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Extraction Process of Essential Oil from *Plectranthus amboinicus* Using Microwave-Assisted Hydrodistillation and Evaluation of It's Antibacterial Activity

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Plectranthus amboinicus oil has many applications in pharmaceutical, medicine and cosmetic industries. Recently, new methods of extracting essential oil used have been increasingly developed to replace traditional methods. In this study, maximization of essential oils yield from *P. amboinicus* was studied by the combination of microwave assisted hydro-distillation (MAHD) and response surface methodology (RSM). We found that that the maximum essential oil yield was 0.1374 % with 94.38 % reliability and influencing factors such as microwave power for this process was 515 W, raw material to water ratio 1.64:1, extraction time at 100.8 min. ANOVA analysis for quadratic model also gives favourable outcome including the high determination coefficient ($R^2 = 0.94$), significant F-value and p-value of coefficients. Under laboratory condition, the obtained yield (0.1374 %) approximated the yield predicted by the quadratic model, suggesting the reasonable soundness of the employed model and RSM when it comes to optimizing the parameters of extraction. In addition, considerable antibacterial activities of extracted essential oil against four bacteria cultures, in particular, *S. aureus* was recognized.

Keywords: *P. amboinicus* oil, Extraction, Microwave-assisted hydrodistillation, Response surface methodology, Antibacterial activity.

INTRODUCTION

Response surface methodology (RSM) is a method devised to optimize a response (output) variable influenced by several inputs. The technique involves designing of a set of experiments, which is a series of tests, to determine the impact magnitude of inputs to the output variable through a statistical model. To achieve this, first or second order polynomial functions are used to describe the system and to investigate empirical conditions for optimal optimization. Typical RSM optimization technique should undergo the following steps: (1) selection of independent variables that significantly influence the system within the limits of that study according to the objectives and experience of the researcher; (2) design experiments and conduct experiments in accordance with a previously defined matrix; (3) mathematical manipulation of empirical data obtained through polynomial function compatibility; (4) assessment of model compatibility; (5) verification of the feasibility and necessity of implementing the shift towards the optimal boundary; (6) conduct experiments based on the optimal results for each variable [1]. The compound design matrix of the central composite (CCD) was first described by Box and Wilson, including the following: (1) Design of fractional factors on coding levels: average (0), low (-1) and high (+1); (2) Design extra points outside the segment, zero from one center; (3) To express of central points to evaluate the repeatability of the method. The application of RSM could both minimize the number of experiments performed and keep the optimal efficiency of the process from being inadequately evaluated. A typical application of RSM is optimization of extraction of essential oil from different material such as Patchouli [2], *P. cubeba* [3], leaves of *Taxus chinensis* [4], *Thevetia peruviana* (yellow oleander) Seeds [5], lemongrass [6] and Iranian *Rosmarinus officinalis* [7]. This had shown that response surface methodology was of great potential in research to replace disadvantaged methods.

Plectranthus amboinicus is a widespread vegetable in Asian cuisine and a long-standing herb in folk medicine for curing of common diseases such as fever, heat cough, inflammation throat, hoarseness, insect bite, treatment of diabetic foot ulcers [8,9]. Studies showed that the *P. amboinicus* had antibacterial activity in high organisms [10-12], therefore essential oils from the plant and their preparations have been increasingly utilized in manufacture of functional foods, pharmaceuticals and cosmetics. *P. amboinicus* oil was commonly obtained from the leaves and stems by extraction. The essential oil content from the plant varies from 0.05-0.12 % and it could be extracted by various methods. Mechanical methods of extraction include pressing or squeezing. Traditional methods often involve distillation with water or with solvent. Recently, extraction by assistance of microwave has been developed and was proved to shorten the distillation time and improve both the composition and the yield of essential oil [13-15].

For the above reasons, the purpose of this research was to use the RSM to optimize the parameters that affect the extraction of *P. ambonicus* oil by microwave-assisted hydrodistillation.

EXPERIMENTAL

Plectranthus amboinicus leaves were purchased in Ho Chi Minh City, Vietnam. The leaves were then preselected, washed, chopped and distilled directly by steam. Anhydrous sodium sulfate was purchased from Sigma Aldrich (US). Deionized water was used as a solvent to extract *P. amboinicus* essential oil by Milli-Q purification system (Millipore, USA).

Microwave-assisted hydrodistillation: A Clevenger type apparatus was connected to a domestic microwave oven MW71E (manufactured by SAMSUNG, Vietnam) for microwave assisted hydrodistillation operation. The power source has the maximum output power of 800 W and voltage of 250 V – 50 Hz. In this operation, water to raw material ratio ranges from 1.4:1 to 2:1 mL/g, microwave power varies from 400 to 600 W and extraction time spread from 30 to 120 min. This maximum duration was justified by the fact that complete MAHD extraction of essential oil from the sample was performed in 2 h [2]. The flask containing 200 g of *Plectranthus amboinicus* and distilled water was placed in the microwave oven cavity. Extracted essential oils were then collected by a condenser set outside the oven.

Single factor investigation: We consider three important factors impacting extraction process of *P. amboinicus* oil including ratio materials to water ratio (mL/g), extraction time (min) and microwave power (W). Following this, optimum conditionis determined based on the microwave-assisted hydrodistillation (MAHD).

Analysis of sample: After extraction, the solution was dried over anhydrous Na_2SO_4. The yield of *P. amboinicus* oil extracted was analyzed to evaluate the performance of MAHD in the extraction of essential oils. Oil yield of an experimental run was calculated using following formula:

$$\text{Yield of essential oil was obtained (\%)} = \frac{\text{Volume of essential oil obtained (mL)}}{\text{Amount of raw materials used (g)}}$$

Experimental design: The central composite design was adopted to determine the optimal parameters of MAHD. Table-1 presented three influencing factor to the extraction yield and corresponding levels. ANOVA analysis, calculation of coefficients and plotting was carried out with Design Expert 7.1.6 software. Predicted yields and actual yields were also compared to evaluate the fitness of the model to the data.

Antibacterial activity: The antibacterial activity of *P. amboinicus* is examined for following bacteria: *Staphyloccous aureus, Salmonella, Escherichia coli* and *Pseudomogas aeruginosa.* Corresponding to each type of bacteria, four 6 mm diameter holes are drilled on an agar test disc. Of the four holes, two of which are reserved for essential oil extracted from the material plant. The two remaining holes contain positive and negative controls, which are 10^{-5} diluted tetracycline and sterile distilled water, respectively. Following that, microbial cultures were inoculated onto the discs and standardized to 0.5 McFarland standard turbidity, which is equivalent to 10^8 cfu/mL. Incubation took place at a temperature of 30 °C for 24 h. Following the incubation period, the diameter of inhibition zone was measured. All operations are conducted in the aseptic environment.

RESULTS AND DISCUSSION

Single factor assays: Single factor investigation of extraction of *Plectranthus amboinicus* oil was illustrated in Fig. 1. For water-to-raw ratio, Fig. 1(a) suggested a clear turning point of the ratio when it comes to promoting oil yield. To be specific, increasing water-to-raw ratio from 1.4 to 1.6 (mL/g) rises yield to approximately 0.125 %. From here, higher dilution of the solution up to 1.8 (mL/g) causes oil yield to decrease sharply before reaching 0.1 %, where the yield began to stabilize. This explained the thermal stress caused by rapid heating of the solution since the microwave power was absorbed effectively due to the presence of excess water [16]. Effects of microwave power and extraction time on yield generally exhibited the positive relationship till a certain point where yield remained steady. For microwave power, with an increase of power from 400 to 500 W, the highest extract oil yield increased from 0.095 to 0.125 %. However, the amount of essential oil remained

TABLE-1
INDEPENDENT VARIABLES MATRIX AND THEIR ENCODED LEVELS FOR RSM MODEL

Code	Name	Units	Levels				
			$-\alpha$	-1	0	+1	$+\alpha$
A	Water to raw material ratio	mL/g	1.26	1.4	1.6	1.8	1.94
B	Microwave power	W	332	400	500	600	668
C	Extraction time	min	40	60	90	120	140

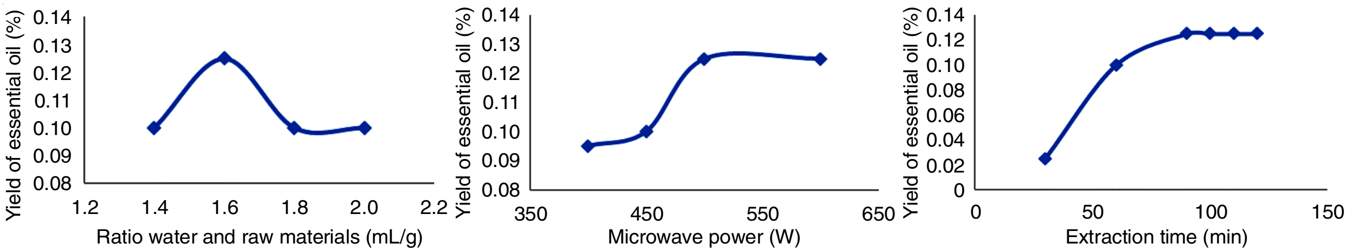

Fig. 1. Effect of three single factors on the extraction of *P. amboinicus*

fixed at 0.125 % when rising power past 500W. The explanation for this is two-fold. First, it could be the heat building up within the material when exposed to increased microwave energy that causes oil to be released more quickly. Second, higher microwave energy levels could exerta higher pressure gradient to the material, rapidly pushing the essential oil out of the glands [17]. For the effect of extraction time, Fig. 1(c) shows the time influence on the oil yields over the course of different durations ranging from 30 to 120 min. Other conditions in this examination including water-to-raw ratio and microwave power were set to 1.6:1 and 500 W, respectively. Visually, most of the oil in the plant, corresponding to the yield of 0.125 %, was rapidly extracted within 90 min of extraction and prolonging extraction time resulted in diminishing rate of extraction. When increasing extraction time past 90 min, oil yield ceases to rise. This phenomenon in MAHD was in line with various studies on different plants [18]. Herein, ratio between water and raw materials 1.6, microwave power 500 W and extraction time 90 min were chosen for following experiment design.

Optimization of MAHD parameters by DX11: To model the effects of water-to-material ratio, extraction time and microwave power on the oil yield, a second-order equation was established and regressed against experimental data. Table-2 presents the experimental parameters produced by CCD design and associated yields. In the design, five levels of variables, including the low (encoded -1), high (encoded +1) and rotatable (encoded ± a), were combined to form 20 runs.

Table-3 shows the ANOVA results for the quadratic model of oil yield. The main terms in the ANOVA table include: effect water to plant material ratio (A), effect of microwave power level (B), effect of extraction time (C), interaction terms (AB, BC, AC) and second order effects(A^2, B^2 and C^2). The Model F-value of 18.67 implies the model was significant. The Lack of Fit F-value of 0.2030 implies the Lack of Fit was not significant relative to the pure error. There was a 94.76 % chance that a "Lack of Fit F-value" this large could occur due to noise. The predicted R^2 of 0.8933 concurred with the adjusted R^2 of 0.8539. A ratio greater than 4 is desirable. Ratio of 12.1931

TABLE-2
MATRIX OF OBSERVED AND PREDICTED VALUES FOR MICROWAVE ASSISTED HYDRO-DISTILLATION (MAHD)

	Experimental parameters			Y (%)			Experimental parameters			Y (%)	
	A	B	C	Actual	Predicted		A	B	C	Actual	Predicted
1	1.4	60	400	0.050	0.0471	11	1.6	40	500	0.050	0.0480
2	1.8	60	400	0.075	0.0767	12	1.6	140	500	0.100	0.0995
3	1.4	120	400	0.075	0.0777	13	1.6	90	332	0.075	0.0780
4	1.8	120	400	0.100	0.0948	14	1.6	90	668	0.100	0.0945
5	1.4	60	600	0.050	0.0569	15	1.6	90	500	0.125	0.1334
6	1.8	60	600	0.075	0.0740	16	1.6	90	500	0.125	0.1334
7	1.4	120	600	0.100	0.1000	17	1.6	90	500	0.125	0.1334
8	1.8	120	600	0.100	0.1047	18	1.6	90	500	0.150	0.1334
9	1.3	90	500	0.075	0.0719	19	1.6	90	500	0.125	0.1334
10	1.9	90	500	0.100	0.1007	20	1.6	90	500	0.150	0.1334

TABLE-3
ANOVA FOR QUADRATIC MODEL

Source	Sum of squares	dF	Mean square	F-value	p-value		Comment
Model	0.0168	9	0.0019	18.67	< 0.0001	Significant	SD = 0.0100
A	0.0010	1	0.0010	10.01	0.0101		Mean = 0.0962
B	0.0032	1	0.0032	31.93	0.0002		CV (%) = 10.40
C	0.0003	1	0.0003	3.280	0.1001		R^2 = 0.9438
AB	0.0001	1	0.0001	0.7793	0.3981		AP = 12.1931
AC	0.0001	1	0.0001	0.7793	0.3981		Adj R^2 =0.8933
BC	0.0001	1	0.0001	0.7793	0.3981		Pred R^2= 0.8539
A^2	0.0040	1	0.0040	39.92	< 0.0001		
B^2	0.0064	1	0.0064	63.90	< 0.0001		
C^2	0.0040	1	0.0040	39.92	< 0.0001		
Residual	0.0010	10	0.0001				
Lack of fit	0.0002	5	0.0000	0.2030	0.9476	Not significant	
Pure error	0.0008	5	0.0002				
Cor total	0.0178	19					

indicated an adequate signal. Therefore, this model could be used to navigate the design space.

The final model in term based on experimental data of extraction yield was proposed as follows:

$$Y = 0.1334 + 0.0086*A + 0.0153*B + 0.0049*C - 0.0031*A*B - 0.0031*A*C + 0.0031*B*C - 0.0167*A^2 - 0.0211*B^2 - 0.0167*C^2$$

The yield of essential oil could be predicted using the above model. To validate the model, residuals of 20 runs and yields of oil were plotted in the Fig. 2(b) and 2(a), respectively. Fig. 2(a) indicated that residuals of experimental yields clearly follow a random pattern. This suggested that there was no violation of assumptions regarding independence of variables and constant variance. Fig. 2(b), which plotted predicted *versus* against actual values, also indicated a close proximity of scattered data points to the 45° line suggesting the reasonable predicting accuracy of the model.

To interpret interaction effects of process variables on oil yield, three-dimensional (3D) response surfaces were plotted showing relationship between the oil yield and three independent factors including water to raw materials ratio (A), time of extraction (B) and power microwave (C). From the graphs (Fig. 3), it could be observed that general trends of the three factors are similar. That is, an increase in any of the three factors induces oil yield to rise until oil yield reaches a certain point, where yield stops rising and eventually, starts diminishing. Optimization of the estimated statistical model yielded following optimal conditions: A= 1.64:1 (mL/g), B= 100.80 min, C= 516.09W corresponding to the *P. amboinicus* oil yield of 0.1374 % and desirability of 94.38 %.

Antibacterial activity: The results of antibacterial assay are reported in Table-4. Overall, the essential oil proved to be effective against all tested bacteria cultures, as demonstrated by the presence of inhibition zones in all discs. Compared to other tested bacteria, *S. aureus*, whose mean inhibition zone is highest, at 35 mm, is the most sensitive organism to the essential oil, considerably exceeding antibiotic zone of 12 mm. For other three bacteria cultures, essential oil exhibited weaker antibacterial activities than tetracycline. To be specific, diameters of essential oil inhibition zone in *E. coli* and *Salmonella* disc are smaller than that of tetracycline inhibition zone by 5.0 and 3.5 mm, respectively. *P. aeruginosa* disc is 11 mm in diameter, which is the smallest diameter and is half as large as the control, suggesting that *P. aeruginosa* has the strongest resistance to antibacterial activity of essential oil among the four bacterial cultures. Our findings are in accordance with previous studies, which reported inhibitory effects of *P. amboinicus* essential oil against *E. coli* and *S. aureus* [19].

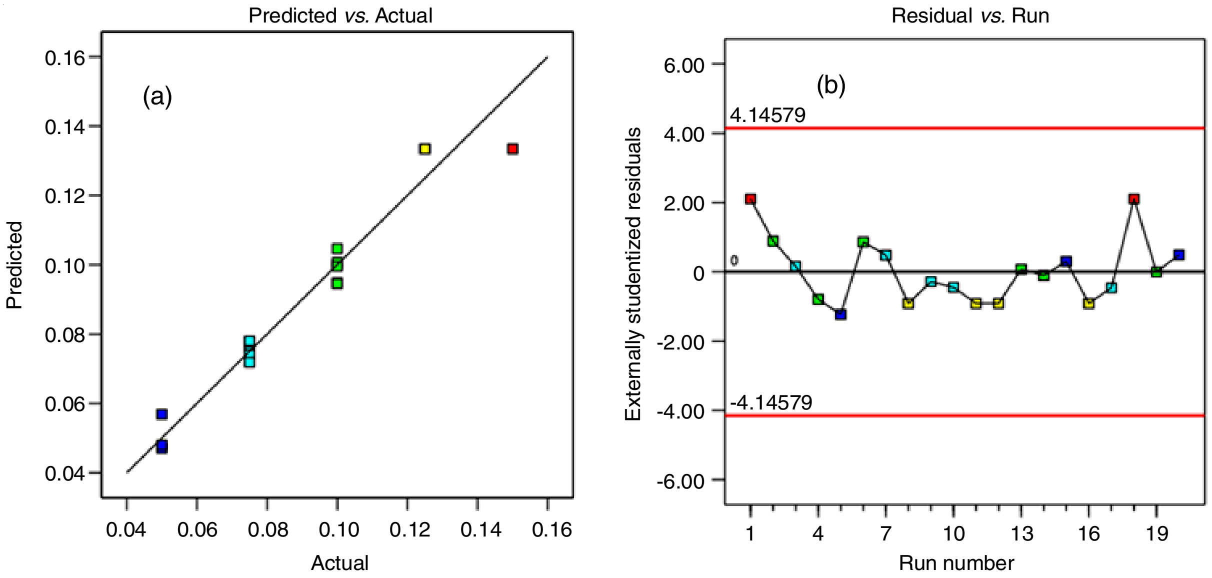

Fig. 2. (a) Comparison between actual values and predicted values and (b) normal plot of residual *vs.* predicted response for yield of *P. amboinicus* oil

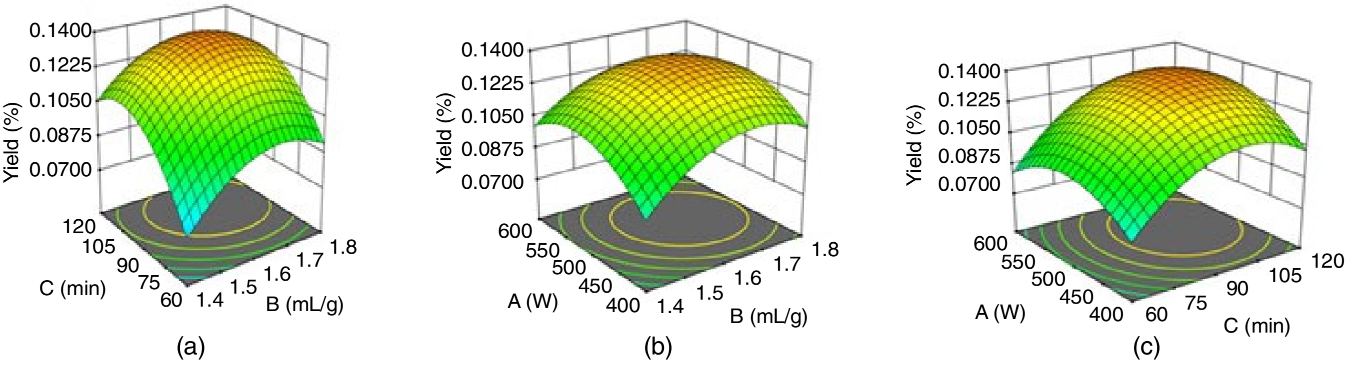

Fig. 3. 3D response surface plots of interaction of Y with (a) B and C, (b) A and B, (c) A and C

TABLE-4
INIHIBITION ZONE OF FOURS BATERIAL
TO ESSENTIAL OIL *P. amboinicus*

Bacteria disc	Mean diameter of antibacterial zone for essential oil (mm)	Diameter of antibiotic zone (mm)
Salmonella	26.5	30
S. aureus	35	12
E. coli	30	35
P. aeruginosa	11	20

Conclusion

Under the optimization performed by RSM, we found that the optimal level of three influencing conditions for extraction of the *P. amboinicus* oil using microwave oven are as follows: the ratio of water to raw materials of 1.64:1 mL/g, the extraction time at 100.80 min and microwave power of 515.09 W with yield of 0.1374 %. RSM. This study also highlighted antibacterial activity of the extracted oil to four bacteria cultures and the convenience of the MAHD method, suggesting the possibility of producing *P. amboinicus* oil more efficiently in terms of both quantity and quality.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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