Physical and Chemical Profile of Essential oil of Vietnamese *Ocimum gratissimum* L.

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**Abstract.** *Ocimum gratissimum* L. essential oil was studied in this research for its physicochemical characteristics and composition. The essential oil extraction performance achieved 0.3-0.45% by hydrodistillation process. The physicochemical properties of the obtained oil were also discovered including specific gravity at 0.9645 g/cm\(^3\), acid index at 13.035 mg KOH/g, ester index at 14.745 mg KOH/g, refractive index at 1.526, and optical rotation at +15.6\(^\circ\). Through gas chromatography /mass spectrometry (GC/MS), it was revealed that the oil is extremely rich in eugenol (59.448 %), trans-\(\beta\)-Ocimene (10.382 %), \(\beta\)-Cubebene (11.783 %), Caryophyllene (6.966 %) and Copaene (2.479 %). Therefore, optimizing the preservation conditions for the essential oils is suggested as potential pathway to extend the application and enhance the value of *Ocimum gratissimum* essential oils.

1. Introduction

In recent years, traditional medicines use of natural products (such as plants, animals, microorganisms, and marine organisms), which are of great importance [1–7]. Products of natural origin have attracted growing interest in the daily life and scientific community. Among all products, essential oils have been used in various fields of production and life such as antimicrobial compounds, medicine, food additives. Essential oils are odorous oily liquids of complex mixtures extracted from several plant organs such as flowers, seeds.
Ocimum gratissimum L. belongs to the Lamiaceae family, which consisted of about 200 species and cultivated mainly in America, Africa, and Asia. The previous study reports that rosemary essential oils play a crucial role in the treatment of different diseases such as diarrhea, headache, fever. Ocimum gratissimum L. is one of spice and medicinal herb which used as vegetable on soup and also enhance the flavor and organoleptic properties. O. gratissimum L. essential oil is powerful antibacterial, antipyretic, anticarcinogenic. Moreover, Ocimum gratissimum L. plays a vital role in food preservative against mycotoxigenic fungi and as a wound healing promoter. There are different highly valuable compounds in the essential oil of O. gratissimum such as eugenol, methyl, eugenol, myrcene [8,9]. Since essential oils tend to be unstable in the presence of heat, light, moisture, the preservation process plays a crucial role in maintaining essential oils.

Chromatography coupled with mass spectrometry (GC-MS) methods help separate and identify different constituent of the essential oil in case of adulterated oil. The hydro-distillation method became more and more popular to extract essential oils from plant materials due to the simplicity of installations, easy of method performing. The previous study demonstrates utilized steam distillation method for essential oils extracted from lavender and artemisia leaves. There have been many studies of oil extraction by hydrodistillation methods, which solve the problem of separation efficiency, composition and utility of essential oils [10,11]. In recent years, cultivating O. gratissimum has been receiving a great deal of public attention due to the essential oil extracted from its flowers and leaves [10,12]. The aim of this study is determining a physicochemical of the O. gratissimum and characterization of its essential oil. Chemical composition analysis through methods gas-chromatography coupled with mass spectrometry (GC-MS).

2. Material and methods

2.1. Plant material and distillation of essential oil
The aerial parts (flower and leaves) of Ocimum gratissimum L were collected in Daklak province in Vietnamese during April - May in the flowering stage. When growing O. gratissimum, depending on the climatic conditions, one year can give 2-4 harvests. On average 1 hectare of O. gratissimum, depending on the density (1.5 m² / cluster), it can provide 12-15 tons of branches and leaves once to harvest, so 1 year can be from 40-60 tons.

![Figure 1](image1.png)

*Figure 1. Material and the process of the O. gratissimum essential oil extraction process*

2.2. Physico-chemical analyses
Some basic physical and chemical parameters of the raw materials have been identified including some criteria for finished oil products analyzed by TCVN: acid index (TCVN 8450: 2010), sensory index (TCVN 8460: 2010) and density of essential oils. The experiments were repeated three times.

*Density:* The proportion of essential oils is the ratio of essential oils at 25°C with the mass of the oil. The same volume of distilled water is also at 25°C.
**Acid index:** The acid index is the number of milligrams of KOH needed to neutralize free acids in 1 gram of fat.

**Ester index:** The soap index is the number of milligrams of KOH needed to neutralize all free acids and acid combined as esters in 1 gram of fat.

### 2.3. Chemical Identification

In order to determine the chemical composition in the essential oil sample, 25 µL 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 C the rate of Division: 30.

### 3. Results and Discussion

The *O. gratissimum* essential oil was obtained with the yield of 0.3 – 0.45 % by steam. The previous study shows the yields of *O. gratissimum* between 0.21 and 0.70 % [13,14]. The values of physicochemical indexes were presented in table 1. The density of *O. gratissimum* essential oils achieved at 0.9645, which is lighter than water and floats on the water. *O. gratissimum* essential oil has two sorts including lighter than water and heavier than water. The acid index is calculated after conducting the titration of essential oils with relatively high KOH (13.035 mg KOH/g). The *O. gratissimum* essential oil contains high fatty acids. On the other hand, a high ester index proves the ester content in *O. gratissimum* essential oil (14.745 mg KOH/g). This is one of the main constituents contributing to the aroma of *O. gratissimum* essential oil. Thus, the process of extracting by the above method rarely changes the properties and composition of essential oils.

#### Table 1. Physical parameters of essential oils from *Ocimum gratissimum* L.

| Organoleptic characteristics | Essential oil Vietnamese                |
|------------------------------|----------------------------------------|
| Phase                        | Liquid                                 |
| Color                        | Light yellow, browning in the air       |
| Odor                         | Specific, Eugenol                       |
| Density 20°C                 | 0.9645                                 |
| Acid index                   | 13.035                                 |
| Ester index                  | 14.745                                 |
| Rotator power                | 15.6                                   |
| Refractive index             | 1.526                                   |

Table 2 and figure 2 shows the results of the GC-MS analysis for *O. gratissimum* essential oil. twenty-five compounds were characterized and identified according to the chromatogram. Five the major constituent were eugenol (59.448 %), trans-β-Ocimene (10.382 %), β-Cubebeine (11.783 %), Caryophyllene (6.966 %) and Copaene (2.479%). The previous study showed the chemical composition of *O. gratissimum* obtained in western Kenya is a methyl eugenol/ocimene chemotype with 64.28% and 10.40% methyl eugenol and β-ocimene, respectively [15]. Moreover, others report have illustrated chemical composition percentages similar ours study with eugenol (57.82%), followed by -bisabolene (17.19%) and thymol (9.8%). Besides, Fandohan et al. (2004) demonstrated that essential oil from *O. gratissimum* of Benin contained p-cymene (22%), terpinene (15%), and thymol (17%) as the major components [16]. The chemical compositions of essential oils are reliant to different factors including seasons, soil structure, texture, and geographical location [17]. The percentage of eugenol, methyl eugenol, caryophyllene oxide, and α-terpineol was higher in the autumn season as compared to the
spring-summer season. The effect of harvesting time and season on the chemical composition of essential oil in other members of family *Lamiaceae* has also been reported [18,19]. These variations indicated that the dynamics of essential composition in aromatic plants is possibly associated with the expression of different genes in different season.

![Figure 2. Chromatogram of components identified in the *Ocimum gratissimum* leaf extract](image)

Table 2. *Ocimum gratissimum* essential oil composition

| Compound         | Retention time | Concentration % |
|------------------|----------------|-----------------|
| Origanene        | 7.157          | 0.425           |
| β-Phellandrene   | 9.143          | 0.484           |
| β-Myrcene        | 10.105         | 0.17            |
| Terpine | 11.298          | 0.203           |
| β-Cymene         | 11.747         | 0.102           |
| trans-β-Ocimene  | 12.678         | 10.382          |
| cis-Ocimene      | 13.211         | 0.572           |
| Moslene          | 13.692         | 0.364           |
| α-Pyronene       | 18.084         | 0.244           |
| L-4-terpineol    | 20.395         | 0.64            |
| α-Cubebe | 27.014          | 0.215           |
| Eugenol          | 27.516         | 59.448          |
| Copaene          | 27.861         | 2.479           |
| β-Bourbonene     | 28.133         | 0.962           |
| β-Elemen         | 28.363         | 0.537           |
| Caryophyllene    | 29.189         | 6.966           |
| Humulene         | 30.151         | 0.528           |
| γ-Cadinene       | 30.842         | 0.347           |
| β-Cubebe | 30.977          | 11.783          |
| Guaiene          | 31.312         | 0.413           |
| α-Murolene       | 31.448         | 0.246           |
| Cadinene         | 32.013         | 1.049           |
| Caryophyllene oxide | 33.33       | 0.269           |
| Torreyol         | 34.418         | 0.075           |
| Calarene epoxide | 34.637         | 0.145           |
Analysis results by GC - MS show that peaks have the greatest intensity with retention time at 27.516 minutes. Through the identification of the chemical structure by mass spectrometry, the compound with a 27.516 minute retention time is Eugenol (C_{10}H_{12}O_{2} molecular weight is 164). The results show that the MS spectrum of the product appears similar: 39; 43; 51; 55; 65; 77; 91; 94; 103; 115; 121; 131; 137; 149; 164 has the same form as the standard spectrum. In addition, an isomer of eugenol with MS spectra of the product also has the same form as the standard spectrum in data banks (figure 3). Eugenol has been demonstrated to have antibacterial property [20,21]. Therefore, activity of the O. gratissimum essential oil observed in present study may be due to the presence of eugenol in good percentage. The previous studies demonstrated that high antifungal activity of eugenol in different fungi species [22]. In the antimicrobial action of essential oil components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of the main importance. The compound that showed antibacterial activity, was identified as eugenol by 1H and 13C NMR, supported by GC/MS, by retention time, and by comparison with an authentic sample. The activity rank of essential oil components is as follows: phenols > aldehydes > ketones > alcohols > ethers > hydrocarbons. The highest activity was reported for phenols – thymol, carvacrol and eugenol, which is explained by the acidic nature of the hydroxyl group, forming a hydrogen bond with an enzyme active center. The eugenol chemical composition different in regions may be due to some environmental factors, the part of the plant used, the age of the plant and the period of the growing season or even to genetic factors Table 3.

![Figure 3. MS spectrum of eugenol after separation](image)

| Place                     | Concentration % | Investigator          |
|---------------------------|-----------------|-----------------------|
| Cyangugu(South-western)   | 11.00%          | Janseen et al [23]    |
| TamilNadu                 | 43.88%          | Devendran et al [24]  |
| Uttarakhand, India        | 53.89%          | Anand et al [9]       |
| Pathumthani (Thailand)    | 25.02%          | Bunrathep et al [25]  |
| Deccan Region, India      | 74.80%          | Sastry et al [13]     |
| Taiwan                    | 84.90%          | Cheng et al [22]      |

4. Conclusion
In this research, fresh *Ocimum gratissimum* L. leaves were used for extraction of essential oil using hydrodistillation method and evaluation of chemical compositions and physicochemical characteristics. The essential oil was obtained yields at 0.3-0.45%. The physicochemical parameters averaged specific gravity (0.9645 g/cm³), acid index (13.035), ester index (14.745), refractive index (1.526) and +15.6° for optical rotation. Thought gas chromatography/mass spectrometry (GC/MS), twenty-five components were identified in the O. gratissimum oils. The concentration of Eugenol achieved 59.448%, followed
by trans-β-Ocimene and β-Cubebene at 10.382% and 11.783%, respectively. It can be concluded that the chemical composition of *O. gratissimum* essential oils correlates with the climatic conditions in which it is grown, as well as genetic variation; thus creating different types of chemistry.

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