Optimization of the Essential Oil Extraction Process from Dong Van Marjoram (*E. winitiana* var. *dongvanensis* Phuong.) by Using Microwave Assisted Hydrodistillation, and the Bioactivities of the Oil Against Some Cancer Cell Lines and Bacteria

Xuan Duy Le¹, Ngoc Mai Pham Thi¹,²,³, Thi Inh Cam¹,², Huu Nghi Do¹, Hong Van Nguyen Thi¹, Tran Dinh Thang⁴, Lai Phuong Phuong Thao¹, Trung Sy Do⁵, Thanh Duong Nguyen⁵, Quoc Long Pham¹,², Tan Phat Dao⁶,⁷, Tri Nhut Pham⁶,⁷ and Quoc Toan Tran¹,²

Abstract

This study reports on the optimization of a microwave-assisted distillation process to obtain Dong Van marjoram essential oil, and the determination of its composition, content of constituents, and cytotoxic and antimicrobial activities. Using the response surface method (RSM), the optimal essential oil distillation conditions were determined as material size 0.74 (cm), water to material ratio 4.14:1 (mL/g), microwave power 302.4 (W), and distillation time 2.1 hours. At optimal conditions, the mass of Dong Van marjoram essential oil obtained was 0.887 ± 0.007 g, corresponding to a content of 0.6% essential oil in the material. GC-MS and GC-FID methods showed that the main chemical constituents of Dong Van marjoram essential oils obtained were rosefuran epoxide (44.9%), caryophyllene (10.8%), germacrene D (2.6%), and α-humulene (1.3%). The essential oil exhibited moderate inhibition against both tested cancer cell lines, with IC₅₀ values of 23.9 µg/mL (for PC3) and 56.2 µg/mL (for A549). However, the oil exhibited strong effectiveness against three bacterial strains, *Escherichia coli*, *Bacillus subtilis*, and *Staphylococcus aureus*, and a yeast strain, *Saccharomyces cerevisiae*, with minimal inhibitory concentration (MIC) values ranged from 50 to 100 µg/mL.

Keywords

Dong Van marjoram (*E. winitiana* var. *dongvanensis* Phuong.), microwave-assisted hydrodistillation (MAHD), response surface methodology, essential oils, chemical composition.

Introduction

Plant essential oils have been shown to exhibit numerous useful properties, including anti-microbial, anti-fungal, antioxidant, anti-free radical, and anti-inflammatory activities.¹,² The composition of essential oils is widely varied and might include terpene and oxide derivatives such as ketones, alcohols, and aldehydes.³-⁵ Dong Van marjoram (*E. winitiana* var. *dongvanensis* Phuong., family Lamiaceae) is an herbaceous plant native to Asian countries such as China, Vietnam, and Thailand.⁶ This species is quite common in the high mountains of Ha Giang province, Vietnam.⁷ Although the plant has been used in the food industry as a seasoning in sausages, baked goods, processed vegetables, and soups, its most prevalent use is in folk medicine for healing.

¹Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
²Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
³Thai Nguyen University of Agriculture and Forestry, Thai Nguyen, Vietnam
⁴Institute of Biotechnology and Food Technology, Industrial University of Ho Chi Minh City, HCM City, Vietnam
⁵Institute of Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam
⁶Institute of Environmental Sciences, Nguyen Tat Than University, Ho Chi Minh City, Vietnam
⁷Faculty of Environmental and Food Engineering, Nguyen Tat Than University, Ho Chi Minh City, Vietnam

Corresponding Authors:
Tri Nhut Pham, Institute of Environmental Sciences, Nguyen Tat Than University, Ho Chi Minh City, Vietnam.
Email: pmnhut@ntt.edu.vn
Quoc Toan Tran, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Ha Noi, Vietnam.
Email: tranquoctoan2010@gmail.com
where the plant is used to treat colds, fever, digestive disorders, sunstroke, rheumatic arthritis, and nyctalopia.\textsuperscript{17}

\emph{Elsholtzia} species produce essential oils with diverse chemical compositions including terpenoids, aldehydes, phenylpropanoids, and phytosterol.\textsuperscript{1} The essential oil of \emph{E. winitiana} is a rich source of citral, containing citral A (34.8\%) and citral B (30.1\%), suggesting its use in the cosmetic and pharmaceutical industries.\textsuperscript{7} Citral is mostly obtained from the upper part of the plant, but its content varies considerably depending on the harvesting time and the quality of the raw materials.\textsuperscript{7} The essential oils of \emph{E. winitiana} also have a high concentration of rosefuran or rosefuran epoxide.\textsuperscript{8,9}

There are many traditional methods to obtain essential oils from plant materials such as hydrodistillation (HD), steam distillation (SD), and organic solvent extraction.\textsuperscript{10} However, the loss of some degradable compounds due to heating or hydrolysis can occur during HD and SD techniques, while organic solvents are toxic. Nowadays, microwave-assisted hydrodistillation (MAHD) is an alternative method for isolating essential oils because of its advantages such as cost- and time-effectiveness, environmental friendliness, and lower energy consumption.\textsuperscript{11-13} Microwave extraction using a microwave oven can improve the performance of the process. This improvement is determined by the energy from microwaves causing internal pressure in the essential glandular cells, which helps the more efficient extraction of the essential oils.\textsuperscript{14}

In this study, we used microwave-assisted hydrodistillation (MAHD) to extract the essential oil from Dong Van marjoram branches (\emph{E. winitiana} var. \emph{dongvanensis} \textsuperscript{Phuong}). Then, we used RSM (Response Surface Methodology) with a central composite design (CCD) to determine the maximum oil distillation performance with four parameters including material size (cm), water to material ratio (mL/g), distillation time (h), and microwave power (W). The quantitative profiles of the essential oils were analyzed using gas chromatography (GC) with flame ionization detection (FID), and GC combined with mass spectrometry (MS). Finally, we evaluated the in vitro bioactivity of the essential oil from branches of Dong Van marjoram (\emph{E. winitiana} var. \emph{dongvanensis} \textsuperscript{Phuong}) obtained by the MAHD method.

**Material and Methods**

\textit{Materials}

\emph{Elsholtzia winitiana} var. \emph{dongvanensis} \textsuperscript{Phuong} plants were collected in December 2020, in Dong Van district of Ha Giang province, Vietnam. A specimen of the plant was botanically identified by Dr Nguyen Quoc Binh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology. After harvesting, the branches were separated and dried to prepare for the extraction of essential oils. The used plant materials were of uniform quality, and free from pests and diseases.

**Extract equipments**

The essential oil extraction system included a microwave model R20A1 produced by SHARP; this equipment acts as the heat source of the extraction process and a Clevenger extraction apparatus. The flask containing the raw material was placed in the microwave chamber. The distillation system was located outside the oven, which condenses and separates the essential oil from the liquid phase.

**The extraction process of essential oils**

Firstly, 150 g of dry plant material was cut into a suitable size and added to a 2 L flask containing water for which the water to material ratio is identified. The sample was soaked for 30 minutes before the Clevenger extraction process was conducted by a microwave oven. Time was measured from when the oven was turned on. After the extraction process, the crude oil and a few condensation products were recovered. The crude essential oil was dried with Na\textsubscript{2}SO\textsubscript{4}, which also completely removed the remaining condensation products. Finally, the mass of essential oil was accurately determined on an analytical balance and stored at 4 °C in a refrigerator until gas chromatography (GC)-flame ionization detector (FID) and GC-mass spectrometry (MS) analyses.

The yield of essential oil obtained (\%) was calculated by the following formula:

\[
\text{The yield (\%) } = \frac{W_1}{W_2} \times 100
\]

where \(W_1\) is the mass of essential oil obtained (g) and \(W_2\) is the mass of dry sample originally used (g).

**Experiment Design for Response Surface Methodology Optimization**

Response surface methodology (RSM) was used to optimize the experimental conditions with respect to maximum oil yield. Four parameters were considered for optimization, namely: material size, water/material ratio, extraction time, and microwave power. First, a series of single-factor investigations was performed by varying one of the considered variables while fixing the others, in the following sequence: material size, water to material ratio, microwave power, and extraction time. Based on the results, experimental levels were determined to include the level center (encoded 0), level low (encoded -1), and level high (encoded +1) to build an experimental design matrix following the Box-Wilson model with Design-Expert 7.0.0 software. Twenty-five sets of experiments were designed, which were then attempted to generate the data for model estimation (Table 1 and 2). The validity of the estimated model was confirmed by performing analysis of variance (ANOVA). The optimized conditions were verified by conducting experiments with those parameters and the masses of essential
oils were compared. The function that describes the mass of the essential oil with respect to experimental parameters is as follows:

\[ Y_k = b_0 + \sum b_j x_j + \sum b_{ju} x_u x_j + \sum b_{jj} x_j^2 \]  

With: \( Y \) and \( X \) are the predicted response and independent variables, respectively; \( b_0 \) is the intercept; \( b_j \), \( b_{ju} \), and \( b_{jj} \) are variable coefficients.

**Phytochemical Screening of Essential Oils**

The essential oil components were analyzed by GC-MS and GC-FID. For GC-MS analysis, the system involved an HP7890A model GC (Agilent Technologies, Santa Clara, CA, USA) equipped with an HP5975C MS detector and an HP5 MS column (60 m × 0.25 mm, film thickness 0.25 µm) (Agilent Technologies, US). Running conditions were GC-MS: carrier gas: helium; flow rate: 1 mL/min; split ratio 100:1; injection volume of sample 1 µL; temperature of injector set at 250 °C. The temperature program began at 60 °C, then increased up to 240 °C, at 4°C/min. The electron impact ionization voltage was 70 eV, the emission current was 40 mA, and the acquisitions scan mass range was 35-450 amu. GC-FID analysis was applied using similar conditions as those used for GC-MS analysis.

The GC-FID peak area was used to calculate the relative percentage of components in the essential oil, without any correction factor. Identification of the constituents was carried out by comparing the obtained retention indices (RI) and mass

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### Table 1. Ranges of parameters determined by experimental design

| Actual variables | Coded variables | \( \Delta \) | \(-\alpha\) | \(-\alpha\) | 0 | 1 | \(+\alpha\) |
|------------------|----------------|-------------|-----------|----------|---|---|--------|
| \( Z_1 \): Material size (cm) | A | 0.5 | 0.29 | 0.5 | 1.5 | 1.71 |
| \( Z_2 \): Water to material ratio (mL/g) | B | 1 | 2.58 | 3 | 4 | 5 | 5.42 |
| \( Z_3 \): Microwave power (W) | C | 120 | 70 | 120 | 240 | 360 | 410 |
| \( Z_4 \): Distillation time (h) | D | 1 | 0.59 | 1 | 2 | 3 | 3.41 |

(Coeficient \( \alpha = 1.414 \))

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### Table 2. Matrix of orthogonal plans and experimental results

| Run | \( Z_1 \) | \( Z_2 \) | \( Z_3 \) | \( Z_4 \) | \( X_1 \) | \( X_2 \) | \( X_3 \) | \( X_4 \) | \( Y \) (The mass of essential oils) |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----------------|
| 1   | 0.5 | 3   | 120 | 1   | -1  | -1  | -1  | -1  | 0.11            |
| 2   | 1.5 | 3   | 120 | 1   | +1  | -1  | -1  | -1  | 0.05            |
| 3   | 0.5 | 5   | 120 | 1   | -1  | +1  | -1  | -1  | 0.14            |
| 4   | 1.5 | 5   | 120 | 1   | +1  | +1  | -1  | -1  | 0.10            |
| 5   | 0.5 | 3   | 360 | 1   | -1  | -1  | +1  | -1  | 0.71            |
| 6   | 1.5 | 3   | 360 | 1   | +1  | -1  | +1  | -1  | 0.50            |
| 7   | 0.5 | 5   | 360 | 1   | -1  | +1  | +1  | -1  | 0.72            |
| 8   | 1.5 | 5   | 360 | 1   | +1  | +1  | +1  | -1  | 0.60            |
| 9   | 0.5 | 3   | 120 | 3   | -1  | -1  | -1  | +1  | 0.15            |
| 10  | 1.5 | 3   | 120 | 3   | +1  | -1  | -1  | +1  | 0.05            |
| 11  | 0.5 | 5   | 120 | 3   | -1  | +1  | -1  | +1  | 0.17            |
| 12  | 1.5 | 5   | 120 | 3   | +1  | +1  | -1  | +1  | 0.06            |
| 13  | 0.5 | 3   | 360 | 3   | -1  | -1  | +1  | +1  | 0.70            |
| 14  | 1.5 | 3   | 360 | 3   | +1  | -1  | +1  | +1  | 0.50            |
| 15  | 0.5 | 5   | 360 | 3   | -1  | +1  | +1  | +1  | 0.72            |
| 16  | 1.5 | 5   | 360 | 3   | +1  | +1  | +1  | +1  | 0.73            |
| 17  | 0.29| 4   | 240 | 2   | -1.414 | 0   | 0   | 0   | 0.80            |
| 18  | 1.71| 4   | 240 | 2   | +1.414 | 0   | 0   | 0   | 0.51            |
| 19  | 1   | 2.58| 240 | 2   | 0    | -1.414 | 0   | 0   | 0.56            |
| 20  | 1   | 5.42| 240 | 2   | 0    | +1.414 | 0   | 0   | 0.65            |
| 21  | 1   | 4   | 70  | 2   | 0    | 0    | -1.414 | 0   | 0.05            |
| 22  | 1   | 4   | 410 | 2   | 0    | 0    | +1.414 | 0   | 0.65            |
| 23  | 1   | 4   | 240 | 0.59| 0    | 0    | 0    | -1.414 | 0.68            |
| 24  | 1   | 4   | 240 | 3.41| 0    | 0    | 0    | +1.414 | 0.71            |
| 25  | 1   | 4   | 240 | 3   | 0    | 0    | 0    | 0    | 0.81            |

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spectra with W09N08, HPCH1607 mass spectral libraries, and the data of NIST Chemistry WebBook.

**Cytotoxicity Assays**

The cytotoxicity of the obtained essential oils was assayed against three established cell lines. Cell lines A549 (CLS 300114) and PC-3 (ATCC CRL-1435) were purchased from the American Type Culture Collection (Manassas, VA, USA), and cell line A-549 (Item number: 300114) from Cell Lines Service GmbH (Eppelheim, Germany). The cells were maintained at the Bioassay Laboratory, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology, Vietnam. Culture media included DMEM (Dulbecco’s modiﬁed Eagle’s medium), EMEM (Eagle’s minimum essential medium, Sigma-Aldrich, St. Louis, MO, USA), and 10% heat-inactivated fetal bovine serum (FBS). The culture was performed in a humidified atmosphere of 95% air and 5% CO₂ at 37 °C. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was used to measure cell viability.17

**Antimicrobial Activity**

Eight microbial strains obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) were used to evaluate the antimicrobial activity, including *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 25923, *Bacillus subtilis* ATCC 27212, *Staphylococcus aureus* ATCC 12222, *Aspergillus niger* ATCC 9763, *Fusarium oxysporum* ATCC 48112, *Saccharomyces cerevisiae* ATCC 16404, and *Candida albicans* ATCC 10231. The antimicrobial activity of the samples was determined by a minimum inhibitory concentration (MIC) assay against the above fungal and bacterial strains. The Gram-positive and -negative bacteria were cultured in tryptic soy broth (TSB; Merck KGaA, Darmstadt, Germany), while fungi were grown in SDB (Merck, Germany) to a final inoculum size of about 150 × 106 colony-forming units (CFU) per mL (or 0.5 McFarland standard at λ = 550 nm). A sample of essential oil at various concentrations ranging from 12.5 to 200 μg/mL was loaded into 96-well microplates containing fresh cultures, and the plates were incubated at 37 °C for 24 hours. Blank controls were prepared identically with essential oils being replaced by 5% DMSO.18 Positive controls included doxycycline for Gram (-) and gentamicin for Gram (+) bacteria, and nystatin for fungi.

**Results and discussion**

**Optimization of experimental conditions using response surface method (RSM)**

Table 3 displays analysis of the ANOVA results for the second order regression model describing essential oil extraction with respect to four factors. Among all the variables, only the terms A, C, AC, A², B², C², D² had coefficients that were statistically significant. The obtained R² value was 0.9923, which is reasonable and in agreement with an Adjusted R² value of 0.9815. In addition, the adequate precision value, which is used to evaluate the interfering signal by 26.829, shows that this model can be used to describe the response and that no further model specification is required. Therefore, it can be concluded that the regression model built by Design Expert 7.0.0 software is appropriate to predict the performance of Dong Van marjoram essential oils extraction. Besides, based on Figure 1, there were

| Source | Sum of Squares | Df | Mean Square | F-value | p-value | Remarks |
|--------|---------------|----|-------------|---------|---------|---------|
| Model  | 1.89          | 14 | 0.14        | 92.14   | <0.0001 | significant |
| A      | 0.11          | 1  | 0.11        | 73.60   | <0.0001 | significant |
| B      | 0.0067        | 1  | 0.0067      | 4.59    | 0.0577  | not significant |
| C      | 1.23          | 1  | 1.23        | 840.63  | <0.0001 | significant |
| D      | 0.00007       | 1  | 0.00007     | 0.048   | 0.8309  | not significant |
| AB     | 0.0004        | 1  | 0.0004      | 0.27    | 0.6131  | not significant |
| AC     | 0.012         | 1  | 0.012       | 8.24    | 0.0166  | significant |
| AD     | 0.0025        | 1  | 0.0025      | 1.70    | 0.2212  | not significant |
| BC     | 0.00002       | 1  | 0.00002     | 0.017   | 0.8988  | not significant |
| BD     | 0.00122       | 1  | 0.00122     | 0.83    | 0.3825  | not significant |
| CD     | 0.00122       | 1  | 0.00122     | 0.83    | 0.3825  | not significant |
| A²     | 0.039         | 1  | 0.039       | 26.70   | 0.0004  | significant |
| B²     | 0.072         | 1  | 0.072       | 49.17   | <0.0001 | significant |
| C²     | 0.4           | 1  | 0.4         | 269.73  | <0.0001 | significant |
| D²     | 0.02          | 1  | 0.02        | 13.62   | 0.0042  | significant |
| Residual | 0.015        | 10 | 0.00146    |         |         |         |
| Cor total | 1.91        | 24 |             |         |         |         |
| Std. Dev. | 0.038       |    | R²           | 0.9923  |         |         |
| Mean    | 0.45         |    | Adjusted R²  | 0.9815  |         |         |
| C.V. %  | 8.55         |    | Adeq. Precision  | 26.829  |         |         |
many data points with predicted values (Predicted) and actual values (Actual) closely distributed to the original straight lines with a slope of 1. In addition, the experimental model was considered a reasonably fit as calculated residuals follow a random pattern, as shown in Figure 2, suggesting that actual responses were approximately close to the value predicted by the regression equation. The second order model is described by the following equation:

\[
\hat{Y} = 0.8 - 0.074A + 0.25C - 0.027AC \\
- 0.07A^2 - 0.095B^2 - 0.22C^2 - 0.05D^2
\]

Response Surface Analysis, Optimization, and Model Verification

Based on a quadratic polynomial model determined from experimental data by reaction surface method, the three-dimensional response surfaces showing the influence of the double interaction effect of the pairs of technological factors on the objective function (the mass of essential oils obtained) are shown in Figure 3. The X- and Y-axes of the three-dimensional response surface represent two factors, for material size and water to material ratio (microwave power 240 W and distillation time 2 h), material size and microwave power (water to material ratio 4:1 (mL/g) and distillation time 2 h), material size and distillation time (water to material ratio 4:1 (mL/g) and microwave power 240 W), water to material ratio and microwave power (material size 1 cm and distillation time 2 h), water to material ratio and distillation time (material size 1 cm and microwave power 240 W), microwave power and distillation time (material size 1 cm and water to material ratio 4:1 (mL/g)). The Z-axes represent the mass of essential oils obtained.

Using the RMS method to optimize the distillation process, the optimal values of the independent variables were obtained by solving second-order regression equations to maximize the mass of essential oils obtained. The results of optimal technological parameters are as follows: Material size 0.74 (cm), water to material ratio 4.14 : 1 (mL/g), microwave power 302.4 (W), and distillation
With optimal conditions, the maximum mass of essential oil obtained was 0.893 g (Table 4 and Figure 4).

From the optimal technological conditions found by RSM, as in Table 4, we repeated the experiment 3 times under these conditions. The average mass of essential oil obtained was 0.887 ± 0.007 (g), whereby the yield of the essential oils obtained was 0.6%. The experimental and theoretical calculation results are almost equivalent, showing that the built model is compatible with the experiment.

**Chemical Composition of the Essential Oils**

The chemical composition of the essential oil obtained by the MAHD method is represented in Table 5. Twenty-five compounds were detected, accounting for 98.6%. The main constituents were rosefuran epoxide (44.9%), followed by caryophyllene (10.8%), germacrene D (2.6%), α-humulene (1.3%), and rosefuran (1.2%). In addition, there was an unknown chemical component, accounting for 26.6% of the oil (m/z 95, 194; RI 1336).

Similar to the oil of Dong Van Marjoram used in this study, that obtained from *E. densa* Benth. in India also had rosefuran as its major component, in this case forming 86% of the total. In North America, of which the main ones were rosefuran epoxide (40.4%) and rosefuran (41.7%). In contrast, rosefuran epoxide was not the main component of the essential oils of some other *Elsholtzia* species in

| Independent variables | Real variables |
|-----------------------|----------------|
| A                     | B              | C      | D      | Material size (cm) | Water to material ratio (mL/g) | Microwave power (W) | Distillation time (h) |
| -0.53                 | 0.14           | 0.52   | 0.1    | 0.74               | 4.14 / 1                       | 302.4               | 2.1                 |

**Figure 4.** Optimum conditions by solution of ramps.

**Table 5.** Chemical composition of the essential oil extracted from *Elsholtzia winitiana var. dongvanensis* Phuong. by the MAHD method.

| Chemical name                   | RI* | RI | Percentage (%) |
|---------------------------------|-----|----|----------------|
| Rosefuran                       | 1095| 1099| 1.2            |
| Linalool                         | 1104| 1103| 0.3            |
| Unknown 1 (95, 166, RI 1175)    | 1175| 1.7 |
| Rosefuran epoxide                | 1177| 1180| 44.9           |
| α-Terpineol                     | 1200| 1200| 0.1            |
| Unknown 2 (96, 168, RI 1213)    | 1213| 4.3 |
| Unknown 3 (95, 166, RI 1222)    | 1222| Nd  |
| Piperitone                       | 1268| 1265| 0.2            |
| Dehydroelsholtzia ketone        | 1306| 1310| 0.2            |
| Unknown 4 (95, 184, RI 1334)    | 1334| Nd  |
| Unknown 5 (95, 194, RI 1336)    | 1336| 26.6|
| α-Copaene                       | 1388| 1389| 0.4            |
| β-Bourbonene                    | 1398| 1400| 0.2            |
| α-Gurjunene                     | 1419| 1425| 0.4            |
| Caryophyllene                   | 1437| 1437| 10.8           |
| α-Humulene                      | 1474| 1471| 1.3            |
| γ-Muurolene                     | 1485| 1490| 0.3            |
| Germacrene D                    | 1497| 1498| 2.6            |
| β-Selinene                      | 1509| 1504| 0.2            |
| Bicyclogermacrene               | 1532| 1514| 0.7            |
| γ-Cadinene                      | 1534| 1530| 0.2            |
| δ-Cadinene                      | 1540| 1536| 0.5            |
| Scapanol                        | 1586| 1594| 0.2            |
| Caryophyllene oxide             | 1607| 1605| 1.2            |
| α-Cadinol                       | 1673| 1674| 0.1            |
| Total                           |     |     | 98.6           |

RI*: Retention index compared between software predictions
Nd: Not detected
positive control* 6.0 9.8 9.4 17.8 6.2 3.0 8.1 2.3
Essential oils 50 >200 50 100 >200 >200 100 >200

(3-methylbut-2-enoyl)-furan, accounting for up to 88.7%.
23 components with the major one being 3-methyl-2-
and neral (39.1%).
22 The essential oil of E. cristata Willd. contained
23 components, the major one being 3-methyl-2-
(3-methylbut-2-enoyl)-furan, accounting for up to 88.7%.
23

The results of this study have contributed to clarifying
the chemical composition of the essential oil of
marjoram from Ha Giang province, Vietnam.

In vitro Bioactivity of Essential Oils

The essential oil obtained by the MAHD method was evaluated
in terms of its anti-proliferative activity on two cancer cell lines
(PC3, and A549), as well as its anti-microbial activity on eight
strains of fungi, yeast, and bacteria.

Moderate inhibition was observed against both tested cell
lines, with IC50 values of 23.3 µg/mL (for PC3) and 56.2 µg/
 mL (for A549) (values for paclitaxel 3.5 and 3.7 ng/mL,
respectively). However, the essential oil showed strong activity
against three bacterial strains (E. coli, B. subtilis, S. aureus), and a yeast
strain (S. cerevisiae) with minimal inhibitory concentration
(MIC) values ranging from 50 to 100 µg/mL (Table 6). In
general, these data show that essential oils from Dong Van
marjoram extracted by the MAHD method have potential in a bio-
logical application.

Conclusion

We have studied and optimized the technological parameters
of the distillation process using microwaves to collect Dong Van
marjoram essential oil. The optimal technological parameters
found were material size 0.74 (cm), water to material ratio
4.14:1 (mL/g), microwave power 502.4 (W), and distillation
time 2.1 (hour). With optimal conditions, the mass of Dong
Van marjoram essential oils obtained was 0.89 ± 0.007 (g),
corresponding to the content of the essential oil of 0.6% in
the material. Dong Van marjoram essential oil was shown to
contain a high content of rosefuran epoxide (44.9%).
The essential oil exhibited only moderate inhibition against
the two tested cancer cell lines (PC3 and A549), but strong effec-
tiveness against three bacterial strains, E. coli, B. subtilis, and
S. aureus, and a yeast strain, S. cerevisiae. These data show that
essential oils from Dong Van marjoram extracted by the
MAHD method have the potential for biological application.

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ORCID iD
Tan Phat Dao https://orcid.org/0000-0002-7836-3624

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