



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

Riavinola Viyoni Pramudiska*, Audi Nur Amartya, Ditta Kharisma Yolanda Putri, Zuhriah Mumtazah, Bkti Palupi

Department of Chemical Engineering, Faculty of Engineering, Jember University, Indonesia

(Submitted: 23 June 2023; Revised: 8 November 2023; Accepted: 9 November 2023)

Abstract. *Piper crolatum* 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 is flavonoids. In this study, red betel leaf was extracted 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 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, surpassing other tropical areas 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, or even yellow. Red betel 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

*corresponding author: riavinolaviyonipramudiska@gmail.com

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 excellent 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. Producing antioxidants is tiny, and the maceration method is still used. The black betel leaf shows that black betel leaf extract has weak antioxidant activity because IC_{50} is only 158.53 ppm. Red betel leaf has better and stronger antioxidant activity than green betel leaf [4]. Extraction processes can be carried out using thermal or non-thermal methods to obtain the chemical constituents of a substance [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, has a greenish-purple hue, and does not produce flowers. Red betel thrives best in shaded areas with limited direct sunlight exposure or in less hot places [3, 8]. Traditional medicines have been made 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, antioxidants are needed to prevent the dangers of these free radicals [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 in red betel leaves is flavonoids, which are 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.

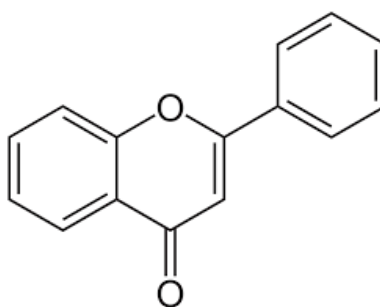


Figure 1. Flavonoid Structure

Table 1. Comparison of Flavonoid Content in Various Leaf Types

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

Table 1 compares 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 separates 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. Traditional 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 it from traditional to modern. 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 by applying mechanical pressure to the cells, resulting in cavitation within the sample. Cell disruption can increase the 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 by forming 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 the 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
<i>Piper betel</i>	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 (IC ₅₀) of 26.73-28.05 mg/mL compared to the diethyl ether fraction (IC ₅₀) of 114.54-28.05 mg/mL.	[21]
<i>Piper betel</i> and <i>Piper crocatum</i>	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]
<i>Piper crocatum</i>	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 IC ₅₀ value of 53.91 ppm. The conclusion of this research study is that the extract has strong antioxidant potential.	[23]
<i>Piper betel</i>	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]
<i>Piper betel</i> and <i>Piper crocatum</i>	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]
<i>Piper crocatum</i>	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 IC ₅₀ , which was found to be 82.71 ppm.	[25]
<i>Piper crocatum</i>	Method: Maceration Solvent: Ethanol Duration: 48 hours Testing method: Measurement of flavonoid content and types of flavonoids using a UV-Vis spectrophotometer from PG	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 (IC ₅₀), the red betel leaf extract showed a value of 47.45 ppm, while vitamin C showed a value of 49.20	[4]

Material	Method and Solvent	Result	References
	Instruments Ltd. Determination of antioxidant content using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay.	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.	
<i>Piper crocatum</i>	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 (IC ₅₀) was conducted using DPPH (2,2-Diphenyl-1-picrylhydrazyl).	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 (IC ₅₀) was found to be 129.11 ± 2.06 ppm.	[10]

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 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, quercetin, ascorbic acid, 1 M 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 50 °C. The dried red betel leaves were ground using a blender and sieved through sieves of 60, 80, and 100 mesh sizes. Determining moisture content in the samples was carried out using the gravimetric method. Gravimetric analysis refers to 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. 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, then 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, then cool 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 8.9%. The following formula was used to determine the percentage of moisture content.

$$\text{Moisture Content (\%)} = \frac{W_1}{W} \times 100\% \dots \dots \dots (1)$$

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 per the design expert into a glass beaker. The crude drug

combined 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.

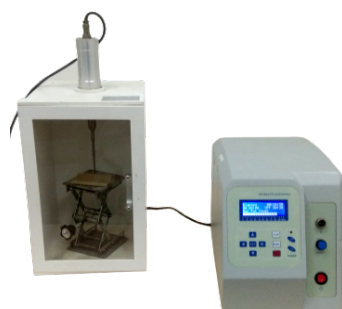


Figure 2. *Ultrasonic Processor*

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 extracting the sample, 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 was 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 1 M 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 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:

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

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:

$$\% \text{ inhibition} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100\% \dots\dots\dots(3)$$

[32].

2.3.3.5 Data Analysis

The data analysis was conducted using Design Expert version 13. Three operating variables are used: 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 treatment runs were carried out as shown in Table 3.

Table 3. Variation of Red Betel extraction data

<i>Run</i>	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

<i>Run</i>	Factor 1 A: Material-to-solvent ratio g/mL	Factor 2 B: Particle size <i>mesh</i>	Factor 3 C: Time min
3	0.06	80	30
4	0.06	100	25
5	0.1	80	25
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. Results 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 essential 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 material stability 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

<i>Run</i>	Factor 1 A: Material-to-solvent ratio g/mL	Factor 2 B: Particle size <i>mesh</i>	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 that the lowest flavonoid content obtained is 102.720 mg QE/g, and the highest 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 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, is more effective in extracting compounds from materials than 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 higher flavonoid, phenol, tannin, and anthocyanin content obtained through Ultrasound-Assisted Extraction than the maceration method [35].

3.2 Quercetin Standard Curve

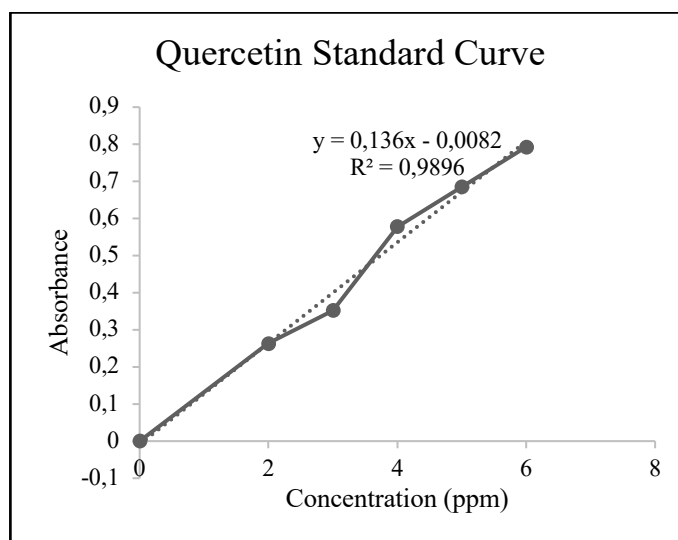


Figure 3. Quercetin Standard Curve

Figure 3 shows the quercetin standard curve that has been plotted from the results of the experiments conducted. From the curve, it can be seen that the result for $R^2 = 0.9896$ indicates a high correlation, and the linear equation is $y = 0.136x - 0.0082$. 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 Statistical Analysis Results

Source	Sum of Squares	df	Mean Square	F-Value	p-value	
Model	41855.79	9	4650.64	50.09	0.0001	<i>Significant</i>

<i>Source</i>	<i>Sum of Squares</i>	<i>df</i>	<i>Mean Square</i>	<i>F-Value</i>	<i>p-value</i>	
A-Material-to-Solvent Ratio	34486.91	1	34486.91	371.42	< 0.0001	
B-Particle Size	10251.22	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	<i>not significant</i>
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. The F-value will be large if the variation between sample means is high compared to the variation within each sample. 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 impact the model [37]. This study obtained a probability value of 0.0001 or less than 0.05, indicating that the model used significantly affected the response [38]. Meanwhile, the probability value for lack of fit obtained was 1.38 or greater than 0.05, which means that this study's discrepancy or lack of fit is insignificant.

Table 6. *Model Summary*

R^2	<i>Adjusted R²</i>	<i>Predicted R²</i>
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.

$$\begin{aligned} \text{Total flavonoid content} = & 447.23 + 156.93A + 27.40B + 12.60C + 6.67AB + 61.00AC \\ & + 20.49BC - 83.56A^2 + 32.70B^2 - 7.74C^2 \dots\dots\dots(4) \end{aligned}$$

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

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, which 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 indicates how well the model can predict new observations, and a value of 0.8637 was obtained, which is quite consistent with the Adjusted R^2 , as the difference is less than 0.2. The relationship between the model and the experimental data is presented in Figure 4.

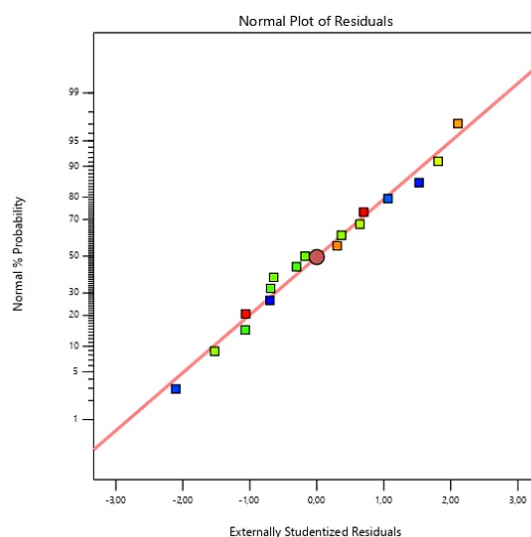


Figure 4. Plot of Data against Trendline

The graph indicates that the model and experimental data are reasonably accurate. The position of the data relative to the trendline suggests the accuracy of the data, where the closer the data is to the trendline, the more accurate the data is. A plot of the data touching the trendline was obtained based on the research results, 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

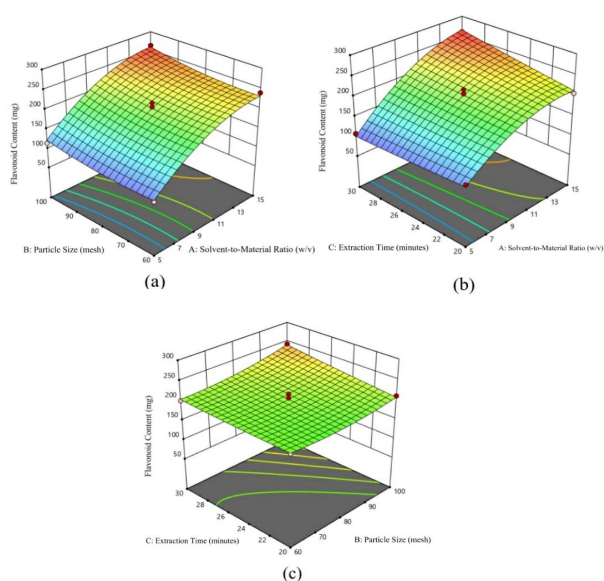


Figure 5. Relationship between flavonoid content and variables (a) particle size (mesh) and solvent-to-material ratio (w/v); (b) solvent-to-material ratio (w/v) and extraction time (minutes); (c) extraction time (minutes) and particle size (mesh).

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. The graph shows a color change, 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, blue 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 impact 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 had the same extraction time, but a smaller solvent volume and 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 impact 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, excessive solvent will require longer extraction times [44].

Figure 5(c) illustrates the relationship between extraction time and particle size. It shows that the longer the extraction time and the smaller the particle size, 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 and a longer extraction time maximize the contact between the material and the solvent, enhancing the extraction efficiency.

3.5 Analysis of Antioxidant Activity Data

Determining 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 more potent antioxidant activity [45].

4. Conclusions

This study aimed to determine red betel leaf extract's total flavonoid content and antioxidant activity using the Ultrasound-Assisted Extraction (UAE) method with 90% ethanol as the solvent. The extraction variables 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%. This research achieves the optimum condition with an extraction time of 25 minutes, particle size of 100 mesh, and material-to-solvent ratio of 0.06 g/mL.

REFERENCES

- [1] Ain, N. R., & Ani, K. (2016). Pertumbuhan Beberapa Jenis Sirih (*Piper Sp.*) pada Berbagai Intensitas Naungan. *Bul. Agrohorti*, 288-297.
- [2] Nisa, G. K., Nugroho, W. A., & Hendrawan, Y. (2014). Ekstraksi Daun Sirih Merah (*Piper Crocatum*) dengan Metode *Microwave Assisted Extraction* (MAE). *Jurnal Bioproses Komoditas Tropis*, 72-78.
- [3] Kopong, M. V., & Wardiniati, N. K. (2022). Review Artikel: Potensi Daun Sirih Hijau (*Piper Betel L.*) dan Daun Sirih Merah (*Piper Corcatum*) Sebagai Antioksidan. *Jurnal Ilmiah Multi Disiplin Indonesia*, 710-729.
- [4] Tonahi, J. M., Nuryanti, S., & Suherman. (2014). Antioksidan Dari Daun Sirih Merah (*Piper Crocatum*). *Jurnal Akademika Kim*, 383-389.
- [5] Puspita, P. J., Safithri, M., & Sugiharti, N. P. (2018). *Antibacterial Activites of Sirih Merah (Piper Crocatum) Leaf Extract* (Aktivitas Antibakteri Ekstrak Daun Sirih Merah). *Current Biochemistry*, 1-10.
- [6] Lestari, A. B., & Dwiatmaka, Y. (2014). Aktivitas Antioksidan Ekstrak Daun Sirih Merah (*Piper Crocatum*) Hasil Optimasi pelarut Etanol-Air. *Jurnal Ilmu Kefarmasian Indonesia*, 74-79.
- [7] Fadillah, M. (2015). *Benefit of Red Betel (Piper Coractum Ruiz & PAV.) as Antibiotics*. *J Majority*, 4(3), 71-75.
- [8] Iqbal, Rustam, N., & Kasman. (2016). Analisis Nilai Absorbansi Kadar Flavonoid Daun Sirih Merah (*Piper Crocatum*) dan Daun Sirih Hijau (*Piper Betel L.*). *Gravitasi*, 15(1), 1-8.
- [9] Kanifah, U., Lutfi, M., & Susilo, B. (2015). Karakterisasi Ekstrak Daun Sirih Merah (*Piper Crocatum*) dengan Metode Ekstraksi Non-Thermal berbantuan Ultrasonik (Kajian Perbandingan Jenis Pelarut dan Lama Ekstraksi). *Jurnal Bioproses Komoditas Tropis*, 3(1), 73-39.
- [10] Prayitno, S. A., Kusnadi, J., & Murtini, E. S. (2018). Karakteristik (Total Flavonoid, Total Fenol, Aktivitas Antioksidan) Ekstrak Serbuk Daun Sirih Merah (*Piper Crocatum Ruiz & Pav.*). *Foodscitech*, 1(2), 26-34.
- [11] Yasa, I. T., Putra, N. K., & Wiadnyani, A. I. (2019). Pengaruh Konsentrasi Etanol Terhadap Aktivitas Antioksidan Ekstrak Daun Sirih Merah (*Piper Crocatum Ruiz & Pav.*) Menggunakan Metode *Microwave Assisted Extraction* (MAE). *Jurnal Ilmu dan Teknologi Pangan*, 8(3), 278-284.

- [12] Susiloningrum, D., & Sari, D. E. (2021). Uji Aktivitas Antioksidan dan Penetapan Kadar Flavonoid Total Ekstrak Temu Mangga (*Curcuma Mangga Valetton & Zijp*) dengan Variasi Konsentrasi Pelarut. *Cendekia Journal of Pharmacy*, 5(2), 119-127.
- [13] Cahyaningsih, E., Sandhi K, P. E., & Santoso, P. (2019). Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Etanol Bunga Telang (*Clitoria ternatea L.*) dengan Metode Spektrofotometri UV-Vis. *Jurnal Ilmiah Medicamento*, 5(1), 51-57.
- [14] Salamah, N., & Widyasari, E. (2015). Aktivitas Antioksidan Ekstrak Metanol Daun Kelengkeng (*Euphoria Longan (L) Steud.*) dengan Metode Penangkapan Radikal 2,2'-Difenil-1Pikrilhidrazil. *Pharmaciana*, 5(1), 25-34.
- [15] Nurmalasari, T., Zahara, S., Arisanti, N., Mentari, P., Nurbaeti, Y., Lestrasi, T., & Rahmiyani, I. (2016). Uji Aktivitas Antioksidan Ekstrak Buah Kupa (*Syzygium Polycephalum*) Terhadap Radikal Bebas dengan Metode DPPH. *Jurnal Kesehatan Bakti Tunas Husada*, 16(1), 61-68.
- [16] Neldawati, Ratnawulan, & Gusnedi. (2013). Analisis Nilai Absorbansi dalam Penentuan Kadar Flavonoid untuk Berbagai Jenis Daun Tanaman Obat. *Pillar of Physics*, 2, 76-83.
- [17] Purnamasari, A., Zelviani, S., Sahara, & Faudi, N. (2022). Analisis Nilai Absorbansi Kadar Flavonoid Tanaman Herbal Menggunakan Spektrofotometer UV-VIs. *Teknosains: Media Informasi Sains dan Teknologi*, 16(1), 57-64.
- [18] Mukhriani. (2014). Ekstraksi, Pemisahan Senyawa, dan Identifikasi Senyawa Aktif. *Jurnal Kesehatan*, 2, 361-367.
- [19] Utami, N. F., Nurdayanty, S. M., & Suhendar, U. (2020). Pengaruh berbagai Metode Ekstraksi Pada Penentuan Kadar Flavonoid Ekstrak Etanol Daun Iler (*Plectranthus Scutellarioides*). *Fitofarmaka Jurnal Ilmiah Farmasi*, 10(1), 76-83.
- [20] Shirsath, S. R., Sonawane, S. H., & Gogate, P. R. (2012). *Intensification of Extraction of natural Products using Ultrasonic Irradiations- A Review of Current Status*. Elsevier, 53, 10-23.
- [21] Handaratri, A., & Yuniati, Y. (2019). Kajian Ekstraksi Antosianin dari Buah Murbei dengan Metode Sonikasi dan Microwave. *Reka Buana: Jurnal Ilmiah Teknik Sipil dan Teknik Kimia*, 4(1), 63-67.
- [22] Suirta, I. W., & Asih, I. R. (2019). Suplemen Ekstrak Daun Sirih (*Piper Betel Lin*) Dalam Menurunkan Kadar *Malondialdehid* Pada Tikus Wistar. *Jurnal Kimia (Journal of Chemistry)*, 13(2), 185-190.
- [23] Manalu, N. Y., & Sinaga, M. S. (2013). Ekstrak Daun Sirih Hijau dan Merah Sebagai Antioksidan pada Minyak Kelapa. *Jurnal Teknik Kimia USU*, 2(1), 37-43.
- [24] Januarti, I. B., Wijayanti, R., Wahyuningsih, S., & Nisa, Z. (2019). Potensi Ekstrak Terpurifikasi Daun Sirih Merah (*Piper Crocatum Ruiz & Pav*) sebagai Antioksidan dan Bakteri. *JPSCR: Journal of Pharmaceutical Science and Clinical research*, 2, 60-68.
- [25] Prayitno, S. A., Kusnadi, J., Murtini, E. S. (2016). *Antioxidant Activity of Red Betel Leaves Extract (Piper Crocatum Ruiz & Pav.) By Difference Concentration of Solvents. Journal of Pharmaceutical Biological and Chemical Sciences*. 7(5), 1836-1843.
- [26] Ahmad, A. R., Juwita., Ratulangi, A. A. D., Malik, A. (2015). Penetapan Kadar Fenolik dan Flavonoid Total Ekstrak Metanol Buah dan Daun Patikala (*Etlintera elatior* (Jack) R. M. SM). *Pharm Sci Res*. 2(1): 1-10.
- [27] Tatiana, W. S., Ria, W. (2020). Uji Aktivitas Antioksidan dengan Metode DPPH dan Uji Sitotoksik Terhadap Sel Kanker Payudara T47D pada Ekstrak Daun Kemangi. *Jurnal Farmasetis*. 9(1): 51-64.

- [28] Kurniasani, A., I. N. Arifina., A. R. Yulianti., M. F. Afif., H. Sholehah., D. B. A. F. Aziz., R. Monica & D. Silviani. (2015). Analisis Kadar Air dan Kadar Abu Metode Gravimetri. *Jurnal ilmiah Pertanian*. 5: 1-9.
- [29] Rohman, A. & Sumantri. (2013). Analisis Makanan. Penerbit Gadjah Mada University Press, Yogyakarta.
- [30] Luginda, R. A., Lohita, B., & Indriani, L. (2018). Pengaruh Variasi Konsentrasi Pelarut Etanol Terhadap Kadar Flavonoid Total Daun Beluntas (*Pluchea indica* (L.) Less) dengan Metode *Microwave-Assisted Extraction* (MAE). *Program Studi Farmasi FMIPA Universitas Pakuan*, 1-9.
- [31] Wirasti. (2019). Penetapan Kadar Fenolik Total, Flavonoid Total, dan Uji Aktivitas Antioksidan Ekstrak Daun Benalu Petai (*Scurrula atropurpurea* Dans.) Beserta Penapisan Fitokimia. *Journal of Pharmaceutical and Medicinal Sciences*. 4(1): 1-5.
- [32] Fatyanti, Salamah. (2017). Penentuan Kadar Total Feenol dan Uji Aktivitas Antioksidan Ekstrak Bunga Sukun (*Artocarpus altilis* L.) *Politeknik Harapan Bersama Tegal*.
- [33] Sholihah, M. (2016). Ultrasonic-Assisted Extraction Antioksidan dari Kulit Manggis. Tesis. Dipublikasikan. Sekolah Pasca Sarjana IPB, Bogor.
- [34] Wiranata, I. G., Sasadara, M. M. V. (2022). Pengaruh Pelarut dan Metode Ekstraksi Terhadap Kandungan Metabolit Sekunder dan Nilai IC50 Ekstrak Umbi Bit (*Beta vulgaris* L.). *Jurnal Integrasi Obat Tradisional*. 2(1): 7-13.
- [35] Anggraini & Kusuma. (2020). Uji Cemarkan pada Ekstrak Etanol Tempe Koro Benguk (*Mucuna Pruriens* L.) sebagai Obat Antidiabetes Terstandar. *Jurnal Ilmiah Cendekia Eksakta*, 5(11), 1-11.
- [36] Sari, B. L., Triastinurmiatiningsih., Haryani, T. S. (2020). Optimasi Metode *Microwave-Assisted Extraction* (MAE) untuk Menentukan Kadar Flavonoid Total Alga Coklat *Pdina Australis*. *ALCHEMY Jurnal Penelitian Kimia*. 16(1): 37-48.
- [37] Pradana, N. D., Saraswati, R., Deviyanti, L. G. A. S. (2022). Optimasi Output Produksi Kakao Bubuk pada Bagian Mixing dengan Menggunakan *Response Surface Methodology- Box Behnken Design* di PT. X. *Journal of Engineering Research*. 1(3): 1-12.
- [38] Hidayat, I. R., Zuhrotun, A., Sopyan, I. (2021). *Design-Expert* Software Sebagai Alat Optimasi Formulasi Sediaan Farmasi. *Majalah Farmasetika*. 6(1). 99-120.
- [39] Marjoni, M. R., Afrinaldi., Novita, A. D. (2015). Anungan Total Fenol dan Aktivitas Antioksidan Ekstrak Air Daun Kersen (*Muntingia calabura* L.). *Jurnal Kedokteran YARSI*. 23(3): 187-196.
- [40] H. Saptari, T., Triastinurmiatiningsih., S. Lohita, B., Sayyidah, I. N. (2019). Kadar Fenolik Dan Aktivitas Antioksidan Ekstrak Etanol Rumpun Laut Coklat (*Padina australis*). *Fitofarmaka*. 9(1): 1-8.
- [41] Sumantining, L. P., Putra, G. P., & Suhendra, L. (2022). Pengaruh Jenis Pelarut dan Ukuran Partikel pada Ekstraksi Kulit Buah Kakao (*Theobroma cacao* L.) menggunakan Metode *Microwave Assisted Extraction* (MAE) terhadap Karakteristik Ekstrak. *Jurnal Rekayasa dan Manajemen Agroindustri*, 10(1), 124-135.
- [42] Putra, I. K. W., Putra, G. P. G., Wrasati, L. P. (2020). Pengaruh Perbandingan Bahan dengan Pelarut dan Waktu Maserasi terhadap Ekstrak Kulit Biji Kakao (*Theobroma cacao* L.) sebagai Sumber Antioksidan. *Jurnal Rekayasa dan Manajemen Agroindustri*. 8(2): 167-176.
- [43] Winata, E. & Yunianta. (2015). Ekstraksi Antosianin Buah Murbei (*Morus alba* L.) Metode *Ultrasonic Batch* (Kajian Waktu dan Rasio bahan dengan pelarut : Pelarut). 3(2), 773-783.

- [44] Zhang, Q. W., Lin, L. G., & Ye, W. C. (2018). *Techniques for Extraction and Isolation of Natural Products: a Comprehensive Review. Chinese Medicine (United Kingdom)*. 13(1): 1-26.
- [45] Yuliantari, N. W. A., Widarta, I. W. R., Permana, I. D. G. M. (2017). Pengaruh Suhu dan Waktu Ekstraksi Terhadap Kandungan Flavonoid dan Aktivitas Antioksidan Daun Sirsak (*Annona muricata L.*) Menggunakan Ultrasonik. *Scientific Journal of Food Technology*. 4(1): 35-42.