

## EXTRACTION AND INVESTIGATION OF THE CHEMICAL COMPOSITION OF ESSENTIAL OIL FROM *Ocimum africanum* L.

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### ABSTRACT

*Ocimum africanum* L. is a distinctive Vietnamese herb, especially common in Tuy Hoa, Phú Yên. This study focuses on extracting and analyzing the chemical composition of the essential oil derived from the leaves of *Ocimum africanum*. The essential oil was extracted using the steam distillation method and its chemical composition was analyzed using gas chromatography-mass spectrometry (GC-MS). The research results indicate that the main component in *Ocimum africanum* essential oil is citral, a compound with a characteristic aroma that exhibits insect-repellent properties and has antimicrobial activity, particularly against pathogenic bacteria.

### 1. INTRODUCTION

*Ocimum africanum* L. belonging to the family of Lamiaceae, is a familiar herb to many Vietnamese people. With its distinctive aroma and ability to thrive in various climates, *Ocimum africanum* has been used in cuisine as a spice and for treating common ailments such as colds, coughs, sore throats, and poor digestion (Do, 2020). Chloroform and methanol extracts, along with white *Ocimum africanum*'s essential oil, have shown inhibitory effects on the growth of *Streptococcus pyogenes* and *Escherichia coli* (Manjula-Bomma *et al.*, 2018). Additionally, the methanol extract of white *Ocimum africanum* leaves demonstrates strong antioxidant activity (IC<sub>50</sub> = 174.04 µg/mL) (Timotius *et al.*, 2021).

In Vietnam, research on *Ocimum africanum* remains limited. Nguyen *et al.* (2021) indicated that *Ocimum africanum*'s essential oil exhibits the strongest effect against gram-positive methicillin-sensitive

*Staphylococcus aureus* and also demonstrates inhibitory effects on gram-negative bacteria such as *E. coli* and *P. aeruginosa*. Phan *et al.* (2023) conducted a research to evaluate the antibacterial and antioxidant activities of fractionated extracts (ethanol, hexane, and ethyl acetate) from *Ocimum africanum*. The results revealed that white basil has potential applications in human disease prevention and treatment

Around the world, several studies have been conducted on essential oil extracted from the *Ocimum africanum* leaves. The chemical composition of *Ocimum africanum* essential oil depends on the harvest time, climate, and extraction method. The two main compounds were founded in *Ocimum africanum* essential oil are geraniol and citral (Padalia *et al.*, 2018; Timotius *et al.*, 2021).

Overall, research on the extraction and chemical composition analysis of *Ocimum africanum* essential oil remains highly limited. In this study, we investigated several

conditions for extracting *Ocimum africanum* essential oil using the steam distillation method. Additionally, the chemical composition of the essential oil was determined using gas chromatography-mass spectrometry (GC-MS).

## 2. MATERIALS AND METHODS

### 2.1. Materials and measurements

*2.1.1. Materials:* *Ocimum africanum* leaves were collected in Ninh Thuan province, Vietnam, in August 2024. Mature, pest-free leaves were selected, washed, removed impurities and stored at 4-8°C to use throughout the experiment.

*2.1.2. Measurements:* Essential oil distillation kit using steam distillation method; gas chromatography-mass spectrometry (GC-MS) machine: Quadrupole 7890A Agilent Technologies; moisture balance HX204.

### 2.2. Methods

#### 2.2.1. Essential oil extraction method

*Ocimum africanum* leaves were pre-processed, weighed, and chopped into small pieces before being placed into a 2 L round-bottom flask. The essential oil was extracted using an essential oil distillation kit through the steam distillation method (Le, 2008). The mixture consists of essential oil and steam, after passing through the condensation system, were collected at the upper layer in the oil collection apparatus. The content of essential oil was calculated using formula (1). The collected essential oil was dehydrated using sodium sulfate and stored in a brown glass jar at a temperature of 3-5°C.

$$\text{The content of essential oil (mL/100 g)} \\ = \frac{V}{m(1-a)} \cdot 100 \quad (1)$$

(V (mL): volume of essential oil; m (g): the mass of the material; a: the humidity of the material)

#### 2.2.2. Identify the sensory evaluation and density of essential oil

*Identify the clarity and color of essential oil:* Prepare a dry, clean, and transparent test tube. Pour 10 mL of essential oil into the test tube. Observe the clarity and color of the essential oil. If the essential oil appears cloudy or not transparent, it indicates the presence of impurities and water.

*Identify the scent of essential oil:* Place a few drops of essential oil onto filter paper. Identify the scent of the essential oil and assess it every 15 minutes. Repeat the process five times. Record observations on the intensity of the scent after each assessment.

*Identify the taste of essential oil:* Prepare a clean, dry glass dish. Add 1.0 gram of granulated sugar. Drop a few drops of essential oil into the dish, mix well, and then use your tongue to identify the taste of the mixture. Record observations on the nature (sweetness and bitterness) and the intensity of the taste after each trial.

*Identify the density of essential oil:* The density of essential oil was identified by using a hydrometer. The density of essential oil was calculated using formula (2).

$$\text{The density of essential oil } d = \frac{P_1 - P}{P_2 - P} \quad (2)$$

(P, P<sub>1</sub>, and P<sub>2</sub> (g) represent the masses of the hydrometer, the hydrometer filled with essential oil, and the hydrometer filled with water, respectively).

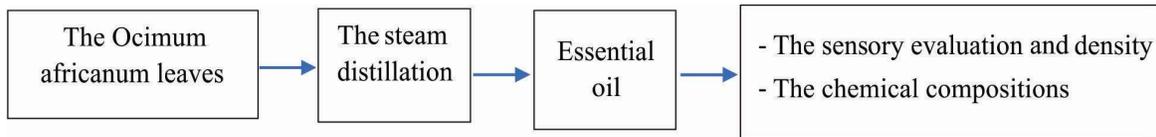
#### 2.2.3. Identify the chemicals of essential oil

The chemical composition of the essential oil extracted from *Ocimum africanum* leaves was analyzed using the gas chromatography-mass spectrometry (GC-MS) method at the Can Tho Center for Standards, Metrology, and Quality Engineering. The GC-MS system used was Thermo Trace GC Ultra - ITQ900.

The TG-SQC column was 30 min length, with an internal diameter of 0.25 mm, and a film thickness of 0.25  $\mu\text{m}$ . Helium was used as the carrier gas. The injector temperature was set at 230°C, and the detector temperature was maintained at 260°C. The oven temperature program started at 60°C (held for 2 minutes),

increased at 4°C/min to 200°C (held for 5 minutes), and then increased at 10°C/min to 260°C (held for 10 minutes). To prevent overloading the chromatography column and MS detector, the essential oil was diluted at a 50:1 ratio in hexane, with a split 20:1 ratio in the injector.

#### 2.2.4. Experimental Arrangement Method



**Figure 1. Experimental setup diagram**

The experiment was designed based on a single-variable model. The factors investigated include the solvent-to-material ratio (mL/g) and distillation time (minutes). The experimental steps were arranged as shown in Figure 1. Each experiment was repeated three times. The average essential oil content among the experiments was compared using the MiniTab 2021 statistical software with a 95% confidence interval.

### 3. RESULTS AND DISCUSSION

#### 3.1. The material's moisture content

Moisture content is a parameter used to determine the essential oil content. The material's moisture content was measured using a moisture analyzer. In this study, the moisture content of the material was determined to be  $88.39 \pm 0.21\%$ . The relatively high moisture content provides favorable conditions for microbial growth, making the material prone to spoilage. Therefore, the material should be stored at an appropriate temperature or dried to reduce moisture.

#### 3.2. Factors affecting the essential oil extraction process

##### 3.2.1. The solvent - material ratio

The experiment was designed based on a single-variable model, with the extraction

time fixed at 240 minutes and the material weight set at 400 g. The results of investigating the effect of the solvent-to-material ratio on essential oil content are presented in Table 1.

**Table 1. Effect of the solvent-material ratio on essential oil content**

No.	The solvent-material ratio (mL/g)	The essential oil content (%)
1	1.0	0.182 <sup>c</sup> ±0.011
2	1.5	0.187 <sup>d</sup> ±0.015
3	2.0	0.195 <sup>c</sup> ±0.009
4	2.5	0.210 <sup>b</sup> ±0.012
<b>5</b>	<b>3.0</b>	<b>0.274<sup>a</sup>±0.018</b>
6	3.5	0.275 <sup>a</sup> ±0.014
7	4.0	0.279 <sup>a</sup> ±0.008

According to these results, as the solvent-to-material ratio increased from 1.0 to 3.0 (mL/g), the essential oil content gradually rose from 0.182 to 0.274 mL/100 g. The solvent impacts the cell membrane, breaking it down and facilitating the release of essential oil. Therefore, an increase in the amount of solvent corresponds to an increase in essential oil content (Le *et al.*, 2023).

However, when the solvent-to-material ratio was further increased from 3.0 to 4.0 mL/g, the essential oil content exhibited no statistically significant difference at the 5% significance level. This indicates that a solvent-to-material ratio of 3.0 mL/g is the most suitable for extracting essential oil from the material. An extraction ratio 3.0 mL/g was selected to optimize solvent usage and minimize costs. These findings align with the research of Le *et al.* (2023), which also examined the effect of solvent-to-material ratio on the essential oil content of *Ocimum basilicum var* leaves.

### 3.2.2. Extraction time

The experiment was designed using a single-variable model, with fixed variables including a material weight of 400 g and a solvent volume of 1200 mL. The results of the investigation into the effect of extraction time on essential oil content are presented in Table 2.

**Table 2. Effect of Extraction Time on essential oil content**

No.	The extraction Time (minutes)	The essential oil content (%)
1	60	0.102 <sup>d</sup> ±0.008
2	90	0.124 <sup>c</sup> ±0.011
3	120	0.176 <sup>b</sup> ±0.014
4	150	0.250 <sup>a</sup> ±0.009
<b>5</b>	<b>180</b>	<b>0.254<sup>a</sup>±0.012</b>
6	210	0.256 <sup>a</sup> ±0.016
7	240	0.259 <sup>a</sup> ±0.007

According to these results, increasing the extraction time from 60 to 150 minutes led to a gradual rise in essential oil content from 0.102 to 0.250 mL/100 g. However, further increasing the extraction time from 150 to 240

minutes did not result in statistically significant differences in the obtained essential oil content.

Short extraction times (less than 150 minutes) are insufficient to extract all the essential oil from the material. An extraction time of 150 minutes is optimal for essential oil extraction. Longer extraction times (greater than 150 minutes) may lead to energy wastage and potentially impact the quality of the essential oil (Le *et al.*, 2023).

### 3.3. The quality of essential oil

#### 3.3.1. The sensory evaluation score and density of essential oil

The essential oil extracted from *Ocimum africanum* leaves using the steam distillation method is in a liquid, transparent state, with an orange-yellow color, a characteristic aroma (similar to the fragrance of *Cymbopogon citratus* essential oil) and a slightly sour taste (Figure 2). The density of *Ocimum africanum* essential oil is  $0.903 \pm 0.001$ .



**Figure 2. The essential oil of *Ocimum africanum* leaves**

#### 3.3.2. The chemical composition of the essential oil

The chemical composition analysis (Table 3) showed that the main components in the essential oil of *Ocimum africanum* include citral, geraniol,  $\beta$ -linalool, 6-methyl-5-heptene-2-one,  $\beta$ -caryophyllene and  $\alpha$ -bisabolene (Figure 3).

**Table 3. The chemical composition of the of *Ocimum africanum*'s essential oil**

No.	The chemical compositions	Content (%)
1	$\alpha$ -Citral	31.03
2	$\beta$ -Citral	23.88
3	<i>trans</i> -Geraniol	5.25
4	<i>cis</i> -Geraniol (nerol)	5.21
5	$\beta$ -Linalool	4.88
6	6-Methyl-5-heptene-2-one	4.61
7	$\beta$ -Caryophyllene	4.47
8	$\alpha$ -Bisabolene	4.42
9	1-Terpinen-4-ol	2.81
10	3-Hexyl hydroperoxide	1.89
11	$\alpha$ -Cayophyllene	1.87
12	Germacrene D	1.57
13	$\alpha$ -Bergamotene	1.35
14	2-Hexyl hydroperoxide	1.31
15	Carane. 4.5-epoxy- <i>trans</i>	1.30
16	$\beta$ - <i>cis</i> -Ocimene	0.93
17	$\alpha$ -Terpineol	0.71

No.	The chemical compositions	Content (%)
18	Caryophyllene oxide	0.60
19	1-Methyl-1-(2-methyl-2-propenyl)cyclopentane	0.54
20	<i>cis</i> -Verbenol	0.52
21	1,1-Dimethyl-2-(2-methyl-2-propenyl)cyclopropane	0.44
22	$\gamma$ -Terpinene	0.41

The main components of the *Ocimum africanum*'s essential oil in this study were similar to the main components of the essential oil of *Ocimum africanum* leaves (Padalia *et al.*, 2018; Kumar *et al.*, 2021) and *Cymbopogon citratus* essential oil (Vázquez-Briones *et al.*, 2015; Hanaa *et al.*, 2019) (Table 4) but differed in the compound content. This difference may be due to the influence of essential oil content in medicinal herbs on factors such as climate, soil, and cultivation methods (Le, 2008).

**Table 4. The main chemical compositions of the essential oil**

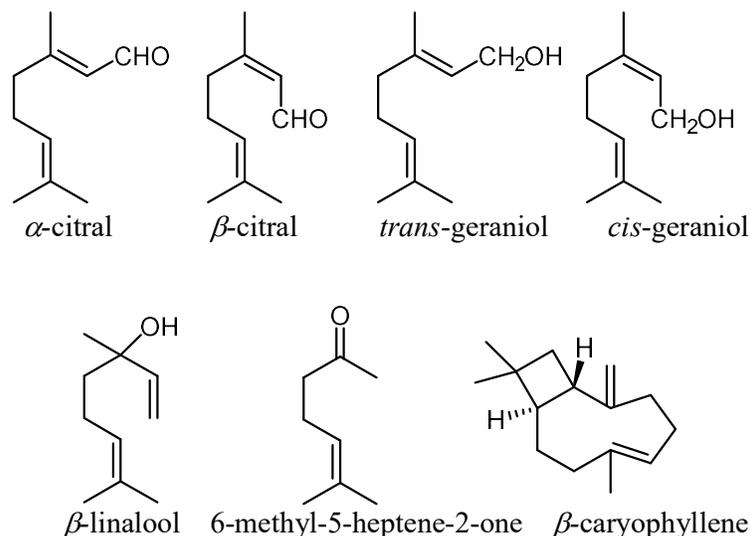
No.	The chemical compositions	The essential oil content (%)				
		<i>Ocimum africanum</i>			<i>Cymbopogon citratus</i>	
		This study	Padalia <i>et al.</i> (2018)	Kumar <i>et al.</i> (2021)	Vázquez-Briones <i>et al.</i> (2015)	Hana <i>et al.</i> (2012)
1	$\alpha$ -citral (geranial)	31.03	40.4	11.04	15.97	40.72
2	$\beta$ -citral (neral)	23.88	33.9	10.91	19.35	34.98
3	<i>trans</i> -geraniol	5.25	4.9	11.30	-	0.53
4	<i>cis</i> -geraniol (nerol)	5.21	1.7	-	-	0.17
5	linalool	4.88	3.7	9.02	0.64	1.03
6	caryophyllene	4.47	1.8	14.12	-	0.28
7	6-Methyl-5-heptene-2-one	4.61	1.6	-	-	-

Citral imparts a characteristic lemon like aroma to the essential oils of lemongrass. Citral possesses many significant bioactivities such as antimicrobial, anti-inflammatory, antiparasitic, allelopathic and mosquito repellent. Citral is the most valuable monoterpene in flavors, fragrances, cosmetics, perfumery and pharmaceuticals. It is used for the synthesis of

vitamin A and  $\beta$ -ionones and other specialty chemicals. A growing number of reports published on the bioactivities of citral, and citral-rich essential oils have reflected their rapidly increasing medicinal and pharmacological significance (Ganjewala *et al.*, 2012). Geraniol is also present in relatively high concentrations in the *Ocimum africanum*'s

essential oil. Geraniol has effects such as anti-inflammatory, antioxidant, antifungal, and analgesic activities (Lei *et al.*, 2019). Geraniol and citral have been shown to be effective insect

repellents against mosquitoes, fleas, and ticks. It is a safer and more natural alternative to synthetic insect repellents (Ganjewala *et al.*, 2012; Lei *et al.*, 2019).



**Figure 3. The structural formula of the *Ocimum africanum*'s essential oil**

#### 4. CONCLUSION

The essential oil content extracted from *Ocimum africanum* leaves was influenced by the solvent-to-material ratio and extraction time. The research results showed that the essential oil content was highest at the solvent-material ratio of 3.0 mL/g; the extraction time was 150 minutes. The obtained essential oil is in liquid form, with a transparent appearance and an orange-yellow color. The essential oil extracted from *Ocimum africanum* leaves has a characteristic aroma (similar to the fragrance of *Cymbopogon citratus* essential oil). The main chemical compositions in the essential oil of *Ocimum africanum* include citral, geraniol,  $\beta$ -linalool, 6-methyl-5-heptene-2-one,  $\beta$ -caryophyllene và  $\alpha$ -bisabolene. Understanding the chemical composition of *Ocimum africanum* essential oil will help determine its potential

applications in cosmetics, pharmaceuticals, and other fields.

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## TRÍCH LY VÀ KHẢO SÁT THÀNH PHẦN HÓA HỌC CỦA TINH DẦU CÂY É TRẮNG (*Ocimum africanum* L.)

### TÓM TẮT

*Cây é trắng (Ocimum africanum L.)* là một loại thảo mộc đặc trưng của Việt Nam, đặc biệt phổ biến ở Tuy Hòa, Phú Yên. Nghiên cứu này tập trung vào việc trích ly và khảo sát thành phần hóa học của tinh dầu thu được từ lá cây é trắng. Tinh dầu này được chiết xuất bằng phương pháp chưng cất lôi cuốn hơi nước và thành phần hóa học của nó được phân tích bằng phương pháp sắc ký khí ghép khối phổ (GC-MS). Kết quả nghiên cứu cho thấy thành phần chính trong tinh dầu cây é trắng là citral, một hợp chất có hương thơm đặc trưng, có đặc tính xua đuổi côn trùng và hoạt tính kháng khuẩn, đặc biệt đối với vi khuẩn gây bệnh.

**Từ khóa:** Citral, GC-MS, *Ocimum africanum*, sự chưng cất lôi cuốn hơi nước, tinh dầu.