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Response Surface Methodology for Optimization Studies of Microwaveassisted Extraction of Sandalwood Oil

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Abstract

Microwave-assisted extraction (MAE) technique was employed to extract the essential oil from sandalwood (*Santalum album* L.). The optimal conditions for microwave-assisted extraction of sandalwood oil were determined by response surface methodology. A central composite design (CCD) was applied to evaluate the effects of three independent variables (microwave power (A: 400–800 W), plant material to solvent ratio (B: 0.10–0.20 g mL⁻¹) and extraction time (C: 40–120 min)) on the extraction yield of sandalwood oil. The correlation analysis of the mathematical-regression model indicated that quadratic polynomial model could be employed to optimize the microwave extraction of sandalwood oil. The optimal extraction conditions of sandalwood oil was microwave power 558.071W, plant material to solvent ratio 0.100274 g mL⁻¹ and extraction time 101.688 min. The maximum sandalwood oil yield was 0.655534 g/100g dry weight under these optimal conditions. Under the extraction condition, the experimental values agreed with the predicted results by analysis of variance. It indicated high fitness of the model used and the success of response surface methodology for optimizing and reflect the expected extraction condition.

Keywords: Extraction, Microwave, RSM, Sandalwood oil, Santalum album L.

1. Introduction

Essential oils, also known as etheric oils (volatile oil) produced by the plant. Essential oils can be obtained from roots, stems, leaves, and flowers of the plant. Essential oils have volatile properties at room temperature without decomposition, have a bitter taste (pungent taste), corresponding smells fragrant with the smell of plants, are generally soluble in organic solvents and insoluble in water. Sandalwood oil is widely used in the cosmetic, perfumery and aromatherapy industries [1].

Sandalwood tree (*Santalum album*) is one of the plants that contain essential oils contained in the stems and roots of sandalwood. Sandalwood oil has a high economic value. Most oil content in the sandalwood tree is located on the terrace which is part of the stem or roots that have a yellow to light brown color and very fragrant flavorful.

Some things that can be used as a solution to improve the quality and quantity of sandalwood oil, among others, is the sandalwood tree cultivation, distillation techniques and equipment used, treatment of raw materials, sandalwood oil refining process as well as product packaging of sandalwood oil. Sandalwood oil is the volatile oil obtained by steam distillation of the commutated dried wood from the trunk and roots of the plant *Santalum album* L. Another alternative to obtain sandalwood oil is to use a solvent extraction technique. But with that method takes quite a long time and further refining to obtain sandalwood oil.

In recent years, the use of microwave-assisted extraction (MAE) of constituents from plant material has shown tremendous research interest and potential [2]. The principle of heating during MAE is based on the direct effect of the microwaves on molecules by ionic conduction and dipole rotation within the processed materials. Ionic conduction caused by the electrophoretic migration and the dipole rotation of realignment of ions under an applied electromagnetic field results in the rise of temperature within the extraction solution. This heating mode obviously enhances the transfer progress of objective solute within material matrix toward solution [3].

Therefore, in this study used methods microwave-assisted extraction (MAE) to obtain sandalwood oil. In addition, the use of methods microwave-assisted extraction (MAE) is also based on the availability of sufficient microwave readily available in the public.

Response surface methodology (RSM) was a collection of statistical and mathematical techniques that has been successfully used to determine the effects of several variables and optimize processes. The main advantage of RSM was to reduce number of experimental trials needed to evaluate multiple variables and their interactions [4]. Response surface methodology (RSM) was applied to optimize the conditions for oil extraction from hemp seed (*Cannabis sativa* L.) by supercritical carbon dioxide extraction with three independent variables namely operating temperature, pressure and particle diameter [5] and *Phaleria macrocarpa* seed by solvent extraction technique using n-hexane with three independent variables namely extraction temperature, time and solvent-to-feed ratio [6].

However, there were no reports available in the literature regarding the optimization of microwave-assisted extraction of essential oils from the sandalwood by RSM. In this study, the microwave-assisted extraction parameters of essential oils from the sandalwood (microwave power, plant material to solvent ratio and extraction time) was firstly investigated and optimized using a three-level, three variable central composite design (CCD).

The objectives of this study were to determine the effect of MAE parameters including microwave power, plant material to solvent ratio and extraction time on the yield of sandalwood oils; and to optimize processing conditions of MAE for the highest yield of sandalwood oils.

2. Experimental

2.1. Materials and chemicals

The main raw material used in this study is sandalwood that comes from the Kupang, East Nusa Tenggara, Indonesia in powder form. All other chemicals and solvents used were of analytical grade.

2.2. Microwave extraction of sandalwood oil

A domestic microwave oven (EMM-2007X, Electrolux, 20 L, 800 W; variable in 200 W increments, 2.45 GHz) was modified for MAE operation. The dimensions of the PTFE-coated cavity of the microwave oven were 46.1 cm x 28.0 cm x 37.3 cm. Three plant material to solvent ratio of sandalwood powder samples (0.10, 0.15 and 0.20 g mL⁻¹) were placed in a 1 L flask containing deionized water (400 mL). The flask was setup within the microwave oven cavity and a condenser was used on the top (outside the oven) to collect the extracted essential oils (Figure 1). The microwave oven was operated at three power level (400 W, 600 W and 800 W) for a period of 2 h. This period was sufficient to extract all the essential oils from the sample. During the first 40 min, the collected essential oils were decanted from the condensate in 8 min intervals. Decantation of the essential oils was then continued with 20 min intervals. To remove water, the extracted essential oils were then dried over anhydrous sodium sulfate, weighed and stored in amber vials at 4 °C until they were used for analysis. All the results are reported in grams of essential oils per 100g of sandalwood powder. The yield of sandalwood oil was found by the following equation

$$y = \frac{V}{W} X \, 100 \tag{1}$$

where y is the sandalwood oil yield (g/100g, w/w), V is the weight or mass of extracted sandalwood oil (g) and W is the weight or mass of sandalwood powder (g).



Figure 1: Schematic representation of the microwave-assisted extraction apparatus used in this study

2.3. Experimental and statistical methodology

RSM is a statistical method that used quantitative date from appropriate experimental design to determine optimal conditions. Therefore, RSM with central composite design (CCD) was used to determine the optimum conditions for microwave-assisted extraction sandalwood oil extraction. The experimental factors were ascertained on the basis of the results of preliminary experiments.

Indonendant variablas	Factor level			
independent variables	-1(-α)	0	1(+α)	
Microwave power (W) (A)	400	600	800	
Plant material to solvent ratio (g mL ⁻¹) (B)	0.10	0.15	0.20	
Extraction time (min) (C)	40	80	120	

Table 1: Independent variables and their levels used in the response surface design

After determining the preliminary range of the extraction variables through the single-factor test, the relationships between the response and the three selected variables (A: microwave power, B: plant material to solvent ratio and C: extraction time) were approximated by the following second order polynomial (Eq. (1)) function:

Extraction yield (g/100g) =
$$\beta_i + \sum_{i=1}^3 \beta_i X_i^2 + \sum_{i=1}^3 \beta_{ii} X_i^2 + \sum_{i< j=2}^3 \beta_{ij} X_i X_j$$
 (2)

where X_i is the corresponding actual value of variable. β_0 is the estimated regression coefficient of the fitted response at the center point of the design; β_i is the regression coefficient for liner effect terms; β_{ij} is interaction effects; and β_{ii} is quadratic effects.

The range of independent variables and their levels is presented in Table 1. The independent variables and their ranges were chosen based on preliminary experiment results. The response function (Y) was extraction yield of sandalwood oil (g/100g).

The fitted polynomial equation is expressed as 3D surface plots in order to visualize the relationship between the response and experimental levels of each factor and to deduce the optimum conditions [7]. According to the analysis of variance, the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. The RSM was applied to the experimental data

using a commercial statistical package, Design-Expert version 9.0.4.1 (Minneapolis, USA). *p*-Values of less than 0.05 were considered to be statistically significant.

The individual and overall optimization procedures were carried out to obtain the optimal levels of three independent variables (A, B and C) leading to obtain the maximum extraction yield of sandalwood oil. For graphical optimization, the reduced response models were expressed as three-dimensional (3D) surface plots to better visualize the interaction effect of main emulsion components on the physicochemical properties studied. It should be noted that the 3D plots were drawn by keeping one variable constant at the center point and varying the other two variables within the experimental range.

3. Results and Discussion

3.1. Effect of single factor on the extraction yield of sandalwood oil

3.1.1. Effect of microwave power

The effect of heating rate on the extraction yield was determined for the different microwave power. Microwave power and temperature are related, because of the high power operation can raise the temperature above the boiling point of the solvent and produce an increase in the extraction yield results. Microwave power acts as a driving force to break up the structure of plant cell membranes, so that the oil can be diffused out and dissolved in a solvent. So the addition of power in general will increase the yield and accelerate extraction time [8].



Figure 2: Effect of microwave power on extraction yield of sandalwood oil by various plant material to solvent ratio for an extraction time of 2 h

The amount and rate of refining operating temperature is affected by the amount of power used. In this study, it can be seen that the greater the power used, the higher the operating temperature. The rise in temperature is a result of the ability of the material and solvent to absorb the energy of the microwaves. Measure of the ability to absorb microwaves is called the dielectric constant. Dielectric properties of a material that is combined with the electromagnetic field generating conversion of electromagnetic energy into thermal energy (heat). And if the electric field at a uniform volume is considered, the amount of power or energy (absorbed per unit volume) is proportional to the electric field strength, frequency, and dielectric loss factor [9]. In general, good power to produce a higher yield is 600 W. Power 600 W to produce a high yield in a shorter time compared with the power of 400 W and 800 W. This is because the greater the power, the operating temperature and the rate of distillation (evaporation) becomes higher. When microwave power than 600 W, the extraction decreased with the further increase of microwave power. These data suggest that applying a higher microwave power for a short time maybe the most effective way to extract essential oil from sandalwood using MAE. However, a higher

microwave power may lead to thermal degradation of the essential oil. This result indicates that microwave power of 600 W is enough to extraction of sandalwood oil in the present work.

3.1.2. Effect of plant material to solvent ratio

In this study, the extraction curves were obtained for different plant material to solvent ratio $(0.10-0.20, \text{g mL}^{-1})$. The results were plotted in Figure 3 which show that much mass of sandalwood and the amount of oil that is obtained, not always positively correlated with increased oil yield obtained. In case of higher plant material to solvent ratio, where amount of water is less, the plant material might have experienced the overheating or charring, resulting in the decreased rate and yield. For high water content, the heat could be wasted in heating up the water which might have reduced the efficiency of the process. Also, hydrolytic effect might have contributed to the lower yield.



Figure 3: Effect of plant material to solvent ratio on extraction yield of sandalwood oil by various microwave power for an extraction time of 2 h

Reduced yield of sandalwood oil as more mass materials can also be caused by factors material density, ie the ratio between the mass of material and volume capacity distiller used. This ratio factor associated with how dense (many) condition of raw materials included in the distillation flask (distiller), so that the process of oil extraction and evaporation can walk perfectly. The density of the material is closely linked to a large room between materials. The density of the material is too high and unevenly can cause the formation of lines "rat holes" that can reduce the yield and oil quality [10]. In addition, the greater the density of the material has also resulted in the rate of oil distillation or evaporation will be slower, because of inhibition of the space to be able to evaporate into the condenser. Thus eventually causing the yield and efficiency of refining decreased. Discussion about the ratio of the mass of material and the capacity of the distillation apparatus is also useful later on for process scale-up tool, the application to determine the ratio of the mass of raw material and volume capacity of the distillation apparatus (kettle flute) which can be used in order to obtain maximum yield.

3.1.3. Effect of extraction time

The extraction process takes place in three different steps: an equilibrium phase where the phenomena of solubilization and partition intervene, in which the substrate is removed from the outer surface of the particle at an approximately constant velocity. Then, this stage is followed by an intermediary transition phase to diffusion. The resistance to mass transfer begins to appear in the solid–liquid interface; in this period the mass transfer by convection and diffusion prevails. In the last phase, the solute must overcome the interactions that bind it to the matrix and diffuse into the extracting solvent. The extraction rate in this period is low, characterized by the

removal of the extract through the diffusion mechanism. This point is an irreversible step of the extraction process; it is often regarded as the limiting step of the process [11].

Based on the effect of the length of extraction time to sandalwood oil yield for a various variables of microwave power and plant material to solvent ratio, it can be seen that the extraction process has reached a diffusion phase. It can be seen from the decrease in the rate of extraction and fewer sandalwood oil obtained during the extraction of entering a time of 90 minutes.



Figure 4: Effect of extraction time on extraction yield of sandalwood oil by various microwave power for a plant material to solvent ratio of 0.10

The amount of sandalwood oil yield is very dependent on the mass of raw materials and the mass of sandalwood oil are obtained. Figure 4 shows the relationship between extraction time to the sandalwood oil yield on the various microwave power for a plant material to solvent ratio of 0.10. It appears that along with increasing extraction time, yield of sandalwood oil produced will increase. The extraction yield of sandalwood oil couldn't reach its maximum in 120 min during the MAE process. If the extraction time was more than 120 min, the extraction yield of sandalwood oil slightly increased with the increase of extraction time. However, the excessive time exposure in the microwave field may cause the degradation of sandalwood oil.

3.2. Statistical analysis and model fitting

The results of the RSM analysis carried out as shown in Section 2.3 are shown in Table 2. Experimental extraction yields were used to determine the coefficients of the response surface equation (Eq. (2)). The regression coefficients of the intercept, linear, quadratic, and interaction terms of the model were calculated using the least square technique and are presented in Table 3. Obtained second-order polynomial equation (Eq. (3)) was found well to represent the experimental data ($R^2 = 0.9594$).

The analysis of variance for the experimental results of the CCD is also shown in Table 4. The R^2 and $adj-R^2$ values were 0.9594 and 0.9229 respectively (Table 4). A high R^2 indicates that the variation could be accounted for by the data satisfactorily fitting the model. Since CV is a measure expressing the standard deviation as a percentage of the mean, smaller values of CV give better reproducibility. The coefficient of variation (CV) of less than 10 indicated that the model was reproducible [12]. The Predicted Residual Sum of Squares (PRESS) for the model, which is a measure of how a particular model fits each point in the design, was 0.0480. The model *F*-value, 26.2700, implied that the model was significant. Adequate precision measures the signal-to noise ratio. A ratio greater than 4 is desirable [13]. For the proposed models, this value was 19.7270, a very good signal-to-noise ratio. All these statistical parameters show the reliability of the models.

Dun		Actual variables		Extraction yield (g/100g)		
Kull	A (W)	$B (g mL^{-1})$	C (min)	Experimental	Predicted	Residue
1	600	0.15	80	0.5144	0.5098	0.0047
2	600	0.15	80	0.5144	0.5098	0.0047
3	400	0.20	40	0.3445	0.3322	0.0123
4	400	0.10	120	0.6295	0.6478	-0.0184
5	600	0.15	40	0.3731	0.3986	-0.0255
6	800	0.20	120	0.5094	0.4989	0.0104
7	400	0.20	120	0.4578	0.4527	0.0051
8	400	0.10	40	0.4737	0.4807	-0.0070
9	800	0.20	40	0.3307	0.3089	0.0218
10	800	0.10	40	0.4245	0.4261	-0.0016
11	600	0.20	80	0.4034	0.4531	-0.0497
12	600	0.15	80	0.5144	0.5098	0.0047
13	600	0.15	80	0.5144	0.5098	0.0047
14	600	0.15	80	0.5144	0.5098	0.0047
15	600	0.15	80	0.5144	0.5098	0.0047
16	400	0.15	80	0.4867	0.4788	0.0080
17	800	0.15	80	0.4528	0.4746	-0.0218
18	800	0.10	120	0.6539	0.6627	-0.0089
19	600	0.10	80	0.6451	0.6093	0.0358
20	600	0.15	120	0.5888	0.5772	0.0116

Table 2: Response surface central composite design and results for extraction yield of sandalwood oil

Table 3: Analysis of variance for the fitted models

Source	DF	Coefficient	Sum of square	Mean square	<i>F</i> -value	<i>p</i> -Value
Model	9		0.150000	0.017000	26.270000	< 0.000100
Residual	10		0.006435	0.000644		
Lack of fit	5		0.006435	0.001287		
Pure error	5		0.000000	0.000000		
Total	19		0.160000			
R^2		0.959400				
$\operatorname{Adj} - R^2$		0.922900				
CV		5.150000				
PRESS		0.048000				
Standard deviation		0.025000				
Adequate precision		19.727000				

The *p*-values were used as a tool to check the significance of each coefficient, which in turn may indicate the pattern of the interactions between the variables. The smaller was the value of *p*, the more significant was the corresponding coefficient. It can be seen from this table that the linear coefficients (A), quadratic coefficients (A², B², C²) and cross product coefficients (AB, AC, BC) were not significant (p > 0.05). The other term coefficient (B and C) was significant, with very small *p* values (p < 0.05).

The predicted values calculated from Eq. (3) were in very good agreement with the experimental values, as shown in Figure 5. Hence, this quadratic model is well suited for this experimental set up.

Table 4: The significance of each response variable effect showed by using F ratio and p value in the nonlinier second order model

	Variables	DF ^a	SS ^b	MS ^c	<i>F</i> -value	<i>p</i> -Value ^d
Linier effects	А	1	0.000044	0.000044	0.068000	0.799700 ns ^e
	В	1	0.061000	0.061000	94.760000	< 0.000100
	С	1	0.080000	0.080000	123.890000	< 0.000100
Quadratic effects	A^2	1	0.003007	0.003007	4.670000	0.056000 ns ^e
	B^2	1	0.001263	0.001263	1.960000	0.191500 ns ^e
	C^2	1	0.001315	0.001315	2.040000	0.183300 ns ^e
Interaction effects	AB	1	0.000490	0.000490	0.760000	0.403400 ns ^e
	AC	1	0.002415	0.002415	3.750000	0.081500 ns ^e
	BC	1	0.001086	0.001086	1.690000	0.223100 ns ^e

^a Degree of freedom

^b Sum of squares

^c Mean sum of squares

 d p values <0.05 were considered to be significant

^e ns: not significant



Actual

Figure 5: Experimental extraction yield vs. the predicted extraction yield under optimum extraction conditions

3.3. Perturbation plot

Perturbation plot shows the comparison between all factors at a selected point in the considered design space. The perturbation plot for the extraction yield of sandalwood oil is shown in Figure 6. The extraction yield response was drawn by changing only one factor over its range while the other factors were held constant. The plot demonstrates the effect of all factors at a central point in the design space (e.g., microwave power, plant material to solvent ratio and extraction time). Not all factors indicated a positive effect on the extraction yield of sandalwood oil. The relatively flat line of microwave power shows lower effect of this factor on the extraction yield of sandalwood oil in the design space. It can be seen from Eq. (3) and Table 4 the perturbation plot that plant material to solvent ratio and extraction time had significant curvature effect. The steep curvature in plant material to solvent ratio behavior demonstrated the response of extraction yield of sandalwood oil g/100g was very rapid to these factors. Through the comparison of coefficients in Eq. (4), the most significant parameter

was determined. In this manner, the order of positive influence of the individual terms on the obtained extraction yield response was microwave power and extraction time.

Extraction yield of sandalwood oil (g/100g) = 0.48430 + 0.00069047A- 4.13712B + 0.00398969C + 0.0007825AB + 0.00000217188AC - 0.005825BC - 0.000000826705A² + 8.57273B² - 0.0000136676C²





Deviation from Reference Point (Coded Units)

Figure 6: Pertubation plot for rate response (for A: microwave power, B: plant material to solvent ratio and C: extraction time)

3.4. Optimization of extraction conditions of sandalwood oil

The best way of expressing the effect of any parameter on the yield within the experimental space under investigation was to generate response surface plots of the equation. Response surfaces were plotted by using Design-Expert software (version 9.0.4.1) to study the effects of parameters and their interactions on extraction yield of sandalwood oil. The results of extraction yield of sandalwood oil affected by microwave power, plant material to solvent ratio and extraction time are presented in Figures 7 and 8.

In Figures 7a and 8a, when the 3-D response surface plot and the contour plot were developed for the extraction yield of sandalwood oil with varying microwave power and plant material to solvent ratio at fixed extraction time 80 min. At a definite microwave power, the yield of extraction decreased slightly with the increase of the plant material to solvent ratio, and nearly reached a peak at the moderate microwave power tested. The highest extraction yield occured at microwave power 558 W and plant material to solvent ratio 0.10 g mL^{-1} .

In Figures 7b and 8b, when the 3-D response surface plot and the contour plot were developed for the recovery of sandalwood oil with varying microwave power and extraction time at fixed plant material to solvent ratio 0.15 g mL^{-1} , it can be seen that maximum recovery of sandalwood oil can be achieved when microwave power and extraction time were 558 W and 101 min, respectively.

Figures 7c and 8c shows the effect of the plant material to solvent ratio and extraction time on the extraction yield of sandalwood oil at a fixed microwave power 600 W. The yield of sandalwood oil was decreasing evidently as the increasing of plant material to solvent ratio and nearly reached a peak at the highest extraction time tested. It can be seen that maximum recovery of sandalwood oil can be achieved when plant material to solvent ratio and extraction time were around 0.10 g mL^{-1} and 102 min.



Figure 7: Response surface (3-D) showing the effect of the microwave power (A), plant material to solvent ratio (B) and extraction time (C) on the sandalwood oil extraction yield





Figure 8: Contour plots showing the effect of the microwave power (A), plant material to solvent ratio (B) and extraction time (C) on the sandalwood oil extraction yield

It can be concluded that optimal extraction conditions of sandalwood oil was microwave power 558.071W, plant material to solvent ratio 0.100274 g mL⁻¹ and extraction time 101.688 min (Figure 9). At this optimized condition, the predicted yield of the sandalwood oil was 0.655534 g/100g.



Figure 9: Graphical optimization of extraction yield of sandalwood oil (g/100g) and its desirability

3.5. Model adequacy checking

Usually, it is necessary to check the fitted model to ensure that it provides an adequate approximation to the real system. Unless the model shows an adequate fit, proceeding with the investigation and optimization of the fitted response surface likely give poor or misleading results [14]. The residuals from the least squares fit play an important role in judging model adequacy [13]. By constructing a normal probability plot of the residuals, a check was made for the normality assumption, as given in Figure 10. The normality assumption was satisfied as the residual plot approximated along a straight line. Figure 11 presents a plot of residuals versus the predicted response. The general impression is that the residuals scatter randomly on the display, suggesting that he variance of the original observation is constant for all values of Y. Both of the plots (Figures 10 and 11) are satisfactory, so we conclude that the empirical model is adequate to describe the sandalwood oil extraction yield by response surface.



Internally Studentized Residuals
Figure 10: Normal probability of internally studentized residuals



Figure 11: Plot of internally studentized residuals vs. predicted response

3.6. Validation of the model

To compare the predicted result with the practical value, the rechecking experiment was performed using some extraction condition. The value obtained from real experiments, demonstrated the validity of the RSM model, since there was no significant (p > 0.05) differences (Table 5). The strong correlation between the real and the predicted results confirmed that the response model was adequate to reflect the expected extraction condition.

Extraction condition			Extraction yield of sandalwood oil (g/100g)		
Microwave	Plant material to	Extraction	Experimentel	Dradiated	
power (W)	solvent ratio (g mL ⁻¹)	time (min)	Experimental		
400	0.10	80	0.5816	0.5861	
400	0.15	40	0.3964	0.3850	
400	0.15	120	0.5268	0.5288	
400	0.20	80	0.4230	0.4143	
600	0.10	40	0.4679	0.4865	
600	0.10	120	0.7384	0.6884	
600	0.20	40	0.2926	0.3536	
600	0.20	120	0.4617	0.5089	
800	0.10	80	0.5761	0.5663	
800	0.15	40	0.3337	0.3461	
800	0.15	120	0.5140	0.5594	
800	0.20	80	0.4488	0.4258	

 Table 5: Predicted and experimental values of the responses at some extraction condition

Conclusions

Response surface methodology has proved to be effective in estimating the effect of three independent variables, microwave power, plant material to solvent ratio and extraction time on the sandalwood oil yield and for predicting the optimal operational conditions. The experimental results showed that linear terms of two independent variables (plant material to solvent ratio and extraction time) had significant effects on the response

value. Based on the analysis of variance and the agreement of the experimental and predicted results, it can be concluded that the generated model was suitable for the simulation of microwave-assisted extraction of sandalwood with any combination of tested variables. The optimal conditions were as follows: microwave power 558.071W, plant material to solvent ratio 0.100274 g mL⁻¹ and extraction time 101.688 min. On the optimum extraction condition the sandalwood oil yield was 0.655534 g/100g dry weight which is reported for the first time.

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