Extraction Bioactive Compound of Pegagan (Centella Asiatica L.) using Solvent-Free Microwave-Assisted Extraction

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Abstract. Gotu Kola (Centella asiatica L.), known as pegagan is a plant with many benefits, including used to repair burns, hypertrophic wound healing, and as an inflammatory agent. C. asiatica grows in tropical Asia and grows in various countries such as Indonesia, Philippines, China, India, Sri Lanka, and Madagascar. C. asiatica has four bioactive components, namely asiatic acid, asiaticoside, madecassic acid, and madecassoside. Microwave-Assisted Extraction (MAE) is one of the methods for extracting bioactive compounds of pegagan leaf. In this study, the extraction of pegagan bioactive compounds carried out using the Solvent-Free Microwave-Assisted Extraction (SFME) method. This research used a box-behnken design model for the optimization process. The variables used are mass of material (20, 40, 60 g), power (150, 300, 450 W), and time of extraction (30, 60, 90 min). The highest yield is 4.5474% at 450 watt microwave power, 20 g mass pegagan leaves, and 60 min extraction time. Mass of material is the factor that has significant effect on yield. Modelling from this research is appropriate which has lack-of-fit > 0.1, and R² 89.67%. The content of bioactive compounds carried out by analyzing the total phenolic content of the product. The most substantial phenol content is 2398.649 μgAGE/ml.

1. Introduction

Indonesia has many natural resources that have not been used optimally. Gotu kola (Centella asiatica L.) has another name Gotu kola or Tiger grass including in the genus Centella, family Apiaceae, kingdom plantae Gotu kola (Centella asiatica L.) Gotu kola plant grows in tropical Asia, such as Indonesia, the Philippines, China, India, Sri Lanka, and Madagascar [1]. In traditional Asian medicine, cc [2]. In addition, C.asiatica has many nutrients, vitamins, minerals, and antioxidants and is useful for improving memory, slowing the aging process, and blood circulation [3]. Gotu kola or C. asiatica has 4 bioactive components, asiatic acid, asiaticoside, madecassic acid, and madecassoside [4]. Centella asiatica has function to repairing small wounds, scratches, burns, hypertrophic wound healing, and functions as anti-photoaging and inflammatory agents [4][2]. Bioactive compounds extraction could be done by several methods, Soxhlet extraction [5], viscozyme [3], subcritical water [4], and microwave-assisted extraction (MAE) [6] [7] methods. In this study, the bioactive compound extraction method will be carried out by using microwave-assisted extraction without using solvents. Microwave-assisted extraction (MAE) technique has many advantages compared to other methods for extraction of bioactive compounds, higher extraction rate, saving in solvent and time, have better quality products with lower costs, and has good performance under atmospheric conditions [6].

From previous studies that have been carried out in the extraction of bioactive compounds by the method of Microwave-assisted extraction (MAE), research conducted by Hiranvarachat [7] the comparison of atmospheric methods and vacuum-assisted microwave extracted extractions to extract...
bioactive compounds from *C. asiatica* plants. In this study, *C. asiatica* plants used were fresh *C. asiatica* leaves and dry *C. asiatica* leaves, while the solvent used was ethanol. The results of the study were that the fresh leaves obtained the optimal yield at 60 kPa, while the dried leaves obtained the best results at atmospheric conditions. Another study states that by using the MAE method with ethanol recruitment, the yield of bioactive compounds is two times greater than soxhlet extraction [5]. This research will use the Solvent-free Microwave-assisted Extraction (SFME) method for extraction of *C. asiatica* plant bioactive compounds, the superiority of the SFME method is that it does not use solvents in the extraction process, the extraction process runs faster, and the extracted components have good quality [8]. Besides, the use of solvents will produce residues in the product so that it will affect the quality of the extract produced and can cause serious health problems if it enters the human body [4]. The objective of this study is to optimize the extraction of *C. asiatica* by using SFME method, the optimal conditions of the extraction process using box-behnken model. All data was analysis using statistical analysis with minitab® software.

2. Material and Method

2.1. Material

Pegagan (*Centella asiatica L.*) was purchased from Banyuwangi, Indonesia. Pegagan leave was harvest every 2 months. In this study used dry leaves for extraction process, the dry leaves have longer storage time than fresh leaves. The leaves were stored at room temperature as required.

2.2. Solvent-free Microwave Extraction

In solvent-free microwave extraction method, we used microwave oven (EMM2308X, Electrolux, maximum delivered power 800 W) with frequency of 2450 MHz. The dimension of the PTTE-coated cavity of the microwave oven were 48.5 x 37.0 x 29.25 cm. The microwave oven was modified by drilling a hole at the top, used a round bottom flask with a capacity 1000 ml was placed inside the oven and was connected to the Clevenger apparatus through the hole. Then the hole was closed with PTFE to prevent any loss of the heat inside. This apparatus design adopted by Heri Septya [9]–[12]. Solvent-free microwave extraction procedure at atmospheric pressure, mass of raw material 20, 40, 60 gram were wetted before extraction process by soaking for 30 minutes, then removed the excess aquadest: Wet material was placed in 1000 ml flask. Power level of microwave (150 W, 300 W, dan 450 W) for 30, 60, and 90 minutes.

2.3. Experimental design

To determine the optimal condition of solvent-free microwaved extraction used Box-Behnken design (BBD) 3-factor 3-level with 5 replicates in center point. Factor parameter are microwave power (A), time extraction (B), mass of material (C) were chosen as independent variable. Experimental parameters for BBD design was perform in table 1. Yield as the response of this study.

| Table 1. Experimental parameters for Box-Behnken design |
|--------------------------------------------------------|
| Factor       | Symbol | Unit | Low | Medium | High |
|--------------|--------|------|-----|--------|------|
| Microwave Power | A      | W    | 150 | 300    | 450  |
| Extraction Time | B      | Min  | 30  | 60     | 90   |
| Mass of Material | C      | g    | 20  | 40     | 60   |
2.4. **Total Phenolic Content Analysis**

Total phenol analysis was carried out to determine the presence of bioactive components in the sample. The total phenol analysis was performed using a spectrophotometer model U-2900 with Gallic Acid standard solution. The wavelength used is 550 nm. The total phenol content will be used to determine the yield of bioactive compounds.

2.5. **Statistical Analysis**

The data were analysed using Analysis of Varian (ANOVA) with confident level 90%. A representative ANOVA analysis to describe normality, homogeneity and significant variables affects the outcomes of this study. All experiment were performed in duplicate.

3. **Result and Discussion**

3.1. **Effect of experimental factor to yield extraction**

In this study, the optimization extraction of pegagan using the Box-Behnken design optimization method, this model will provide smaller number of experimental runs and can determine the factors that contribute significantly to the product. This research focused on the optimal conditions in the Solvent-free Microwave Extraction (SFME) process. From the BBD model with 3-factor 3-level with five replications at the center point, a combination is generated. In table 2 we can see Box-Behnken experimental design and their response.

| Run | A (W) | B (min) | C (g) | Yield (%) |
|-----|-------|---------|------|-----------|
| 1   | 150 (-1) | 30 (-1) | 40 (0) | 2,3317    |
| 2   | 450 (+1) | 60 (0)  | 60 (+1) | 3,9978    |
| 3   | 300 (0)  | 60 (0)  | 40 (0)  | 2,2252    |
| 4   | 300 (0)  | 60 (0)  | 40 (0)  | 1,8671    |
| 5   | 450 (+1) | 30 (-1) | 40 (0)  | 2,6707    |
| 6   | 300 (0)  | 90 (+1) | 60 (+1) | 1,6143    |
| 7   | 150 (-1) | 60 (0)  | 20 (-1) | 4,5474    |
| 8   | 450 (+1) | 60 (0)  | 20 (-1) | 5,2254    |
| 9   | 300 (0)  | 60 (0)  | 40 (0)  | 2,4841    |
| 10  | 450 (+1) | 90 (+1) | 40 (0)  | 0,9865    |
| 11  | 300 (0)  | 90 (+1) | 20 (-1) | 2,6761    |
| 12  | 150 (-1) | 60 (0)  | 60 (+1) | 1,6515    |
| 13  | 300 (0)  | 30 (-1) | 20 (-1) | 3,6709    |
| 14  | 150 (-1) | 90 (+1) | 40 (0)  | 1,8343    |
| 15  | 300 (0)  | 30 (-1) | 60 (+1) | 1,9459    |

The effect of experimental factors on yield was analyzed using ANOVA with a confident level of 90% or $\alpha = 0.1$, the results of the analysis indicated that the factors affected yield if the p-value <0.1. Table 3 shows summarize the ANOVA result of the quadratic model. Microwave power is one of the factors used in this study. Microwave power is a critical factor because it can affect the yield of bioactive compounds. The effect of microwave power in the extraction process is, the higher the power used, the
more the solubility and diffusivity levels of the extracted raw material will increase. Besides, high microwave power will also increase the pressure in the cell wall to stimulate release of target compounds [13]. The result of ANOVA on power has a p-value of 0.225 where the p-value is more than $\alpha$, which microwave power in this study does not have a significant effect on yield. The other factor, extraction time, which is also a crucial factor in the SFME process. In the extraction process, the extraction time is mostly proportional to the yield produced [14]. ANOVA results showed that the extraction time did not significantly affect yield with a p-value of 0.112. The last factor is the mass of the material used. The mass of the material used is related to the density factor of the material. The material density factor is the ratio of the raw material to the volume capacity of the distiller flask, the mass of the material used is also very influential on the yield produced [9]. From ANOVA analysis, the mass of raw materials is a factor that has a significant effect on yield with a p-value of 0.013 where this value is less than the value of $\alpha$. So from this study, it can be concluded that the factors that have a significant effect on yield are the mass of raw materials $>$ extraction time $>$ microwave power.

### Table 3. ANOVA data and the effects of SFME condition on the response of bioactive compound yield

| Source          | DF | Adj SS   | Adj MS   | F-Value | P-Value |
|-----------------|----|----------|----------|---------|---------|
| Model           | 9  | 17,9458  | 1,99397  | 4.82    | 0.049   |
| Linear          | 3  | 8,2982   | 2,76606  | 6.69    | 0.033   |
| A               | 1  | 0.7910   | 0.79100  | 1.91    | 0.225   |
| B               | 1  | 1.5382   | 1.53821  | 3.72    | 0.112   |
| C               | 1  | 5.9690   | 5.96897  | 14.44   | 0.013   |
| Quadratic       | 3  | 8,4897   | 2,82991  | 6.85    | 0.032   |
| A*A             | 1  | 1,2046   | 1,20456  | 2.91    | 0.149   |
| B*B             | 1  | 2,4080   | 2,40798  | 5.82    | 0.061   |
| C*C             | 1  | 4,4044   | 4,40441  | 10.65   | 0.022   |
| 2-Way Interaction| 3  | 1,1579   | 0,38595  | 0.93    | 0.490   |
| A*B             | 1  | 0,3521   | 0,35209  | 0.85    | 0.398   |
| A*C             | 1  | 0,6958   | 0,69581  | 1.68    | 0.251   |
| B*C             | 1  | 0,1100   | 0,10996  | 0.27    | 0.628   |
| Error           | 5  | 2,0670   | 0,41339  |         |         |
| Lack-of-Fit     | 3  | 1,8750   | 0,62499  | 6.51    | 0.136   |
| Pure Error      | 2  | 0,1920   | 0,09599  |         |         |
| Total           | 14 | 20,0127  |         |         |         |

3.2. **Response Surface Methodology Modeling**

Data from the experiment using BBD were evaluated by regression analysis. Table 3 is the result of ANOVA analysis with a quadratic model. The F-test and p-value determined the significance of the suggested statistical model. The F-value is obtained by dividing the mean square by the residual mean square. That means a large F-value with a small p-value indicates the significance of the factor used and illustrates the appropriate relationship between the actual conditions and the results predicted by the model [15]. The results obtained show a large F-value with a p-value $<$ 0.1; this indicates that the model used is appropriate. Lack-of-fit variance is another statistic to determine the accuracy of the suggested model. If the p-value of the lack-of-fit is more than $\alpha$ ($\alpha = 0.1$), then the model used is correct and can
be used to predict the experimental results. The statistical analysis found that the p-value of lack-of-fit was 0.136, where the p-value was > 0.1, so it could be said that the model in this study was appropriate. The distribution point determines the suitability of the predicted and actual values of the model on a straight line, which indicates that the quadratic model can be used to predict the actual value. The acceptable value of $R^2$ is higher than 0.75 [16]. $R^2$ value for this study were found of 89.67%, which higher than 75%. Thus this model is reliable and precise, and the quadratic model was sufficient to represent the experimental data and can be shown in the following equation:

$$Y = 8.74 - 0.0147A + 0.1018B - 0.3199C + 0.000025A^2 - 0.000897B^2 + 0.002730C^2 - 0.00066AB + 0.000139AC + 0.000276BC$$

(1)

3.3. Optimization of SFME extraction to yield

Figure 1 shows the main effect plot for the three factors with three levels. This figure shows a visualization of the primary influence of several factors used, from Figure 1 it can be seen that the optimal conditions of each factor used on the yield. In factor A, the highest microwave power yield was found at 450 W microwave power. The higher the microwave power, the higher the evaporation rate so that the extracted yield will also be higher [17]. Factor B is time, the optimal extraction time is 40 - 60 min, it can be seen extraction time curve in Figure 3, the extraction time of 90 minutes there is a decrease in yield, this can cause the extract yield with increasing temperature variations which can cause thermal decomposition of some of the volatile compounds [17]. Whereas for factor C, the mass of raw materials, the smallest mass of raw material, 20 g, produced the largest yield. The mass of the material used is related to the density factor of the material. The material density factor is the ratio of the raw material to the volume capacity of the distiller flask. The mass of the material used is also very influential in the yield produced. When the density of raw materials is too high causes rat holes, which can lead to a decrease in the yield of bioactive compounds [14]. The optimal operating conditions of this study are at a microwave power of 450 W, extraction time of 40 - 60 minutes, and the mass of raw materials 20 grams with a yield of 5.2254%. In comparison, the optimization of the RSM model gave optimal results at a microwave power of 450 W, extraction time of 43.33 minutes, and a mass of 20 g of raw material with a predicted yield of 4.867%. From the two optimization results above, the yield is not too far away between the actual and predicted results, so it can be concluded that using the RSM approach can be used and can be accepted to predict future results.

3.4. Bioactive Compound in Centalla Asiatica Extract

The content of bioactive compounds represented by the total phenolic content in the sample was analyzed using the spectrophotometric method at the UPT Integrated Laboratory and Technology Innovation Center (CDAST), University of Jember. The analysis of total phenol content was carried out because phenolic compounds, the most abundant secondary metabolites in plants [18]. C. asiatica plant extracts revealed various biochemical constituents such as polyphenols and carotenoids [19]. From the analysis of total phenol content, the highest total phenol content was 2398.69 $\mu$g GAE / mL or equivalent to 2.39869 mg GAE / g. Meanwhile, from other studies, it was found that the total phenol content was 9.03 mg GAE / g [19]. From the results obtained from the research we conducted, when compared to other studies, it has a large enough difference, this is because in this study we did not use solvent, while other studies used solvent to extract bioactive compounds in raw materials. The function of this solvent has a significant effect, the mixture of ethanol and water as a solvent shows excellent performance to extract polyphenols from plant raw materials. Apart from that, research has shown that polyphenols are more comfortable to extract using polar solvents [19].
Figure 1. Main effects plot to yield

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