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Optimization of Microwave-Assisted Extraction of Essential Oil from Vietnamese Basil (*Ocimum basilicum* L.) Using Response Surface Methodology

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Abstract: Basil plant is a common source for linalool and estragole. However, it has been showed that the chemical composition of basil varies considerably depending on many factors including method of extraction, cultivar of the plant or geographical location. In this study, we attempted to extract essential oil from Vietnamese basil and analyze the chemical composition of the obtained oil using gas chromatography–mass spectrometry (GC-MS). The extraction method of choice was microwave-assisted hydro-distillation (MAHD) and the process was optimized with Response Surface Methodology (RSM) with regard to four experimental parameters including raw material size, raw material to water ratio, extraction time and microwave power. The results showed that ground basil leaves, when extracted with optimal conditions of water-to-material ratio of 3.2:1, extraction time of 97 (min) and microwave power of 430 (W), gave the actual essential oil yield of 0.6%. Regarding ANOVA results of the quadratic model, high determination coefficient (R^2 = 0.9077), significant F-value of 10.92 and the *p*-value of less than 0.05 indicate that this model is significant between experimental and predicted variables, and should be fixed. In addition, GC-MS analysis revealed that major components of Vietnamese Basil were Estragole (87.869%), α-Bergamotene (2.922%), τ-Cadinol (2.770%), and Linalool (1.347%).

Keywords: Basil (*Ocimum basilicum* L.); microwave-assisted extraction; response surface methodology; yield and composition of essential oils

1. Introduction

Substances derived from plant ingredients, especially essential oils, have been extensively used in many industries including cosmetics, food and pharmaceuticals [1]. Essential oils are complex, volatile compounds characterized by strong odors. The chemical composition of the essential oil is derived from terpenes and oxidizing compounds, or fatty compounds, in which each of these compounds produce different properties of essential oils [2].

Ocimum basilicum L., commonly referred to as basil, is a plant that belongs to the Lamiaceae family [3]. Basil is native to India and China due to the favorable climatic conditions, but is now commonly cultivated in many tropical and temperate countries in Asia, Africa, Central and South America [4]. In fresh form, basil leaf is often used as a daily spice and food ingredient. In traditional medicine, thanks to the anti-inflammatory, anti-oxidant and antimicrobial properties of the plant, basil is also used to promote digestion, stimulate respiratory circulation, relieve cold symptoms and alleviate digestion issues [5].

The essential oils extracted from basil organs are of light yellow color with a subtle aroma [6]. Similar to the fresh plant and basil-derived products, basil essential oils also exhibited numerous valuable pharmaceutical properties, most notably antimicrobial and anti-fungal activities [7–11]. Regarding chemical composition, basil oil is a complex mixture composed of many chemical components, few of which are found at relatively high concentrations including citral, 1,8-cineole, linalool, estragole, eugenol, methyl eugenol and methyl cinnamate [12]. However, empirical investigations have shown that chemical compositions of basil vary considerably. For example, while basil leaves collected in Turkey and Iran were found to be abundantly composed of estragole (52.6% in Basil sample obtained in Turkey, 52.4% and 40.4% in Iranian purple basil leaves and in green basil leaves respectively) [13,14], other studies indicated that other cultivars such as Brazilian and Pakistani leaves contain mostly linalool with concentrations ranging from 56.7 to 69.3% [10,15]. Such differences with regard to composition could be due to various factors, including seasonal variation [10], geographical location of the plant, extraction method [16] and most strikingly, cultivar of the basil [17].

When it comes to extracting essentials oils from plant materials, apart from the conventional hydro-distillation method (HD) used in the aforementioned composition studies of basil, microwave-assisted hydro-distillation (MAHD) has recently been considered as a preferable method due to advantages over conventional ones regarding cost and time effectiveness, environmental friendliness and reduced energy consumption [18]. In addition, microwave-assisted methods in essential oil extraction could improve both the yield and quality of the produced oils. This enhancement effect is attributable to the internal pressure inside the oil gland cell wall exerted by microwave energy, which effectively leaches out the oil contained inside [18]. Similar to HD, the MAHD process also requires careful selection of experimental parameters. However, most studies for basil generally compare results of solvent-free microwave extraction (SFME) with HD extraction [16,19] or analyze chemical composition of Basil oils after HD extraction, and therefore, lack rigorous optimization for the extraction process. An exception to this is one particular work where MAHD processing of Mexican basil is optimized with Response Surface Methodology (RSM) [20]. However, chemical composition of the basil extract was not clearly reported in this study.

Given these notions, the aim of this study is two-fold. First, we continue this research direction by adopting MAHD process to extract essential oils from another basil cultivar, Vietnamese basil, as to the best of our knowledge, the essential oil extraction of this plant has not yet been attempted. In addition, to achieve the maximum oil yield, Response Surface Methodology (RSM) is applied. RSM is a technique that is widely utilized in optimization of many processes including removal of contaminants from wastewater [21–25], extraction of natural compounds from plants [26–28] or gelatin from animal by-products [29]. In this study, optimization of the extraction process is conducted with regard to three parameters including water-to-material ratio, microwave power and extraction time. Second, by examining chemical composition of the extracted Vietnamese Basil essential oils, we compare that with other studies to propose the use of Vietnamese basil in possible large scale production.

2. Materials and Methods

2.1. Plant Sample Preparation

Fresh basil leaves of approximately 3 cm were purchased in the local market in Go Vap District, Ho Chi Minh City, Vietnam. After purchasing, the materials were washed with water several times to remove impurities. All raw materials are stored in a desiccant bag, stored in a refrigerator (LC-1416B, Alaska, Ho Chi Minh City, Vietnam) at temperatures below 10 °C.

2.2. Extraction Method

The system for basil oil extraction comprises a domestic microwave oven (model ME71A, Samsung, Ho Chi Minh City, Vietnam), acting as the heat source for extraction, and a Clevenger distillation apparatus (Bach Khoa Ltd., Ho Chi Minh City, Vietnam). The flask containing the plant material is placed inside the oven cavity and is connected to the apparatus outside the oven for condensing and separating the oil and aqueous phase.

2.3. Extraction Process

The process of basil essential oil extraction is shown in Figure 1. First, fresh basil leaves were washed, then cut into pieces with size of around 1 cm or ground in accordance with the experiment. The processed sample was then weighed to 100 g with an electronic scale and introduced into the 1 L flask containing water following a pre-specified water-to-raw ratio. Following that, Clevenger extraction was conducted by the microwave oven. Time is measured immediately after turning the oven on. After the extraction period, the raw essential oil is removed and a small amount of condensate is obtained. Lastly, the extracted oil is dried with Na_2SO_4 (Sigma-Aldrich, St. Louis, MO, USA) to completely remove the remaining condensate.

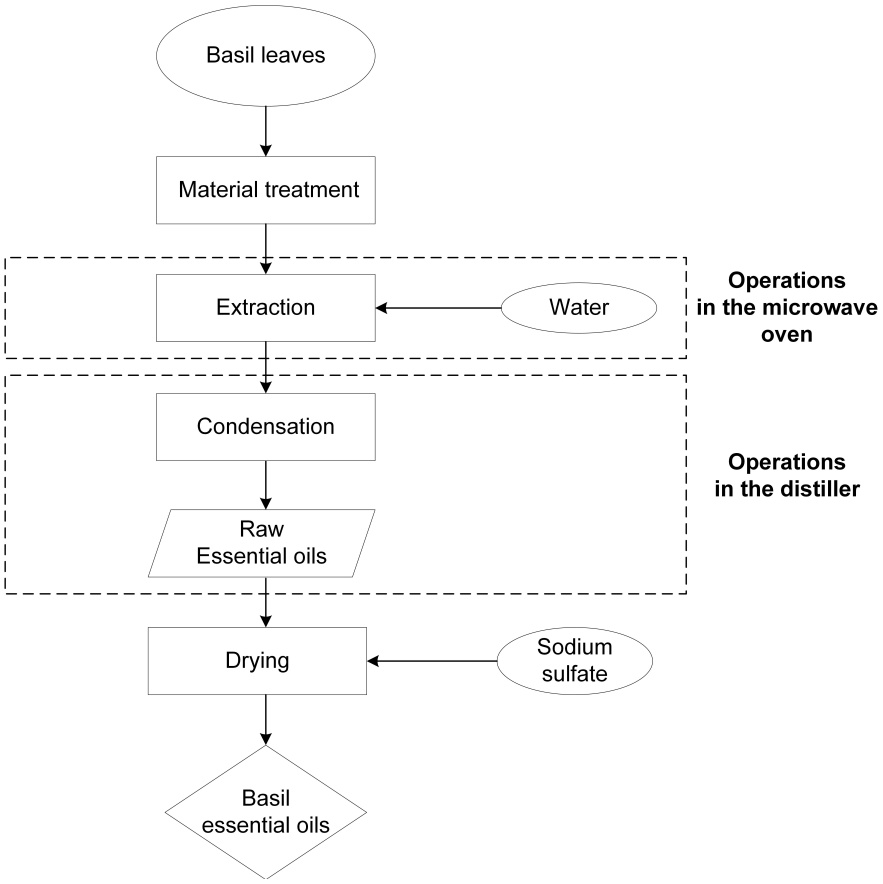


Figure 1. *Cont.*

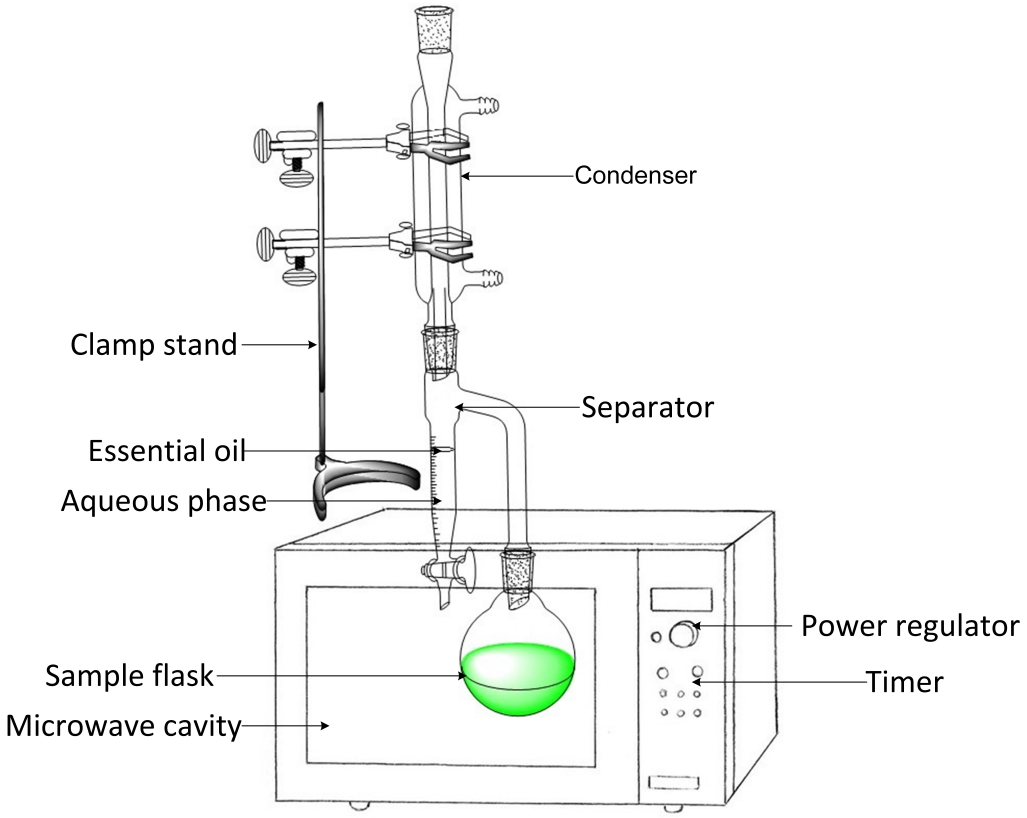


Figure 1. (**a**) The process and (**b**) sketch diagram of the Basil essential oil extraction process.

2.4. Optimization of the Extraction Process Using RSM Procedure

First, we conduct single factor investigation of four factors including material size, water-to-material ratio, time, and microwave power by individually varying one factor while keeping other variables at a fixed value. Based on our acquaintance with the basil essential oil, fixed values were chosen as water-to-material ratio of 3:1, 60 min of extraction time and 450 W of microwave power. Obtained set of values by single factor investigation will then be used in Central Composite Design (CCD) to produce multiple sets of experiment conditions (See Tables 1 and 2. For CCD results). Following that, 20 experiment attempts will be conducted following the specified conditions to generate the oil yield data for estimation of a second-order quadratic model. Estimation results will be tested with ANOVA (Analysis of Variance) to confirm model validity. Lastly, optimal conditions are calculated from the final model and verified by an actual experiment attempt.

Essential oil yield, measured in %, is determined from the following the formula: $Y = \frac{V}{m} \times 100$, where V and m are volume of attained oil (mL) and weight of used basil leaves (g) respectively. CCD, ANOVA and calculation of optimal conditions were executed using Design-Expert software (Stat-ease Inc., Minneapolis, MN, USA). All experimental attempts were conducted in triplicate where the highest value would be recorded.

Table 1. Levels and Independent factors of the basil essential oil extraction process.

Levels	Independent Factors		
	Ratio of Water and Basil Leaves A (mL/g)	**Extraction Time B (min)**	**Microwave Power C (W)**
$-\alpha$	1.32:1	39.55	197.73
-1	2:1	60	300
0	3:1	90	450
1	4:1	120	600
$+\alpha$	4.68:1	140.45	702.27

Table 2. Details of experimental attempts employed in the Response Surface Methodology (RSM) optimization.

No.	Parameters			Yields		No.	Parameters			Yields	
	Ratio (A)	Time (B)	Power (C)	Actual	Predicted		Ratio (A)	Time (B)	Power (C)	Actual	Predicted
1	2	60	300	0.40	0.38	11	3	39.5	450	0.40	0.41
2	4	60	300	0.50	0.49	12	3	140	450	0.50	0.53
3	2	120	300	0.50	0.49	13	3	90	198	0.50	0.53
4	4	120	300	0.60	0.57	14	3	90	702	0.50	0.51
5	2	60	600	0.40	0.41	15	3	90	450	0.70	0.67
6	4	60	600	0.50	0.48	16	3	90	450	0.70	0.67
7	2	120	600	0.50	0.67	17	3	90	450	0.70	0.67
8	4	120	600	0.50	0.50	18	3	90	450	0.60	0.67
9	1.32	90	450	0.40	0.41	19	3	90	450	0.60	0.67
10	4.68	90	450	0.50	0.53	20	3	90	450	0.70	0.67

2.5. Identification of Components by Gas Chromatography-Mass Spectrometry (GC-MS)

To determine the chemical composition in the oil sample, 25 µL of essential oil taken from the optimized process was mixed in 1.0 mL n-hexane and dehydrated with Na_2SO_4. The instrument was GC Agilent 6890 N (Agilent Technologies, Santa Clara, CA, USA), MS 5973 inert, HP5-MS column, head column pressure of 9.3 psi. GC-MS were obtained under the following conditions: carrier gas He; flow rate 1.0 mL/min; split 1:100; injection volume 1.0 µL; injection temperature 250 °C. From the initial hold at 50 °C for 2 min, oven temperature progressed to 80 °C at 2 °C/min, from 80 °C to 150 °C at 5 °C/min, from 150 °C to 200 °C at 10 °C/min, from 200 °C to 300 °C at 20 °C/min and was maintained at 300 °C for 5 min.

3. Results and Discussion

3.1. Single Factor Investigation

The factors that affect the yield of basil essential oil in the extraction process are shown in Figure 2. Figure 2a indicated that the yield of basil essential oil increased as the material size decreases. When the basil leaves size changes from whole to ground leaves, the yield of the essential oil increases from 0.2 to 0.5%. This decline could be explained as follows. As the material is cut smaller, cells containing the oil are broken in larger quantities, making water diffusing into the oil sacs of basil more quickly. This rapidly pushes the essential oils out under the influence of microwave energy, leading to higher performance. Therefore, ground basil leaves have been chosen in subsequent investigations.

In the second survey, the water-to-material ratio factor was investigated. From Figure 2b, the extract yield increased from 0.2 to 0.5% when increasing the ratio from 1:1 to 3:1. Since water is absorbed into the material easily, ingredients could be dissolved more efficiently with higher amount of solvent. Therefore, adding more water to extraction process would cause greater diffusion of essential oil into the water, leading to increased yield of the essential oils. However, increasing this ratio from 3:1 to 4:1 caused the yield of the essential oil to decrease from 0.5 to 0.4% because excess water could dissolve or emulsify the oil. Therefore, the ratio of water to raw materials of 3:1 (mL/g) is selected for the best result of 0.5% yield for subsequent experiments.

Similarly, Figure 2c shows the time extraction effect to the yield of basil essential oil. When the extraction time increases from 30 to 90 min, the yield of the extracted essential oil gradually increases from 0.2 to 0.7% at 90 min, but it decreased to 0.4% at 105 min because of denaturation of some substances in the oil caused by prolonged exposure with high temperature. Regarding the effect of microwave power, Figure 2d shows that high microwave power leads to better performance of the extraction, but only to a certain extent, where corresponding yield would decrease thereafter. Increased temperature, caused by microwave-induced movement of molecules, could affect oil yield in two ways. First, magnetic wave heats water within the cells, exerting internal pressure and rupturing

oil glands. Second, high temperature also impairs the surface tension and, in turn, viscosity of water, causing quicker heat transfer from outside into the materials. However, at a very high temperature, some temperature-sensitive substances in the essential oil could decompose, adversely affecting the extraction yield, the oil quality, and the cost of production due to the increased consumption of energy. Therefore, the extraction time and microwave power in the survey were chosen as 90 min and 450 W, respectively.

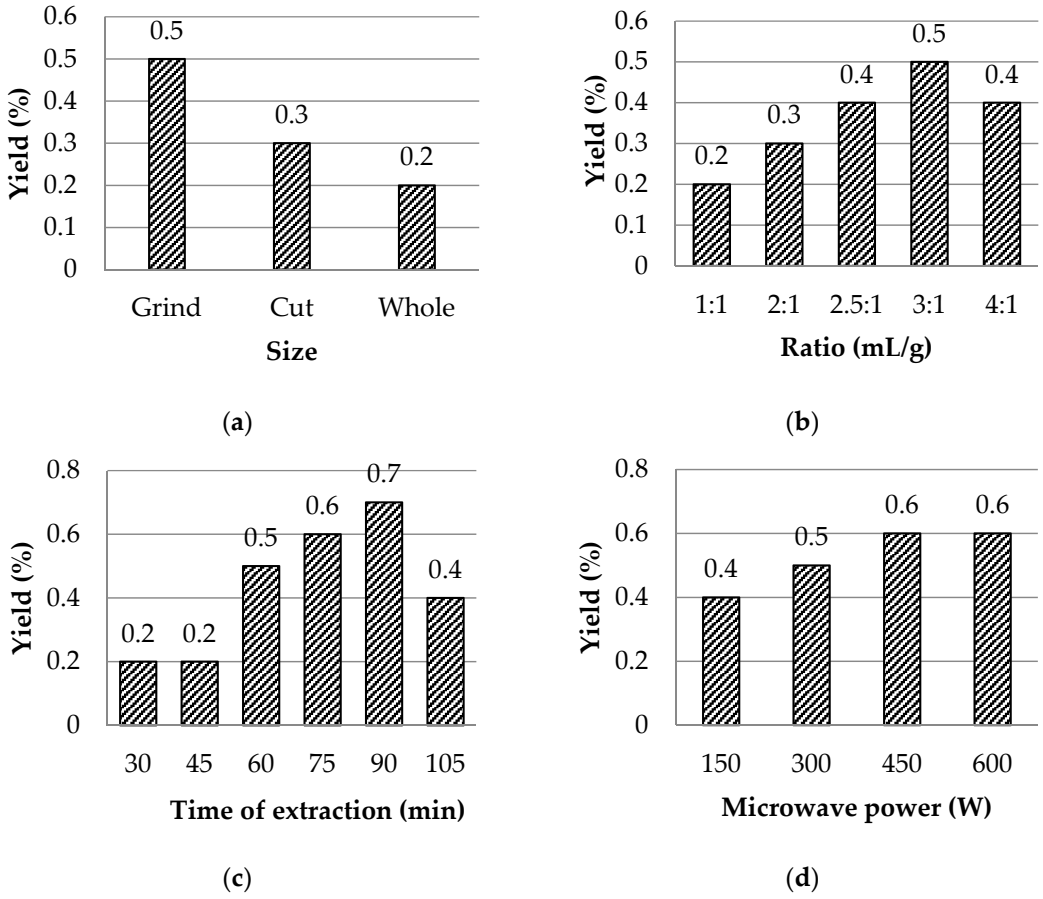


Figure 2. Effect of (**a**) size of the materials; (**b**) water-to-material ratio; (**c**) time of extraction; and (**d**) microwave power to the yield of the essential oil.

3.2. Optimization of Experimental Conditions Using RSM

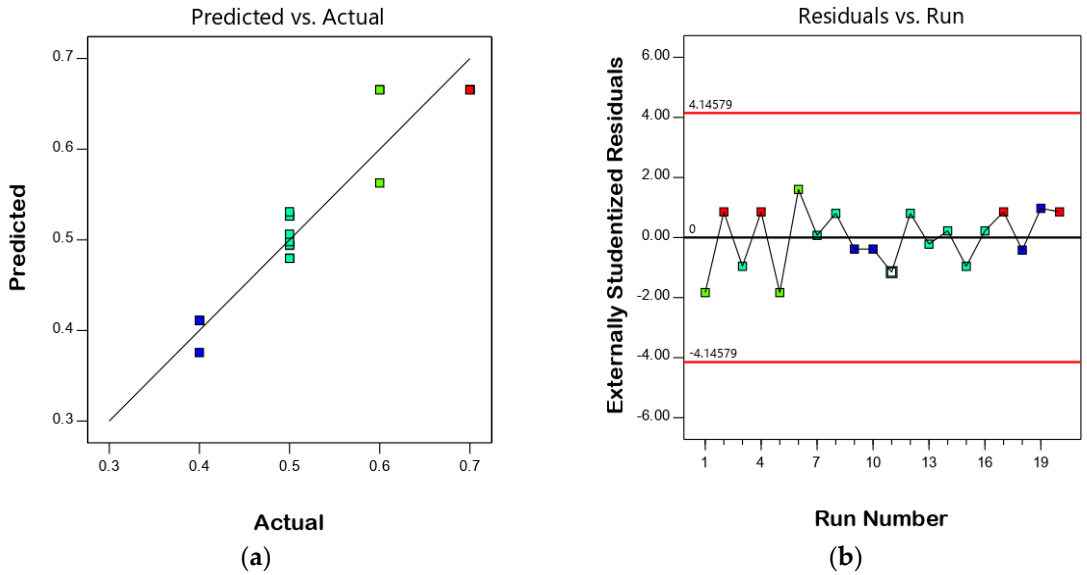
Table 3 displayed the results of ANOVA for the second order regression model of extracted basil essential oil from which three factors were considered and analyzed. The F-mode value of 10.92 implied that the model produced by Design-Expert software is significant. There is only a 0.04% chance that an F-value this large could occur due to noise. *p*-values less than 0.0500 indicated that model terms are significant. In this case, A, B, A^2, B^2, C^2 are significant model terms. The Lack of Fit F-value of 0.42 implies the Lack of Fit is not significant relative to the pure error. There is an 81.67% chance that a Lack of Fit F-value this large could occur due to noise. The R^2 of 0.6895 is in reasonable agreement with the Adjusted R^2 of 0.8246. The Adeq. Precision of 9.4159 indicates an adequate signal and this model can be used to navigate the design space. Therefore, no further specification of the model is required and it can be asserted that the yield of the extraction model produced by the software is fixed and suitable. In addition, the experimental model was considered reasonably fit since calculated residuals follow a random pattern as shown in Figure 3a. Figure 3b demonstrated that data points corresponding to predicted and actual values were scattered across the 45-degree line with close proximity, suggesting that the actual results are accurately predicted from the factor values. Thus,

based on the data analysis of the oil yield from experiments, the optimization of the predicted model results in optimized parameters as A = 3.237:1 (mL/g), B = 97.074 (min), and C = 430.870 (W), obtaining the yield of 0.674% with 90.77% reliability. The final quadratic model is described as follows:

$$Y = 0.6656 + 0.0343A + 0.0343B - 0.0073C - 0.0125AB - 0.0125AC - 0.0125BC - 0.0696A^2 - 0.0696B^2 - 0.0520C^2 \quad (1)$$

Table 3. ANOVA for the quadratic model.

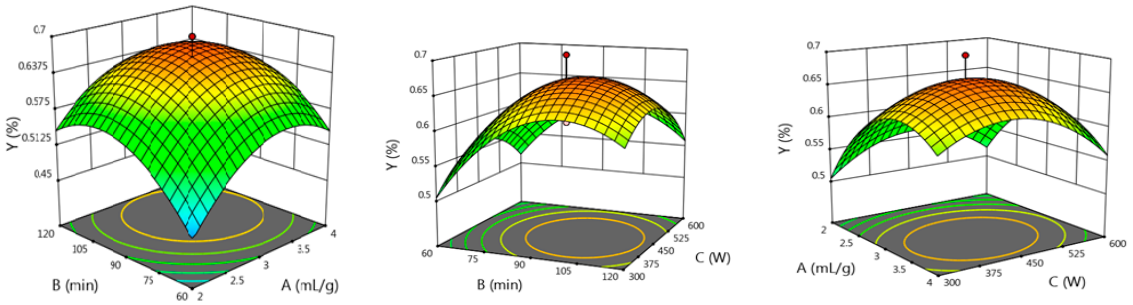
Source	Sum of Squares	Df	Mean Square	F-Value	*p*-Value	Remarks
Model	0.1865	9	0.0207	10.92	0.0004	significant
Water-to-material Ratio (A)	0.016	1	0.016	8.46	0.0156	significant
Extraction time (B)	0.016	1	0.016	8.46	0.0156	significant
Microwave Power (C)	0.0007	1	0.0007	0.386	0.5483	not significant
AB	0.0013	1	0.0013	0.6589	0.4359	not significant
AC	0.0013	1	0.0013	0.6589	0.4359	not significant
BC	0.0013	1	0.0013	0.6589	0.4359	not significant
A^2	0.0699	1	0.0699	36.85	0.0001	significant
B^2	0.0699	1	0.0699	36.85	0.0001	significant
C^2	0.0389	1	0.0389	20.52	0.0011	significant
Residual	0.019	10	0.0019	-	-	-
Lack of Fit	0.0056	5	0.0011	0.4229	0.8167	not significant
Pure Error	0.0133	5	0.0027	-	-	-
Std. Dev.	0.0436	-	R^2	0.9077	-	-
Mean	0.535	-	Adjusted R^2	0.8246	-	-
C.V. (%)	8.14	-	Predicted R^2	0.6895	-	-
-	-	-	Adeq. Precision	9.4159	-	-



(**a**) (**b**)

Figure 3. (**a**) The plot comparing actual values and predicted yield values; (**b**) Normal plot of Residuals with Run number. Points denoted as square dots correspond to actual experiment runs. Blue, cyan, green, and red color represent increasing order of actual yields corresponding to presented data points.

Based on the optimized parameters, Figure 4 shows the mutual interactions of the factors and interaction of factors with the yield of the lemon oil obtained. Visually, the efficiency of the attained oil increases proportionally with condition parameters. However, as these conditions exceed the optimal point (3.237:1 mL/g, 97.074 min, and 430.870 W), the obtained basil essential oil content ceases to rise, and eventually, starts diminishing. The parameters predicted by the software were then used to perform actual extraction for verification. After a set of triplicate experiments, the yield is determined at 0.6% which approximates the predicted yield from the model (0.674%). Given this result, it is suggested that empirical values were accurately predicted by the quadratic model.



(**a**) (**b**) (**c**)

Figure 4. The 3D plots representing factors influencing yields of basil essential oil include (**a**) effect of extraction time and ratio; (**b**) effect of extraction time and microwave power; and (**c**) effect of ratio and microwave power.

Regarding yield, comparison of yields in various studies was showed in Table 4. We found that optimized yield derived from Vietnamese basil leaves (0.6%) was higher than optimized yield derived from MAHD extraction of Mexican basil leaf (0.45%). In comparison with studies of SFME with no optimization, our yield was also higher. However, it is inconclusive as to whether or not MAHD is more efficient than HD, in regard to basil oil extraction, since the reported yields for HD extraction varied wildly and were at variance with MAHD yields.

Table 4. Comparison of oil yields.

	This Study	[10]	[13]	[16]	[17]	[19]	[20]
Method	MAHD	HD	HD	SFME, HD	HD	SFME, HD	MAHD
Materials	Vietnamese leaves	Pakistani leaves	Turkish leaves	French leaves	Italian leaves	Egyptian leaves	Mexican leaves
Yield (%)	0.6	0.5 to 0.8 depending on seasonal variations	1.00	0.028 for SFME, 0.029 for HD	0.3 to 0.8 depending on shape, color and size of leaves	0.48 for both methods	0.45

3.3. Results of GC-MS

The results of GC-MS analyses revealing composition of essential oil are given in Table 5 and GC-MS chromatogram obtained for a sample of basil essential oil is illustrated in Figure 5. At first glance, it is indicated that the Vietnamese basil leaf was rich in estragole, as demonstrated by very high estragole content, at 87.9%, which is followed by α-Bergamotene, τ-cadinol and linalool respectively at 2.922%; 2.770% and 1.347%. Besides genetic differences and nutritional status of the plants, the abundance of estragole constituent in the oil could be explained by the effect of microwave. To be specific, microwave radiation causes polar molecules containing oxygen, such as water and estragole molecules, to spin rapidly. As such, polar compounds in oil bags could easily be separated from the material, leading to higher content. In contrast, hydrocarbons, which are nonpolar, are less prone to magnetic wave and are therefore less likely to be isolated [30]. For α-Bergamotene, τ-Cadinol and linalool, GC-MS results indicated that the presence of these substances is consistent with results from French, Italian, Egyptian and Iranian basil plants. Overall, the abundance of estragole suggests that Vietnamese basil leaf is a suitable material for production of flavoring and smelling agents.

Table 5. Constituents of the extracted essential oil and comparison with those of several studies relating to basil oil extraction.

R.T. (min)	Constituent	Content (This Study)	Comparison with Regard to Method of Extraction, Material and Composition						
			[15]	[10]	[13]	[16]	[17]	[19]	[14]
			HD Brazilian Leaves	HD Pakistani Leaves	HD Turkish Leaves	SFME, HD French Leaves	HD Italian Leaves	SFME, HD Egyptian Leaves	HD Iranian Aerials
11.988	Limonene	0.254	nd	tr to 0.3	13.64	nd	nd to 0.58	nd	nd
12.103	1,8-Cineole	0.239	3.55	0.2 to 1.1	nd	1.5 to 5.8	0.9 to 12.9	6.8 to 7.3	nd to 2.4
16.380	Linalool	1.347	69.33	56.7 to 60.6	nd	25.3 to 39.1	41.1 to 76.2	43.5 to 48.4	nd to 20.1
18.680	L-camphor	0.290	nd	1.1 to 3.1	nd	0.3	0.10 to 0.83	0.3 to 0.4	nd
21.713	Estragole	87.869	nd	nd	52.60	nd	nd to 41.40	13.3 to 14.3	40.5 to 52.4
22.392	Fenchyl acetate	0.379	nd	nd	12.29	nd	nd to 0.56	0.1 to 0.2	nd
24.912	Acetic acid	0.217	nd	nd	nd	nd	nd	nd	nd
28.384	β-Elemene	0.693	nd	nd	nd	2.4 to 3.2	0.12 to 0.66	0.7 to 0.9	nd
29.189	Caryophyllene	0.246	nd	1.2 to 1.7	nd	nd	0.09 to 0.80	0.1	nd
29.681	α-Bergamotene	2.922	nd	nd	nd	6.0 to 7.6	nd to 3.37	2.5 to 2.7	0.5 to 5.2
29.764	α-Guaiene	0.167	nd	nd	nd	nd	nd to 0.27	tr	nd
30.183	α-Caryophyllene	0.292	nd	nd	nd	nd	nd	nd	nd
30.967	Germacrene-D	0.205	nd	1.1 to 3.3	0.47	nd	0.72 to 2.11	0.8 to 0.9	nd to 0.8
31.072	β-Farnesene	0.234	nd	nd	nd	nd	nd	0.4	nd
31.615	α-Bulnesene	0.302	1.07	nd	nd	nd	nd	nd	nd
31.824	γ-Cadinene	0.991	1.58	3.2 to 5.4	nd	2.2 to 3.1	0.38 to 1.37	1.1 to 1.3	nd to 1.8
33.236	(−)-Spathulenol	0.213	nd	tr to 0.5	nd	nd	nd	0.4 to 0.6	nd to 0.9
33.947	Cubenol	0.370	nd	nd	nd	nd	nd	nd	nd
34.407	τ-Cadinol	2.770	nd	nd	nd	5.6 to 6.7	1.76 to 7.55	0.1	nd to 5.9

nd: not detected. tr: trace amounts.

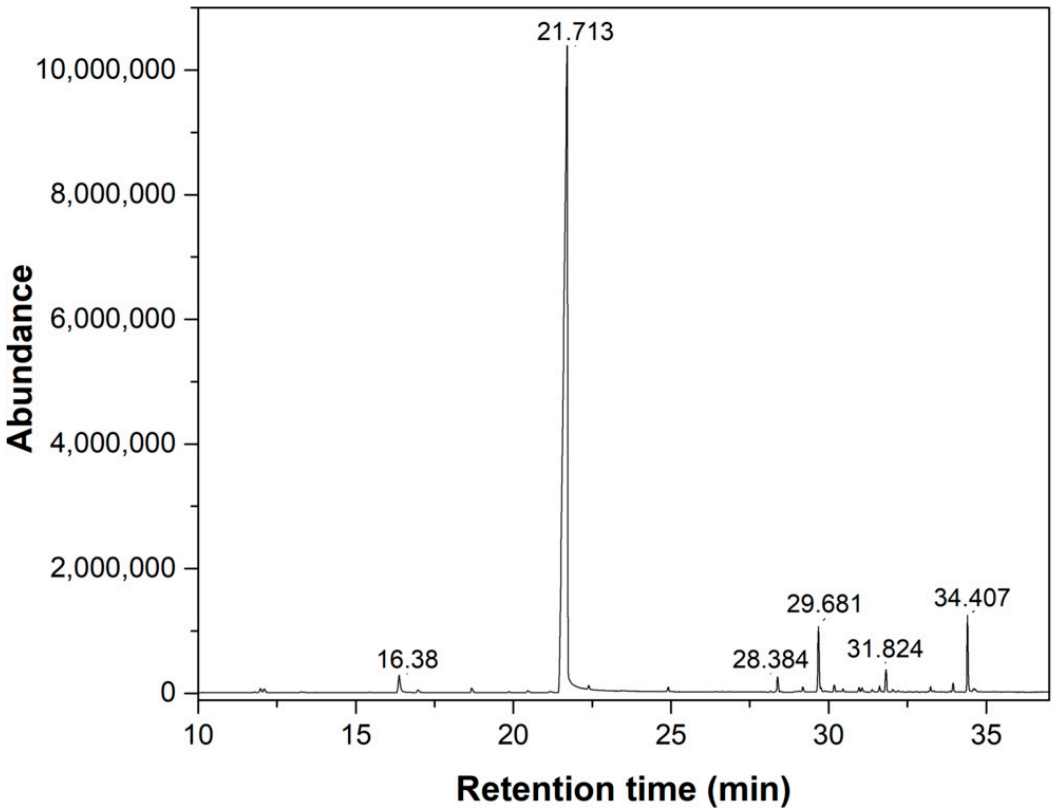


Figure 5. The result of chromatography of basil essential oil.

4. Conclusions

We have successfully applied and optimized MAHD extraction of essential oils from Vietnamese basil leaves. Results from RSM suggested that optimal conditions for this extraction include water to basil ratio of 3.237:1 (mL/g) time of extraction of 97.074 (min), and microwave power of 430.087 (W). After verifying the predicted optimal yield, we determined that the actual yield is 0.6%, which is higher than results of related studies applying MAHD and SFME. GC-MS results also revealed that Vietnamese basil is very rich in estragole. Therefore, it is possible to assert that the combination of RSM and microwave distillation makes the research direction faster, more economical and efficient than traditional methods.

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