# FACTORS INFLUENCING CONTENT AND COMPOSITION OF ESSENTIAL OIL OF *ELSHOLTZIA CILIATA* (THUNB.) HYLAND COLLECTED IN DAK LAK AND ANTIBACTERIAL ACTIVITY OF THE OBTAINED ESSENTIAL OIL

## MỘT SỐ YẾU TỐ ẢNH HƯỞNG ĐẾN HÀM LƯỢNG VÀ THÀNH PHẦN TINH DẦU KINH GIỚI *ELSHOLTZIA CILIATA* (THUNB.) HYLAND THU HÁI TẠI ĐẮK LẮK VÀ HOẠT TÍNH KHÁNG KHUẨN CỦA TINH DẦU

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**ABSTRACT**

From the fresh leaves of the *Elsholtzia ciliata* (Thunb.) Hyland, an essential oil was obtained by hydrodistillation. Through GC-MS analysis, a total of 45 compounds were detected, of which the main components of the essential oil are Z-Citral (28.36), Carveol (27.88%), *cis*-Verbenyl acetate (9.46%), Germacrene D (5.1%), *δ*-3-Carene (5.36%) and *trans*-Caryophyllene (4.41%). Factors influencing the content of essential oil such as the time of the day on the quantity and quality of essential oil, the length of dry-down or partial wilt and time of distillation were investigated. The results showed that conditions for the highest amount of essential oil are time of collection at 13 o'clock, partial wilt in 7 hours, distillation in 3 hours 30 minutes. The antibacterial activity of *Elsholtzia ciliata* (Thunb.) Hyland essential oil on *E. coli* was investigated. The results showed that the essential oil had strong antibacterial activity with approximately 98.2% inhibition and the sterile ring diameter for *E. coli* at 53.0mm.

***Keywords:*** *Elsholtzia ciliata* (Thunb.) Hyland*, Essential oil, GC-MS, antibaterial, E. coli.*

**TÓM TẮT**

Từ lá tươi của cây *Elsholtzia ciliata* (Thunb.) Hyland, thu được tinh dầu bằng phương pháp chưng cất thủy phân. Qua phân tích GC-MS đã phát hiện tổng cộng 45 hợp chất, trong đó thành phần chính của tinh dầu là Z-Citral (28,36), Carveol (27,88%), *cis*-Verbenyl acetate (9,46%), Germacrene D (5,1%), *δ-3*-Carene (5,36%) và *trans*-Caryophyllene (4,41%). Các yếu tố ảnh hưởng đến hàm lượng tinh dầu như thời gian trong ngày đến số lượng và chất lượng tinh dầu, thời gian khô hoặc héo và thời gian chưng cất đã được nghiên cứu. Kết quả cho thấy điều kiện để thu được lượng tinh dầu cao nhất là thời điểm thu hái lúc 13 giờ, héo trong 7 giờ, chưng cất trong 3 giờ 30 phút. Hoạt tính kháng khuẩn của tinh dầu *Elsholtzia ciliata* (Thunb.) Hyland trên vi khuẩn *E. coli* đã được nghiên cứu. Kết quả cho thấy, tinh dầu có hoạt tính kháng khuẩn mạnh với khả năng ức chế khoảng 98,2% và đường kính vòng vô trùng đối với *E. coli* là 53,0mm.

***Từ khóa****: Elsholtzia ciliata (*Thunb.*)* Hyland*, Tinh dầu, GC-MS, kháng sinh, E. coli.*

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## 1. INTRODUCTION

*Elsholtzia ciliata* (Thunb.) Hyland (*E. ciliata*) belongs to the Lamiaceae family - a large family contains about 236 genera and more than 6000 species [1], and is native throughout Europe and Asian countries such as China, Korea, Japan and Vietnam [2-4]. In Vietnamese traditional medicine, *E. ciliata* has been used for the treatment of cold, fever, flu, headache, dizziness, rheumatism, pharyngitis, abdominal pain, diarrhea, vomiting, measles and itchy sores [1, 5]. *E. ciliata* is known to be a rich source of phenols as well as flavonoids, steroids, and triterpenes in essential oil [4]. The extracts of different polarity and isolated compounds from this plant exhibited numerous biological activities, including antioxidants [1,5], antibacterial, anticancer, anti-inflammatory [6], antiviral [5], antiarrythmic [7] and vasorelaxant effects [8].

Despite the fact that there were several publications on chemical composition and biological activities of essential oil from *E. ciliata* in the world [9-11] in some provinces in Vietnam namely Vinh, Hue and Ho Chi Minh city [12-14], the composition and biological investigation of essential oil from *E. ciliata* collected in Buon Ma Thuot city, Vietnam has not been reported. In general, the relative composition of essential oil varies significantly with climatic and geographical variations. Therefore, in this

research, we described the composition, antibacterial and antioxidant activities of essential oil from *E. ciliata* collected in Buon Ma Thuot city. The results showed that some components of Buon Ma Thuot *E. ciliata* essential oil differed from those previously reported. The essential oil obtained also revealed higher antibacterial and antioxidant activities.

Fig. 1. *Elsholtzia ciliata* (Thunb.) Hyland collected in Dak Lak Province

## 2. MATERIALS AND METHODS

### 2.1. Plant material

The fresh leaves of *E. ciliata* (Thunb.) Hyland were collected from Tan Tien commune, Buon Ma Thuot city, Dak Lak province, Vietnam in 2022. The sample was identified by Dr. Nguyen Quoc Binh (Vietnam National Museum of Nature, Vietnam Academy of Science and Technology). A voucher specimen, KG-BMT-03, is deposited at Faculty of Natural Science and Technology, Tay Nguyen University, Buon Ma Thuot city, Dak Lak province, Vietnam.

### 2.2. Essential oil extraction

The fresh leaves of *E. ciliata* (Fig. 1) were cleaned, cut into small, and subjected to steam-distillation in a Clevenger-type apparatus for 4h. Anhydrous $Na_2SO_4$ was added to the crude essential oil to remove all water residue The anhydrous essential oil was stored in a sealed vial at 10°C in the dark prior to analysis.

### 2.3. Essential oil analysis

GC-MS analysis of *E. ciliata* leave essentail oil was performed on a Thermo Trace GC Ultra-ITQ900 gas chromatograph. Data interpretation was performed using the MassFinder 4.0 software. A fused-silica capillary TG-SQC column (30m x 0.25mm ID, 0.25µm film thickness) was used for separation.

GC operation conditions: the injector temperature was 250°C; the detector temperature was 260°C; the oven temperature was programmed from 60 to 260°C at 4°C/min. Helium was used as carrier gas at a flow rate of 1.0mL/min. A 1µL of the oil sample was injected using split mode with a split ratio of 1:10.

GC-MS operation conditions: The mass spectrometer was operated in electron-impact (EI) mode, the ionization energy was 70eV, the interface temperature was 280°C, the ion source temperature was 230°C, the MS quadrupole temperature was 200°C, and the scan range was 35 - 650 amu. The GC operation conditions were identical to those described above for GC.

### 2.4. Identification and quantification of essential oil constituents

Retention indices of oil constituents were determined on the HP-5 MS column using standard C7-C30 straight chain hydrocarbons (Aldrich Chemical Company, USA). Individual compounds in the oil were identified by comparison of their mass spectra and retention indices with those in GC-MS libraries (NIST 08, Wiley 09$^{th}$ Version) and/or with those reported in literatures. The relative percentage amounts of the separated compounds were computed from GC data without the use of correction factors.

### 2.5. Antibacterial activity assay

#### *2.5.1. Microbial strain*

The Gram negative strain - *Escherichia coli* (ATCC 25922) from laboratory stock cultures was used in the evaluation of the antibacterial activity of the leaves oil of *E. ciliata*.

#### *2.5.2. Inhibition zone*

The antibacterial activity of the sample was determined using agar disc diffusion. The microorganism liquid ($10^7$CFU/mL) was coated on solid medium and placed on 6 mm circular filter paper pieces in the middle of a Petri dish. Ethanol was removed from the essential oil by steaming. Then, the essential oil was dissolved in 10% DMSO. The *E. ciliata* essential oil (40µL) was drawn onto the filter paper using 10% DMSO as the negative control. The dishes were sealed and cultivated. The inhibition zones were used as a measure of antibacterial activity. The assays were performed in triplicate.

#### *2.5.3. Determination of Minimum Inhibitory Concentration (MIC)*

Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the test sample that inhibits a visible growth of bacteria. It is a measure of the antibacterial effect of the antibacterial agents. The essential oil was dissolved in ethanol, and serial two fold dilutions of the essential oil was prepared in a 96-well plate. 20µL bacteria broth medium were added into each well to produce a series of solution concentration range of 0.5 - 2.0mg/mL. Assays were performed at a pH value in the range of 7.4 - 7.6, and the micro plates were incubated at 37°C for 24h.

Each assay was conducted in triplicate to ensure reproducibility.

## 3. RESULTS AND DISCUSSION

### 3.1. Factors influencing the content of *E. ciliata* essential oil

#### *3.1.1. Distillation time*

The amount of essential oil depends strongly on distillation time, the longer the distillation time, the higher the essential oil amount obtained. However, when extending the time to a certain limit, the amount of essential oil does not increase and may negatively affect essential oil

quality. Therefore, it is necessary to determine the appropriate distillation time. *E. ciliata* samples (preliminary leaf selection) were collected at 7 o'clock, wilted for 2 hours then chopped and distilled with water for different periods of time: 1 hour 30 minutes, 2 hours 30 minutes, 3 hours 30 minutes and 4 hours 30 minutes. The results are shown in Fig. 2.

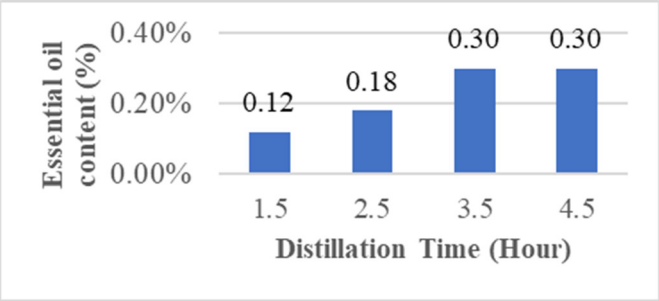


Fig. 2. Effect of distillation time on the content of essential oil

The investigation results showed that essential oil yield (%) is directly proportional to extraction time. At distillation time of 3 hours 30 minutes and 4 hours 40 minutes, the amount of essential oil obtained was the same and the highest (0.5mL - 0.3%). It is obvious that the longer distillation time, the more energy was consumed for heating. Therefore, the appropriate time of distillation was 3 hours 30 minutes

***3.1.2. Harvest time***

Raw materials were collected at different times of the day: 7 o'clock, 10 o'clock, 13 o'clock and 16 o'clock and distilled with 550 mL of water for optimum distillation time of 3 h 30 min. The results of the survey are shown in Fig. 3.

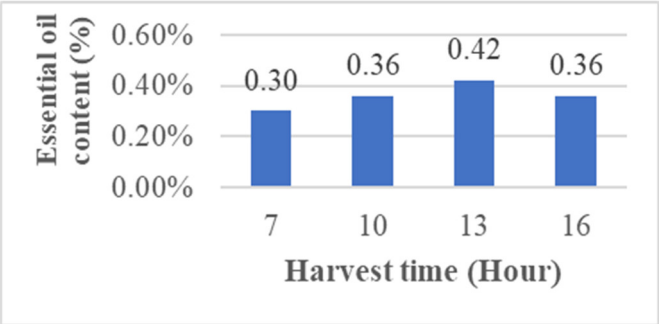


Fig. 3. Effect of harvesting time on the yield of essential oil

As a result, *E. ciliata* leaves collected at 13 o'clock gave the highest essential oil content of 0.42% (0.7mL). This can be explained that at 13 o'clock, the air humidity is the lowest, the amount of light shining on the plant is the highest, the plant photosynthesis is strong, and the water vapor in the plant escapes quickly, so the amount of essential oil accumulated in the plant is the most.

The harvest time at 13 o'clock was chosen to conduct further survey.

***3.1.3. Time of wilting***

Some plants continue to produce essential oil postharvest, others reduce the amount of essential oil in the material, so the time of wilting has an important effect on the amount of essential oil obtained. To survey the influence of the time of wilting, samples were collected at 13 o'clock, left to wilt indoors for different time periods: 2 hrs, 7 hrs, 12 hrs and 17 hrs.

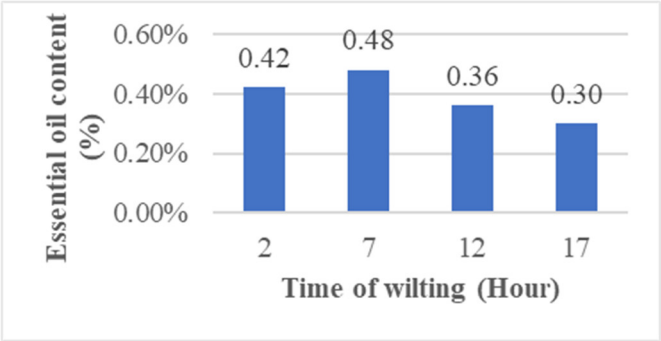


Fig. 4. Effect of time of wilting on the yield of essential oil

The experimental results showed that the optimal wilting time was 7 hours when the content of essential oil was the highest as the amount of water in the plant is greatly reduced. However samples with longer time of wilting had lower amount of essential oil as essential oil evaporated together with water.

**3.2. Composition of the essential oil of *E. ciliata* leaves**

The obtained *E. ciliata* essential oil is light yellow in color and has a mild fragrance.

The GC-MS chromatograms of *E. ciliata* essential oil are shown in Fig. 5. The chemical composition of the essential oil is presented in Table 1.

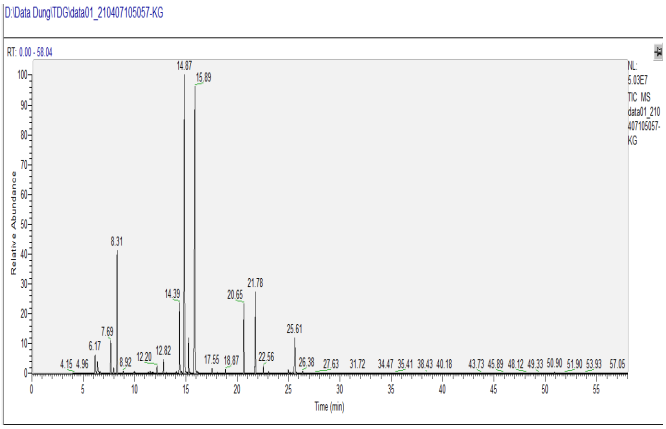


Fig. 5. GC-MS chromatogram of *E. ciliata* essential oil

Table 1. Chemical compositions of *E. ciliata* essential oil

| No. | Compounds | Content (%) |
|---|---|---|
| 1 | *β*-Longipinene | 0.01 |
| 2 | *cis*-Verbenyl acetate | **9.46** |
| 3 | Panaxydol | 0.12 |
| 4 | (-)-Myrtenol | 0.7 |
| 5 | (-)-Isopulegol | 0.03 |
| 6 | *β*-Longipinene | 0.11 |
| 7 | 2-β-Pinene | 0.15 |

| | | |
|---|---|---|
| 8 | **δ-3-Carene** | **5.36** |
| 9 | α-Humulene | 0.18 |
| 10 | **Carveol** | **27.88** |
| 11 | **Z-Citral** | **28.36** |
| 12 | α-Sinensal | 0.05 |
| 13 | ***trans*-Caryophyllene** | **4.41** |
| 14 | **(+)-Germacrene D** | **5.1** |
| 15 | γ-Muurolene | 0.12 |
| 16 | δ-Cadinene | 0.03 |
| 17 | (-)-Germacrene D | 0.33 |
| 18 | Verrucarol | 0.12 |
| 19 | *trans*-Valerenyl acetate | 3.3 |
| 20 | Dehydroaromadendrene | 0.18 |
| 21 | β-Guaiene | 0.04 |
| 22 | (1R)-*cis*-Verbenol | 0.03 |
| 23 | Verbenol | 0.91 |
| 24 | *trans*-2-Ethyl-2-hexen-1-ol | 2.01 |
| 25 | 6-Camphenol | 1.21 |
| 26 | Ethyl-2 methyl-3- pentene-1 | 0.3 |
| 27 | Camphene | 2.96 |
| 28 | β-Phellandrene | 0.31 |
| 29 | Benzoylformic acid | 0.29 |
| 30 | Sabinene | 0.23 |
| 31 | isopropylidenecyclopropyl methyl ketone | 0.09 |
| 32 | Chavicol | 0.16 |
| 33 | *trans*-2-caren-4-ol | 0.19 |
| 34 | Ylangene | 0.08 |
| 35 | Myrtenyl formate | 2.11 |
| 36 | Methyl -4,6-tetradecadiynoate | 0.13 |
| 37 | Methyl chrysanthemate | 0.45 |
| 38 | Methyl -2,4-tridecadiynoate | 0.06 |
| 39 | Testosterone | 0.07 |
| 40 | Panaxjapyne A | 0.01 |
| 41 | α-Pinene | 0.22 |
| 42 | trans-Z-α-Bisabolene epoxide | 0.06 |
| 43 | β-Longipinene | 0.04 |
| 44 | 3β,17β-dihydroxyestr-4-ene | 0.06 |
| 45 | Falcarinol | 0.03 |
| 46 | Other compounds | 1.01 |
| **Total** | | 100% |
| **Hydrocarbon** | | 30.51%, |
| **Derivatives containing oxygen** | | 68.46% |

Through analysis, the chemical composition of *E. ciliata* essential oil has 43 identified compounds. The main chemical compositions of essential oils are *Z*-Citral (28.36), Carveol (27.88%), *cis*-Verbenyl acetate (9.46%), Germacrene-D (5.1%), δ-3-Carene (5.36%) and *trans*-Caryophyllene (4.41%).

The results of this study are different from those previously reported by Le Ngoc Thach [13] with 34 compounds were detected from *E. ciliata* collected in Ho Chi Minh City. In which, the main constituents are geraniol (16.49%) and neral (13.68%). However, in our study, these two compounds were not present. In another study, the chemical composition of *E. ciliata* essential oil collected in Hue is mainly geraniol (28.385%), neral (21.679%), β-Ocimen (22.992%).

Especially, the study of Elena A. Korolyuk [15] show that there is a big difference. The main constituents of *E. ciliata* essential oil are dehydro-elsholtzia ketone (66.1 - 72.4%), α-dehidro-elsholtzion (2.0 - 5.7%), elsholtzia ketone (3.3 - 19.3%), perillen (2.1 - 3.9%) and humulen (1.5 - 3.8%). Meanwhile, these components are not present in this study as well as the studies of the above authors.

The results show that the chemical composition of essential oils will be different if the geographical location and soil conditions are different.

### 3.3. Antibacterial activity of *E. ciliata* essential oil

The antibacterial activity of the oil of *E. ciliata* against Gram-positive and Gram-negative bacteria strains is shown in Table 2. *E. ciliata* essential oil exhibited variable degree of antibacterial activity against all microorganisms tested. The results of this research show that *E. coli* was the most sensitive bacteria among microorganisms tested, with inhibition zones of 18.1 and 54.0mm, respectively, and the concentration of 2.0mg/ml, the antibacterial circle was 53.0mm and the ability to inhibit *E. coli* was 98.2% (Table 2). This results also showed that the oil exhibited significantly higher activity against Gram-positive bacteria than Gram-negative bacteria. In our research, the antibacterial activities of the essential oil might be described to the higher content of oxygenated monoterpenes. The oxygenated monoterpenes such as carveol [16] and Z-Citral [17] in our essential oil can be related to antibacterial properties.

Table 2. Antibacterial ability of *E. coli* of *E. ciliata* essential oil

| Bacteria density | Concentration of essential oil (mg/ml) | Diameter of sterile ring (mm) | Resistance ability |
|---|---|---|---|
| $10^7$ CFU | 2,00 | 54mm, 54mm, 54mm | 100% (*bacteria* do not grow all over the agar plate) |
| $10^7$ CFU | 1,75 | 53mm, 53mm, 53.1mm | 98,20% |
| $10^7$ CFU | 1,50 | 44.5mm, 44.5mm, 44.08mm | 82,15% |
| $10^7$ CFU | 1,00 | 32.42mm, 32.5mm, 32.5mm | 60,14% |

| | | | |
|---|---|---|---|
| $10^7$ CFU | 0,75 | 25.5mm, 25.7mm, 25.5mm | 47,34% |
| $10^7$ CFU | 0,50 | 18.1mm, 18.13mm, 18.2mm | 33,60% |

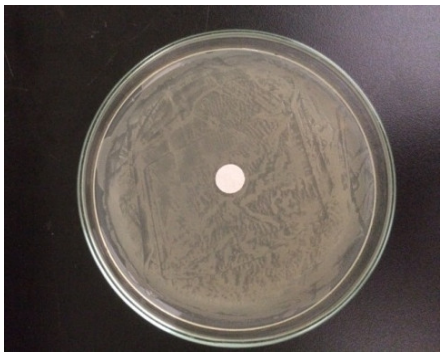

Fig. 6. Negative control (DMSO 2%)

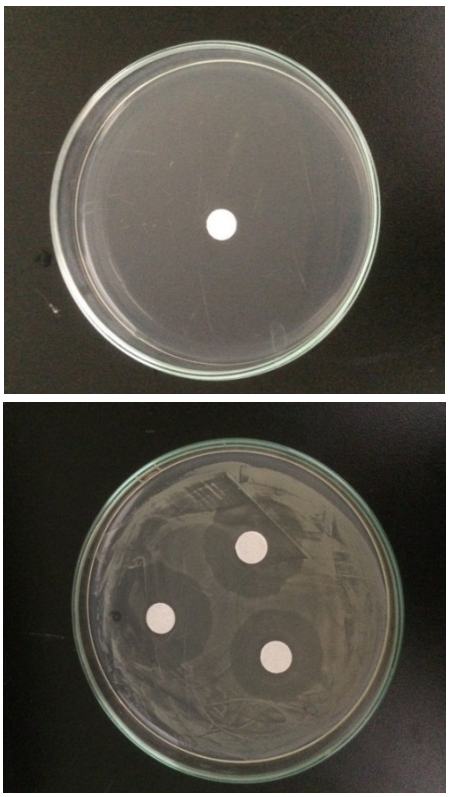

Fig. 7. Antibacterial ability of *E. coli* of *E. ciliata* at the basil essential oil with concentration (2mg/ml and 0,5mg/ml)

With a dilute concentration of 1.0mg/mL and an antibacterial ring whose diameter measured against the resistance ring of *E. coli* at 32.47mm, the inhibitory ability is 60.14%. The resistance ability of *E. coli* bacteria increases with concentration gradually from 0.5 mg/ml to 2.0 mg/ml; when it reaches 2.0mg/ml, bacteria will not grow entirely on the agar plate. At a concentration of 1.75mg/mL, the antibacterial ring is 53.0mm and the *E. coli*-inhibiting ability is 98.2%. This explains why *E. ciliata* essential oil has the potential to be a natural antibacterial agent.

## 4. CONCLUSION

*E. ciliata* (Thunb.) Hyland collected in Buon Ma Thuot city, Dak Lak province contained from 0.12 - 0.48% essential oil. The main chemical components of the essential oil are *Z*-Citral (28.36), Carveol (27.88%), *cis*-Verbenyl acetate (9.46%), Germacrene D (5.1%), *δ*-3-Carene (5.36%) and trans-Caryophyllene (4.41%). Optimal conditions for extracting essential oils are harvest time at 13 o'clock in the dry season, 7 hrs of wilting and 3 hrs 30 min of distillation. The investigation of the antibacterial effect of the essential oil revealed effective inhibition against *E. coli* bacteria, with an inhibitory ability of 98.2% and a sterile ring diameter at 53.0mm. These results indicate potential application of *E. ciliata* essential oil in pharmaceutical industry.

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**THÔNG TIN TÁC GIẢ**

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