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Optimization of Terpenoid Extraction from Bay (*Syzygium polyanthum*) Leaves using Ethanol as a Solvent by an Ultrasonic Wave Technology

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ABSTRACT

Bay is one of the medicinal plants. Bay leaves have been used in the pharmaceutical and food industry as an antimicrobial. The chemical compounds in the leaves that served as an antimicrobial were terpenoids. This study aims to determine the optimum extraction conditions of bay leaves using a response surface methodology (RSM). Ten grams of dried and mashed bay leaves was extracted for 60 minutes using ethanol as a solvent. The extraction was conducted at various temperatures (60, 70, 80 ^{o}C) and various ratios of the starting material to solvent (1: 8, 1:10, 1:12 g/g) for 60 min. The optimum extraction condition based on the RSM results was achieved at 70 °C with a ratio of the starting material to solvent of 1:12 g/g resulting in the extract yield of 9.69%. The results of the GC-MS analysis on the obtained extracts showed that 33% of terpenoids was obtained from the extraction at the optimum condition.

Keywords: bay leaf; RSM; terpenoids; ultrasonic extraction.

1. INTRODUCTION

Bay leaf is abundantly available in Indonesia with a total plantation area of 47,530 ha and export value of 57,428 tons in 2016 (Central Bureau of Statistics, 2016). Bay leaves are efficacious as a crop and food flavoring. Bay leaves have been utilized in the pharmaceutical and food industries as antimicrobials (Dewanti & Wahyudi, 2011). It has been reported that bay leaf extract could inhibit the growth of *Escherichia coli* and *Staphylococcus aureus* by 40% and approximately 5%, respectively (Fitriani et al., 2005). The ethanol-extracted bay leaves with a concentration of 1% was reported to be able to kill C. *albicans* on a disc paper (Dewanti et al., 2011). The compounds that typically showed an antimicrobial property were phenolics (e.g. flavonoids and tannins), terpenoids, fatty acids, and alkanoids (Rahayu, 1999). The bay leaf extract contained terpenoids (32.87%), alcohols (20.35%), esters (25.85%), and eugenol (21.56%) (Guenther, 1990). Terpenoids was reported as the most abundant component in the bay leaf extract due to the existence of their derived compounds such as sesquiterpene, monoterpene, and diterpene.

Ethanol could extract bay leaves with a yield of 11.5% for a 24-hours extraction time (Murhadi et al., 2007). The more solvent used; the more filtrate would be. The extraction using ethanol as a solvent produced higher yields of extract than that using water in the same condition (Setyawan et al., 2012). The ultrasonic-aided extraction of bay leaves has not been reported before. The use of ultrasonic-aided extraction could increase the yield of the extract as previously reported (Tian et al., 2012) compared to the Soxhlet and supercritical extractions. Moreover, it was also reported that the ultrasonic-aided extraction could give a faster extraction time (Fuadi et al., 2012). In this study, the ultrasonic-aided extraction was conducted to shorten the extraction time and improve the yields of terpenoids from bay leaves.

The optimum condition of the extraction of bay leaves was determined using a Response Surface Technology (RSM). This technique was reported as an efficient method in the optimization of the variables affected the corresponding process. This technique was combined with mathematical and statistical techniques for the design of the extraction experiments of bay leaves and used to analyze the problems where the independent variables (i.e. temperatures and ratios of feedstock-to-solvents) would affect the response variable of bay leaves extract.

2. METHODS

The extraction experiments used an ultrasonic wave and were conducted at various temperatures of 60, 70, 80 ^{o}C with the ratios of starting material to solvent of 1: 8, 1:10, and 1:12. An ultrasonic cleaning bath was used in the extraction experiments. A simple sketch of ultrasonic-aided apparatus used in this study is presented in **Figure 1**. A blender, a sieve of 60 mesh, a digital scale, a 200 mL one-neck boiling flask, a set of ultrasonic-aided extraction apparatus, a 25 mL measure pipette, filter papers, and watch glass were used during the preparation of the bay leaves as the starting material and/or the extraction experiments.

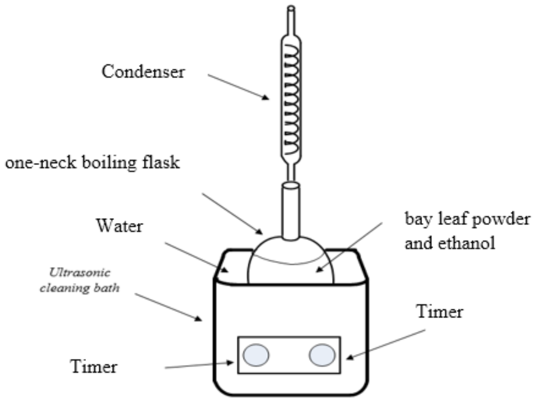

Figure 1. Schematic diagram of the ultrasonic-aided extraction apparatus used in this study.

Bay leaves from Sampangan market, Central Java, Indonesia were used in the extraction experiments. The leaves used were old bay leaves with dark-green color. This kind of bay leaves would give more bioactive compounds (Bahriul et al., 2014). The increase in the content of bioactive compounds was probably due to the exposure of the leaves to direct sunlight during the synthesis of bioactive compounds (Ghulamahdi et al., 2008).

The bay leaves were washed with water 2-3 times and dried under sunlight. The dried leaves were crushed and sieved using a 60-mesh sieve. A10 g of bay leaf powder was added with various weight of ethanol (80-120 g) in a 200 mL one-neck flask. A set of an ultrasonic-aided extraction apparatus was prepared as is shown in Figure 1. The extraction experiments were carried out at various temperatures (60-80°C) for 60 minutes. The mixture obtained from each experiment was filtered using a filter paper and separated between the residue and filtrate. Furthermore, the filtrate was separated from the solvent using a rotary vacuum evaporator at 40 kPa and 35 °C. The extract obtained was separated from the solvent and recovered. The weight of extract obtained was designated as “yield”. The yield of the obtained extract was determined using equation (1).

$$\text{Yield (\%)} = \frac{\text{weight of extract recovered}}{\text{weight of bay leave feedstock}} \text{ x } 100\% \quad (1)$$

An optimization of the extraction condition was also studied using a Response Surface

Technology (RSM) technique. The first step of this technique was determining the suitable approximation to express the relationship between dependent Y and (chosen) independent (X) variable). An optimum region that would be used in the experiments would be obtained from this step. A 2k design was used as first order model design. A polynomial regression model was formulated using equation (2), where Y is response, k is variable number, x_i is independent variable, I could be 1,2,3,....k, β_0 is a constant, β_i is predictor coefficient, and ϵ is an error.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \epsilon \quad (2)$$

If a curvature was found in the system, a second order polynomial model was used. This second order model would allow the determination of points with an optimum response. Equation (3) was used for this case.

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j}^{k} \beta_{ij} x_i x_j + \epsilon \quad (3)$$

The extract from the experiment at the optimum condition was analyzed using a Perkin Elmer gas chromatograph equipped with a mass detector to determine the components of the leaf extract. A Rtx-5 MS capillary column with 30 m length, interval diameter of 0.25 mm and 0.25 µm thickness was used in the gas chromatograph. The analysis using the gas chromatograph was performed using helium as a carrier gas (20 mL/min) with an injector temperature of 280°C and an initial column temperature of 70°C. The column temperature was ramped up with a heating rate of 10°C/min to 280°C. A Perkin Elmer Elite-5 MS library was used in the MS detector to identify the separated compounds in the GC.

3. RESULTS AND DISCUSSION

In this study, the extraction of bay leaves was carried out using an ultrasonic wave extraction method. Dried and ground bay leaves were used as the feedstock. The drying process would reduce the water content of the leaves close or less than 10% and allow the active compounds to be extracted by the solvent (Febriani et al., 2015). Grinding process would allow an increase in the surface area that enables a more effective interaction between the solvent (ethanol) and the feedstock.

3.1. Effect of Temperatures and Ratios of Feedstock to Solvent on the Extract Yields

The use of solvents will greatly affect the acquisition of bay leaf extract. The extraction of bay leaves in this study was conducted at various temperatures (i.e. 60, 70, 80 °C) and ratios of feedstock to solvent (i.e. 1:8, 1:10, 1:12 g/g). The yields of bay leaf extract at various extraction temperatures and ratios of starting material to solvent can be seen in **Figure 2**. The highest extract yield was achieved by the extraction at 70°C with a ratio of feedstock to solvent of 1:12.

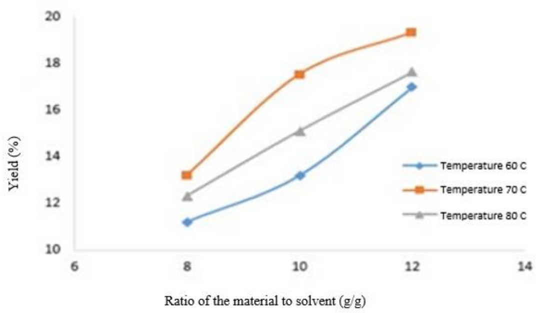

Figure 2. Effect of weight ratios of feedstock to solvent on the extract yields at different extraction temperatures in the ultrasonic-aided extraction with an ultrasonic power of 40 kHz.

The data in **Figure 2** indicated the increase in the extract yield by increasing the amount of the solvent used. The highest extract yield was obtained in the ultrasonic-aided extraction with a weight ratio of feedstock to solvent of 1:12 at all temparatures studied. The amount of solvent used in the extraction would affect the contact area of the solid feedstock with the solvent. The more the solvent used, the greater the contact area between the solid feedstock and the solvent. As a result, the interaction of the solvent to the solid is more evenly distributed so that the resulting yield was even greater (Jayanudin, 2014). In the liquid-solid extraction, the greater surface interaction would allow a significant diffusion process of the solvent toward the solid feedstock resulting a more cell-fluid discharge (Treyball, 1980). The addition of a solvent enables the expansion of the pores of the solid feedstock and then dissolves the active components in the feedstock by diffusing out the surface of the solid particles and moving to the film layer around the solid, subsequently to the liquid body (Putri et al., 2016).

An extraction at a higher temperature would allow the solvent to diffuse to the pores of the solid feedstock (Putri et al., 2016). The highest extract yield was obtained at the extraction temperature of 70°C at all weight ratios of feedstock to solvent studied. A further increase in the extraction temperature might cause the structure of compounds in bay leaf extract to be easily evaporated. This was possibly due to the high susceptibility of sesquiterpene (one of terpenoids) to degrade by increasing temperature (Robinson, 2005).

3.2. Optimization of the Extracton of Bay Leaves Using Response Surface Methodology (RSM)

In this study, the optimization of extraction was conducted using a Design-Expert software i.e. Response Surface Methodology (RSM) with a Central Composite Design (CCD). The RSM with CCD was used to optimize the number of experiments need to be done on the extraction of bay leaves. The results of the variation ratio of the ratios of feedstock to solvent and extraction temperatures at this RSM can be seen in **Table 1.**

Table 1. The results of the variation ratio of the ratios of feedstock to solvent and extraction temperatures in the RSM.

Variable	lowest coefficient (-1)	0	highest coefficient (1)
Temperature (°C)	60	70	80
Ratio of feedstock to solvent (g/g)	1:8	1:10	1:12

Table 2. Experimental and predicted yields of the bay leaf extract at various temperatures and ratios of feedstock to solvent using an ultrasonic-aided extraction results and response surface methodology.

Run	A: Temperature	B: ratio of feedstock to solvent	Yield by experiment (%)	Yield by model (%)
1	0	-1	13.202	13.97
2	0	0	17.528	17.3
3	-1	0	13.198	14,11
4	1	0	15.115	15.33
5	0	0	17.528	17.3
6	0	0	17.528	17.3
7	0	0	17.528	17.3
8	1	-1	12.326	12.12
9	0	0	17.528	17.3
10	-1	-1	11.211	10.66
11	0	1	19.317	19.69
12	1	1	17.624	17.6
13	-1	1	16.987	16.62

The experimental and predicted yields of the bay leaf extract (as is presented in **Table 2**) showed a good suitability. The highest yield of bay leaf extract was obtained in the ultrasonic-aided extraction at a ratio of feedstock to solvent of 1:12 and a temperature of 70°C which yielded an extract yield of 19.317%, with a good suitability with the predicted yield (19.69%) that were analyzed using the Design-Expert software version 10.1. The suitability of the regression model and the significance of the estimated variables were determined by the Analysis of

Variance (ANOVA). In the ANOVA data, several values affected the compatibility between the experimental and the model prediction results. The condition of the model was not suitable according to the experiment if the value of lack of fit was less than alpha ($\alpha = 0.05$). If the p-value was less than alpha ($\alpha = 0.05$), so that it would be concluded that there was a lack of fit in the model (Octaviani et al., 2017). The α-value is the value of failure allowed in the experiments. The smaller the chance of failure or error, the greater the value of confidence in the decision. The condition of the model is suitable according to the experiment if the value of lack of fit is greater than alpha ($\alpha = 0.05$), then the p-value is significant. A summary of the ANOVA results is shown in **Table 3**.

Table 3. Data analysis of variants (ANOVA) for the ultrasonic-aided extraction of bay leaves at various temperatures and ratios of feedstock to solvent.

source	Sum of Squares	Df	mean Square	F Value	p-value Prob> F
Model	76.69	5	15.34	45.73	<0.0001
A-temperature	2.24	1	2.24	6.69	0.0361
B-ratios	49.24	1	49.24	146.81	<0.0001
AB	0.057	1	0.057	0.17	0.6922
A2	18.32	1	18.32	54.62	0.0002
B2	0.62	1	0.62	1.84	0.2173
residual	2.35	7	0.34		
Lack of Fit	2.35	3	0.78		
Pure Error	0000	4	0000		
Cor Total	79.04	12			
R-Squared	97.03%				

A p-value on the model was less than 0.0001 (as is shown **Table 3**), which means p-value less than the significance level α of 0.05. It can be concluded that the model of the ultrasonic-aided extraction of bay leaves at various temperatures and ratios of feedstock to solvent well fitted the experiment results. The accuracy of this method can be seen from the coefficient of determination with R^2 value of 97.03%, indicating that the estimated yields from the model showed a good approximation to those obtained from the experimental results. This indicated that 97.03% of the total variation in the results obtained was well represented in the model (Arofisma et al., 2013).

The mathematical model obtained to predict the value of the yields of bay leaf extract in this study is written in equation (4).

$$Y = 17.30 + 0.61 (A) + 2.86 (B) - 0.12 (AB) - 2.58 (A2) - 0.47 (B2) \quad (4)$$

The significance of each coefficient was determined by the p- and f-values. A more significant coefficient on the model was assigned by the greater f-value and the smaller p-value. B (feedstock to solvent ratios) variable was the most influential variable due to its largest f-value and its smallest p-value. On the other hand, A (temperatures) variable, AB, A2, and B2 showed a less effect on the extract yields.

The results of model predictions (marked as “predicted”) were presented as a straight line and the experimental results (marked as “actual”) were symbolized by boxes. It was clearly shown by Figure 3 that the scattered actual values showed a good approximation to the predicted value (straight line). This model showed a low deviation and better precision toward the experimental data with a standard deviation and precision of 0.58 and 17.59, respectively. A low standard deviation value indicated a good accuracy for the corresponding model.

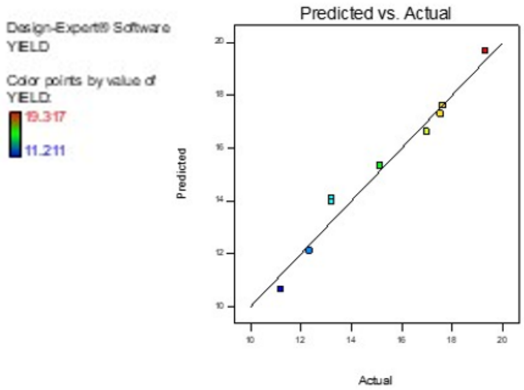

Figure 3. Relationship of the actual and predicted yields of the extract in the ultrasonic-aided extraction of bay leaved at 70°C with a ratio of feedstock to solvent of 1:12 g/g.

Figure 3 explained the extract yields obtained from the Design Expert software calculation (straight line) compared to the actual results from the conducted experiments (scattered data). The actual results (from the experimental data) were in the straight-line region. This indicated that the experiments carried out have good precision values. The results of the optimization of the extraction conditions performed using the software is shown in **Figure 4**.

Contour chart in **Figure 4** described the relationship between the parameters that influenced of the extract yields (i.e. extraction temperatures and weight ratios of feedstock to solvent) indicated by the different colors. This chart mainly indicated the relationship between the parameters of the extraction process conditions.

The red part in the graph showed the highest interaction between variable (19.317%) while the blue part was showing the lowest interaction between variable (11.211%). The points on the same curved line gave the same response value even though the process conditions were varied. Based on the results of the optimization that has been done using RSM, it was obtained that the optimum condition was achieved in the ultrasonic-aided extraction at 70°C with a weight ratio of raw material to solvent of 1:12 g/g with an extract yield of 19.693%.

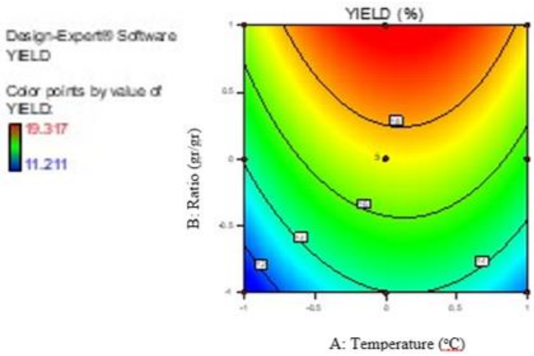

Figure 4. Optimization contour graph of the yields of extract in the ultrasonic-aided extraction of bay leaves at various temperatures and ratios of feedstock to solvent.

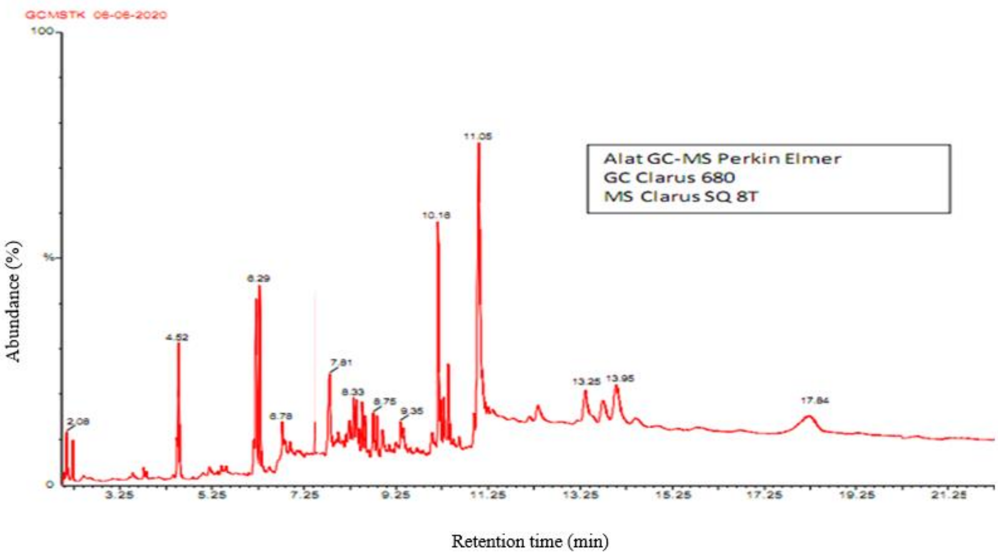

Figure 5. Chromatogram of the bay leaf extract in the ultrasonic-aided exraction at 70°C with a ratio of feedstock to solvent of 1:12 g/g and an ultrasonic power of 40 kHz.

Table 4. Identified terpenoids in the bay leaf extract obtained from the ultrasonic-aided extraction at 70°C with a ratio of feedstock to solvent of 1:12 g/g and an ultrasonic power of 40 kHz.

Retention time (min)	Identified compound	Relative concentration (%)
8.331	α-Himachalene	4
8.386	Eremophilene	4
8.386	δ-Cadinene	4
10.162	γ-Selinene	2
10.162	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	14
10.387	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	5

3.3. Qualitative Analysis of Terpenoids from Bay Leaf Extract Using Gas Chromatography Mass Spectroscopy (GC-MS)

The bay leaf extract obtained from the ultrasonic-aided extraction at at 70°C with a ratio of feedstock to solvent of 1:12 g/g and an ultrasonic power of 40 kHz was analysed by using a gas chromatograph equipped with a MS detector. The total ion chromatogram of the extract is presented in **Figure 5** and the identified terpenoids (based on the library) are listed in **Table 4**. **Table 4** clearly showed that the relative concentration of terpenoids was 33% of the total GC detectable compounds.

4. CONCLUSIONS

This study showed that the ultrasonic-aided extraction of bay leaves reached an optimum condition at 70°C with a weight ratio of feedstock to solvent of 1:12. This condition was able to result in an extract yield of 19.317%. The GC-MS result indicated that 33% of terpenoids were able to be recovered from the extract.

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