Optimization of microwave assisted hydrodistillation of essential oil from lemon (*Citrus aurantifolia*) leaves: Response surface methodology studies

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Abstract. In this paper, microwave-assisted hydro-distillation (MAHD) of essential oils from the lemon (*Citrus aurantifolia*) leaves was attempted and optimized. Optimization of MAHD was performed by using response surface methodology. In the optimization, selected parameters consisted of water and material ratio, microwave power and extraction time were. It is also indicated that quadratic polynomial model could be employed to optimize the microwave extraction of essential oil from Lemon (*Citrus aurantifolia*) leaves. The optimal extraction conditions included microwave power of 523.89W, water to material ratio of 3.27 mL/g, and extraction time of 84.47 minutes. The optimized yield (0.76%) approached predicted yield predicted by the model, implying that the model is suitable to predict behavior of the process.

1. Introduction
Medicinal plants have been the source of therapeutic agents since time immemorial. These plants have been a backbone in the process of discovering new drugs and played an important role in providing healthcare in developing countries. *Citrus aurantifolia* L., is a small citrus fruit that belongs to the family Rutaceae. *Citrus aurantifolia* L. is used in beverages, food additives, and cosmetic industries. The fruit holds an important economic value in the total agricultural production in Vietnam and is ranked fourth in terms of cultivation area [1–5]. Undoubtedly, the fruit and leaves of *C. aurantifolia* are of great economic importance, but these are widely used by herbalists worldwide as traditional medicines for skin care, weight loss, improved digestion, nausea, urinary disorders, and for gum diseases. Many *Citrus*
species have been widely utilized in foods, beverages and as fragrances in cosmetics because of their excellent refreshing flavor of the essential oils as well as their sweet and delicious taste [6–8]. Essential oils, also known as etheric oils (volatile oil) is a substance that is produced by various organs including stems, leaves, flowers and roots of plants. The essential oil accounts for more than 75% of weight of the lemon leaves. The component with the highest content is Citral (which accounts for more than 80% of citronella oil content) which is very unstable and could be easily oxidized and denatured by external conditions such as light, heat and pH. At ambient temperature, essential oils are volatile, soluble in organic solvents, insoluble in water; carry a bitter taste and smell of the original plants. The application of essential oil is well established and ranges from flavors and aromatic agent in cosmetics and food preservatives to therapeutic agents in biomedical applications [9,10]. Traditionally, isolation of essential oils from plants has been performed via hydro-distillation or mechanical pressing. However, recent advances have showed that microwave-assisted extraction (MAE) have tremendous potential and experimental attempts with MAE have been conducted to isolate valuable constituents from plant materials. The advantages of using MAE over conventional methods are numerous. It has been proved that MAE could offer better yield and quality; reduce extraction time and solvent consumption. A previous study focusing on isolation of polysaccharides and comparing with results from convention techniques showed that MAE could drastically reduce extraction time and solvent consumption while improve quantity of polysaccharide from plant materials [11–13].

A common technique for optimization and quantification of effects of parameters on a desired response is response surface methodology (RSM). The technique rests on a number of statistical techniques to describe a desired response with respect to various experimental parameters. Since the method could drastically reduce number of experimental attempts required to obtain reliable estimation of the response function, it has been widely adopted in optimization of different processes [14–21]. In this study, microwave-assisted extraction (MAE) was used to obtain essential oil from Lemon (Citrus aurantifolia) leaves. Moreover, the use of methods microwave-assisted extraction (MAE) is also based on the availability of sufficient microwave readily available in the public. In addition, the optimum values (microwave power, plant material to solvent ratio and extraction time) for the process variables was determined using a three-level, three variable central composite design (CCD).

2. Materials and methods

2.1. Plant samples
Lemon leaves were harvested from Tien Giang province, Vietnam. After transportation to the laboratory, lemon leaves were picked, washed, grinded by grinding equipment (Sunhouse SHD4322, 200W, Vietnam) and distilled directly by steam.

2.2. Extraction method
The essential oil obtained was extracted with a microwave-assisted hydro-distillation system comprising a Clevenger apparatus and a domestic microwave oven MW71E (manufactured by SAMSUNG, Vietnam) operating at the maximum power source of 800W and voltage of 250v-50Hz. The flask containing 100g lemon leaves and distilled water was placed within the microwave oven cavity. The condenser was placed on the top of the oven to collect extracted oil, which was subsequently subjected to anhydrous sodium sulfate (Na$_2$SO$_4$, Sigma Aldrich) to remove excess water. Performance of MAE was evaluated through oil yield from lemon leaves. To be specific, yield of essential oil (Y) in every experiment attempt was calculated following the equation (1):

\[
Y \, (\%) = \frac{V}{W} \times 100
\]

where y is the lemon leaves oil yield (mL/100g material), V is the volume of essential oil obtained (mL), and W is the amount of lemon leaves originally used (g).

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2.3. Experimental design with RSM
Effects of material-to-water ratio, microwave power, and extraction time on the yields of essential oil were investigated in the RSM design. Table 1 lists parameters of interest and their corresponding coding for CCD inputs. Initial levels of parameters were determined by preliminary experimental results. Each independent factor varies over five levels: minus and plus alpha (axial point), minus and plus 1 (factorial point) and the center point. The condition A, B, C were set from 2:1 to 4:1 mL/min, 300 to 600W, and 60 to 120 minutes, respectively.

Table 1. Independent variables and their encoded levels for RSM model

| Symbols | Independent variables                  | Coded levels |
|---------|----------------------------------------|--------------|
| A       | Water-to-material ratio (mL/g)         | -α 1 -1 0 1 4 | α 4.68 |
| B       | Microwave power (W)                   | 198 300 450 600 702 |
| C       | Extraction time (min)                 | 40 60 90 120 140 |

3. Results and discussion
A second-order equation was established to model the effects of three parameters namely water-to-material ratio, microwave power, and extraction time on the essential oil yield. Table 2 presents the attempt parameters generated using the central composite design (CCD) and their corresponding yields. The resulting model is tested using three tests including significance of the regression model, significance on individual coefficients and lack of fit (LOF).

Table 2. The coded variables, yield (experimental and predictive) for each run

| Std order | Run | Coded variables | Extraction yield (%) | Experimental | Predictive |
|-----------|-----|----------------|----------------------|--------------|------------|
| A         | B   | C             |                      |              |            |
| 1         | 10  | -1 -1 -1      | 0.4000               | 0.4060       |            |
| 2         | 6   | 1 -1 -1       | 0.6000               | 0.5839       |            |
| 3         | 15  | -1 1 -1       | 0.4500               | 0.4546       |            |
| 4         | 1   | 1 1 -1        | 0.6500               | 0.6574       |            |
| 5         | 13  | -1 1 1        | 0.6500               | 0.6378       |            |
| 6         | 3   | 1 -1 1        | 0.6500               | 0.6406       |            |
| 7         | 18  | -1 1 1        | 0.7000               | 0.7113       |            |
| 8         | 16  | 1 1 1         | 0.7500               | 0.7392       |            |
| 9         | 4   | -α 0 0        | 0.5500               | 0.5419       |            |
| 10        | 12  | α 0 0         | 0.7000               | 0.7149       |            |
| 11        | 2   | 0 -α 0        | 0.5500               | 0.5665       |            |
| 12        | 8   | 0 α 0         | 0.7000               | 0.6902       |            |
| 13        | 19  | 0 0 -α        | 0.4500               | 0.4466       |            |
| 14        | 9   | 0 0 α         | 0.7000               | 0.7102       |            |

Repeated runs:
| 15        | 11  | 0 0 0         | 0.7000               | 0.7415       |            |
| 16        | 20  | 0 0 0         | 0.7500               | 0.7415       |            |
| 17        | 5   | 0 0 0         | 0.7500               | 0.7415       |            |
| 18        | 14  | 0 0 0         | 0.7500               | 0.7415       |            |
| 19        | 7   | 0 0 0         | 0.7500               | 0.7415       |            |
| 20        | 17  | 0 0 0         | 0.7500               | 0.7415       |            |

Table 3 displays the analysis of variance results for the quadratic model describing lemon leaves oil yield and consisting of aforementioned tests. F-value of the model was 69.98, which is highly significant.
at 5%, indicated that the model is significant. Similarly, based on p-values, seven variables including water-to-materials ratio (A), effect of microwave power (B), effect of extraction time (C), the interaction of water-to-materials ratio and extraction time (AC), the second order effect of the water-to-materials ratio (A²), the second order effect of the water of microwave power (B²) and second order effect of the extraction time (C²) were found to be statistically significant terms at p-value>5%. Regarding magnitude, it was found that effect of water-to-materials ratio was most influential to the yield of lemon leaves oil. This could be explained by the polar nature of water. As water presents excessively, interaction of the mixture to microwave could be enhanced, thus improving the efficiency of the extraction process. Based on the value of coefficients, the significance of the factors are as follows: A > B > C > AC > A² > B² > C².

The Lack-of-Fit value of 0.7809 implies that the probability of occurrence of the LOF due to noise is 60.37%. The R²-value was calculated to be 0.9844, which approximates 1 and well agrees with the adjusted R² of 0.9703. For adequate precision, a value of higher than 4 would be desirable for navigation of design space. The results showed that the ratio of 24.6292 indicated an adequate signal.

### Table 3. Analysis of variance table for quadratic model

| Source       | Sum of Squares | dF | Mean Square | F-value | p-value        |
|--------------|----------------|----|-------------|---------|----------------|
| Model        | 0.2337         | 9  | 0.0260      | 69.98   | <0.0001 Significant |
| A-A          | 0.0361         | 1  | 0.0361      | 97.33   | <0.0001         |
| B-B          | 0.0185         | 1  | 0.0185      | 49.79   | <0.0001         |
| C-C          | 0.0839         | 1  | 0.0839      | 226.15  | <0.0001         |
| AB           | 0.0003         | 1  | 0.0003      | 8.423   | 0.3803          |
| AC           | 0.0153         | 1  | 0.0153      | 41.27   | <0.0001         |
| BC           | 0.0003         | 1  | 0.0003      | 8.423   | 0.3803          |
| A²           | 0.0230         | 1  | 0.0230      | 62.09   | <0.0001         |
| B²           | 0.0230         | 1  | 0.0230      | 62.09   | <0.0001         |
| C²           | 0.0230         | 1  | 0.0479      | 129.13  | <0.0001         |
| Residual     | 0.0037         | 10 | 0.0004      |         |                |
| Lack of Fit  | 0.0016         | 5  | 0.0003      | 0.7809  | 0.6037 not significant |
| Pure Error   | 0.0021         | 5  | 0.0004      |         |                |
| Cor Total    | 0.2374         | 19 |             |         |                |
| Std. Dev     | 0.0193         |    | R²          | 0.9844  |                |
| Mean         | 0.6475         |    | Adjusted R² | 0.9703  |                |
|              |                |    | Predicted R² | 0.9333  |                |
|              |                |    | Adel Precision | 24.6292 |                |

Values obtained from Design Expert 11

The final model with respect to coded factors could be described as follows (2):

\[
Y = 0.7415 + 0.0514*A + 0.0368*B + 0.0784*C + 0.0062*AB - 0.0438*AC + 0.0062*BC - 0.0400*A^2 - 0.0400*B^2 - 0.0577*C^2 (2)
\]

The model could be used to predict the yield of lemon leaves oil given any set of experimental parameters. Figure 1 demonstrated plot of probability and corresponding residuals and plot of residuals versus the predicted response. Visually, it is revealed that the residuals were distributed closely to the 45-degree line, which suggests that errors follow normal distribution and least-square fit is adequate to produce residuals. Figure 1B displays that data points follow no clear pattern and situate above and below x-axis equally. These results suggest adequacy of the estimated model and no violation of the independence or constant variance assumption.
Figure 1. Normal probability plot of residual (A) and plot of residual versus the predicted response for yield of lemon leaves oil (B)

Figure 2 shows three-dimensional response surfaces and corresponding two-dimensional contour plots show interaction effect of process variables on lemon leaves oil production. Both plots showed pairwise relationship between the essential oil yield and three independent factors including water-to-material ratio (A), microwave power (B), and extraction time (C). It is recognized that the trends in the three plots are generally similar. To be specific, the relationship between an experimental factor and the yield was positive until yield reaches a peak, from which the yield starts to diminish.
Verifications runs were conducted with slightly altered optimal conditions for convenience. To be specific, actual conditions in these runs include water-to-material ratio of 3:1 mL/g, microwave power 450W and extraction time of 90 min. We attempted experiments in triplicate and compared with the results of the model. The results are shown in Table 4. It was observed that the difference between predicted and actual yield was marginal, suggesting reliability of the employed model and the suitability in optimizing the extraction process.

|          | A (Ratio, mL/g) | B (Power, W) | C (Time, min) | Y (Yield,%) | Error (%) |
|----------|----------------|--------------|---------------|-------------|-----------|
| Predicted| 3.27           | 523.89       | 84.47         | 0.762       |           |
| Experiment 1 | 3.3           | 525.00       | 84.50         | 0.75        | 4.75      |
| Experiment 2 | 3.3           | 525.00       | 84.50         | 0.75        | -1.6      |
| Experiment 3 | 3.3           | 525.00       | 84.50         | 0.77        | -1.6      |
| Average Exp | 3.3           | 525.00       | 84.50         | 0.77        | 1.04      |

4. Conclusions

Through the experimental process, the surface response methodology was considered as a tool of computer software application for designing the experiments for optimizing the extraction conditions of essential oils from the lemon leaves. A RSM, in conjunction with a CCD, was used to quantify and optimize the three different parameters namely water-to-material ratio, microwave power, and extraction time on the extraction yield. Model value of $R^2$ (98.44%) indicated high agreement between the experimental and predicted value. The results indicated that a quadratic model adequately represented the experimental data. Calculated optimal parameters included 3.27 mL/g of water and material ratio, 523.89W of microwave power, and 84.47 minutes of extraction time, corresponding with the predicted extraction yield 0.762%. Further experiment confirming these optimal conditions solidified the results and suggested the employed model reliable for predicting the yield.

Acknowledgement

This research is funded by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam.
References

[1] Le N T T, Bach L G, Nguyen D C, Le H T X, Pham K H, Nguyen D H and Hoang T T T 2019 Evaluation of Factors Affecting Antimicrobial Activity of Bacteriocin from Lactobacillus plantarum Microencapsulated in Alginate-Gelatin Capsules and Its Application on Pork Meat as a Bio-Preservative Int. J. Environ. Res. Public Health 53 43-52

[2] Nguyen T V L, Nguyen M D, Nguyen D C, Bach L G and Lam T D 2019 Model for Thin Layer Drying of Lemongrass (Cymbopogon citratus) by Hot Air, Processes 7 21

[3] Huynh M C, Nguyen T S V, Le T H N, Nguyen D C and Bach L G 2019 Evaluation of Conditions Affecting Properties of Gac (Monmordica CoCochinesis Spreng) Oil-Loaded Solid Lipid Nanoparticles (SLNs) Synthesized Using High-Speed Homogenization Process Processes 7 90

[4] Mollazadeh H, Mahdian D and Hosseinizadeh H 2018 Medicinal plants in treatment of hydertiglyceridemia: A review based on their mechanisms and effectiveness Phytomedicine 53 43-52

[5] Phan A N Q, Bach L G, Nguyen D T and Le T H N 2019 Efficient Method for Preparation of Rutin Nanosuspension Using Chitosan and Sodium Tripolyphosphate Crosslinker J. Nanosci. Nanotechnol 19 974–984

[6] Ajikumaravan S, Ramani K S, Akhil S N and Sabulal B 2018 Citrus peels prevent cancer Phytomedicine 50 531-537

[7] Al-Aamri M S, Al-Abousi N M, Al-Jabri S S, Alam T and Khan S A 2018 Chemical composition and in-vitro antioxidant and antimicrobial activity of the essential oil of Citrus aurantifolia L. leaves grown in Eastern Oman J. Taibah Univ. Med. Sci. 13 108–112

[8] Tran T H, Le K H, Nguyen D C, Dao T P, Le T H N, Nguyen D H, Nguyen T D, Vo N D V, Tran Q T and Bach L G 2019 The Study on Extraction Process and Analysis of Components in Essential Oils of Black Pepper (Piper nigrum L.) Seeds Harvested in Gia Lai Province, Vietnam Processes 7 56

[9] Dao T P, Nguyen D C, Nguyen D T, Tran T H, Nguyen P T N, Le T H N, Nguyen D H, Nguyen V D V and Bach L G 2019 Extraction Process of Essential Oil From Pletranthus amboinicus Using Micro-wave-Assisted Hydrodistillation and Evaluation of It’s Antibacterial Activity Asian J. Chem. 31 977–981

[10] Megawati, Fardhyanti S D, Sediawan B W and Hisham A 2019 Kinetic of mace (Myristicae arillus) essential oil extraction using microwave assisted hydrodistillation: Effect of microwave power Ind. Crops Prod. 131 315-322

[11] Ibrahim N A and Zain M A A 2018 Microwave-assisted solvent extraction of castor seeds Chin. J. Chem. Eng. 26 2516-2522

[12] Franco-Vega A, Ramire-Corona N, Lopez-Malo A and Palou E 2019 Studying microwave assisted extraction of Laurus nobilis essential oil: Static and dynamic modelling J. Food Eng. 247 1-8

[13] Nguyen T C D, Le T N H, Do T S, Pham V T, Tran D L, Ho T T V, Tran V T, Nguyen D C, Nguyen D T, Bach L G, Huynh K P H and Doan V T 2019 Metal-Organic Framework MIL-53(Fe) as an Adsorbent for Ibuprofen Drug Removal from Aqueous Solutions: Response Surface Modeling and Optimization J. Chem 2019 11

[14] Van Tran T, Bui Q T P, Nguyen T D, Le N T H and Bach L G 2017 A comparative study on the removal efficiency of metal ions (Cu2+, Ni2+, and Pb2+) using sugarcane bagasse-derived ZnCl2-activated carbon by the response surface methodology Adsorpt. Sci. Technol. 35 72–85
[16] Tran V T, Bui P T Q, Nguyen D T, Ho T T V and Bach L G 2017 Application of response surface methodology to optimize the fabrication of ZnCl$_2$-activated carbon from sugarcane bagasse for the removal of Cu$^{2+}$ Water Sci. Techno. 75 2047-2055

[17] Tran V T, Bui T P Q, Nguyen D T and Bach L G 2017 Application of response surface methodology to optimize the fabrication of ZnCl$_2$-activated carbon from banana peels Surf. Interfaces 6 209-217

[18] Tran T H, Nguyen P T N, Pham T N, Nguyen D C, Dao T P, Nguyen T D, Nguyen D H, Vo D V N, Le X T, Le N T H and Bach L G 2019 Green technology to optimize the extraction process of turmeric (Curcuma longa L.) oils IOP Conf. Ser. Mater. Sci. Eng. 479 012002

[19] Tran T H, Nguyen P T N, Ho V T T, Le T H N, Bach L G and Nguyen T D 2019 Using soft computing approaches for orange (Citrus nobilis Lour. var. nobilis) oils extraction process IOP Conf. Ser. Mater. Sci. Eng. 479 012015

[20] Nhan N P T, Hien T T, Nhan L T H, Anh P N Q, Huy L T, Nguyen T C T, Nguyen D T and Bach L G 2018 Application of Response Surface Methodology to Optimize the Process of Saponification Reaction from Coconut Oil in Ben Tre-Vietnam Solid State Phenom. 279 235–239

[21] Bach L G, Van Tran T, Nguyen T D, Van Pham T and Do S T 2018 Enhanced adsorption of methylene blue onto graphene oxide-doped XFe$_2$O$_4$($X =$ Co, Mn, Ni) nanocomposites: kinetic, isothermal, thermodynamic and recyclability studies Res. Chem. Intermed. 44 1661–1687