas(g)}} \times 100\%$$

### 2.3.3 Coffee Leaves Extraction

Coffee leaves that have been weighed according to the ratio is prepared and put into a beaker glass. After that, a solvent is added according to the specified ethanol volume with a concentration of 96% and put into a beaker mixed with the sample. Extraction was carried out using the UAE (Ultrasound Assisted Extraction) method according to the table below. The results of the extraction were filtered using paper, so that the filtrate was obtained from the extraction (Gede *et al*, 2021). Simplicity extraction was carried out by screening data to determine the experimental run. Run data that has been processed with test points from BBD (Box-Behnken Design) can be seen in table 2 below:

**Table 2.** Box-Behnken Design test point screening data

No	Time (Minute)	Ratio (g/mL)	Amplitude (%)
1	30	0,15	70
2	20	0,15	60
3	20	0,2	50
4	20	0,15	60
5	30	0,2	60
6	10	0,15	70
7	10	0,2	60
8	20	0,1	50
9	30	0,15	50
10	10	0,1	60
11	20	0,2	70
12	30	0,1	60
13	20	0,15	60
14	10	0,15	50
15	20	0,15	60
16	20	0,15	60
17	20	0,1	70

### 2.3.4 Total Phenol Analysis

#### 2.3.4.1 Preparation of 100 ppm gallic acid solution

Making a 100 ppm gallic acid solution is by weighing 0.01 grams of gallic acid with the addition of 1 mL of ethanol and adding distilled water until the volume reaches 100 mL (Devi *et al.*, 2017).

#### 2.3.4.2 Determination of the maximum wavelength of gallic acid

Determining the maximum wavelength of gallic acid is by taking 1 mL of gallic acid mother liquor with a concentration of 100 ppm in a test tube and adding 1 mL of Folin-Ciocalteu reagent. The mixed solution was shaken until homogeneous and then left for 4 to 8 minutes at room temperature. The next process was adding 4 mL of 10% Na<sub>2</sub>CO<sub>3</sub> solution to the test tube, then shaking until homogeneous and allowed to stand for 15 minutes. Then the solution was analyzed with a UV-VIS spectrophotometer with a wavelength range of 700-800 nm (Devi *et al.*, 2017).

#### 2.3.4.3 Preparation of gallic acid calibration curve with Follin-Ciocalteu reagent

Preparation of the calibration curve begins with taking 1 mL, 3 mL, 5 mL, and 7 mL of 100 ppm gallic acid mother liquor. Then it was diluted to 10 mL with concentrations of 10 ppm, 30 ppm, 50 ppm, and 70 ppm respectively. Each solution was taken 0.2 mL and put into a test tube. 1 mL of Folin-Ciocalteu reagent was added, then homogenized and allowed to stand for 5 minutes. Then 2 mL of 10% Na<sub>2</sub>CO<sub>3</sub> was added, homogenized and allowed to stand for 5 minutes. In the final step, the solution was added with distilled water until it reached a volume of 10 mL and allowed to stand for 40 minutes and then the wavelength was measured until a calibration curve was obtained with the regression equation  $y = ax+b$  (Devi *et al*, 2017).

#### 2.3.4.4 Determination of total phenol content by the Follin-Ciocalteu method

To determine the total phenol content, the initial step was to take 2 mL of extract and add 8 mL of distilled water and add 1 mL of Folin-Ciocalteu reagent, then shaken until the mixture was homogeneous, then allowed to stand for 8 minutes. The next step was 3 mL of 10% Na<sub>2</sub>CO<sub>3</sub> into the mixture, shaken again until the solution was homogeneous and then allowed to stand for 40 minutes at room temperature. Then the absorption was measured using a UV-VIS spectrophotometer at the maximum wavelength that was obtained (Devi *et al*, 2017).

#### 2.3.4.5 Observed Parameters

The parameters observed were the determination of water content and total phenol analysis using the UV-VIS Spectrophotometry method (Devi *et al*, 2017).

### 3. Result and Discussion

#### 3.1 Extraction of Total Phenolic Content from Coffee Leaves (*Coffea Sp.*)

The research was conducted from October 2022 to December 2023 at the Chemistry and Bioprocess Laboratory, Chemical Engineering Study Program, Department of Mechanical Engineering, Faculty of Engineering, University of Jember. This study used coffee leaves (*Coffea Sp.*) to be extracted and tested for their total phenolic content using the ultrasonic wave extraction method. Determination of the value of the water content using the gravimetric method where the value of the water content must be less than 10%. The water content value obtained from coffee leaves is 7.5% (Wijaya *et al*, 2022).

The factors that affect the extraction of coffee leaves are the extraction time, the ratio of solvents, and the power of the ultrasonic device. According to Ibrahim *et al* (2015) states that time affects the results of extraction. Time that is too long and exceeds the optimum limit can

cause changes in structure, in this condition an oxidation process occurs for bioactive compounds so that it will affect the extraction results which tend to decrease (Yuliani et al, 2019). Gonzales-centeno et al (2014) stated that the application of power to the ultrasonic device can increase the yield efficiency of the extraction process. This happens because of the cavitation effect which makes it easier for the solvent to diffuse into the solid material during the extraction process (Dzah et al, 2020). The linear equation for the gallic acid standard curve used to determine the total phenolic content of the sample is  $y = 0.0763x - 0.0993$  ( $R^2 = 0.9758$ ).

**Table 3.** Results of analysis of the total phenolic content of coffee leaves

No	Time (minutes)	The Ratio of Ingredients to Solvents (g/mL)	Amplitude (%)	Total Phenolic Content (mg GAE/g sampel)
1	30	0,15	70	0,138
2	20	0,15	60	0,132
3	20	0,2	50	0,097
4	20	0,15	60	0,142
5	30	0,2	60	0,101
6	10	0,15	70	0,125
7	10	0,2	60	0,108
8	20	0,1	50	0,200
9	30	0,15	50	0,131
10	10	0,1	60	0,207
11	20	0,2	70	0,098
12	30	0,1	60	0,192
13	20	0,15	60	0,130
14	10	0,15	50	0,135
15	20	0,15	60	0,131
16	20	0,15	60	0,136
17	20	0,1	70	0,209

The table above shows that the lowest total phenolic content obtained was 0.097 mg GAE/g and the highest total phenolic content was 0.209 mg GAE/g. The lowest total phenolic content was obtained from a combination of parameters of 20 minutes, a ratio of 0.2 g/mL of substance to a solvent, and an amplitude of 50%. The highest total phenolic content was obtained from a combination of parameters of 20 minutes, a ratio of 0.1 g/mL of substance to

solvent, and an amplitude of 70%. The difference in the total phenolic content produced is due to differences in extraction conditions, variations, and other factors that affect the extraction process, one of which is sample preparation prior to extraction (Kadek *et al*, 2020).

### 3.2 Statistical Analysis

The total phenolic content data was then analyzed by Analysis of variance (ANOVA) to prove that the parameters used in the extraction process can affect the total phenolic content. The ANOVA results are presented in the table below.

**Table 4.** ANOVA Results

Source	Sum of Squares	df	Mean Square	F-value	p-value	
<b>Model</b>	0.0218	9	0.0024	56.39	< 0.0001	significant
A-Time	0.0000	1	0.0000	0.4759	0.5125	
B-Ratio	0.0204	1	0.0204	473.41	< 0.0001	
C-Amplitude	8.002E-06	1	8.002E-06	0.1859	0.6794	
AB	0.0000	1	0.0000	0.3019	0.5998	
AC	0.0001	1	0.0001	1.68	0.2358	
BC	0.0000	1	0.0000	0.2845	0.6103	
A <sup>2</sup>	8.326E-07	1	8.326E-07	0.0193	0.8933	
B <sup>2</sup>	0.0013	1	0.0013	31.09	0.0008	
C <sup>2</sup>	8.337E-06	1	8.337E-06	0.1936	0.6732	
<b>Residual</b>	0.0003	7	0.0000			
Lack of fit	0.0002	3	0.0001	2.64	0.1855	not significant
Pure Error	0.0001	4	0.0000			
<b>Cor Total</b>	0.0221	16				

Parameters can be said to be significant if the probability value (p-value) from the analysis results is  $\leq 0.05$  or 5%, and the lack of fit value with a p-value  $\geq 0.05$  (Sari *et al*, 2020). The p-value generated in this study was <0.0001, so the parameters of extraction time, ratio, and amplitude of coffee leaves with solvents significantly affected the response, namely the total phenolic content (Rohmah *et al*, 2022). Meanwhile, the p-value for lack of fit is 0.1855 or



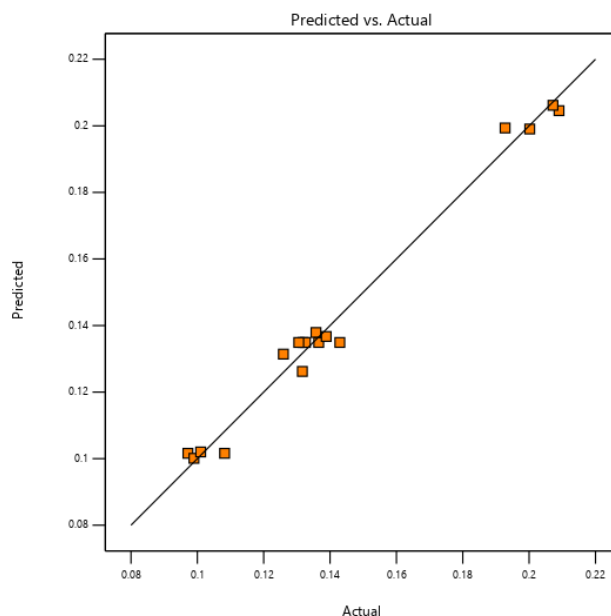
18.55% which is not significant. Lack of fit is a discrepancy or deviation between experimental data and predictive model data (Pertiwi, 2018 & Rahmawaty, 2014)

Table 5. Summary Models

$R^2$	$R^2_{adjusted}$	$R^2_{predicted}$
0.9864	0.9689	0.8482

The research results can be stated according to the model if the resulting  $R^2$  value exceeds 0.75 or is close to 1 (Haryani, 2019 & Marjoni, 2015). The ANOVA results yield an  $R^2$  value of 0.9864 which indicates that the model is in accordance with the research results. The resulting adjusted  $R^2$  value of 0.9689 indicates that there is a relationship between extraction time, ratio, and amplitude of coffee leaves and solvents to the response to total phenolic content (Rohmah et al, 2022). The total phenolic content as a response to the extraction parameters is modeled using the following equation =  $0,1349 - 0,0016 A - 0,0505 B + 0,0010 C + 0,0018 AB + 0,0043 AC - 0,0018 BC - 0,0004 A^2 + 0,0178 B^2 - 0,0014 C^2$

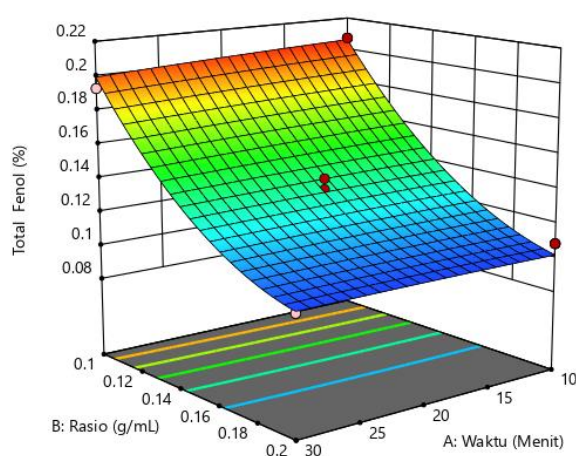
The values of A, B and C respectively are the variables of extraction time, ratio and amplitude of coffee leaves. If the variable coefficient is negative, it indicates a decrease in value in the total phenolic content and vice versa (Rohmah et al, 2022). Based on the equation above, the extraction ratio variable affects the total phenolic content. The relationship between experimental data and model data is presented in the figure below.



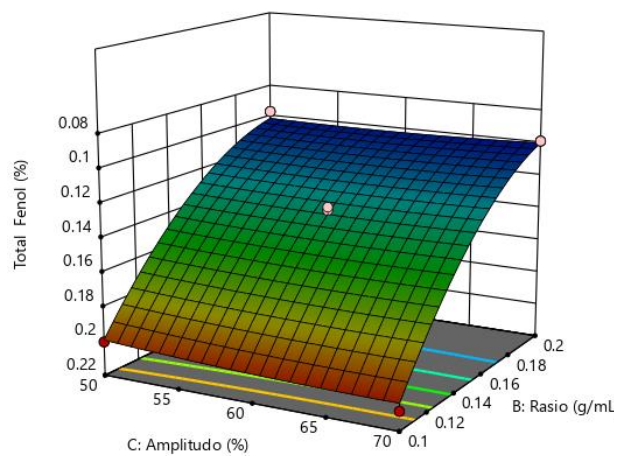
**Figure 1.** Relationship between experimental data and predictive data

The picture above shows that the graph of experimental data with model prediction data is quite accurate, there is a strong correlation between experimental data and model data. The distance between the location of the data and the trendline shows the accuracy of the data, the closer the data is to the line, the more accurate the data (Yilmaz et al, 2017). Based on the research results, the data plot touches the line which shows that the experimental data is close to the model data with an  $R^2$  value of 0.9864.

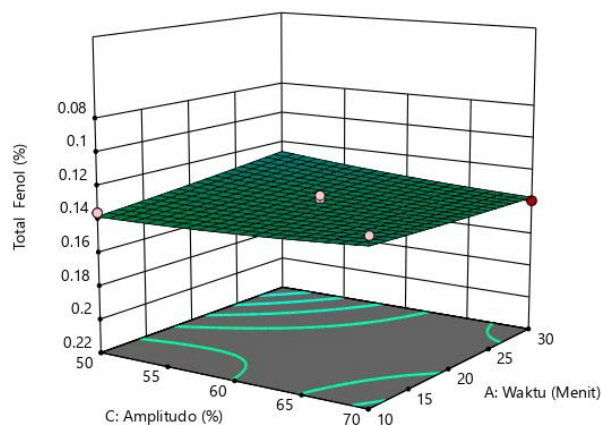
### 3.3 Effect of Extraction Parameters on Total Phenolic Content



**Figure 2.** Effect of interaction between solvent ratio and time



**Figure 3.** Effect of amplitude interaction with the ratio of solvents



**Figure 4.** Effect of amplitude interaction with time

The image above is a graph showing the effect of the variable ratio of ingredients and extraction time on the total phenolic content. The image contains a combination of parameters that affect the response value through a color change. The red color indicates the highest total phenolic content, while the dark blue color indicates the lowest total content. The longer the extraction time and the higher the ratio of ingredients, the lower the total phenolic content. The optimum total phenolic content resulting from this study was 0.209 mg GAE/g sample. Based on the total phenolic content obtained, the smaller the particle size, the greater the contact with the solvent so that the material is easier to extract (Yuliani et al, 2019). This is consistent with research conducted by Xiumin et.al (2020), the variable ratio of coffee leaves to solvents shows that the higher the ratio of coffee leaves to solvents, the higher the total phenolic content obtained. The results of this study showed an increase in yield when using a ratio of materials to solvents from 10:1 to 20:1 g/mL. However, using a ratio of material to solvent that is too high will require a long extraction time (Zhang et.al, 2018). The low total phenolic from the extraction process is also affected by the presence of unwanted components.

#### 4. Conclusions

Based on the research that has been done, it can be concluded that the extraction parameters (time, ratio of material to solvent, and amplitude) have an effect on the total phenolic content. The total phenolic content equation model obtained  $Y = 0.1349 - 0.0016 A - 0.0505 B + 0.0010 C + 0.0018 AB + 0.0043 AC - 0.0018 BC - 0.0004 A^2 + 0,0178 B^2 - 0.0014 C^2$  ( $R^2 = 0.9758$ ) with the optimum total phenolic content of the study located on the 17th running, which is 0.209 mg GAE/g.

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