

Application of Response Surface Methodology for the Optimization of Essential Oils from Pomelo [*Citrus grandis* (L.) Osbeck] Leaves using Microwave-Assisted Hydrodistillation Method

TAN PHAT DAO¹, THIEN HIEN TRAN¹, DUY TRINH NGUYEN^{1,2}, DUY CHINH NGUYEN¹, DAI HAI NGUYEN^{2,3}, NHAN THI HONG LE⁴, DO TRUNG SY⁵, NGUYEN THI THANH HUONG⁶ and BUI LE MINH^{1,*}

¹NTT Hi-High Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

²Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

³Institute of Applied Materials Science, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

⁴Department of Chemical Engineering, Ho Chi Minh City University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam

⁵Institute of Chemistry, Vietnam Academy of Science and Technology, Hanoi, Vietnam

⁶Institute for Tropical Technology, Vietnam Academy of Science and Technology, Hanoi, Vietnam

*Corresponding author: E-mail: blminh@ntt.edu.vn

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Pomelo leaves oil has many applications regarding hair nourishment and stimulation. Recently, new methods of extracting essential oil used have been increasingly developed to replace traditional methods. In this research, maximization of essential oils yield from *Citrus grandis* was studied by the combination of microwave assisted hydro-distillation (MAHD) and response surface methodology (RSM). We found that the maximum essential oil yield was 0.3197 % with 91.3 % desirability corresponding to factors such as material and water ratios of 3.04:1, extraction time at 62.76 min and microwave power of 482.17 W. ANOVA analysis for quadratic model also gives favourable outcome including the high determination coefficient ($R^2 = 0.9443$), significant F-value and p-value of coefficients. All these values indicate that this model is significant between experimental and predicted variables.

Keywords: *Citrus grandis*, Pomelo leaves, Microwave-Assisted Hydrodistillation, Response surface methodology.

INTRODUCTION

Safety concerns about chemical additives have pressed the demand for natural preservatives and, in turn, driven scientific interest to essential oils and extracts of plant origin [1-4]. Among abundant sources of herb materials containing essential oils with bioactivities, citrus fruits peels are a familiar and rich source of essential oils. Pomelo (*Citrus grandis* (L.) Osbeck), belonging to the Rutaceae family, is the native genus of tropical and subtropical areas in Asia. Nowadays, it is one of the most widely cultivated crops and represents a large proportion of fruit production worldwide [5,6]. The plant is also recognized as one of the most potent herbs in traditional medicine.

Essential oils from citrus species, residing mostly in the fruits and the leaves, contain a wide variety of biologically active secondary metabolites such as flavonoids, limonoids,

coumarins and furano coumarins, sterols, volatile oils, organic acids and alkaloids. The essential oils are also rich in biologically active compounds, which are recognized for their medicinal, physiological and pharmacological values. As demonstrated by many studies, citrus essential oil has antimicrobial, anti-septic, antibacterial, antioxidant, anticancer, antifungal, anti-inflammatory and hypoglycemic properties [7-9].

Essential oils contain highly volatile substances that are isolated through various methods of extraction from plants of a single botanical species. Most of the plant oils could be extracted using a certain solvent that would enter into the plant cells, dissolving and releasing the oil. Method of oil extraction could vary from distillation to solvent extraction. Recently, microwave heating has been applied in the extraction of essential oils and is considered an efficient method to improve both the composition and the yield of essential oil from the material [10-13].

The aim of this paper is to optimize the conditions affecting yield of essential oils from the Pomelo [*Citrus grandis* (L.) Osbeck] leaves extracted via microwave-assisted hydrodistillation method. Considered process variables include microwave power, particle to water ratio and time. We adopted response surface methodology (RSM) in conjunction with central composite design (CCD) to achieve the objective. Lastly, based on the validation data, the optimal solution produced by RSM was statistically verified by the coefficient of determination (R^2) [14-17].

EXPERIMENTAL

Pomelo leaves are taken from Thu Duc wholesale market, Ho Chi Minh City, Vietnam. The material is washed several times with water to remove impurities and is allowed to dry. The ingredients was then ground using a grinder (about 2-3 mm) and distilled directly by steam. Anhydrous sodium sulfate was purchased from Sigma Aldrich (US). Deionized water was used as a solvent to extract pomelo leaves 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. This maximum duration was justified by the fact that complete microwave-assisted hydrodistillation extraction of essential oil from the sample was performed in 2 h [18]. The flask containing 25 g pomelo leaves and distilled water was placed in the microwave oven cavity. Extracted essential oils were then collected by a condenser set outside the oven. Finally, the solution was dried and dehydrated to obtain pure essential oils.

Analysis of sample: After the extraction process, the solution was dried over anhydrous sodium sulfate, remove water. The yield of essential oil extracted was analyzed to evaluate the performance of microwave-assisted hydrodistillation in the

extraction of pomelo leaves oil. Oil yield of an experimental run was calculated by following formula:

$$\text{Yield (\%)} = \frac{\text{Volume of attained essential oil (mL)}}{\text{Amount of raw materials used (g)}} \quad (1)$$

Experimental design: The response surface methodology (RSM) was used to optimize the three factors on the yield of pomelo oil including the effect of material and water ratios (A), extraction time (B) and microwave power (C). The central composite design from RSM was adopted to determine the optimal parameters of microwave-assisted hydrodistillation and response variable is yield of essential oil (Y), which were shown Table-1. ANOVA analysis, calculation of coefficients and plotting was carried out with Design Expert 11 software. The experimental yields and predicted yields were also compared to evaluate the fitness of the model to the data.

RESULTS AND DISCUSSION

The experiments were designed according to the design method of complex CCD center. Experimental results (20 experiments) predictions by Design-Expert 11 are shown in Table-2.

Based on the results of Table-2 we find that the response factor (yield) is strongly dependent on process variables (power microwave, material and water ratios, time extraction). The highest efficiency was achieved at 0.35 % when testing at 450 W for 60 min with a 1:3 ratio of material and water. Evaluation results from DX11 software for the equation express the relationship between response and independent variables:

$$Y = 0.32 + 3.661E-003A + 0.022B + 0.017C + 6.250E-003AB - 6.250E-003AC - 0.031BC - 0.042A^2 - 0.086B^2 - 0.033C^2 \quad (2)$$

Table-3 shows that all the model is significant because the Prob value > F of the model less than 0.05. The response factors B, BC, A², B², C² are significant. Furthermore, adeq precision (AP) of the response model is 0.35 %. This number

TABLE-1
INDEPENDENT VARIABLES MATRIX AND LEVELS OF THE OPTIMIZATION FACTORS

Code	Independent factors	Units	Levels				
			$-\alpha$	-1	0	+1	$+\alpha$
A	Material and water ratios	mL/g	1.3	2	3	4	4.7
B	Microwave power	W	198	300	450	600	702
C	Extraction time	Min	9.6	30	60	90	110

TABLE-2
RESULTS OF ACTUAL AND PREDICTED VALUES FOR THE RSM MODEL

S. No.	Experimental parameters			Y (%)		S. No.	Experimental parameters			Y (%)	
	A	B	C	Actual	Predicted		A	B	C	Actual	Predicted
1	2.0	30	300	0.10	0.083	11	3	9.6	450	0.00	0.037
2	4.0	30	300	0.10	0.090	12	3	110	450	0.15	0.110
3	2.0	90	300	0.15	0.180	13	3	60	198	0.20	0.200
4	4.0	90	300	0.20	0.210	14	3	60	702	0.25	0.250
5	2.0	30	600	0.20	0.190	15	3	60	450	0.30	0.320
6	4.0	30	600	0.20	0.170	16	3	60	450	0.30	0.320
7	2.0	90	600	0.15	0.160	17	3	60	450	0.35	0.320
8	4.0	90	600	0.15	0.117	18	3	60	450	0.35	0.320
9	1.3	60	450	0.20	0.190	19	3	60	450	0.30	0.320
10	4.7	60	450	0.20	0.210	20	3	60	450	0.30	0.320

TABLE-3
ANOVA FOR QUADRATIC MODEL

Source	Sum of squares	dF	Mean square	F-value	p-value	Comment
Model	0.0168	9	0.016	18.65	< 0.0001	Significant
A	0.0010	1	1.831E-004	0.21	0.6563	SD = 0.030
B	0.0032	1	6.690E-003	7.69	0.0197	Mean = 0.21
C	0.0003	1	4.012E-003	4.61	0.0573	CV (%) = 14.22
AB	0.0001	1	3.125E-004	0.36	0.5624	R ² = 0.9443
AC	0.0001	1	3.124E-004	0.36	0.5624	AP = 13.407
BC	0.0001	1	7.810E-003	8.98	0.0134	Adj R ² = 0.8943
A ²	0.0040	1	0.025	28.55	0.0003	Pred R ² = 0.7053
B ²	0.0064	1	0.110	121.67	< 0.0001	
C ²	0.0040	1	0.015	17.69	< 0.0018	
Residual	0.0010	10	8.703E-003	8.703E-004		
Lack of Fit	0.0002	5	5.370E-003	1.074E-003	1.61	0.3068
Pure error	0.0008	5	3.33E-003	6.667E-004		Not significant
Cor Total	0.16	19				

Lack of Fit, Pure error, significant p < 0.05, not significant p > 0.05

is greater than to 4.0 indicating that the suitable signal for the models may be used for the direction for the design space. The Lack of Fit F-value of 1.61 implies the Lack of Fit is not significant relative to the pure error. Probability of for Lack of Fit to achieve this results is 30.68 %, smaller than 50 %. R² value = 0.9443 specifies that the model has good compatible with the experiment data.

This is a model that can be used to predict the performance of pomelo leaves oil in laboratory scale. Normal probability plot of residual and plot of residuals. The predicted response for yield of *Citrus grandis* oil is shown in Fig. 1(a) and 1(b). The points in Fig. 1(a) usually fall on a straight line indicating that the error is not significant. Fig. 1(b) shows that there is no clear pattern and abnormal structure. The dispersed points are almost equal in the upper and lower x-axis. This suggests that the proposed models are sufficient and there is no reason to suspect any independent breach or constant variance assumption. 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, 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 = 3.04:1 (mL/g), B = 62.76 min, C = 482.17 W corresponding to the pomelo leaves oil yield of 0.3197 % and desirability of 94.43 %.

Table-4 shows the optimum conditions for the microwave power, material/water ratio and extraction time after applying the DX11 optimization software. Accordingly, at the ratio of 3.04, the time of 62.72 min and 482.06 operating power, highest yield efficiency is achieved at 0.31626 %. This number approximates to the actual yield, conducted with almost identical conditions, of 0.3 %.

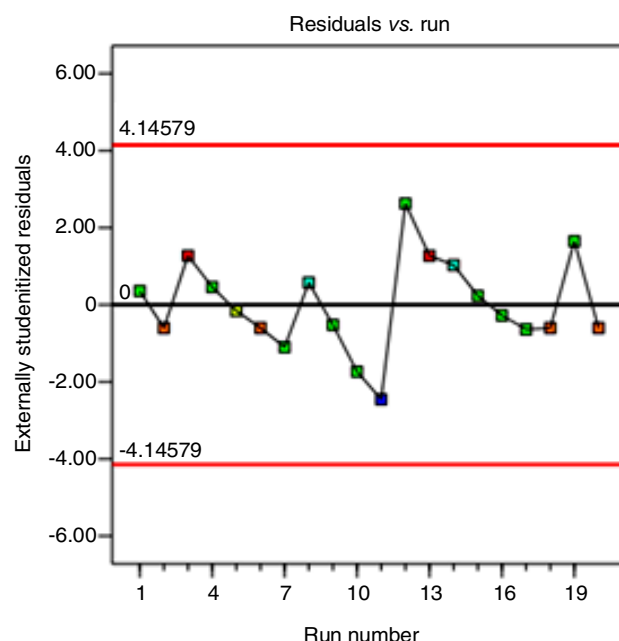
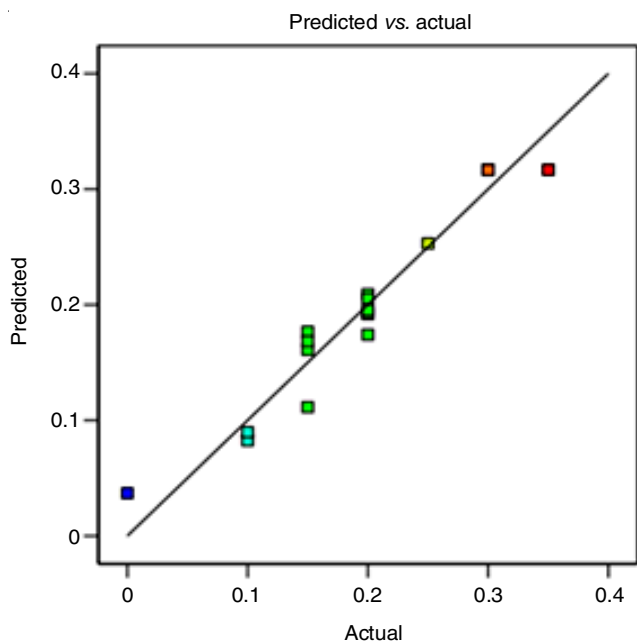


Fig. 1. (a) Comparison between actual values and predicted values; (b) Normal plot of residual vs. predicted response for yield of Pomelo leaves oil

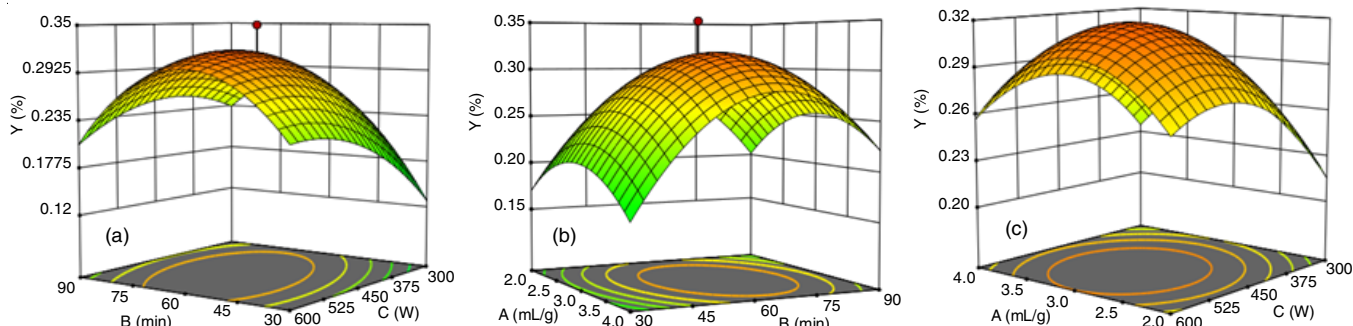


Fig. 2. 3D response surface plots of interaction relationship of Y with (a) B and C, (b) A and B, (c) A and C. In Fig. 2. Factor A (microwave power), B (ratio water and raw materials), C (extraction time)

TABLE-4
EXPERIMENTAL RESULTS USING OPTIMUM CONDITION IN COMPARISON WITH PREDICTED RESULTS

	Material and water ratios (g/mL)	Extraction time (min)	Microwave power (W)	Yield of essential oil (%)
Predicted	3.04	62.72	482.06	0.31626
Actual	3	63	485	0.3

Conclusion

This work reports the optimization of the performance of extraction processing by the response surface methodology. It is found that the optimal conditions of extraction of Pomelo leaves oil using microwave oven heat are as follows: materials and water ratios 3.04:1 mL/g at 62.72 min; and microwave energy of 482.06 W with yield of 0.32 % RSM. From there, it is possible to assert that the combination of response surface methodology and microwave assisted distillation makes the study direction faster, more economical and efficient than traditional methods.

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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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