Extraction and analysis of chemical composition of *Ocimum gratissimum* L essential oil in the North of Vietnam

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**Abstract.** *Ocimum gratissimum* L. is a fragrant plant of the Lamiaceae family. *O. gratissimum* has been used in folk medicine, cosmetics, and food industry. In this study, herbs of *O. gratissimum* were collected in the summer in Northern Vietnam and extracted for essential oils by hydrodistillation, followed by gas chromatography - mass spectrometry (GC-MS) analysis. The efficiency of essential oil extraction was 0.2258% ± 0.0735. According to the analysis results, twenty-seven components were identified. The main ingredients include Eugenol (65.135%), Ocimene <(Z)-b-> (7.20%), Caryophyllene <(E)-> (6.64%), and Germacrene D (12.03%). This result suggests that the *O. gratissimum* essential oil is a potential source of antimicrobial, preservative and flavoring agent.

1. Introduction

Essential oil (EOs) is a natural product that can be extracted from different plant organs including leaves, stems, flowers, bark and roots [1-8]. EOs are widely distributed in nature, widely applied in the pharmaceutical, aromatherapy, food, and cosmetic industries. The amount of essential oil in a plant depends on the climatic and soil conditions. Plants grown in the tropics have more oil content than in temperate regions. Even within the plant, the compositions and amounts of essential oils obtained from different parts may differ. In addition, the amount of EOs depends on the habitat in which the plant grows, methods of harvesting, preserving, and extracting of the plant material.

Vietnam is located in the tropical monsoon area with high temperature, heavy rain and relatively high humidity, creating a diverse flora rich in natural products, especially essential oils (lemongrass, basil, rosemary, orange, lemon, jasmine ...) [9-13]. Of which, *Ocimum gratissimum* has been used extensively in folk medicine to treat various conditions including flu, cough and headache. Notable components existing in *O. gratissimum* EOs composition included eugenol and methyleugenol [14-16]. *O. gratissimum* is a light-demanding shrub, with a fairly wide ecological amplitude, which can be adapted to hot and humid tropical climates as well as subtropical regions. At present, *O. gratissimum* are mostly distributed in Central, South Africa and Southeast Asia. In Vietnam, *O. gratissimum* thrives in the plains, midlands and low mountains. At altitudes above 1,000 m, the tree grows slowly. *O. gratissimum* figures for the ability to regenerate shoots. The height of the plant could vary from 0.5 to 1.5 m. The square body has egg-shaped, hairy leaves that grow diagonally. Inflorescences are often situated in the interstitial leaves or branches of the plant.

EOs of *O. gratissimum* exhibits antibacterial, antifungal and anti-cancer activities, as suggested by the composition rich in eugenol, thymol, citral, Geraniol and linalool [17-19]. EOs is a homogeneous product
obtained by distillation of hydro-distillation, which is the most commonly used method. Despite the fact that this method is simple and brings about numerous advantages, including ease of parameter control, shortened production time, and lower costs, it has yet been employed for *O. gratissimum* EO extraction. Therefore, the purpose of this research is to conduct the extraction *O. gratissimum* EOs using hydrodistillation. The phytochemical compounds in the obtained essential oil was determined through analysis by gas chromatography-mass spectrometry (GC-MS). The results are expected to provide an essential insight to the use of *O. gratissimum* essential oils in production of cosmetics and food.

2. Materials and methods

2.1. Plant material and chemicals preparation

*O. gratissimum* is collected in Dong Chau, Tien Hai in Thai Binh province in July 2019. After that, the material was pretreated (remove the leaves, wash, drain) and leaves were chopped into the size of 1-2 mm for subsequent extraction process. Chemicals of analytical grade, including anhydrous sodium sulfate (Na$_2$SO$_4$), n-hexane (Sigma-Aldrich, USA) and water.

2.2. Essential oil extraction

A total of 100 g of raw material and distilled water was placed in 1.0 L flask and fixed onto the distillation apparatus to commence oil recovery by hydrodistillation. Each distillation run was performed continuously for 3 hours. The upper layer of essential oil was collected using a funnel and dried with Na$_2$SO$_4$. Obtained essential oils were stored in dark, closed jars and analyzed through GC-MS analysis.

2.3. GC-MS Analyses

Chemical composition of the *O. gratissimum* EO was determined by GC-MS analysis [20]. A GC Agilent 6890 N instrument was coupled with HP5-MS column and MS 5973 inert. The head column was 9.3 psi of pressure. The Essential Oil was added with n-hexane and dehydrated with Na$_2$SO$_4$. The flow rate was kept constant at 1 mL/min. The temperature of injector was set at 250°C and the rate of division was 30. Thermal program for samples began at 50 °C for 2 min, then continued to rise from 80 °C, 150 °C, 200 °C and finally reached 300 °C. The compounds were determined by comparing retention times (R.T.) with the Wiley library or with published mass spectra (Web NIST, GMD).

3. Results and discussion

3.1. Essential oil extraction

The achieved oil yield was 0.2258% ± 0.0735 and has pale yellow color. This result is at variance with previous reports involving essential oil extraction of *Ocimum gratissimum* L. harvested from different geographical areas. To be specific, while essential oil extraction of *O. gratissimum* collected from eastern Kenya and from Bénin resulted in relatively high yield ranged from 0.49% to 0.78% [16, 21], the plant materials in Ceará (Brazilian) region produced a low yield of 0.17% [22]. In addition, it was also found that dried *O. gratissimum* led to significantly higher extraction efficiencies, at 1.07% and 1%, respectively indicated by Pino et al. and Keita et al. [23, 24]. These results suggested the harvest stage of *O. gratissimum* may play in important role in determining extraction efficiencies where pre-flowering plants will give less essential oil than full flowering plants. Moreover, the yield of EOs obtained from the plant harvested in the afternoon is lower with yields from the plants harvested in the morning, presumably due to the impact of sunlight at that time [21].

3.2. Analysis of phytochemical content

The GC-MS analysis of the obtained *O. gratissimum* essential oil from Thai Binh province has identified its chemical content. As shown in Table 1 and Figure 2, it was found that there were 27 different retention time values, corresponding to 27 compounds existing in the EOs. The main compound was Eugenol, a phenylpropanoid compound with the content of 65.135%. The high peak intensity of eugenol suggests its role as the main component determining the characteristics of *O. gratissimum* essential oil. In addition, monoterpenes and Sesquiterpenes accounted for relatively high levels in EOs with notable compounds including Germacrene D (12.03%), Ocimene <(Z) -b-> (7.20%) and Caryophyllene <E-> (6.64%). The chemical structure of Eugenol, Ocimene <(Z) -b->, Caryophyllene <E-> and Germacrene D were shown in Figure 2.
On the other hand, the remaining compounds were ranged from 0.18-0.9%, suggesting the lower contents. However, the GC-MS analysis of essential oil of *O. gratissimum* harvested in the Eastern region of Kenya showed Eugenol content of 68.8% and 74.1%, which were was higher than the eugenol content in the current study [25]. In contrast, Lemos et al. (2005) analyzed samples of *O. gratissimum* EOs collected in Brazil and found that the main ingredient content of Eugenol (57.82%) was relatively lower than those harvested in neighboring areas [22]. For *O. gratissimum* samples in Benin, analysis results showed that the main components in essential oils are mainly monoterpenes including: ρ-cymene (28.08 - 53.82%), thymol (3.32 – 29.13%), γ-terpinene (1.11 - 10.91%), α-thujene (3.37–10.77%), and β-myrcene (4.24–8.28%) and almost no Eugenol content was detected [21]. Composition of essential oils is very vulnerable to climate, weather and soil types, which in turn determines the quality of essential oils. Season and time of harvest and weather may also alter the smell of the essential oil. It was found that harvesting plant sources in the early morning or late afternoon is also important in ensuring the quality of essential oils, directly affecting the composition and performance of essential oils [26].

Table 1. Chemical compositions of the EOs of *O. gratissimum*.

| Retention Time (Min) | Compounds                  | %   |
|----------------------|----------------------------|-----|
| 8.06                 | Hex-3-en-1-ol <Z->         | 0.12|
| 10.10                | Thujene <a->               | 0.20|
| 11.56                | Sabinene                   | 0.33|
| 11.97                | Myrcene                    | 0.20|
| 13.51                | Ocimene <(Z)-b->           | 7.20|
| 13.88                | Ocimene <(E)-b->           | 0.42|
| 14.37                | Terpinene <g->             | 0.15|
| 14.67                | Sabinene Hydrate <cis>     | 0.28|
| 15.66                | Linalool                   | 0.12|
| 18.62                | Terpenin-4-ol              | 0.41|
| 24.81                | Eugenol                    | 65.13|
| 25.54                | Copaene <a->               | 0.90|
| 25.89                | Bourbonene <b->            | 0.71|
| 25.98                | Elemene <cis-b->           | 0.79|
| 26.98                | Copaene <b->               | 0.15|
| 27.05                | Caryophyllene <E-> (=Caryophyllene <b->) | 6.64|
| 27.30                | Gurjunene <b-> (=Calarene) | 0.26|
| 28.12                | Humulene <a->              | 0.57|
| 28.71                | Muurolene <g->             | 0.17|
| 28.96                | Germacrene D               | 12.03|
| 29.31                | Amorphene <g->             | 0.18|
| 29.42                | Muurolene <a->             | 0.21|
| 30.10                | Cadinene <d->              | 0.74|
| 32.11                | Caryophyllene oxide        | 0.77|
| 33.65                | Muurolol <epi-a- (=T-Muurolol) | 0.36|
| 33.75                | Muurolol <a- (=Cadinol <d->) | 0.18|
| 34.02                | Cadinol <a->               | 0.48|
| **Total**            |                            | 99.70|
Figure 1. GC-MC of the essential oil extracted from *O. gratissimum* by hydrodistillation

![Eugenol](image1)

![Germacrene D](image2)

![Ocimene <(Z)-b->](image3)

![Caryophyllene <b->](image4)

Figure 2. The structure of the main constituent is determined in *O. gratissimum* essential oil

4. Conclusion
The results obtained in this study show that *O. gratissimum* contains important compounds that contribute to the essential properties of *O. gratissimum* essential oil. Hydrodistillation seems to be the suitable procedure, as it gives good yield and the oil component is equivalent to the reports in the published literature. *O. gratissimum* EOs is obtained with a yield of 0.2258% ± 0.0735 (w / w), with the presence of eugenol being the highest component itself. Twenty-seven components were identified with major compounds were Eugenol 65.135%, Ocimene <(Z)-b-> 7.20%, Caryophyllene <E-> 6.64%, and Germacrene D 12.03%. Further
research should involve evaluation of antibacterial and antioxidant activities O. gratissimum EOs to establish their use in food and cosmetic preservatives.

5. References

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