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Extraction of Flavonoid Compounds from Red Betel (*Piper crocatum*) Using Ultrasound-Assisted Extraction (UAE) Method

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Abstract. *Piper Crocatum* or red betel is one of the medicinal plants known for its beneficial antioxidant content in maintaining overall health. One of the types of antioxidants found in red betel leaf it was flavonoids. In this study, the extraction of red betel leaf was conducted using the Ultrasound-Assisted Extraction (UAE) method to analyze the flavonoid content and antioxidant activity in the red betel leaf. A 90% ethanol solvent was used, and the extraction variables included the ratio of material to solvent, particle size, and extraction time, which were determined using Design Expert 13 software. The flavonoid test was performed using a UV-Vis spectrophotometer at a wavelength of 431 nm. The analysis of antioxidant activity was conducted using DPPH (2,2-Diphenyl-1-picrylhydrazyl). The highest flavonoid content obtained was 263.676 mgQE/g under the optimum operating conditions with a particle size of 100 mesh, a material-to-solvent ratio of 0.06, and an extraction time of 25 minutes.

Keywords: *Red betel leaf, Piper crocatum, Ultrasound Assisted Extraction, Flavonoid, Antioxidant.*

1. Introduction

Indonesia has a remarkably high biodiversity that is distributed across various regions. There are approximately 30,000 plant species, far surpassing other tropical regions such as West Africa and South America. It is known that around 9,600 species have medicinal properties, and about 200 of them are essential medicinal plants for the traditional medicine industry. One of the well-known medicinal plants with numerous benefits and uses is betel (*Piper* sp.). There are many different types of betel plants, distinguished by the color of their leaves, which can be green, red, black, and even yellow. Red betel, in particular, is highly sought after due to its healing properties for various ailments and its ornamental value [1].

Red betel leaves are known to grow abundantly in various regions of Indonesia and are recognized as one of the medicinal plants [2]. Red betel can be found in different areas of

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Indonesia, such as Papua, Aceh, Yogyakarta, West Java, and others [3]. In Indonesia, red betel needs to be continually developed because apart from its medicinal use, it is also often utilized as an ornamental plant. Therefore, the cultivation of red betel has great potential for development in Indonesia [4]. Red betel is known to be used as a treatment for diabetes, gout, hypertension, hepatitis, cancer, and even as an anti-diabetic agent [5]. The medicinal properties of red betel leaves are attributed to the presence of antioxidants in several active compounds such as flavonoids, alkaloids, polyphenols, tannins, and essential oils [6,7]. From the results of research conducted by Zulhizar et al (2022) yellow betel leaves contain more alkaloids, terpenoids, saponins, and phenolics than red and green betel leaves. The process to produce antioxidants is very small and still uses the maceration method. In black betel leaf shows that black betel leaf extract has weak antioxidant activity because IC_{50} is only 158.53 ppm. In red betel leaf extraction has better and stronger antioxidant activity than green betel leaf.[4]. To obtain the chemical constituents of a substance, extraction processes can be carried out using either thermal or non-thermal methods [9]. One non-thermal extraction method that can be employed is Ultrasound-Assisted Extraction (UAE).

The red betel, known by its Latin name *Piper crocatum*, belongs to the Piperaceae family [10]. The red betel has wavy leaves with a combination of green, pink, and silver colors on the upper surface, and a purplish-red color on the lower surface of the leaves [11]. Its stem is round and has a greenish-purple hue, and it does not produce flowers. Red betel thrives best in shaded areas with limited exposure to direct sunlight or in places that are not overly hot [3,8]. Various traditional medicines have been produced using natural medicinal plants, including red betel. Herbal concoctions derived from these plants have been proven effective in treating various ailments [5].

In health, free radicals are the cause of many degenerative diseases. Therefore, to prevent the dangers of these free radicals, antioxidants are needed [12]. Antioxidants are compounds that can prevent the formation of free radicals in the body, thus inhibiting cell damage [13]. Antioxidants can be obtained from both inside and outside the body. Based on their source, antioxidants are divided into natural and synthetic antioxidants. Synthetic antioxidants have been thoroughly tested for their toxic reactions, but some may become toxic after long-term use [14]. Therefore, natural antioxidants are used to prevent the development of free radicals in the body and repair damaged cells [15]. Natural antioxidants can be found in most plants, such as kedawung plants, red betel, guava, and bay leaves. The most important

phenolic compounds in natural antioxidants are flavonoids and phenols [14]. One beneficial chemical compound found in red betel leaves is flavonoids, which function as antioxidants. According to (Tonahi et al., 2014), apart from flavonoids, red betel leaves also contain other phytochemical compounds such as alkaloids, saponins, tannins, and polyphenols [4]. Flavonoids are reducing compounds that can effectively inhibit oxidation, both enzymatically and non-enzymatically. Flavonoids act as scavengers of superoxide and hydroxyl radicals, protecting lipid membranes from damaging reactions. The antioxidant activity of flavonoids is mainly due to the presence of phenolic hydroxyl groups in their molecular structure. Flavonoids have a carbon backbone consisting of 15 carbon atoms, with two benzene rings, A and B (C_6), attached to the C(C_3) propane chain, forming a C_6-C_3-C_6 arrangement. These three structures can be seen in Figure 1.

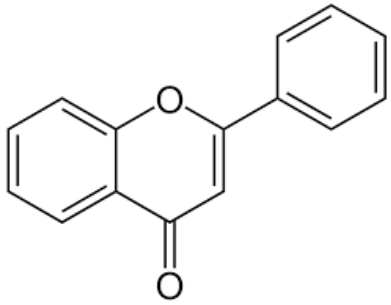

Figure 1. Flavonoid Structure

Table 1. Comparison of Flavonoid Content in Various Leaf Types

Plant Species	Average Absorbance	Flavonoid Content	References
Sauropus androgynus	0.389	13.1101 µg/ml	[16]
Monstera deliciosa	0.806	26.7137 µg/ml	
Annona muricata L.	0.831	27.5027 µg/ml	
Piper crocatum	1.195	39.3778 µg/ml	
Piper betel	0.829	11.857 µg/g	[8]
Piper crocatum	3.204	45.771 µg/g	
Hibiscus rosa-sinensis	0.320	28.890 ppm	[17]
Ceiba pentandra	0.280	25.970 ppm	
Piper crocatum	0.421	36.262 ppm	

Table 1 shows a comparison of flavonoid content in various types of leaves, and the highest flavonoid content is found in red betel or *Piper crocatum*. Analysis was conducted to

determine the levels of flavonoids and antioxidant activity in red betel. Extraction was performed first to obtain an extract from red betel, followed by testing. Extraction is a process of separating a substance from its mixture using an appropriate solvent [18]. The different polarities of antioxidant components can affect extraction efficiency. Factors influencing extraction efficiency include temperature, extraction time, type and concentration of solvent, ratio of material to solvent, and particle size of the plant material. Extraction methods are divided into two types: conventional and modern. Conventional extraction methods include maceration, reflux, percolation, and Soxhlet. Meanwhile, modern extraction methods include Ultrasound-Assisted Extraction (UAE) and Microwave-Assisted Extraction (MAE) [19]. Previous studies often used conventional methods for extracting red betel. The maceration method is commonly employed using ethanol and acetone solvents, which diffuse into the plant cells, causing the release of active compounds due to osmotic pressure. The maceration process is relatively inexpensive and easy, but it requires a considerable amount of time, and the flavonoid content and yield obtained are not free from organic solvents. Therefore, to address the limitations of the conventional extraction method, it is necessary to update the extraction method from conventional to modern extraction methods. One of these methods is Ultrasound-Assisted Extraction (UAE). Research conducted by (Utami et al., 2020) revealed that the extraction process of Plectranthus scutellarioides leaves using Ultrasound-Assisted Extraction (UAE) was more optimal compared to maceration and reflux methods, as the flavonoid content obtained through Ultrasound-Assisted Extraction (UAE) was higher than that obtained through maceration and reflux methods [19].

Ultrasound-Assisted Extraction (UAE) is a modified maceration method that utilizes ultrasound signals with a frequency of 20 kHz. This is done to apply mechanical pressure to the cells, resulting in cavitation within the sample. Cell disruption can lead to increased solubility of compounds in the solvent and improve extraction efficiency [18]. Ultrasound-Assisted Extraction (UAE) is a non-thermal extraction method that enhances mass transfer rate and breaks down cell walls through the formation of numerous microcavities, thereby reducing the extraction time and optimizing solvent usage [19]. The increased contact speed between the extract and the solvent facilitates better penetration towards the cell walls and release of cellular components. Another advantage of UAE is the ability to extract compounds from the matrix without damaging the structure of the extract. The low temperature helps minimize heat loss

and prevents the loss or degradation of compounds with low boiling points [20]. The comparative results of various extraction methods previously used can be seen in Table 2.

Table 2. Extraction of Red Betel Leaves (*Piper crocatum*) using Various Methods

Material	Method and Solvent	Result	References
Piper betel	Method: Maceration Solvent: Ethanol 96% Testing Method: DPPH (2.2-Diphenyl-1-picrylhydrazyl)	The antioxidant activity test results indicated that the n-hexane fraction exhibited higher antioxidant activity (IC50) of 26.73-28.05 mg/mL compared to the diethyl ether fraction (IC50) of 114.54-28.05 mg/mL.	[21]
Piper betel* and *Piper crocatum	Method: Extraction using a hotplate and filtration Solvent: Ethanol 96% Testing Method: Peroxide value testing to determine antioxidant resistance by adding coconut oil	The best peroxide value result obtained was 55.13% for the red betel material under the optimum conditions with a solvent volume of 150 ml, extraction time of 75 minutes, and stirring speed of 300 rpm.	[22]
Piper crocatum	Method: Digestion Solvent: Aquadest (distilled water) Test Method: DPPH (2.2-Diphenyl-1-picrylhydrazyl)	The test results of the concentrated extract of red betel leaf obtained an IC50 value of 53.91 ppm. The conclusion of this research study is that the extract has strong antioxidant potential.	[23]
Piper betel	Method: Ultrasound-Assisted Extraction (UAE) Solvent: Ethanol Testing Method: DPPH (2.2-Diphenyl-1-picrylhydrazyl)	The results showed that UAE (Ultrasound-Assisted Extraction) yielded significantly better results compared to the maceration method. The total flavonoid content was 21.50 mgRE/gDW and the antioxidant activity was 94.99% for UAE, while the maceration method resulted in a flavonoid content of 13.48 mgRE/gDW and antioxidant activity of 78.12%.	[24]

Material	Method and Solvent	Result	References
Piper betel and Piper crocatum	Method: Maceration Solvent: 96% Ethanol Test Method: Quantification of flavonoid content and identification of flavonoid types using UV-Vis spectrophotometer.	The highest absorbance values were obtained on day 1, where it was 3.204 for red betel leaves and 0.829 for green betel leaves. The flavonoid content generated for red betel leaves was 45.77 µg/g, while for green betel leaves it was 11.857 µg/g.	[8]
Piper crocatum	Method: Maceration Solvent: Ethanol Test Method: DPPH (2.2-Diphenyl-1-picrylhydrazyl) and Quercetin using UV-Vis spectrophotometer.	The highest flavonoid content obtained was 197.96±12.51 mgQE/g using 90% ethanol as the solvent, while the lowest flavonoid content was obtained when using 50% ethanol as the solvent, which was 159.32±4.71 mgQE/g. The antioxidant activity was expressed as IC50, which was found to be 82.71 ppm.	[25]
Piper crocatum	Method: Maceration Solvent: Ethanol Duration: 48 hours Testing method: Measurement of flavonoid content and types of flavonoids using a UV-Vis spectrophotometer from PG Instruments Ltd. Determination of antioxidant content using DPPH (2.2-Diphenyl-1-picrylhydrazyl) assay.	The red betel leaf extract tested positive (+) for the presence of flavonoids and tannins, indicated by the formation of a yellow-orange color. As for the antioxidant activity test (IC50), the red betel leaf extract showed a value of 47.45 ppm, while vitamin C showed a value of 49.20 ppm. Based on these values, it can be concluded that the red betel leaf extract is classified as a very strong antioxidant as it is below 50 ppm.	[4]
Piper crocatum	Method: Maceration Solvent: Ethanol Duration: 3 days Testing method: The analysis of total flavonoids was performed with a UV-Vis spectrophotometer. The analysis of antioxidant activity	The obtained results showed a total phenol content of 157.61 ± 4.18 mg GAE/g. The total flavonoid content was 168.33 ± 4.23 mg QE/g. The antioxidant activity (IC50) was found to be 129.11 ± 2.06 ppm.	[10]

Material	Method and Solvent	Result	References
	(IC50) was conducted using DPPH (2.2-Diphenyl-1-picrylhydrazyl).		

The working principle of the Ultraviolet-Visible (UV-Vis) spectrophotometer is based on the absorption of light, which involves the interaction of light with atoms and molecules. Incident light hits the surface of the substance, and the light that passes through the substance is measured. The measurable quantity is the ratio of the intensity of the incident light to the intensity of the light after passing through the sample. The measurement of flavonoid content using a UV-Vis spectrophotometer is based on the maximum absorption of flavonoid extracts. The spectrum of flavonoids can be determined in solutions using solvents such as methanol and ethanol [8]. The measurement of flavonoid content is performed using a standard quercetin solution at a wavelength of 431 nm [26], while the antioxidant activity of the red betel leaf extract is tested using 2,2-Diphenyl-1-picrylhydrazyl (DPPH). DPPH acts as a free radical that is scavenged by antioxidants in the test sample. This reaction can cause a color change that can be measured using a UV-Vis spectrophotometer in the visible light range at 517 nm [27], allowing the determination of the free radical scavenging activity in the sample. This study aims to determine the optimal operating conditions for extracting flavonoids from red betel using the Ultrasound-Assisted Extraction (UAE) method with the Box-Behnken Design (BBD) model in Design Expert 13 software, with the variables used, which are the material-to-solvent ratio, particle size, and extraction time.

2. Materials and Methods

2.1 Materials

The materials used are 90% ethanol, distilled water, 10% aluminum chloride solution, quercetin solution, ascorbic acid, 1M sodium acetate, DPPH, and red betel leaves obtained from Batu, Malang.

2.2 Equipment

The equipment used in the research on the extraction of red betel leaves includes an Ultrasound Processor model CSBJZQFS-150N0001V2 with a power of 80 watts, blender, spatula, measuring glass, beaker glass, dropper pipette, cup, desiccator, analytical balance,

weighing scale, stirring rod, filter paper, oven, glass funnel, volumetric pipette, vial bottles, UV-Vis spectrophotometer, aluminum foil, and thermometer.

2.3 Methods

2.3.1 Preparation of Herbal Material

Samples of red betel leaves were collected from Batu, Malang in fresh condition and sorted. The red betel leaves were then cut into small pieces and dried using an oven at a temperature of 50ºC. The dried red betel leaves were further ground using a blender and sieved through sieves with sizes of 60, 80, and 100 mesh. The determination of moisture content in the samples was carried out using the gravimetric method. Gravimetric analysis refers to the process of isolating and measuring the weight of specific compounds or elements. The principle of determining moisture content with this method is through the evaporation of water in the sample by heating, and then the sample is weighed until a constant weight is achieved, indicating that all the water has been evaporated. The first step involves heating an empty cup in an oven at a temperature of 105°C for 15 minutes, followed by cooling it in a desiccator. Then, the cup is weighed and filled with 2 grams of the red betel leaf sample, and the cup with the sample is weighed again. The next step is to heat the cup containing the sample in the oven for one hour, followed by cooling it in a desiccator. Finally, the final weight of the cup and its contents is measured [28]. In this study, the moisture content of the herbal material used was found to be 8.9%. The following formula was used to determine the percentage of moisture content.

$$Moisture\ Content\ (\%) = \frac{W1}{W}\ x\ 100\% \quad \text{.....(1)}$$

W = Weight of the sample before drying (gr)

W1 = Weight loss after drying (gr)

[29].

2.3.2 Sample Extraction

Sample preparation is done by mixing the material or crude drug and 90% ethanol solvent in the predetermined ratio as per the design expert into a beaker glass. The crude drug mixed with the solvent is then homogenized first by stirring it using a stirring rod. The equipment used for the extraction can be seen in Figure 2.

Figure 2. *Ultrasonic Processor*

Extraction is carried out using an Ultrasound Processor model CSBJZQFS-150N0001V2 with a power of 80 watts (100%) for a specific duration according to the predetermined variables. After the sample is extracted, it is filtered using filter paper to separate the filtrate from the sample residue.

2.3.3 Analysis of Flavonoid Content

2.3.3.1 Preparation of Blank Solution

0.1 mL of 10% aluminum chloride solution is added to a 5 mL volumetric flask, then 0.1 mL of 1 M sodium acetate solution is added. Distilled water is added to the flask up to the mark and mixed well. The blank solution is incubated for 30 minutes.

2.3.3.2 Preparation of Quercetin Standard Curve

A series of quercetin standard solutions were prepared at concentrations of 2, 3, 4, 5, and 6 ppm from a 100 ppm standard solution. 0.1-0.3 mL of the 100 ppm standard solution was pipetted and added to a 5 mL volumetric flask. Then, 0.1 mL of 10% aluminum chloride solution and 0.1 mL of 1M sodium acetate solution were added. Distilled water was added to reach the mark. The solution was then mixed well until homogeneous. Incubation was carried out for 30 minutes, and the absorbance was measured at the maximum wavelength of 431 nm [26, 30].

2.3.3.3 Determination of Total Flavonoid Content in the Extract

0.1 mL of the sample was added to a 5 mL volumetric flask, followed by the addition of 0.1 mL of 10% aluminum chloride solution, 0.1 mL of 1 M sodium acetate solution, and distilled water to the mark on the volumetric flask. The solution was mixed well until homogeneous, then transferred to a test tube and incubated for 30 minutes. The absorbance was measured at the maximum wavelength of 431 nm [26]. The obtained absorbance value was then

inserted into the regression equation obtained from the quercetin standard curve. The total flavonoid content was calculated using the following formula:

$$Total\ flavonoid = \frac{C \times v \times fp}{g} \quad \text{.....(2)}$$

C = Phenolic concentration (x value)

V = Volume of extract used (mL)

Fp = Dilution factor

g = Weight of the sample used (gr)

[31].

2.3.3.4 The Observed Parameters

The observed parameters are the moisture content of the material, analysis of flavonoid content, and the percentage of antioxidant inhibition using the UV-Vis spectrophotometer method. The percentage of DPPH inhibition in each extract and vitamin C can be calculated using the following formula:

$$\%\ inhibition = \frac{Absorbance\ of\ control - absorbance\ of\ sample}{Absorbance\ of\ control} x100\% \quad \text{.....(3)}$$

[32].

2.3.3.5 Data Analysis

The data analysis was conducted using Design Expert version 13. There are 3 operating variables used, which are particle size, extraction time, and material-to-solvent ratio. Extraction was carried out by screening data to determine the experimental run. Data modeling or variation was performed using the Box-Behnken Design (BBD) method, 17 runs of treatment were carried out as shown in Table 3.

Table 3. Variation of Red Betel extraction data

Run	Factor 1 A: Material-to-solvent ratio g/mL	Factor 2 B: Particle size *mesh*	Factor 3 C: Time min
1	0.1	60	20
2	0.1	100	20
3	0.06	80	30
4	0.06	100	25
5	0.1	80	25

Run	Factor 1 A: Material-to-solvent ratio g/mL	Factor 2 B: Particle size *mesh*	Factor 3 C: Time min
6	0.1	60	30
7	0.1	100	30
8	0.1	80	25
9	0.1	80	25
10	0.06	80	20
11	0.06	60	25
12	0.1	80	25
13	0.2	80	20
14	0.2	60	25
15	0.2	80	30
16	0.2	80	25
17	0.2	100	25

3. Result and Discussion

3.1 The Extraction of Flavonoid Compounds from Red Betel Leaf (*Piper crocatum*)

This study aimed to determine the content or concentration of flavonoid compounds in red betel leaves through the extraction process using the Ultrasound-Assisted Extraction (UAE) method, followed by testing the flavonoid compound content using a UV-Vis spectrophotometer. Before the extraction process, pretreatment was performed on the red betel leaves, including drying and measuring the moisture content of the raw material or crude drug. Measuring the moisture content is important because if a material has a moisture content exceeding 10%, it can affect the quality of the material, promote fungal growth, and cause hydrolysis of chemical constituents present in the red betel leaf crude drug. The moisture content obtained was less than 10%, ensuring optimal stability of the material and reducing microbial growth, thereby facilitating the extraction process [33]. In this study, the moisture content of the red betel leaf material was measured, and the result showed a moisture content of 8.9%.

Calculations were performed to determine the flavonoid content in each sample. The calculation results are as follows:

Table 4. Analysis Results of Flavonoid Content in Red Betel Leaf

Run	Factor 1 A: Material-to-solvent ratio g/mL	Factor 2 B: Particle size *mesh*	Factor 3 C: Time min	Flavonoid content mgQE/gr
1	0.1	60	20	196.25
2	0.1	100	20	209.607
3	0.06	80	30	260.367
4	0.06	100	25	263.676
5	0.1	80	25	205.931
6	0.1	60	30	201.029
7	0.1	100	30	242.573
8	0.1	80	25	193.431
9	0.1	80	25	216.102
10	0.06	80	20	205.404
11	0.06	60	25	239.779
12	0.1	80	25	200.906
13	0.2	80	20	118.651
14	0.2	60	25	102.720
15	0.2	80	30	108.112
16	0.2	80	25	196.372
17	0.2	100	25	114.485

Table 4 shows the lowest flavonoid content obtained is 102.720 mg QE/g and the highest flavonoid content is 263.676 mg QE/g. The lowest flavonoid content was obtained in the 14th run with an extraction time of 25 minutes, a solvent-to-material ratio of 0.2 g/mL, and a particle size of 60 mesh. Meanwhile, the highest flavonoid content was obtained in the 4th run with the same extraction time of 25 minutes, a solvent-to-material ratio of 0.06 g/mL, and a particle size of 100 mesh. The flavonoid content obtained in this study is higher compared to the flavonoid content obtained in a previous study conducted by Prayitno et al. (2018) using the same material, red betel leaf, with a maximum flavonoid content of 168.33 ± 4.23 mg QE/g through a maceration extraction process for 3 days using ethanol as the solvent. Another study conducted using the same material and maceration extraction method with 90% ethanol as the solvent resulted in a lower flavonoid content of 197.96 ± 12.51 mg QE/g [25]. The difference in flavonoid content is attributed to the different extraction methods used, operational variables, extraction conditions, and other factors that can affect the extraction process. Ultrasound-Assisted Extraction or modern extraction methods are more effective in extracting compounds from materials compared to maceration or conventional extraction processes. The effect of ultrasound can cause the cell wall to become thinner and enhance the solvent penetration ability,

allowing the solvent to extract the chemical substances from the cells effectively [34]. This is consistent with a study conducted by Wiranata et al. (2022) on the influence of extraction methods on the content of secondary metabolites in beetroot extracts. The results showed that the flavonoid, phenol, tannin, and anthocyanin content obtained through Ultrasound-Assisted Extraction were higher compared to the maceration method [35].

3.2 Quercetin Standard Curve

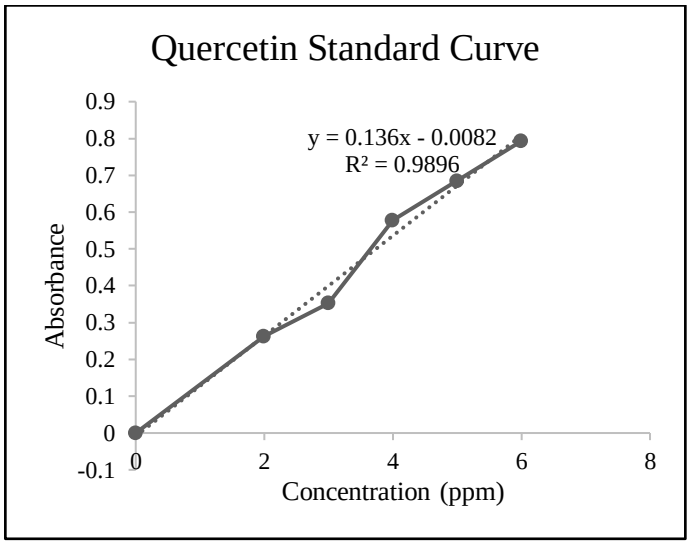

Figure 3. Quercetin Standard Curve

Figure 3 shows the quercetin standart curve that has been plotted from the results of the experiments conducted. From the curve, it can be seen that the result for $R^2 = 0.9896$ indicating a high correlation, and the linear equation $y = 0.136x – 0.0082$. This standard curve will be used to determine the flavonoid content.

3.3 Statistical Analysis

The flavonoid content data were analyzed using Analysis of Variance (ANOVA) to demonstrate that the variables used in the extraction process can influence the magnitude of the flavonoid content produced. Table 5 shows the results of the ANOVA statistical analysis for this experiment.

Table 5. ANOVA Statictical Analysis Results

Source	*Sum of Squares*	*df*	*Mean Square*	*F-Value*	*p-value*	
Model	41855.79	9	4650.64	50.09	0.0001	*Significant*

Source	*Sum of Squares*	*df*	*Mean Square*	*F-Value*	*p-value*	
A-Material-to-Solvent Ratio	34486.91	1	34486.91	371.42	< 0.0001	
B-Particle Size	1025l.22	1	1025.22	11.04	0.0127	
C-Time	843.97	1	843.97	9.09	0.0195	
AB	36.80	1	36.80	0.3963	0.5490	
AC	1072.64	1	1072.64	11.55	0.0115	
BC	198.62	1	198.62	2.14	0.1870	
A^2	3996.17	1	3996.17	43.04	0.0003	
B^2	298.77	1	298.77	3.22	0.1159	
C^2	8.16	1	8.16	0.0879	0.7754	
Residual	649.96	7	92.85			
Lack of Fit	330.83	3	110.28	1.38	0.3694	*not significant*
Pure Error	319.13	4	79.78			
Cor Total	42505.75	16				

Based on the table, the F-value is inversely proportional to the P-value. The F-value is either the sample means or the within-sample variation. If the variation between sample means is high compared to the variation within each sample, the F-value will be large. Variables or parameters can be considered significant if the probability value (P-value) obtained from the ANOVA analysis is < 0.05, and the P-value of lack of fit is > 0.05 [36]. Lack of fit refers to the discrepancy or deviation in the model to determine whether the variables used have an impact on the model [37]. In this study, a probability value of 0.0001 or less than 0.05 was obtained, indicating that the model used has a significant effect on the response [38]. Meanwhile, the probability value for lack of fit obtained was 1.38 or greater than 0.05, which means that the discrepancy or lack of fit in this study is not significant.

Table 6. *Model Summary*

R^2	*Adjusted* R^2	*Predicted* R^2
0.9847	0.9650	0.8637

Mathematically, the equation model for total flavonoid content as a response of extraction variables can be modeled by equation 4.

$$Total\ flavonoid\ content = 447.23 + 156.93A + 27.40B + 12.60C + 6.67AB + 61.00AC$$
$$+20.49BC - 83.56A^2 + 32.70B^2 - 7.74C^2 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (4)$$

The Values of A, B, and C, respectively, represent the variables of material-to-solvent ratio, particle size, and extraction time.

The correlation or relationship between experimental data and the response is determined using the coefficient of determination (R^2). R^2 is a value that indicates the proportion of variability in the response variable that can be explained by the combination of variables used [30]. The study is considered appropriate to the model when the R^2 value approaches 1 [39], the summary for the R^2 values obtained from this experiment can be seen in Table 6. In this study, the ANOVA results for R^2 were found to be 0.9847, indicating that the model is in accordance with the research findings or close to linearity [40]. As for the Adjusted R^2, it can be used to measure the confidence level of adding variables to improve the predictive power of the model, and a value of 0.9650 was obtained, indicating a relationship among the process variables used, namely extraction time, solvent-to-material ratio, and particle size. Additionally, the Predicted R^2 is used to indicate how well the model can predict new observations, and a value of 0.8637 was obtained, which is quite consistent with the Adjusted R^2as the difference is less than 0.2. The relationship between the model and the experimental data is presented in Figure 4.

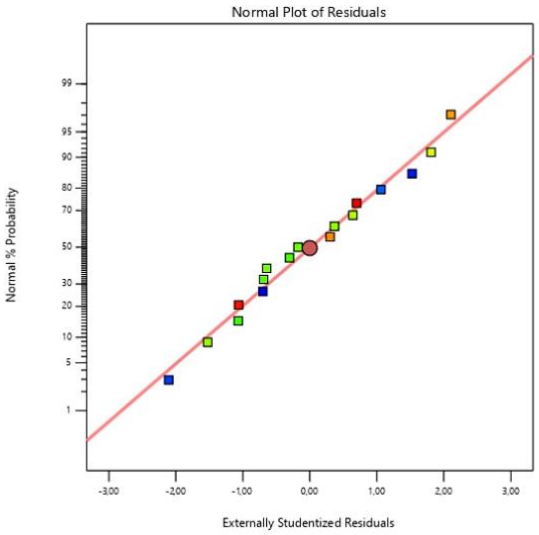

Figure 4. Plot of Data against Trendline

The graph indicates that the model data and experimental or experimental data are fairly accurate. The position of the data relative to the trendline indicates the accuracy of the data, where the closer the data is to the trendline, the more accurate the data is. Based on the research results, a plot of the data touching the trendline was obtained, indicating that the experimental data is close to the model data with an R^2 value of 0.9847.

3.4 The Effect of Extraction Parameters on Flavonoid Content

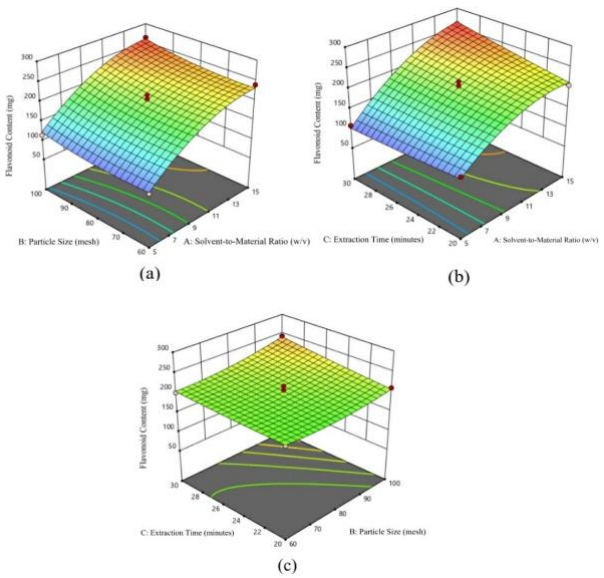

Figure 5. Relationship between flavonoid content and variables (a) particle size (mesh) and solvent-to-material ratio (w/v); (b) solvent-to-material ratio (w/v) and extraction time (minutes); (c) extraction time (minutes) and particle size (mesh).

Figure 5 shows the graphs of each variable (particle size, solvent-to-material ratio, and extraction time) in the extraction of flavonoids from red betel leaves. There is a color change in the graph, indicating the relationship between the parameters or variables used. The red color indicates the highest flavonoid content of 263.676 mgQE/g with a particle size of 100 mesh. Meanwhile, the blue color represents the lowest flavonoid content of 102.720 mgQE/g at a particle size of 60 mesh.

From Figure 5(a), it can be observed that the solvent-to-material ratio and particle size have an impact on the flavonoid content. In run 4, the highest flavonoid content of 263.676 mgQE/g was obtained using the highest solvent-to-material ratio and the smallest particle size. On the other hand, in run 5, a lower flavonoid content of 205.931 mgQE/g was obtained with a lower solvent-to-material ratio of 0.1 g/mL and a larger particle size of 80 mesh, while keeping the extraction time constant at 25 minutes. The lowest flavonoid content of 102.720 mgQE/g was obtained in run 14, which also had the same extraction time, but a smaller solvent volume and larger particle size. From the data, it can be concluded that increasing the solvent volume and reducing the particle size result in higher flavonoid content. A smaller particle size provides a larger contact area between the material and the solvent, allowing for more efficient extraction of the flavonoid compounds. The broken cell membranes facilitate the solvent to extract compounds from within the cells, making the diffusion process easier [41]. The solvent-to-material ratio also influences the extraction process, as a higher amount of solvent allows for the maximum contact between the material and the solvent, enabling more efficient absorption or binding of the compounds present in the material and thus maximizing the flavonoid content [42].

In Figure 5(b), it can be observed that the extraction time and solvent-to-material ratio have an impact on the flavonoid content. This can be seen in run 3, where the highest flavonoid content of 260.367 mgQE/g was obtained using a particle size of 80 mesh, the highest solvent-to-material ratio of 0.06 g/mL, and the longest extraction time of 30 minutes. On the other hand, in run 8, with the same particle size of 80 mesh, a lower solvent-to-material ratio of 0.1 g/mL, and a shorter extraction time of 25 minutes, a lower flavonoid content of 193.431 mgQE/g was obtained. In run 13, with the same particle size but the lowest solvent volume of 0.2 g/mL and the shortest extraction time of 20 minutes, a lower flavonoid content of 118.651 mgQE/g was obtained compared to the experiments that used higher solvent volumes and longer extraction times. From the data, it can be concluded that the longer the extraction time, the higher the

quantity of extracted material due to a greater opportunity for contact between the material and the solvent, resulting in increased flavonoid content until reaching the saturation point of the solution [43]. The extraction time is related to the duration of contact between the material and the solvent until a certain point where the extracted compounds are fully diffused from the material [41]. Once the extraction process has reached the optimal time or the extracted compounds have fully diffused, further extension of the extraction time does not significantly increase the flavonoid content. Increasing the amount of solvent used allows for the extraction of more compounds. However, if the ratio is too high, using excessive solvent will require longer extraction times [44].

Figure 5(c) illustrates the relationship between the extraction time and particle size. It shows that the longer the extraction time and the smaller the particle size used, the higher the flavonoid content obtained. This effect can be observed in run 1, where a solvent-to-material ratio of 0.1 g/mL, the largest particle size of 60 mesh, and the shortest extraction time of 20 minutes resulted in a flavonoid content of 196.25 mgQE/g. This value is lower than the flavonoid content obtained in run 5, which used the same solvent volume but a smaller particle size of 80 mesh and a longer extraction time of 25 minutes, resulting in a flavonoid content of 205.931 mgQE/g. A further increase in flavonoid content was observed in run 7, with the same solvent-to-material ratio of 0.1 g/mL, the smallest particle size of 100 mesh, and the longest extraction time of 30 minutes, yielding a higher flavonoid content of 242.573 mgQE/g. Hence, it can be concluded that using smaller particle sizes and longer extraction times leads to higher flavonoid content. The smaller particle size, combined with a longer extraction time, maximizes the contact between the material and the solvent, enhancing the extraction efficiency.

3.5 Analysis of Antioxidant Activity Data

The determination of antioxidant activity using DPPH is expressed as the percentage of DPPH inhibition (% inhibition), where a higher inhibition value indicates a higher antioxidant activity. From the antioxidant activity testing results, the sample with the highest flavonoid content in run 4, with a concentration of 263.676 mgQE/gr, exhibited an inhibition percentage of 27.458%. On the other hand, the sample with the lowest flavonoid content in run 14, with a concentration of 102.720 mgQE/gr, showed an inhibition percentage of 14,328%. This indicates a relationship between flavonoid content and antioxidant activity, where higher flavonoid levels in a sample correspond to stronger antioxidant activity [45].

4. Conclusions

This study aimed to determine the total flavonoid content and antioxidant activity of red betel leaf extract using the Ultrasound-Assisted Extraction (UAE) method with 90% ethanol as the solvent. The extraction variables used were the solvent-to-material ratio, particle size, and extraction time. The lowest flavonoid content obtained was 102.720 mgQE/gr with an inhibition percentage of 14.328%, while the highest flavonoid content obtained was 263.676 mgQE/gr with an inhibition percentage of 27.458%. In this research, the optimum condition is achieved with an extraction time of 25 minutes, particle size of 100 mesh, and material-to-solvent ratio of 0.06 g/mL.

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