Optimization of supercritical fluid extraction of *Mariposa Christia Vespertilionis* leaves towards antioxidant using response surface methodology

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**Abstract.** *Mariposa Christia Vespertilionis* (MCV) or also known as butterfly wing leaves, is popular in the traditional medicine practice. The objective of this study is to optimize the antioxidant components (based on antioxidant activity analysis) from the MCV leaves extract using Supercritical Fluid Extraction (SFE) based on different temperature, pressure, and particle size via Response Surface Methodology (RSM). Besides that, it is also to evaluate the optimal condition of different extracting antioxidant activity from MCV leaves using Design Expert 10 software. The condition range for both pressure and temperature were between 150-350 bar and 30-70 °C with constant extraction time. The size of particle samples were between 63 µm-1000 µm. The antioxidant was analyzed using UV–vis Spectrophotometer (UV-vis). The lowest antioxidant activity was 8.20 % and higher antioxidant activity was obtained at 49.76 %. Lower absorbance indicated highest free radical scavenging activities. Maximum percentage (%) indicated high scavenging activities, thus higher 1,1-diphenyl-2-picrylhydrazyl (DPPH) is a good indication for antioxidant. The predicted RSM optimal condition of antioxidant activity was at temperature 50 °C, pressure 282 bar, and particle size 500 µm, which the predicted antioxidant was 50.62 %. Upon verification through experiment, it was found out that the antioxidant activity value was close to the predicted value with an average error of less than 1%.

1. Introduction

Antioxidants play an important role to protect the body from risk of diseases including chronic diseases such as cancer and heart diseases [1] [2]. Antioxidant is defined as a compound that can delay, inhibit, or prevent the oxidation of oxidizable material by scavenging free radical and diminishing oxidative
stress [3] [4]. Based on a recent study, *Mariposa Christia Vespertilionis* (MCV) leaves, or also known as butterfly wing leaves, is popular with the characteristics of antimalarial and anticancer component [5] [6] [7]. In addition, it can also treat snake bites, tuberculosis, heal fractures, increase blood circulation, and cold in term of traditional medicine method [7].

There are many methods of extracting fluid from the plant to produce high quality product but the factor of environmental issues also need to be considered. The conventional extraction method [8] is limited and less sufficient because the method only extract few components from the plants [9]. It is because some plants are sensitive with the condition of high temperature during extraction process [10]. Besides that, it also consumes more time and use large amount of solvent. Thus, it will generate considerable amount of waste and will contaminate the sample [11] as well as contribute to environmental issues.

According to the research, almost 90 production industries [12] use Supercritical Fluid Extraction (SFE) in their production process. SFE method is favorable in industries because the extract obtained by SFE has higher quality extraction compared to other methods such as conventional organic solvent extraction [13]. Besides that, SFE method also has other advantages over conventional method in term of time reduction, less organic solvent volume, and more selective extraction [14][15].

Response surface methodology (RSM) is a statistical technique used to evaluate the effect of multiple factors [16] [17] [18]such as temperature, pressure, and particle size in order to identify the optimal condition for a selected response while minimizing the number of experiment required [19]. UV–vis Spectrophotometer (UV-vis) is known as a component analysis [20] and favored by most laboratories with identification and measurement of organic and inorganic compound [21]. The advantages of UV-vis are wide application, high sensitivity, good accuracy and ease, as well as convenient to analyze.

The aim of this study was to optimize the antioxidant components (based on antioxidant activity analysis) from the MCV leaves extract utilizing SFE equipment and analysed using Response Surface Methodolgy (RSM) in the Design Expert 10 software. The extraction factors which are temperature, pressure, and particle size, were chosen as the independent variables for the extraction and their influence on the antioxidant activities was studied through a Central Composite Design (CCD). The content of the antioxidant activities was determined using UV-vis analysis with a validated method. There were few previous studies related to the MCV extractions using SFE [22][23] but none of them were focusing on the optimization using RSM. If optimization was not carried out in any process, it would trigger unnecessary waste to be generated from the extraction process, thus raising the energy utilization and manufacturing cost [24].

2. Materials and methods

2.1. Apparatus and instruments

Instruments, such as Perkin Elmer (Lambda 25) UV-vis was used to analyze antioxidant activity and Memmert oven to remove the moisture content in the MCV leaves. AS200 sieve shaker was used to separate the raw material into uniform sizes. Analytical balance was also used to weigh the mass of grinded samples for SFE unit. The model of analytical balance was Shimadzu. Besides that, laboratory-scale SFE unit was also used to extract the MCV leaves fluid. The model was Thar SFC which is available in the university.

2.2. Design of experiment using response surface methodology

In this experiment, three factors of manipulated parameter were used to evaluate the antioxidant activity. The manipulated parameters were Temperature (°C), Pressure (bar), and Particle Size (µm). The design parameters are shown in Table 1. Design Expert version 10 (DE 10) was used and parameters were inserted into DE 10 and analyzed by using the Central Composite Design (CCD) method.

| Table 1. The design parameters |
2.3. Sample preparation of MCV leaves

MCV leaves were collected from the local area and rinsed with deionized water. To ensure that the leaves had been dried completely, they were left in the oven at 35 °C for two days. The moisture content in the sample should be below than 10 % in order to ensure no difficulty and problem arisen during the SFE extracting process which might affect the antioxidant activity [25]. The MCV leaves were grinded and sieved into different sizes from 63 µm to 1000 µm (63 µm, 125 µm, 250 µm, 500 µm, and 1000 µm).

2.4. Supercritical fluid extraction

The manipulated parameters to run the SFE were obtained from DE10. The range of pressure and temperature were between 100-300 bar and 30-70 °C with constant extraction time which was 1 hour per sample. The sizes of particles samples for SFE were between 63 µm -1000 µm. The co-solvent ethanol amount was 10 % per sample. The CO$_2$ flow rate was 20 g/min.

2.5. Analyzed sample using UV-vis spectrophotometer

Antioxidant activity was measured by using UV–vis Spectrophotometer. Sample analysis was prepared by mixing up 0.024 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) with 100 ml of ethanol in a volumetric flask to produce 0.6 Mm DPPH solution. Then, the DPPH solution was shaken vigorously to ensure a thorough mixing and stored in a dark place for 30 minutes. After 30 minutes, 1.5 ml of extracted sample was added into 1.5 mL of DPPH solution in the volumetric flask. The solution was mixed well and covered with aluminum foil and stored in a dark room for 30 minutes. Each sample was made into triplicate and stored in the dark place. Absorbance of all samples was recorded at a wavelength of 517 nm. The absorbance of sample and control were calculated in term of antioxidant activity in percentage, based on the formula below:

\[
\% \text{ DPPH} = \frac{(A_{\text{control}} - A_{\text{sample}})}{A_{\text{control}}} \times 100
\]

\[A_{\text{control}} = \text{Absorbance of control}
\]

\[A_{\text{sample}} = \text{Absorbance of sample}
\]

3. Results and discussions

3.1. Optimization of antioxidant activity by using response surface methodology (RSM)

RSM is commonly used because of its effectiveness and convenience in optimization process. The study was conducted using central composite design (CCD) with RSM. Three independent (manipulated parameter) variables were selected and used in the study which were temperature, pressure, and particle size. The analysis data for antioxidant activity (AA) is presented in Table 2. The lowest antioxidant recorded experimentally was 8.20 % and the highest antioxidant was 49.76 %. Lower absorbance indicates higher free radical scavenging activities. Maximum percentage (%) indicates high scavenging activities, thus higher DPPH is good indication for antioxidant. The antioxidant activity was high with higher free radical scavenging abilities.
Table 2. List of independent variables level in CCD, experimental and RSM predicted results.

| Run | Temperature (°C) | Pressure (bar) | Size (µm) | Experimental AA (%) | RSM Predicted AA (%) |
|-----|------------------|----------------|-----------|---------------------|----------------------|
| 1   | 50               | 250            | 250       | 47.14               | 44.91                |
| 2   | 70               | 250            | 250       | 8.20                | 8.28                 |
| 3   | 50               | 250            | 250       | 42.17               | 44.91                |
| 4   | 40               | 300            | 500       | 31.37               | 33.01                |
| 5   | 50               | 250            | 250       | 37.09               | 44.91                |
| 6   | 40               | 300            | 125       | 27.30               | 28.20                |
| 7   | 50               | 250            | 250       | 48.90               | 44.91                |
| 8   | 60               | 200            | 125       | 14.79               | 12.41                |
| 9   | 60               | 300            | 500       | 41.92               | 44.91                |
| 10  | 50               | 250            | 250       | 49.76               | 44.91                |
| 11  | 30               | 250            | 250       | 24.49               | 24.33                |
| 12  | 50               | 250            | 63        | 22.14               | 29.85                |
| 13  | 50               | 250            | 1000      | 43.07               | 42.09                |
| 14  | 50               | 250            | 250       | 48.61               | 44.91                |
| 15  | 40               | 200            | 500       | 44.05               | 45.45                |
| 16  | 60               | 300            | 125       | 18.57               | 17.99                |
| 17  | 50               | 150            | 250       | 22.14               | 25.01                |
| 18  | 50               | 350            | 250       | 23.09               | 20.72                |
| 19  | 50               | 250            | 250       | 47.56               | 44.91                |
| 20  | 40               | 200            | 125       | 36.45               | 32.92                |

3.2. Model analysis
The relationship between independent variables which were temperature, pressure and size of particle were well fitted with the quadratic model in RSM to optimize the antioxidant activity. The quadratic regressions model for antioxidant activity from CCD in term of factors is presented in the Equation below and the predicted AA values are as per Table 2. The coded stand for AA = Antioxidant activity (%), A = Temperature (°C), B = Pressure (bar), C= Size of particle (µm).

Equation:

\[
AA = + 55.71 +8.49* A - 7.94* B + 6.12* C + 10.29 * AB +27.48* AC - 9.65 * BC - 28.6 0* A^2 - 22.05 * B^2 -19.74 * C^2
\] (2)

The significant of analysis of variance (ANOVA) of the model is found to be significant with value of probability (P > 0.0002) as shown in Table 3. P-values smaller than 0.05 imply that model terms are significant while greater than 0.1000 imply that model terms are not significant [26][27]. Thus C, AC, A^2, B^2, and C^2 are also significant model term. The determination of correlation coefficient (R^2) for the test model in CCD method and quadratic model was 0.9251. The Lack of Fit F-value was 1.30 which implies the Lack of Fit is not significant relative to the pure error. The higher the number Lack of Fit F-value, there is more possibility that the model does not fit the data effectively, and hence, not-significant means it was good for the model.

Table 3. ANOVA analysis
3.3. Effect of parameters
The relationship between temperature, pressure, and particle size was illustrated from the Design Expert 10 in 2D and 3D response surfaces. The illustrated figures are shown in Figure 1 which is about relationship between pressure and temperature, Figure 2 about relationship between sizes and temperature, and lastly Figure 3 about effect of pressure and sizes. Apparently, the three variables show quadratic trends where the optimal region lay in the middle of the curve instead of plateau.

![Figure 1. Response surface plot for the effect of temperature and pressure.](image)
Figure 2. Response surface plot for the effect of temperature and size.

Figure 3. Response surface plot for the effect of pressure and size.

3.4. Validation of the model
Numerical optimization was used to predict the maximum antioxidant activity from the combination of independent variable. The best local maximum point was selected based on the maximized desirability which is close to 1.000. Based on the recommended optimal condition, the predicted antioxidant activity
(AA) was 50.62 % at temperature of 50 °C, pressure at 282 bar, and particle size of 500 µm. In order to test the accuracy of the predicted optimal, validation runs were carried out and the average AA obtained was 50.14 % and thus, the average error was 0.95 %. The verification result analysis indicated that the experimental value was close to the predicted value. Thus, the model proposed was satisfactory and accurate even though there was a small error which might due to some noise during measurement of the MCV leaves sample during the sample preparation and extraction process.

4. Conclusion

Different antioxidant activities were evaluated according to the different condition of parameters. The higher antioxidant activity was obtained from the experimental condition which was 49.76 %. After the optimization using numerical optimization, the predicted optimal condition was compared with the experimental condition. The optimized experimental antioxidant activity value was close to the predicted value with an average error of less than 1 %. The optimum conditions were temperature at 50°C, pressure at 282 bar, and particle size of 500 µm.

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