The optimization of ultrasonic-assisted extraction of antioxidant compounds from butterfly pea flower (Clitoria ternatea L.) by using response surface methodology

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Abstract. Butterfly pea flower (Clitoria ternatea L.) contains many phytochemical compounds that are beneficial to human health, one of which is incorporated in the group of phenolic compounds. Generally, the extraction process of an organic compound requires heat and takes a relatively long time. Therefore, the application of the ultrasonic method for the extraction of butterfly pea flower is studied. This study aims to obtain the best-operating conditions in the ultrasonic-assisted extraction of the phenolic compound of the butterfly pea flower. The optimization of operating conditions during the extraction process was performed by using Response Surface Methodology (RSM) with the application of Design Expert 10 software. The results of the optimum operating conditions are 62.063 ml/g of solvent and flower ratio, 60% ultrasonic power output, and 15 minutes of extraction time. These operating conditions result in 55.834 ml of extract, 0.874 mg/ml of total phenolic content (TPC), and 10.42 mg/l of total anthocyanins (TA).

Keywords: Clitoria ternatea L; Ultrasonic Extraction; Phenolic Compound; Total Anthocyanin; Response Surface Methodology.

1. Introduction
Herbal medicinal products are now increasingly demanded around the world. The current issues about health and the harm caused by synthetic chemicals in the products on the market make herbal based products are more favorable than the standard chemical products. Besides, issues related to the environment, such as global warming and pollution, engage the trend of "back to nature" which increasingly is in demand by the community. One of the medicinal plants having the potential attribute as raw material for making herbal products is the Butterfly pea flower (Clitoria ternatea L).

Butterfly pea flower has been widely studied on its health beneficial properties, such as anti-allergy [1], anti-diabetic, anti-inflammatory, analgesic [2], anti-microbial [3], [4], and contains anthocyanin compounds with high antioxidant activity [5-7]. In Indonesia, brewing of butterfly pea flower is believed to heal baby eye-related problems. This belief is reinforced by the results of research confirming that the Butterfly pea flower has antibacterial properties, including eye infection bacteria. Also, older people in ancient times used the brewing water of Butterfly pea flower to clean their eyes as they believe that this way can prevent myopic eyes, and can reduce the risk of blindness due to cataracts [8]. Kusrini et al. (2017) has studied the anti-cataract ability from this flower; the result showed that the extract of the petal flower has more active in order to dissolve calcium and natrium as...
the main composition of the model cataract [8]. Besides, the flower is also known as an antioxidant source that are very beneficial to human health, such as phenol and various derivatives such as anthocyanin [9].

Various studies of antioxidant properties of butterfly pea have been conducted. Wang & Stoner (2008) reported the anthocyanin's ability to prevent cancer [10]. Other studies have suggested the ability of anthocyanin to inhibit aging, to prevent neurological disease, to heal inflammation, diabetes, and bacterial infections [11]. Some researchers report that the anthocyanin contained in this flower is considered to possess a potential antioxidant, as in a study worked by Vankar and Srivastava (2010), who conducted a study of antioxidant activity extracted from 15 flowers [5]. The results showed that anthocyanin antioxidant activity of butterfly pea was ranked second highest after *Mirabilis jalapa* with the ability to restrict the DPPH radical by 86%. Other researchers reported that flower extract had better anti-oxidation ability compared to *Eclipta prostrata* L [12,13].

The extraction process with a particular solvent is required to gain one or more active ingredients. Generally, the process requires heat and takes a relatively long time. Study on optimization of anthocyanin extraction showing optimum operating conditions until 70 minutes. High consumption of energy can cause a higher production cost. Therefore, a more efficient method of extraction is required. The use of non-thermal technology for the extraction process has been widely studied, such as the application of electric field [14,15], magnetic field [16], and microwave [17,18]. Another way of extraction recognized as a cheap, fast, simple, and more efficient is the ultrasonic extraction method [19-23]. The condition regarding the optimization of the extraction process is necessary to obtain the best extract results.

In this study, the extraction variables (ratio of solvent, ultrasonic power, and extraction time) are optimized by using Response Surface Methodology (RSM) for maximum desire responses, extract volume, total phenol, and total anthocyanin.

2. Material and Methods

2.1 Materials

This research used Chrom-Tech B03 Ultrasonic processor to assist extraction, blender, rotary evaporator, Spectrophotometer UV-Visible Spectrophotant Pharo 300, vortex, Whatman 40 paper (8μm), vacuum filter, Buchner funnel, and glassware laboratory. The materials in the research were: dried petal of butterfly pea flowers, distilled water, and chemicals with p.a quality, such as potassium chloride, hydrochloric acid, sodium acetate, Gallic acid, Na₂CO₃, and Folin-ciocalteau reagent.

2.2 Extraction of Phenol Compounds using Ultrasonic

The dried Butterfly pea flower (*Clitoria ternatea* L) was crushed with a dry blender. A total of 200 ml solvent (water) was placed in a beaker glass, then was filled with the blended flower in a various ratio of the solvent ratio. The extraction process was performed by emitting ultrasonic waves into a mixture of flower and water, with variations of power output and reaction time, and the depth of the probe was 2 cm. The depth of the ultrasonic probe was equated to the treatment of each sample uniformly. The independent variables used in the extraction process consisted of 3 factors and three levels, as shown in Table 1.

| Table 1. Factors and levels | Unit | Low (-1) | Center 0 | High (1) |
|-----------------------------|------|----------|----------|---------|
| Solvent Ratio               | ml/g | 50       | 75       | 100     |
| Power output ult.           | %    | 20       | 60       | 100     |
| Extraction Time             | min  | 5        | 10       | 15      |
The suspension of ultrasonic extraction was filtered by using Whatman 40 (8 μm) filter paper assisted by a vacuum filter. Then the filtrate was dried up by using a rotary evaporator at 40 °C. The concentrated extract was placed in a container covered by aluminum foil to prevent degradation of the compounds in the extract due to external exposure before analysis. Volume measurement, total phenol, and total anthocyanin analysis were then conducted.

2.3 Determination of TPC and TA
The determination of total phenol content was performed by Folin-Ciocalteau method with Gallic acid as a standard [20]. Whereas, the determination of total anthocyanin was adopted by Lee, Durst, & Wrolstad (2005) [24]. The monomeric anthocyanin content was expressed as cyanidin-3-glycoside content, as measured by the differential pH method.

2.4 Optimization of operating conditions of ultrasonic extraction
The optimization of the extraction process was conducted by the Response Surface Method (RSM) and was executed under Box-Behnken design. As three factors were studied, 13 variations should be tested. Besides, it took at least three replications at the center point (0,0,0). In this study, the five replications were performed at the center point; therefore, the number of experimental variations became 17. Each factor consisted of 3 levels representing the lower (-1), center (0), and upper limit (1), according to Box-Behnken design. The running order in the experiment was done in line with the design. The experimental variations are presented in Table 2. Data processing was then conducted by using Design Expert 10 software to obtain optimum operating extraction conditions.

| Std | Run | Solvent Ratio (ml/g) | Power output (%) | Extraction Time (min) |
|-----|-----|----------------------|------------------|-----------------------|
| 3   | 1   | 50                   | 100              | 10                    |
| 17  | 2   | 75                   | 60               | 10                    |
| 14  | 3   | 75                   | 60               | 10                    |
| 12  | 4   | 75                   | 100              | 15                    |
| 5   | 5   | 50                   | 60               | 5                     |
| 16  | 6   | 75                   | 60               | 10                    |
| 9   | 7   | 75                   | 20               | 5                     |
| 13  | 8   | 75                   | 60               | 10                    |
| 7   | 9   | 50                   | 60               | 15                    |
| 11  | 10  | 75                   | 20               | 15                    |
| 10  | 11  | 75                   | 100              | 5                     |
| 6   | 12  | 100                  | 60               | 5                     |
| 2   | 13  | 100                  | 20               | 10                    |
| 8   | 14  | 100                  | 60               | 15                    |
| 4   | 15  | 100                  | 100              | 10                    |
| 1   | 16  | 50                   | 20               | 10                    |
| 15  | 17  | 75                   | 60               | 10                    |

3. Results and Discussion

3.1 Optimization of ultrasonic-assisted extraction
In this study, the extraction process runs optimally if the measuring variables/ responses (extract volume, total phenol, and total anthocyanin) reach the maximum value. However, the maximum value for each response could not achieve in the same treatment; therefore, data processing is necessary for the extraction optimization. The data processing was carried out by using Box-Behnken design combined with Response Surface Methodology (R.S. M).

The result of the ultrasonic extraction is presented in Table 3. The table contains the extraction process variables (solvent ratio, ultrasonic power output, extraction time) and experimental data consisting of extract volume, total phenol, and total anthocyanin from the 17-extraction process, including five repetitions at the center point.

Table 3. Data Result of ultrasonic-assisted extraction

| Run | Solvent ratio (ml/g) | Power output (%) | Extraction Time (min) | Extract volume (ml) | Total Phenol (mg/ml) | Total Anthocyanin (mg/l) |
|-----|----------------------|------------------|-----------------------|---------------------|----------------------|------------------------|
| 1   | 50                   | 100              | 10                    | 55                  | 0.77                 | 8.93                   |
| 2   | 75                   | 60               | 10                    | 35                  | 0.68                 | 8.77                   |
| 3   | 75                   | 60               | 10                    | 44.9                | 0.78                 | 9.52                   |
| 4   | 75                   | 100              | 15                    | 51.4                | 0.87                 | 3.76                   |
| 5   | 50                   | 60               | 5                     | 22.9                | 0.5                  | 2.67                   |
| 6   | 75                   | 60               | 10                    | 38.2                | 0.75                 | 11.44                  |
| 7   | 75                   | 20               | 5                     | 43.2                | 0.55                 | 4.34                   |
| 8   | 75                   | 60               | 10                    | 40                  | 0.67                 | 7.6                    |
| 9   | 50                   | 60               | 15                    | 45                  | 0.62                 | 6.1                    |
| 10  | 75                   | 20               | 15                    | 46.8                | 0.57                 | 9.85                   |
| 11  | 75                   | 100              | 5                     | 36.8                | 0.71                 | 8.1                    |
| 12  | 100                  | 60               | 5                     | 38.4                | 0.63                 | 8.6                    |
| 13  | 100                  | 20               | 10                    | 49.1                | 0.59                 | 6.85                   |
| 14  | 100                  | 60               | 15                    | 34.8                | 0.68                 | 9.69                   |
| 15  | 100                  | 100              | 10                    | 46                  | 0.61                 | 4.93                   |
| 16  | 50                   | 20               | 10                    | 21                  | 0.47                 | 1.5                    |
| 17  | 75                   | 60               | 10                    | 34.5                | 0.7                  | 9.35                   |

The research data on Table 3 is analyzed by RSM using Design Expert 10 software including ANOVA (Analysis of Variance) to find an appropriate equation model which correlates each response (extract volume, total phenol, total anthocyanin) used in the experiment as well as the involved factors (solvent ratio, ultrasonic power output, and extraction time). Besides, the relationships of each response are shown in the form of a two-dimensional graph (contour) and a 3-dimensional graph. The two and 3-dimensional graphs are marked with different colors on the contours and the surface responses from blue, green, yellow to red. A red area represents the maximum value of the response.

3.2 RSM Analysis of Extract Volume

The result of RSM analysis on the extract volume shows that the three studied factors influence the extract volume following the suggested model in the software, such as a 2-factor interaction (2FI) with a p-value of 0.0448 (significant). However, according to p-value on each factor, it indicates that there is still a factor that is not significant with p-value, such as the B.C. factor (interaction between ultrasonic power output and extraction time). Thus, the researcher decided to eliminate this factor.
Therefore, the selected model for the extract volume is the reduced 2-factor interaction (reduced 2FI). The equation model which associates the extract volume with all the factors is in the following equation:

\[ Y_1 = 40.18 + 3.05A + 3.64B + 4.59C - 9.28AB - 6.43AC \]

with:
- \( Y_1 \) = extract volume (response) (ml)
- A = solvent ratio (ml/g)
- B = ultrasonic power output (%)
- C = extraction time (minutes)

![Graph: Predicted vs. Actual](image)

**Figure 1.** Experimental results vs model predictions data of extract volume

The conformity of model prediction with experiment result data is presented on Figure 1, which correlates the experimental with the predicted extract volume based on model. This graph also includes the results of the Design Expert 10 software. From Figure 1, the value of \( R^2 \) is 0.6448, which means that 64.48% of the total data can be explained by the model, while the model does not represent 35.52% of the total experimental data. This result indicates that the model for the extract volume parameter is not yet ideal for representing all data; however, in this research, the extract volume parameters are less critical for the optimization of the extraction process. Thus, it is acceptable that even the model has not adequately represented the experimental data.

The resulting model shows the influence and interaction between factors and the amount of extract volume. The coefficient of each factor shows how it affects the response. The most significant coefficient shows the most considerable influence on the extracted value. In the model, the most significant influence is the interaction between solvent ratio and ultrasonic power output, as followed by the interaction between solvent ratio and extraction time. However, both coefficients are negative, meaning that the extract volume will be high if one of the interacting factors is either maximum or minimum. In contrast, the extract volume will be low if the two interacting factors are equal to the maximum or minimum value.

According to the factor level, the lowest factor is the factor with the most significant number of dried flowers. Therefore, based on the negative coefficient in the equation, the value of the response (or in this case is the volume of the extract) will be maximized if one of the interacting factors, which
is the solvent ratio, has a minimum value and other factors have a maximum value such as the ultrasonic output power and the extraction time. The minimum value of the solvent ratio means that the extracted flower bar is most dominant. In contrast, the extract volume is in the minimum value if the solvent ratio and other factors of ultrasonic power output and the length of extraction are equal to minimum or maximum value. Exact information in determining the optimum conditions of the response is obtained by looking at the contour and response surface in Figure 2.

![Response surface and contour plots showing effect of solvent ratio (A) and extraction time (B) on volume extract](image)

**Figure 2.** Response surface and contour plots showing effect of solvent ratio (A) and extraction time (B) on volume extract

The contour and response surfaces of Figure 2 shows that the extract volume reaches a maximum point (indicated by red area) when the extraction process is run with: minimum solvent ratio (50 ml/g), maximum ultrasonic power output (100%), and maximum extraction time 15 minutes). The maximum conditions of the extract will be achieved when more amounts of Butterfly pea flower (*Clitoria ternatea* L) are used (with minimum solvent ratio), and the higher ultrasonic output power will emit the longer extraction time (approximately for 15 minutes).

The total volume of measured extracts is derived from the solvent (water). In each extraction process, a 200 ml of solvent is used with solvent ratio (water) per flower (ml/g) of 50, 75, and 100, respectively, and the flower mass varies at 4 g, 2.67 g, and 2 g. The difference of the flower mass of Butterfly pea flower creates a various amount of the obtained extract volume for each treatment. Differences in the volume of the extract are also due to the evaporation process. Evaporation is
performed for 1 hour with different concentration levels of extracts due to different solvent ratios, affecting a difference in the boiling point of the extract. Consequently, the amount of water vaporized is similar, but the remaining volume of the extract left is different. The differences of extract volume are also due to the lack of maximization of the filtration process by using vacuum filter. It is because, at the end of the separation process, the remaining filtrate is absorbed in the supernatant, thus affecting the volume of the extract produced.

3.3 RSM Analysis of Total Phenol

The result of RSM analysis to total phenol shows that total phenol, which is influenced by three factors, has followed the suggested model, which is a quadratic factor with a p-value of 0.0019 (significant). However, as presented by the p-value of each quadratic factor, there is still a factor having a p-value of more than 0.1 indicated as an insignificant factor (p > 0.1), which in this case is B² (p = 0.4615) and C² (p = 0.4079). Therefore, the selected model for total phenol is reduced quadratic. The equation model, which connects the total phenol with all the studied factors, is presented in the following equation:

\[ Y_2 = 0.70 + 0.019A + 0.098B + 0.044C - 0.070AB - 0.018AC + 0.03BC - 0.089A^2 \]

with:

\( Y_2 \) = Total phenol (response) (mg/ml)

Similarly, in the extract volume, the suitability of the model prediction for the total phenol parameters with the experimental results data is demonstrated by the graph relating both the total phenol data of the experimental results and the total phenol from the model prediction (Figure 3).

![Predicted vs. Actual](image_url)

Figure 3. Experimental results vs model predictions data of total phenol

From the plot graph of the actual data and the model prediction on Figure 3, it is apparent that the value of R² is 0.8759, which means that 87.59% of the overall data can be explained by the model, while the model does not represent 12.41% of the total experimental data. Similarly, in the equation model for extract volumes, the recommended software model for total phenol is not considered ideal for modeling the process; because the value of R² is not close to 1. However, in this study, the main
The goal is the optimization of operating conditions based on the best (highest) results of each parameter/response, including total phenol. Thus, this model can still be used to assist in predicting the total phenol of yield extraction.

The equation model for the total phenol in the above equations has a complicated relationship between the responses and all the studied factors. Therefore, the contour and response surface in Figure 4 is presented to see the effect of each factor on total phenol.

![Figure 4. Response surface and contour plots showing the effect of solvent ratio (A) and power output (B) on total phenol](image)

From the contour and response surface on Figure 4, it is apparent that the total phenol reaches a maximum (marked with the red area) when the extraction process is run with: minimum solvent ratio (50 ml/g), maximum ultrasonic power output (100%), and maximum extraction time (15 minutes). Thus, it is clear that the maximum condition of the total phenol extract will be achieved when the mass of Butterfly pea flower (*Clitoria ternatea* L) is used in a higher amount (with minimum solvent ratio) along with ultrasonic output power as well as more excellent extraction time.

Ultrasonic output power also has a significant effect on extracted phenol content, indicates that the phenol content in the organic material is relatively stable to the temperature caused by ultrasonic wave emission. By the study of [25] states that one of the contents of phenol compounds is flavanone glycoside in *Citrus paradisi* fruit, which will be damaged if heated at 120°C for 90 minutes or at 150 °C for 30 minutes. While in this study, the temperature caused by the highest ultrasonic emission reaches only 85 °C; thus, the damage in most of the phenol compounds is preventable. Another study even mentioned that heating at 120°C for 120 minutes increased the total phenol content of orange peel extract [26]. This study further strengthens the evidence that most of the phenol compounds in organic material extracts are relatively stable when exposed to heat effects.
3.4 RSM Analysis of Total Anthocyanin

Results of RSM analysis of total anthocyanin show that three studied factors mostly influence total anthocyanin following the suggested model (quadratic) with a p-value of 0.0070 (significant). However, if p-value is seen on each quadratic factor, there are still factors having p-value greater than 0.1; therefore, subsequent removal (reduction) factors are not significant (p > 0.1) wherein this case are C² (p = 0.1928) and AC (p = 1,000). Therefore, the selected model for the total anthocyanin is a reduced quadratic. The equation which links among the total anthocyanin with all the studied factors is presented in the following equation:

\[ Y_3 = 9.05 - 2.65A + 0.63B + 0.77C + 0.34AB - 1.75BC - 2.39A^2 - 1.59B^2 \]

with \( Y_2 \) is equal to total anthocyanin (response) (mg/l)

![Predicted vs. Actual](image)

**Figure 5.** Experimental results vs. model predictions data of total anthocyanin

Similarly, in the extract volume and the suitability of the model predictions for the total anthocyanin parameters with the experimental results, data can be seen in the graph linking the total anthocyanin data of the experimental results with the total anthocyanin from the model predictions (Figure 5). The R² is 0.8798, which means that the model can explain 87.98% of the overall data. Judging from the value of the coefficient of determination (R²), the model suggests software for total anthocyanin, which is considered better than the model for extract volume. For modeling, this R² value is still not ideal because it is not yet close to 1. However, in this study, the main objective is the optimization of operating conditions based on the best results (highest) of each parameter/response; therefore, this model helps predict the total results of extraction anthocyanin.

The resulting model is a quadratic model that shows the number of influences in each factor, the interaction between factors, and the squared value of each factor to the number of anthocyanin levels in the extract. Similarly, the equation model for the previous extract volume, as marked by coefficients in each factor, indicates how it affects the response. In the equation model for this total anthocyanin, the association of anthocyanin levels with all the studied factors shows a more complicated correlation than the equation model on the extract volume, which is difficult to interpret the factors influencing the anthocyanin levels. Therefore, the influence of each factor on anthocyanin levels is displayed in the contour and response surface in Figure 6.
Figure 6. Response surface and contour plots showing the effect of solvent ratio (A) and power output (B) on total anthocyanin.

From the contour and the response surface in Figure 6, it is apparent that the anthocyanin content reaches the maximum (marked with the red area) when the extraction process is run with minimum solvent ratio (50 ml/g), minimum ultrasonic power output (20%), and time maximum extraction (15 minutes). Thus, it is clear that the maximum condition of the anthocyanin content of the extract will be achieved when more telescope is used (with minimum solvent ratio) as well as the longer extraction time. It is interesting, however, that the maximum anthocyanin levels are achieved precisely when ultrasonic power output is at a minimum level. It is likely due to the heat effect as caused by the ultrasonic waves themselves. The heat effects resulting from ultrasonic wave emissions with 20, 60, and 100% of output power respectively range from 35°C – 41°C, 46°C – 69°C to 63°C – 85°C. The effects of excessive exposure to heat during extraction or storage may cause degradation of anthocyanin compounds resulting in low levels of produced anthocyanin extract [27-29].

Mei et al. (2014) examined the stability of anthocyanin extracted from maize seeds resulting in the increased anthocyanin levels in the first 30 minutes of storage with temperatures of 30 °C, 50 °C, and
60 °C [30]. However, anthocyanin levels decreased gradually after 30 minutes of storage time. As for storage temperature above 60 °C, the anthocyanin levels continued to decrease from the beginning of storage, even above 80 °C, decreasing the anthocyanin levels to 40% within 2 hours.

Although heat treatment can cause anthocyanin degradation, exposure to heat with the right temperature and time can assist in the inactivation of enzymes having the characteristic of degrading anthocyanins such as peroxidases, phenoloxidases, polyphenol oxidases, and glycosidases raising the anthocyanin content in the extract [30,31].

3.5 Selection Process and Optimization of Operating Conditions

The extraction process becomes optimal if the volume of extract and total phenol reaches a maximum level. Analysis of optimum operating conditions of the extraction process can be engaged by observing the contour result and red-colored response surface from each response (Figures 2, 4 and 6). The observation result of the optimum operating condition in each response is summarized in Table 4.

Table 4. Optimal Operating Conditions of the Extraction Process in Each Response

| Factor                  | Optimum condition coordinates (red area) |
|-------------------------|----------------------------------------|
|                         | Extract volume | Total Phenol | Total Anthocyanins |
| Solvent Ratio (ml/g)    | 50-75          | 50-90        | 50-90               |
| Power output ult. (%)   | 60-100         | 70-100       | 70-100              |
| Extraction time (min)   | 10-15          | 10-15        | 10-15               |

Table 4 provides responses which will reach a maximum level at the same range of factor only in extraction time; however, the optimum condition in ultrasonic power output and solvent ratio factors reach a different range of factor. This condition causes the determination of optimum condition to be delayed. Therefore, the determination of optimum operating conditions is conducted by using RSM analysis by Design Expert 10 software.

The RSM analysis result proceeded from software shows that the extract volume followed the linear equation model with a 2-interaction factor, while total phenol content follows the quadratic equation model. Furthermore, an equation model connects responses to obtain each factor. All p-value has a smaller value than 0.05, meaning that all test parameter values are significant, while the entire fit values have shown more than 0.05, meaning that at a significant level of 5%, the models are appropriate.

In this experiment, the measured parameters are extract volume, total phenol, and total anthocyanin as automatically tested by its correlation by using software to find out the correlation between responses. The result of the correlation test has shown that there is no strong correlation among them. Thus, it is necessary to have a priority arrangement for process optimization. A high total of anthocyanin is chosen as the most critical factor (priority) because it is the most abundant antioxidant flower, which is thought to play a role in the cure/prevention of cataracts. The second priority of the total phenol and the one chosen for the lowest priority is the extract volume because this parameter is the least essential compared to the other two parameters. From that priority, the software will arrange several combinations of treatments until proper treatment is found, producing the extracts with the most optimum response parameters according to the priorities. By observing the most exceptional desirability value, the optimal combination of treatment for extraction in this study is obtained with the condition of a solvent ratio of 62.181 ml/g, ultrasonic power output of 50.238% and extraction time for 15 minutes.

The optimum operating conditions produce an ultrasonic power output of 50.238%. The value of power output is not performed with the available ultrasonic equipment, because the existing power output variations are only 20, 60, and 100%. Therefore, the adjustment was made to the priority setting stage with the specific goal for the ultrasonic power output factor adjusted to the available equipment, which is 60%. This value is the ultrasonic power output value, which is closest to the optimum
operating conditions as obtained in the previous stage. The selection of importance is selected based on the greatest value for the targeted ultrasonic power output.

By following the given limits, the software will arrange the combination of treatments to find out the proper treatment, which produces the extract with the most optimum response parameters according to the priorities made. The combination of treatments resulting in the best response of the results of Design Expert 9.0.3 software is presented in Table 5. From the results of running software (Table 5), the optimal operating conditions with the highest desirability are the solvent ratio of 62.063 (ml/g), 60% of ultrasonic power output (target), and extraction time of 15 minutes.

**Table 5. Solution to Optimal Operating Conditions (the result of software running)**

| No | Ratio a/t | Power output (%) | Extraction time (min) | Extract Volume (ml) | Total Anthocyanin (mg/l) | Total phenol (mg/ml) | Desirability |
|----|-----------|------------------|-----------------------|---------------------|--------------------------|---------------------|--------------|
| 1  | 62.063    | 60               | 15                    | 46.510              | 10.556                   | 0.717               | 0.634        |
| 2  | 62.279    | 60               | 15                    | 46.481              | 10.554                   | 0.718               | 0.634        |
| 3  | 61.659    | 60               | 15                    | 46.565              | 10.558                   | 0.716               | 0.633        |
| 4  | 62.624    | 60               | 15                    | 46.435              | 10.551                   | 0.719               | 0.633        |
| 5  | 63.263    | 60               | 15                    | 46.348              | 10.542                   | 0.721               | 0.632        |
| 6  | 61.127    | 60.076           | 15                    | 46.654              | 10.557                   | 0.714               | 0.632        |
| 7  | 63.035    | 60.125           | 15                    | 46.405              | 10.541                   | 0.721               | 0.631        |
| 8  | 62.329    | 60               | 14.936                | 46.374              | 10.544                   | 0.717               | 0.630        |

The verification process was conducted by repeating the optimum extraction for five times repetitions; the results of the extract are then analyzed and are compared to the predicted models. As a result, it is found that the average value of each response generated is still within the range as predicted by the model (Table 6), which proves that the optimum obtained condition is already representative of the extraction process in this research.

**Table 6. Extraction process results at optimum conditions (solvent ratio of 62 ml / g, 60% ultrasonic power output, and 15 min extract time)**

| Replication | Extract Volume (ml) | Total anthocyanin (mg/l) | Total phenol (mg/ml) |
|-------------|---------------------|--------------------------|----------------------|
| 1           | 44.3                | 9.685                    | 0.728                |
| 2           | 41.7                | 10.854                   | 0.706                |
| 3           | 50.2                | 9.518                    | 0.733                |
| 4           | 45.9                | 11.522                   | 0.714                |
| 5           | 47.8                | 10.520                   | 0.723                |
| Mean        | 45.98               | 10.420                   | 0.721                |
| Deviation Std. | 3.251            | 0.832                    | 0.011                |
| Std. Error  | 1.454               | 0.372                    | 0.005                |
| Model Prediction | 46.51 ± 6.555 | 10.56 ± 1.392             | 0.717 ± 0.049        |
| Response Range | 39.955 – 53.065   | 9.168 – 11.952           | 0.668 - 0.766        |

3.6 The Comparison of Extract Result of Ultrasonic Extraction with Conventional Methods

The preparation of the extract by the conventional method is carried out by immersing dry flowers with the same solvent as in ultrasonic extraction, which is aquadest. There are two types of conventional methods performed, which are cold maceration (without heat treatment) and heat
maceration. The operating conditions are made equal to the optimum operating conditions resulting from the optimization of ultrasonic extraction operation conditions, which is the solvent ratio of 62 ml/g and the extraction time of 15 min. The temperature for heat maceration is 50°C. Each treatment is repeated two times.

A comparison of extract result of ultrasonic extraction method (optimum) with the result of the extract with conventional method is conducted to know whether the quality of extract obtained by ultrasonic method is better than that with the conventional extraction method.

| Table 7. Comparison of ultrasonic and conventional method extraction result |
|---------------------------------|-----------------|-----------------|-----------------|
| **Extraction Parameter (Response)** | **Ultrasonic extraction result** | **Maceration (without any temperature treatment)** | **Maceration (50 °C)** |
| Extract volume (ml) | 45.98 | 45.8 | 44.9 |
| Total phenol (mg/ml) | 0.721 | 0.204 | 0.500 |
| Total Anthocyanin (mg/l) | 10.42 | 1.920 | 5.218 |

Table 7 provides the difference of yield between ultrasonic extraction, maceration (without temperature treatment), and maceration (with temperature treatment for 50 °C). There is not much difference in the volume of the extract because the solvent ratio is similar; therefore, the obtained volume of extracts is also nearly the same. A slight difference has been occurred in the extract volume, due to the effectiveness of the solid phase during the filtration process by using a conventional vacuum filter. The lowest volume occurs in the extract with a heat maceration temperature of 50 °C; this occurs because the use of heat in the extraction process can lead to solvent evaporation. The difference in extract volume is relatively small because the extraction time is only 15 minutes.

Significant differences are noted in total phenol in extracts resulting from ultrasonic extraction and conventional extraction. The total phenol extracted from ultrasound is much better than the result of maceration conducted with or without heating treatment. This result is following research of Vinatoru, (2001), pointing out that the influence of ultrasonic waves occurs on the extraction process of some herbal ingredients such as fennel and mint [32]. The result shows that during the ultrasonic waves, extraction yield increases to 34% for fennel fruit, and 0.3% for mint. Other studies suggest conducting treatment with ultrasonic exposure of 0.15 W/cm2 to increase the extraction yield by 16.5% on the extraction of genipocide compounds in the gardenia fruit [33]. Leonelli & Mason (2010) stated that ultrasonic waves contribute to the destruction of plant cell walls creating the compounds in the cell to dissolve into the solvent readily [34]. Also, exposure to ultrasonic waves might raise the penetration of solvents into the cells, increasing the extraction yield.

Although excessive exposure to heat may damage the phenol compounds, the maceration extraction at 50 °C produces an extract with a higher total phenol compared to maceration without heating temperature treatment with a similar time of extraction of 15 minutes (Table 7). This result is following the research conducted by Choi et al. (2011), stating that phenol compounds still show an increase at a temperature of 120 °C in the orange peel extraction process [26].

All parameters (responses) of extraction with ultrasonic waves (Table 7), show better results compared to conventional extraction processes, reinforcing evidence that ultrasonic extraction is an efficient method of extraction compared to conventional methods [32].

4. Conclusion
In this research, the extraction of the phenolic compound from Butterfly pea flower (Clitoria ternatea. L) is carried out by using ultrasonic waves. Ultrasonic wave extraction is proven to shorten extraction time compared to that with maceration. The optimum operating conditions of the optimization result are solvent and flower ratio of 62 ml/g, 60% ultrasonic output power, and 15 minutes of extraction time. Under these operating conditions, extracts are produced with a volume of 55.834 ml, 10.42 mg/l of anthocyanin, and total phenol of 0.874 mg/ml.
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