OPTIMIZATION APPROACH OF MICROWAVE ASSISTED EXTRACTION ANTHOCYANINS PIGMENTS BUTTERFLY PEA FLOWOERS (Clitoria Ternatea L.) Using OFAT (One-Factor-At-a-Time) METHOD

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ABSTRACT

***Purpose**: The purpose of this study is to optimize the extraction of anthocyanin pigments from butterfly pea flowers (Clitoria ternatea L.) to enhance the efficiency and stability of these valuable phytochemical compounds for potential applications in functional food products. **Methodology**: A preliminary optimization was conducted using Microwave-Assisted Extraction (MAE) with a 0.75% aquadest-tartaric acid solvent system. The One-Factor-At-a-Time (OFAT) method was applied to investigate the influence of three independent variables: solvent-material ratio (1:10,1:15,1:20,1:25,1:30 and 1:35 g/mL), extraction time (3,6,9,12,15 and 18 minutes), and microwave power (90,180,270,360,450 and 540 watts) on anthocyanin yield (mg/L). **Results**: The results demonstrate that increasing the solvent-material ratio up to a certain point improves pigment recovery. However, excessive exposure to microwave energy and prolonged extraction time may lead to degradation of anthocyanins. **Findings:** The extraction of anthocyanins using MAE resulted in the highest yield of 33.89 ± 0.47 mg/L under optimal conditions at 450 W for 15 minutes with a solvent-to-material ratio of 1:15. **Novelty**: The use of aquadest–tartaric acid as a solvent in MAE represents an alternative approach that is more environmentally friendly and potentially more effective in preserving anthocyanin stability. **Originality**: This research highlights a less-explored combination of weak organic acid and water as a green solvent system, which has not been widely applied in the extraction of Clitoria ternatea L. using MAE. **Conclusion**: The application of MAE using tartaric acid-based solvents demonstrates a promising strategy for optimizing the recovery of anthocyanins while supporting sustainable extraction practices. **Type of paper:** Research paper.*

***Keywords**: Butterfly pea flower; Anthocyanin, Tartaric acid, Microwave Assisted Extraction,*

INTRODUCTION

Use of synthetic dyes in food and beverages can have negative impacts, such as toxic and carcinogenic effects. One alternative to replace synthetic dyes is to use natural pigments such as anthocyanins found in butterfly pea flowers (*Clitoria ternatea L.*). According to

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Pracima (2015), anthocyanins are an attractive alternative to replace synthetic dyes amaranth (FD & C Red No. 2), which have been banned in the United States and several other countries. Anthocyanins not only function as natural dyes but also have antioxidants and antibacterial properties that are beneficial in food (Migliorini et al, 2019). Several clinical studies have shown that consumption of anthocyanins from certain foods has the potential to protect the body from the risk of cancer (Rifqi, 2021). One method of obtaining anthocyanins from butterfly pea flowers (*Clitoria ternatea L.*)

Important steps in the separation, identification and purification of anthocyanins can be done by extraction. According to Anggriani (2019) explains that extraction methods can be carried out in several ways including maceration, ultrasound, percolation, microwave, sochlet, reflux, and steam distillation. *Microwave Assisted Extraction* (MAE) is an extraction with the help of microwaves which is based on the absorption of microwave energy by polar molecules which heat the solvent and increase the penetration of the solvent into the sample matrix, making it easier for the compound to be extracted. Studies of MAE in increasing the yield and effectiveness of extraction have been widely applied. MAE extraction utilizes microwave energy to accelerate the release of target compounds from the sample matrix into the solvent. This process works by inducing rotation of molecular dipoles, which causes disruption of hydrogen bonds. This disruption increases the movement of dissolved ions and helps the solvent penetrate deeper into the sample matrix, thereby increasing extraction efficiency (Izirwan et al, 2020).

Several factors of the extraction method with MAE are microwave power, sample to solvent ratio, solvent pH, solvent concentration, and irradiation time (Belwal et al, 2020). Anthocyanins tend to be stable in acidic conditions, so the use of solvents such as distilled water and tartaric acid is the right choice. The optimal concentration of tartaric acid for anthocyanin extraction from butterfly pea flowers is 0.75%, which can produce a total anthocyanin of 0.82 mg/mL with a yield of 24.21% using the maceration method (Hartono, 2013). The disadvantages of the maceration method itself lie in the length of the process and the high costs used due to the large amount of solvent used. A higher solvent volume will usually increase the extraction yield in conventional extraction methods. The same technique cannot be applied to the MAE method because it causes lower extraction yields. The application of microwave energy was carried out by Izirwan et al (2020) found the highest anthocyanin content in butterfly pea flowers of 30.475 mg/L. Generally, the solvent ratio that is often used ranges from 1:10 to 1:50 (w:v). In addition to the ratio, the time and power of

MAE extraction have a major influence on extraction. Hasdar (2021) explained that extraction time that is too long or too short can affect the physical and chemical properties of the extracted material. Research from Thuy et al (2021) stated that after MAE extraction at 3 and 5 minutes with a power of 400W using aquades as a solvent, the anthocyanin content of butterfly pea flower extract (*Clitoria ternatea L*) increased with extraction time from 32.86 to 39.60 mg/l. Microwave power and extraction time work together to influence each other so that both factors must be appropriate (Salve and Ray, 2020). To obtain optimal conditions from the combination of three variables and the type of 0.75% aquades-tartaric acid solvent, an approach study is needed to determine the upper and lower limits before further optimization is carried out using statistical methods such as *Response Surface Methodology* (RSM).

RESEARCH METHODOLOGY

The method used in this study is One-Factor-At-a-Time (OFAT) is where one factor is tested in a variety of ways while other factors are maintained at a fixed value (Muttaqin, 2019). This approach is used to find the optimum conditions of each factor, namely the ratio (b/v), extraction time (minutes) and power (watts) with 2 repetitions to ensure the validity of the results with a response in the form of anthocyanin levels (ppm), so that the treatment is obtained as in **Table 1.** Experimental data were analyzed using the Multiple Linear Regression ANOVA method to evaluate the simultaneous and partial effects of each independent variable on the response. Testing was carried out at a significant level of 5% to see the significance of the influence between variables, as well as to determine which variable gave the most dominant contribution to the total anthocyanin response produced.

The determination of the treatment and value of each factor is determined based on preliminary research from various optimum treatments in each literature, including a combination of 400 watts of power, a ratio of 1:20 and a time of 6 minutes using aquades solvent (Thuy et al, 2021), an optimum treatment of 3 minutes, a ratio of 1:20 and a power of 30% with 96% ethanol solvent (Azharini et al, 2021) and a combination treatment of a ratio of 1:15, a time of 15 minutes and a temperature of 60^{o}C with 96% ethanol (Izirwan et al, 2020).

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Table 1. Total Treatment Approach

Treatment (P)	Ratio (w/v)	Time (minutes)	Power (Watt)
P1	10	-	-
P2	15	-	-
P3	20	-	-
P4	25	-	-
P5	30	-	-
P6	35	-	-
P7	+	3	-
P8	+	6	-
P9	+	9	-
P10	+	12	-
P11	+	15	-
P12	+	18	-
P13	+	+	90
P14	+	+	180
P15	+	+	270
P16	+	+	360
P17	+	+	450
P18	+	+	540

Description: (-) constant/fixed value chosen by the researcher to find the optimum factor variable, (+) optimum value obtained in one variable used.

Materials and Tools

Butterfly pea flowers of the double petal variety with a harvest age of 3-4 months originating from Gondang Village, Gondang District, Sragen Regency (dry conditions), tartaric acid, distilled water, aluminum foil, cotton, KCL-HCL buffer (0.1M and pH 1) and acetate buffer (pH 4.5).

The tools used in this study were analytical balance, digital balance, 80 mesh sieve, filter paper (Whatman), food dehydrator (10 Tray FDH-10), UV-Vis spectrophotometer (DUAL BEAM UV Vis Genesys 10S UV-Vis), vacuum rotary evaporator (Buchi Rotavapor® R-300), microwave, porcelain cup, pH meter, beaker glass (Duran), 500 ml Erlenmeyer flask (Herma), dropper pipette (One-med), volume pipette (Iwaki), spatula, waterbath shaker, desiccator, test tube (One-med), funnel (Herma), micropipette (Joanlab) and measuring cup (Iwaki).

Making Butterfly Pea Flower Simple Powder

The initial stage in this study refers to previous studies that are almost similar with differences in the type of solvent (Husna et al., 2022) where butterfly pea flowers are dried using a food dehydrator at a temperature of 60°C for 6 hours until the water content decreases. After the drying process is complete, the butterfly pea flowers (including flower petals) are

ground using a blender to produce a fine powder. The powder that has been obtained is then sieved with a size of 80 mesh to obtain more uniform particles.

Water content testing is carried out to ensure that the water content of the butterfly pea flower simple powder is appropriate less than 10% (BPOM, 2014). Butterfly pea flower powder that has met the standard is stored in a dark container at room temperature with the addition of silica gel to prevent moisture and avoid direct exposure to sunlight.

Anthocyanin Extraction of Butterfly Pea Flowers

The modified extraction method (Thuy et al. 2021) was carried out by weighing 10g of butterfly pea flower powder and putting it into a 500 mL Erlenmeyer flask, then adding 0.75% aquades-tartaric acid solvent with a ratio of 1:10 to 1:35 (w/v). The Erlenmeyer flask was covered with cotton and aluminum foil, then shaken using a water bath for 5 minutes at a speed of 150 rpm to allow solvent penetration into the material.

Next, the mixture was put into a microwave oven with the power adjusted based on the trial design between 90–540 watts and an extraction time of 3–18 minutes (Stop and go system = 15 and 5 minutes). After the extraction process was complete, the sample was cooled to room temperature, then filtered using a vacuum pump with filter paper to obtain a dregs-free filtrate. The filtrate obtained was then stored in a dark vial container at low temperature to be analyzed for its total anthocyanin content.

Water Content Analysis

Empty cup and sample to be used Then weighed and dried, the dried cup plus a 5g sample is dried in an oven at 105°C for 3 hours then dried and cooled in a desiccator Then weighed, repeated until a constant weight is obtained and calculated using the formula (AOAC, 2012).

$$Water\ Content = \frac{W - (W1 - W2)}{W}\ X\ 100\ \%$$

Description:

W = Sample weight before drying (gr)

W1 = Sample weight and dry cup (gr)

W2 = Empty cup weight (gr)

Total Anthocyanin Content Analysis

Total anthocyanin is measured using the pH difference method (Rafi et al. 2018). Anthocyanin extract samples were pipetted as much as 0.8 ml and put into 2 test tubes. The first test tube was added with 7.2 ml of KCl-HCl buffer solution (0.2 M, pH 1) and the second test tube was added with 7.2 ml of sodium acetate buffer solution (0.2 M, pH 4.5). The

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absorbance of each solution was measured at a wavelength of 510 nm and 700 nm after incubation for 15 minutes at room temperature. The total anthocyanin content in the sample can be calculated using the following formula:

$$Anthocyanin\ (\frac{mg}{l}) = \frac{A \times MW \times FP \times 10^3}{\varepsilon \times \ell}$$

Description:

A = (A_{520}nm – A_{700}nm) at pH 1.0 - (A_{520}nm – A_{700}nm) at pH 4.5

MW = 449.2 g/mol (cyanidin-3-glucoside)

FP = dilution factor,

ℓ = cuvette path length (cm)

ε = 26,900 molar extinction coefficient ($L \times mol^{-1} \times cm^{-1}$)

10^3 = conversion factor from grams to milligrams.

RESULT AND DISCUSSION

Samples of butterfly pea flowers (*Clitoria ternatea L*) with a harvest age of 3-4 months from Gondang Village, Gondang District, Sragen Regency were conventionally dried with sunlight for 3 days until the water content of the fresh flower petals was reduced. However, after drying, the percentage of water content was still quite high and still far above the standard water content of simplicia, which is less than 10% (BPOM, 2014). The drying process was carried out for 6 hours at a temperature of 60°C according to the literature (Sukmawati et al, 2024). The following are the results of the water content of the butterfly pea flower powder in Table 2**.**

Table 2. Analysis of Water Content of Butterfly pea Flower Powder

Drying Method	Average Water Content ± STDEv (%)
Food Dehydrator	5,32 ± 0,07
Conventional	12,89 ± 0,02

From the data in Table 2, the results of the analysis of the water content of the butterfly pea flower powder after drying with a Food Dehydrator were obtained at around 5.32 ± 0.07% where the powdered simplicia is said to have met the standards set by BPOM. High water content in the powdered simplicia can increase the risk of bacterial and microbial growth, so content analysis is important to ensure the quality and safety of the ingredients. Following the appearance of the butterfly pea flower powder using 2 different drying methods in Figure 1.

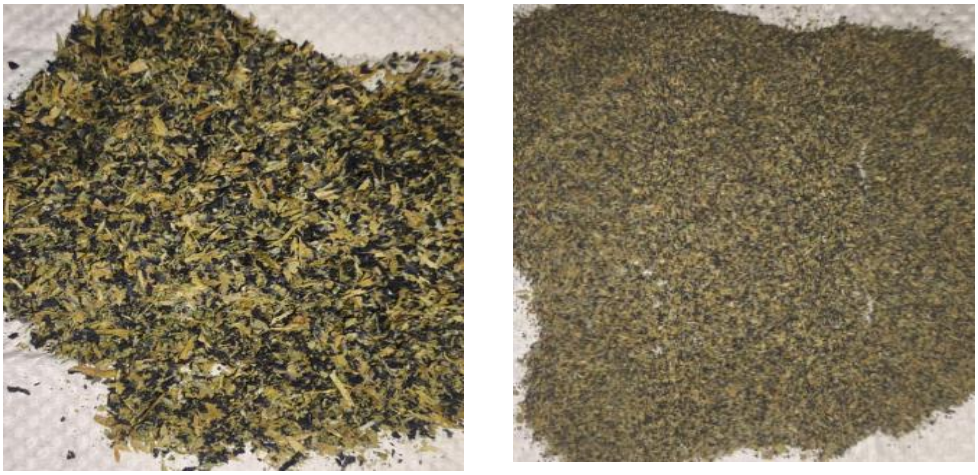

Figure 1. Appearance of Drying Using the Conventional Solar Heat Method for 3 days (a) and Food Dehydrator for 6 hours at a temperature of 60ºC (b)

Excessive water content can accelerate the degradation process, causing the material to be more easily damaged and not durable during storage. These results also clarify that the higher the temperature and the longer the drying, the lower the water content of the butterfly pea flowers produced (Hariadi *et al*, 2023). Conventional drying methods, particularly sun drying, is among the oldest and most accessible preservation methods traditionally utilized by farmers to process various food materials (Inyang et al, 2017). Commonly practiced in developing countries by exposing food items directly to sunlight on surfaces such as mats, rooftops, or drying floors (Bindu et al., 2016). Although cost-effective, sun drying is time-consuming and exposes the material to potential microbial contamination due to poor hygienic conditions (Yarkwa and Uvir, 2015). Specifically for butterfly pea flowers, conventional drying methods such as sun drying often result in higher residual moisture content, which may compromise the stability and efficacy of phytochemical compounds such as anthocyanin.

Effect of Ratio, Time and Power on Anthocyanin Levels of Butterfly Pea Flowers

Extraction of anthocyanin pigments from butterfly pea flowers using the Microwave-Assisted Extraction (MAE) method is influenced by several parameters. Considering that research with similar types of solvents has never been conducted, this research approach examines the variation of the ratio of materials to solvents, extraction time, and microwave power used. The ratio of materials to solvents is one of the important parameters in the extraction process because it affects the solubility and efficiency of target compound acquisition. The following is the effect of the ratio on anthocyanin levels which can be seen in Figure 1.

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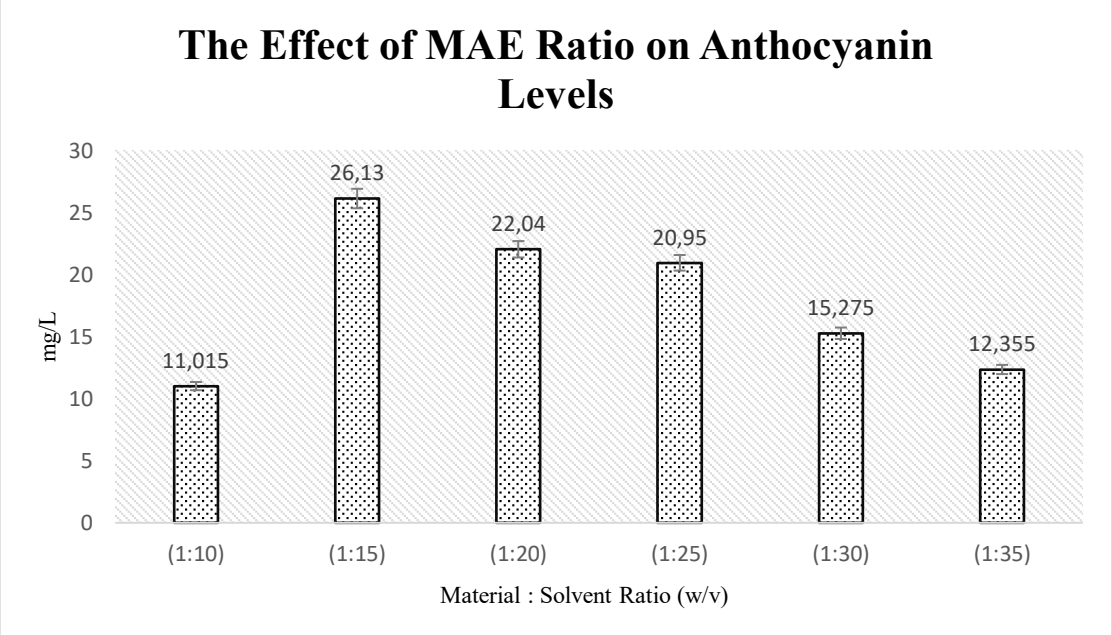

Figure 1. Effect of the Ratio of Material to Solvent on the Anthocyanin of Butterfly Pea

Based on Figure 1, the most optimal material to solvent ratio condition is in the P2 treatment with a ratio of 1:15, while at a ratio of 1:10 only anthocyanin levels of 11.02 ± 0.4 mg/L are obtained. Although according to Llompart et al. (2019) the selection of solvent volume depends on the type and size of the sample, the most common is about 10 times lower than that used in conventional extraction, but in this study, it is suspected that the solvent ratio of 1:10 has not been able to dissolve the entire sample. So that the extraction process does not run optimally.

From these results it can be explained that to determine the minimum limit value for the ratio factor is 1:15 while the maximum limit is 1:25. In addition to the material to solvent ratio, extraction time is a crucial factor in determining the efficiency of anthocyanin release during the MAE extraction process. Optimal extraction time allows more effective solvent penetration into the material matrix, thereby increasing the acquisition of anthocyanin pigments. The results of the effect of extraction time on the anthocyanin pigments of butterfly pea flowers can be seen in Figure 2 below.

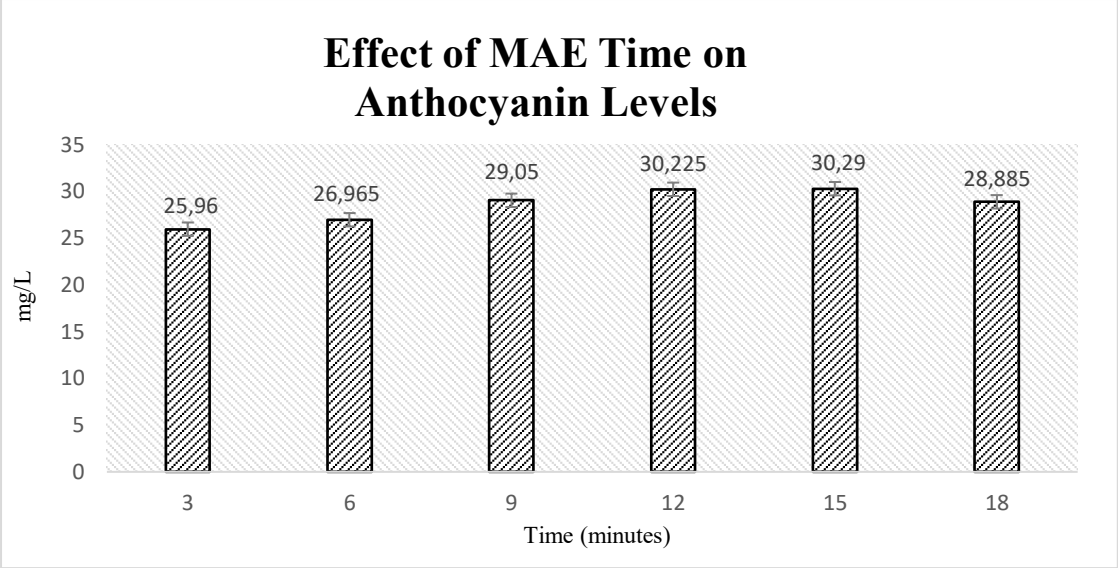

Figure 2. Effect of MAE Extraction Time on Anthocyanin Content of Butterfly Pea Flowers

Based on the results of Figure 2. The optimum treatment of the time variable is in the P11 treatment with a duration of 15 minutes. The extraction time itself affects the results of the anthocyanin content of the butterfly pea flower, where the longer the extraction time, the more anthocyanin content will be obtained. However, at a certain time interval if it is too long, the anthocyanin pigment will undergo a degradation process due to the heat generated by collisions between particles which are getting longer during the MAE extraction process.

Increasing temperature during heating can trigger decomposition and changes in pigment structure, which leads to color fading (Fartoosi et al., 2025). In addition, heating also has the potential to activate enzymes such as anthocyanase, polyphenol oxidase, and peroxidase, which play a role in changing the color of anthocyanins through oxidation mechanisms. This oxidation process causes degradation of anthocyanins, converting them into colorless compounds, namely carbinol bases (Enaru *et al*, 2021).

Microwave power and extraction time work together to influence each other so that both factors must be appropriate (Salve and Ray, 2020). However, too high a power risks causing thermal degradation of anthocyanins due to excessive temperature increases. Therefore, optimizing MAE power is a crucial aspect in maximizing anthocyanin yield. The following is the observation data regarding the effect of MAE power variations on the levels of anthocyanins produced which can be seen in Figure 3.

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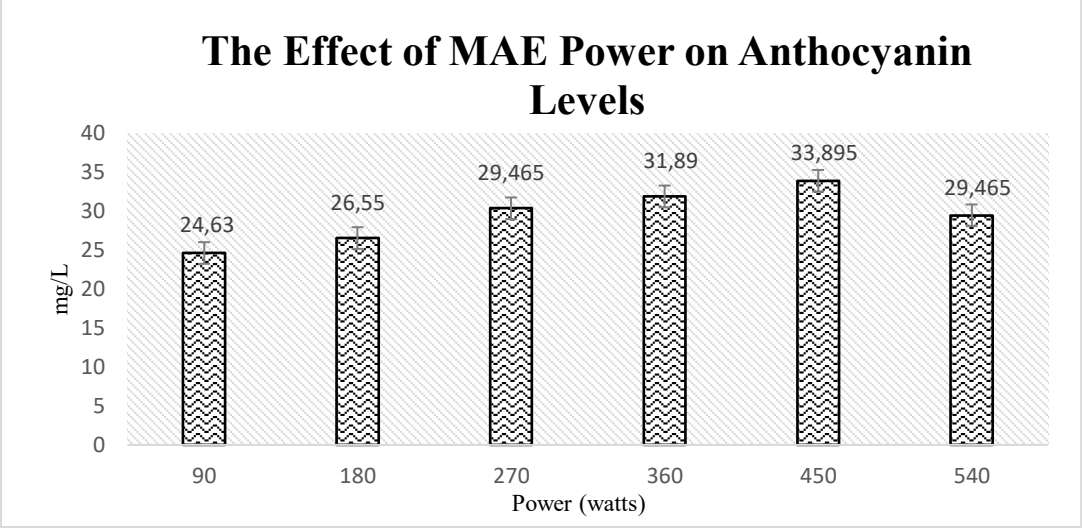

Figure 3. Effect of MAE Extraction Power on Anthocyanin Content of Butterfly Pea Flowers

Based on the results shown in Figure 3, the highest power effect on anthocyanin levels was achieved in the P17 treatment, which used 450 watts of power. However, there was a decrease in anthocyanin levels when the power increased to 540 watts. This indicates that in this power variable approach study, the maximum point of anthocyanin acquisition was found at the 450 watt level. This finding is in line with the literature presented by Damayanti et al. (2020), which states that increasing power tends to increase extraction results. Complete data on OFAT treatments are presented in Table 3.

Table 3. Results of the Combination of OFAT Method Treatments on Anthocyanin Levels

Treatment (P)	Ratio (w/v)	Time (min)	Power (watt)	Average ± (mg/L)
P1	10	3	270	11,02 ± 0,47
P2	15	3	270	26,13 ± 0,59
P3	20	3	270	22,04 ± 0,94
P4	25	3	270	20,95 ± 0,35
P5	30	3	270	15,27 ± 0,82
P6	35	3	270	12,35 ± 0,47
P7	15	3	270	25,96 ± 0,35
P8	15	6	270	26,96 ± 0,82
P9	15	9	270	29,05 ± 0,23
P10	15	12	270	30,22 ± 0,23
P11	15	15	270	30,29 ± 0,14
P12	15	18	360	28,88 ± 0,23
P13	15	15	90	24,63 ± 0,35
P14	15	15	180	26,55 ± 0,70
P15	15	15	270	30,36 ± 0,57
P16	15	15	360	31,89 ± 0,70
P17	15	15	450	33,89 ± 0,47
P18	15	15	540	29,47 ± 0,82

Result of multiple linear regression analysis show that the regression model formed has a coefficient of determination (R^2) value of 0.639 in Table 5. Indicating that 63.9% of the variation in the total anthocyanin response can be explained by the variables of the ratio of material to solvent (X_1), extraction time (X_2), and microwave power (X_3), while the remaining 36.1% is influenced by other factors outside the model. The results of the ANOVA test in Table 4. show a significance value (p-value) of 0.002 (<0.05), so it can be concluded that the three factors simultaneously have a significant effect on the total anthocyanin response.

Table 4. Results of ANOVA Analysis

	df	*SS*	*MS*	*F*	*Significance F*
Regression	3	500,1615222	166,7205074	8,267805	0,002067633
Residual	14	282,3103706	20,16502647		
Total	17	782,4718928			

	Coefficients	*Standard Error*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	18,63139932	6,492575903	0,012361	4,706208955	32,55659
X_1	-0,31485981	0,197012399	0,132323	-0,737409381	0,10769
X_2	0,713716042	0,209392729	0,004241	0,264613304	1,162819
X_3	0,01622195	0,01174585	0,188903	-0,008970393	0,041414

Table 5. Multiple Linear Regression

Regression Statistics	
Multiple R	0,79950423
R Square	0,639207014
Adjusted R Square	0,561894231
Standard Error	4,490548571
Observations	18

However, partially, only the extraction time variable (X_2) has a significant p value (0.004 <0.05), indicating that time is a dominant factor in increasing anthocyanin extraction. This is in line with research by Widhianto and Estiasih (2021) which states that more optimal contact time can increase solvent diffusion into the material matrix and increase the release of bioactive pigments such as anthocyanins. Meanwhile, the solvent ratio and microwave power did not show a statistically significant effect, possibly because the interaction between the two is still in a range that is not extreme enough to produce significant changes to bioactive components (Brady, 2014).

CONCLUSION

Based on the research that has been done using the OFAT method, it is known that the ratio of material to solvent, time and MAE extraction power affect the total content of

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anthocyanin pigments in butterfly pea flowers. This study also showed that by drying the food dehydrator at a temperature of 60ºC for 6 hours, a lower water content of 5.32 ± 0.07% can be obtained, in addition, by adding acid to the aquades-tartaric acid solvent 0.75% makes anthocyanin more stable during the extraction process. From this study, it was found that the optimum treatment was obtained by a ratio of 1:15, extraction time of 15 minutes and power of 450 watts with anthocyanin results of 33.89 ± 0.47 mg/L.

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