Optimization of Microwave-assisted extraction and compositional determination of essential oil from leaves of *Eucalyptus globulus*

T H Tran 1,*, T C Q Ngo,1 T P Dao,1 P T N Nguyen,1 T N Pham,1 T D Nguyen1,4, H T K Linh5, N H Nguyen6, M H Cang7

1 NTT Hi-Tech Institute, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
2 Faculty of Chemical Engineering and Food Technology, Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam
3 Department of Chemical Engineering, HCMC University of Technology, VNU-HCM, Ho Chi Minh City, Vietnam
4 Graduate University of Science and Technology, Vietnam Academy of Science and Technology, Ha Noi, Viet Nam
5 BKU Institute of Advanced Applied Science and Technology (BKIST), Ho Chi Minh City, Vietnam
6 Institute of Applied Materials Science, Vietnam Academy of Science and Technology, 01 TL29, District 12, Ho Chi Minh City, Vietnam
7 Nong Lam University, Ho Chi Minh City, Vietnam

*E-mail: tthien@ntt.edu.vn, labasm2013@gmail.com

Abstract: The extraction of essential oil extracted from *Eucalyptus globulus* leaves in Southern Vietnam by microwave-assisted extraction (MAE) was investigated. The experimental study was conducted to determine the effect of different extracting parameters such as size of material, water to material ratio (1 mg/mL to 5 mg/mL), time (20 min to 80 min) and microwave power (100W to 600W). The essential oil content is determined by Gas Chromatography-Mass Spectrometry GC-MS method. The main ingredients of *Eucalyptus globulus* essential oils were Eucalyptol (38.771%). The highest essential oil content of 2.65 mg/L was achieved with the ground material, at the ratio of raw materials to water of 1:3 mL/g, extraction time of 60 min, and microwave power of 450W. In conclusion, MAHD method is considered an efficient extraction process, saving time and cost of materials and heating.

1. Introduction

In the history of the exploitation and use of several properties of some active phyto-compounds from herb plants through the extraction process have produced several highly active drugs for medical purposes perhaps as old as the history of humanity. Nowadays, herbal medicines has been receiving a great deal of public attention [1–6]. This may be thanks to the recognition that modern synthetic drugs often produce unwanted side effects, which ultimately cause more problems than the actual disease itself. Herbal medicine gives a glimmer of hope through its phyto-compounds, on the one hand which is said to bring about a great sense of healing without actually having unwanted side effects, on the other hand its quality guaranteed.
However, the process of extraction has received less attention and study. Plants can contain thousand secondary metabolites that are used in the medical field, making the need to develop fast and high yield extraction methods an absolute necessity [7]. However, the traditional method takes longer extraction time which causes thermal degradation for most phyto-compositions [8]. To respond to the above requirements, recent years have experienced the inheritance and development of new extraction techniques with decreased solvent consumption, reduced extraction time, raised concerns about prevention of environmental pollution and particular interest in bioactive components including microwave-assisted hydrodistillation (MAHD), extraction with pressure solvent (PSE), and supercritical fluid extraction (SCFE).

There are abundant studies about the using of microwaves to extract organic compounds and pesticide residues from the natural product [9–13]. Therefore, using heat from the microwave was applied by researchers to extract polar compounds in plants. In 2004, Stashenko and his colleagues developed the process of microwave-assisted hydrodistillation (MAHD) [14]. Difference essential oil from aromatic plants was extracted by microwave technique, for example, the conditions for removal of Vietnamese Basil (Ocimum basilicum L.) is 3:2:1 (mL/g) water: material ratio, 97 minutes, 430 (W) microwave power, which obtained the 0.6% essential oil yield [15]. as another example, Moreover, the previous study provided that microwave power 500W at leaf and material ratio 0.33 gives the highest yield in less time is 1.41% w/w dry basis printed 50 minutes in Piper betle L [16]. The result can be concluded that using the process of extracting biological compounds with the support of viewed microwaves is highly cost effective as well as environmental impact [17]. Essential oils have appeared and developed according to the civilization of humanity, which contains many aromatic compounds characterized by each source of raw materials provided. Nowadays, essential oils are applied in different fields such as food, cosmetics, pharmacy and so on [18–19].

The purpose of this study was investigated and optimize the conditions that affect the extraction of essential oils from E. globulus leaves. Moreover, this research was used a microwave-assisted extraction method to obtain essential oils contained high content of 1,8-cineole ingredient.

2. Materials and Methods

2.1. Plant Sample Preparation

Eucalyptus globulus leaves were purchased in Ninh Binh province, Vietnam. After collecting, the leaves were washed with water. All raw materials are kept in a refrigerator below 10°C.

2.2. Extraction Method

The system for Eucalyptus globulus oil extraction comprises a microwave oven, Clevenger distillation apparatus, the flask. The microwave plays a vital role in heat generation. Plant material are placed in the flask which is put in the microwave oven. The apparatus separates the oil and aqueous phase outside the oven.
Figure 1 illustrates the process of Eucalyptus globulus leaves essential oil extraction. First, fresh leaves were washed and ground. Second, 100 g leaves were mixed with 1 L water. Then, Clevenger extraction was operated by the microwave oven. Next, the raw essential oil was separated to obtain the extraction oil which was dried with Na$_2$SO$_4$.

2.3 Single factors investigation

The factors selected for the optimal extraction of Eucalyptus globulus leaves essential oils include water to material ratio, material size, time, and power of the microwave oven were presented in Table 1. In order to evaluate the optimum between experiments, the yield of the Eucalyptus globulus leaves essential oil obtained was examined through equation (1)

$$Yield\ of\ E.G\ leaves\ oil\ (\%) = \frac{the\ volume\ of\ E.G\ leaves\ oil\ obtained\ (mL)}{the\ amount\ of\ E.G\ leaves\ originally\ used\ (g)}$$ (1)

| Table 1. Factors affecting the extraction process |
|-----------------------------------------------|
| Size                                          |
| (Leaves, Cut, Grind)                         |
| Water to material ratio (mL/g)                | 3:01  | 50   |
| Extraction time (min)                         | 50    |
| Extraction power (W)                          | 450   |
| Water to material ratio (mL/g)                | (1:01-5:01) |
| Extracting time (min)                         | Select the previous experiment |
| Extracting power (W)                          | 450   |
| Extracting time (min)                         | (20-80) |
| Extracting power (W)                          | Select the previous experiment |
| Extracting power (W)                          | (200-600) |
2.4. Identification of Components by Gas Chromatography-Mass Spectrometry (GC-MS)

In order to determine the chemical composition in the essential oil sample, 25µL of essential oil from the optimized process was added with 1.0 mL n-hexane and dehydrated with Na₂SO₄. The instrument was GC Agilent 6890 N, HP5-MS column, MS 5973 inert, head column pressure of 9.3 psi.

The gas chromatography-mass spectroscopy (GC-MS) was employed to analyze the chemical composition of the essential oil samples. GC-456 SQ with SCION performance RESTEK Rxi-5ms (30 m x 0.25 mm, 0.25 μm df, bring the gas Helium constant flow rate: 1 mL/min. Injector temperature is 250° C the rate of Division: 30

3. Results and Discussion

3.1 The effect of the material size

The size of the Eucalyptus globulus leaves is divided into three parts using a sieve (Original, Cut fiber, Grind). Figure 2A illustrates the effect of the leaves size on the yield extraction of the essential oil. With the reduction in leaves size of the sample, the yield extraction of the essential oil is significantly improved. It may involve an increase in the contact surface area that allows more exposure of the sample to the solvent while increasing the better penetration of the heat from the microwave [20]. Moreover, the amount of water starts to evaporate because it cannot tolerate high microwave intensity during longer extraction times. In general, water plays a vital function in distillation to limit heat-degraded raw materials and also act as an essential carrier in the evaporation process before condensation takes place. As can be seen from Figure 2A, ground samples obtained the highest yield of essential oil, achieved 2.5%.

3.2 The effect of solvent and solvent to materials ratio

The figure 2B illustrates the effect of solvent to materials ratio on essential oil extraction. As shown in Figure 2B, the yield of the essential increased when the ratio of water and materials rose (raised from 1.3% to 2.6%). This result is explained that with the increase of water to materials ratio, the contact area between material and water also increased, making essential oil more fully dissolved out from material. However, this ratio continued to increase, which was no significant difference in the yields of the essential (from 2.6% to 2.5%).

3.3 The effect of the extraction time

At the ratio of water to raw materials of 3:1 and at a constant operating microwave power of 450 W, Figure 3C illustrates the yield of essential oils extracted from eucalyptus leaves at different times. It can be seen that the increase time from 20 minutes to 60 minutes and the yield rose steadily, in particular at 20, 30, 40, 50, and 60 minutes achieved 1.0%, 1.2%, 1.4%, 2.5%, and 2.65% respectively. When the extraction time was 60 min, MAHD was able to produce the highest yield of 2.65%. However, as the extraction time was increased beyond 60 min, the yield can be seen to be no longer increased significantly. This may cause the plant cell began to degrade when the raw material is exposed to the heat. Thus, the essential oil is released to the environment. In conclusion, based on the results obtained in Figure 2C, 60 minute had been selected as the optimal time for extracting Eucalyptus globulus leaves essential oil by MAHD.

3.4 The effect of the extraction power

Figure 2D shows the influence of microwave power from 100W to 600W region on Eucalyptus globulus essential oil yield. When the microwave power is increased from 100W to 450W, the yield of the essential oil increases significantly from 1.4% to 2.65%. The increase in microwave power not only increases the irradiated thermal energy of the microwave but also enhances the mass transfer of solvent from penetrating into the plant cells. Water is an excellent polar, which can efficiently and quickly transport the natural compounds of plants through molecular interaction with magnetic fields.
However, too high power can lead to volatile oil loss and degradation of compounds in essential oils. When microwave power exceeds 450W, extracting essential oils from eucalyptus leaves does not increase significantly from 2.65% to 2.4%, this may be due to the extraction of compounds that achieve balance and decomposition in this situation. In summary, the optimal microwave power is defined as 450W for the extraction of *Eucalyptus globulus* leaves essential oil.

![Graphs showing yield vs. size, ratio of water to raw material, extraction time, and extraction power](image)

**Figure 2.** The effect of the factors on the *Eucalyptus globulus* leaves essential oil: A) leaves size  B) water to raw material ratio  C) extraction time  D) extraction power

### 3.5 Chemical composition of *E. globulus* oil

In plants, the formation of essential oils depends on many factors including genetic differences in plants, location, and the elements of agriculture. The composition and content of the compounds in *Eucalyptus globulus* essential oils are listed in Table 2. It was found that thirty-five compounds in eucalyptus essential oils were identified by GC-MS analysis, represented 99.863% of the total oil, in which Eucalyptol (38.77%), α-Pinene (14.94%) and α-Terpineol (6.354%) were the main components of the Eucalyptus oil. As mentioned above, in *Eucalyptus globulus* essential oil, 1,8-cineole has antibacterial properties like *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Therefore, *Eucalyptus globulus* essential oil is applied to the field of medicine and humanity treatment thanks to these ingredients.
The purpose of the present article was to apply microwave-assisted extraction (MAE) methods of pigments Eucalyptus globulus leaves in Southern Vietnam to extract the essential oil. The highest essential oil achieved at 2.65 mg/L. The optimal extraction conditions for the extraction of essential oil of Eucalyptus globulus leaves were achieved at ground material, the ratio of raw materials to water (1:3 mL/g), extraction time (60 min), and microwave power (450W). With microwave support, the quantity and quality of Eucalyptus globulus essential oil improved significantly with a content of 2.65% containing 35 components found. Thought gas chromatography/mass spectrometry (GC/MS), the result revealed that the oil is extremely rich in Eucalyptol (38.77%).

| Peak | R.T. (min) | Compounds | Molecular Formula | Content (%) |
|------|------------|-----------|-------------------|-------------|
| 1    | 7.429      | α-Pinene  | C10H16            | 14.94       |
| 2    | 7.92       | α-Fenchene| C10H16            | 0.305       |
| 3    | 7.972      | Camphene  | C10H16            | 0.756       |
| 4    | 9.259      | β-Pinene  | C10H16            | 8.787       |
| 5    | 11.768     | α-Cymene  | C10H14            | 2.244       |
| 6    | 12.145     | Eucalyptol| C10H18O           | 38.771      |
| 7    | 13.682     | γ-Terpinene| C10H16          | 0.638       |
| 8    | 16.923     | Fenchol   | C10H18O           | 2.364       |
| 9    | 18.272     | 1R)-(+)Norinone | C9H16O       | 0.214       |
| 10   | 18.356     | L-pinocarveol| C10H18O      | 3.127       |
| 11   | 18.816     | β-Terpineol| C10H18O         | 0.351       |
| 12   | 19.621     | Pinen-3-one| C10H18O         | 1.178       |
| 13   | 19.81      | Borneol   | C10H18O           | 2.811       |
| 14   | 20.395     | 4-Terpinenol| C10H18O      | 1.877       |
| 15   | 20.96      | trans-p-mentha-1(7),8-dien-2-ol | C10H18O     | 0.707       |
| 16   | 21.106     | α-Terpineol| C10H18O         | 6.354       |
| 17   | 21.242     | Myrtenal  | C10H18O           | 0.434       |
| 18   | 21.305     | Myrtenol  | C10H18O           | 1.268       |
| 19   | 22.33      | Fenchyl acetate | C12H20O      | 0.398       |
| 20   | 22.685     | Cyclohexanol| C10H16O         | 0.341       |
| 21   | 24.86      | Bornyl acetate| C12H20O      | 0.251       |
| 22   | 27.035     | α-Terpineol acetate | C12H20O    | 1.964       |
| 23   | 29.148     | β-Caryophyllen| C15H24         | 0.549       |
| 24   | 29.723     | β-Neoclovene| C15H24         | 1.635       |
| 25   | 30.35      | Aromadendrene| C15H24        | 0.554       |
| 26   | 31.343     | Elixene   | C15H24           | 0.299       |
| 27   | 32.828     | Epiglobulol| C15H20O         | 0.508       |
| 28   | 32.985     | Maaliol   | C15H20O           | 0.312       |
| 29   | 33.205     | Spathulenol| C15H20O         | 0.855       |
| 30   | 33.341     | Globulol  | C15H20O           | 3.737       |
| 31   | 33.487     | Viridiflor | C15H20O         | 0.334       |
| 32   | 33.508     | Cubeban-11-ol | C15H28O       | 0.247       |
| 33   | 33.696     | β-Eudesmol| C15H20O           | 0.382       |
| 34   | 34.062     | β-Selinol  | C15H20O           | 0.326       |
| 35   | 34.564     | 2-Naphthalenemethano| C15H20O    | 0.065       |
Acknowledgment
This study was supported by Nguyen Tat Thanh University, Ho Chi Minh City, Vietnam

References

[1] Mai H, Nguyen T, Le T, Nguyen D and Bach L 2019 Evaluation of Conditions Affecting Properties of Gac (Momordica Cocochinensis Spreng) Oil-Loaded Solid Lipid Nanoparticles (SLNs) Synthesized Using High-Speed Homogenization Process Processes 7 90

[2] Pham T N, Tran B P, Tran T H, Nguyen D C, Nguyen T N P, Nguyen T Q, Vo D V N, Le N T H, Le X T, Nguyen T D and Bach L G 2019 Response surface modeling and optimizing conditions for anthocyanins extraction from purple sweet potato (Ipomoea batatas (L.) Lam.) grown in Lam Dong province, Vietnam IOP Conf. Ser. Mater. Sci. Eng. 479 012012

[3] 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

[4] 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 pro cess IOP Conf. Ser. Mater. Sci. Eng. 479 012015

[5] Dao T P, Nguyen D C, Tran T H, Nguyen D T, Nguyen D H, Vo D V N, Le X T, Le N T H and Bach L G 2019 Extraction Process of Essential Oil from Plectranthus amboinicus Using Microwave Assisted Hydrodistillation and Evaluation of It’s Antibacterial Activity Asian J. Chem. 31 977–81

[6] Tran T H, Ha L K, Nguyen D C, Dao T P, Nhan L T H, Nguyen D H, Nguyen T D, Vo D-V N, 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

[7] Nyiredy S 2011 Separation strategies of plant constituents – current status J. Chromatogr. B 812 35–51

[8] Luque A M D 1998 Soxhlet extraction of solid materials : an outdated technique with a promising innovative future Anal. Chim. Acta 369 1-10

[9] Afoakwah A N and Teye E 2012 Review Article Microwave Assisted Extraction (MAE) of Antioxidant Constituents In Plant Materials G.J.B.B. 1 132–40

[10] Zuloaga O, Etxebarria N, Ferna L A, Madariaga J M, Saila K A, Unibertsitatea E H and Bilbao E 1999 Optimisation and comparison of microwave-assisted extraction and Soxhlet extraction for the determination of polychlorinated biphenyls in soil samples using an experimental design approach Talanta 50 345–357

[11] Sanghi R and Kannamkumarath S S 2004 Comparison of Extraction Methods by Soxhlet, Sonicator , and Microwave in the Screening of Pesticide Residues from Solid Matrices J. Anal. Chem. 59 1032–1036

[12] Eskilsson C S and Bjorklund E 2000 Analytical-scale microwave-assisted extraction J. Chromatogr. A 902 227–50

[13] Parera J, Santos F J and Galceran M T 2004 Microwave-assisted extraction versus Soxhlet extraction for the analysis of short-chain chlorinated alkanes in sediments J. Chromatogr. A 1046 19–26

[14] Mandal V, Mohan Y and Hemalatha S 2006 Microwave Assisted Extraction - An Innovative and Promising Extraction Tool for Medicinal Plant Research Phcog Rev. 1 7-18

[15] Tran T H, Nguyen H H H, Nguyen D T, Nguyen T Q, Huynh T, Le T H N, Nguyen D H, Tran D L, Do S T and Nguyen D T 2018 Optimization of Microwave-Assisted Extraction of Essential Oil from Vietnamese Basil (Ocimum basilicum L.) Using Response Surface Methodology Processes 6 206

[16] Amareesh A, P G, Khan S and R Zari S 2017 Comparative Study of Microwave Assisted Hydro
ro-Distillation with Conventional Hydro-Distillation for Extraction of Essential Oil from Piper betle L. *Biosci. Biotechnol. Res. Asia* **14** 401–7

[17] Farhat A, Ginies C, Romdhane M and Chemat F 2009 Eco-friendly and cleaner process for isolation of essential oil using microwave energy Experimental and theoretical study *J. Chromatogr. A* **1216** 5077–85

[18] Calo J R, Crandall P G, O’Bryan C A and Ricke S C 2015 Essential oils as antimicrobials in food systems - A review *Food Control* **54** 111–9

[19] Turek C and Stintzing F C 2013 Stability of essential oils: A review *Compr. Rev. Food Sci. Food Saf.* **12** 40–53

[20] Šavikin K, Jankovic T, Pljevljakušić D, Zdunic G and Nada C 2016 Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique *Food Chem.* **194** 135–42

[21] Dorthe A M 1999 Optimization by factorial design of focused microwave assisted extraction of polycyclic aromatic hydrocarbons from marine sediment *J. Anal. Chem.* **364** 228–37