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# Biochemical Features of *Ocimum canum* Sims Grown in Ukrainian Southern Steppe Conditions

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*Ocimum canum* Sims is an aromatic, essential oil, and medicinal plant. Although this species is not as well studied as other members of the genus, such as *Ocimum basilicum*, there is evidence of high biological activity of extracts and use for certain diseases. The essential oil composition, according to previous data, is valuable but depends on numerous factors. This study aimed to evaluate economically valuable features of *O. canum* f. 17–35 in the flowering stage grown in the South of Ukraine (Kherson Oblast). Plants were investigated for morphometric features such as plant height (cm), plant diameter (cm), length and width of leaves (in cm), inflorescence length (cm), the number of shoots (first and second order), inflorescences (per plant), and described the colour of shoots, lamina, and flowers. The essential oil was obtained by the hydrodistillation method (Ginsberg's method) on the Clevenger apparatus. The study of the component composition of the essential oil was carried out by the high-performance gas-liquid chromatography method. It was found the average values of plant height (55.17 cm), plant diameter (44.6 cm), leaf length (6.98 cm), leaf width (4.1 cm), inflorescence length (26.55 cm), inflorescence number (82.43), shoot number of first order (9.88), and shoot number of second order (5.73). The 43 compounds were determined in the essential oil, and 41 were identified. The main components of the *O. canum* f. 17–35 essential oil of this sample are linalool (58.99%), methyl chavicol (10.66%), *epi*-α-cadinol (5.14%), eugenol (4.71%), and geraniol (4.35%) Thus, the obtained data concerning the *O. canum* showed differences in essential oil content and selected morphometrical parameters of plants. Also, the essential oil of *O. canum* f. 17–35 contains the principal chemicals that can be used in further pharmacological investigations, cosmetic, and food industries

**Keywords:** American basil, essential oil, terpenoids, morphometry

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## Introduction

The genus *Ocimum* L. includes more than 70 species, which are distributed in tropical and subtropical regions on almost all continents. Some species are used in traditional medicine, cosmetology, and cooking (Nascimento et al., 2011; Shanaida and Cherevko, 2024). Species of this genus are a source of aromatic essential oils, which have antimicrobial, antiemetic, antidiabetic, antiasthmatic, antistress, and anticancer effects (Selvi et al., 2015).

*Ocimum canum* Sims is a small-used species of this genus, and it is distributed in Africa, the Indian subcontinent, China, and Southeast Asia. The plant has a pungent, aromatic taste and is usually cultivated for culinary purposes (Aluko et al., 2012; Shanaida and Cherevko, 2024). *O. canum* is also used to treat various types of diseases: headaches, colds, fever, and joint inflammation (Aluko et al., 2012). *O. canum* plant extracts exhibited antidiabetic, anthelmintic properties, and high antioxidant and antileishmanial activity (Nyarko et al., 2002; Dash et al., 2014; Selvi et al., 2015; Silva et al., 2018; Tshilanda et al., 2019). *O. canum* extract has hepatoprotective properties against oxidative stress caused by alcohol (George and Chaturvedi, 2008). Also, the essential oil of plants of this species has high bactericidal activity against *Plasmodium falciparum* and *Anopheles funestus* larvae in the mature stage. Therefore, it is recommended in the development of natural biocides to combat malaria vector larvae and for the isolation of natural products with antimalarial activity (Ntonga et al., 2014).

*O. canum* essential oil is used to preserve food against insect damage (Weaver et al., 1991). One way to use it in insect control is in the form of aerosol bombs (Belong et al., 2013). The essential oil of *Ocimum canum* showed high antibacterial activity against two food bacteria, *Listeria monocytogenes* and *Salmonella enterica* serotype *Typhimurium*. Moreover, it is higher in the daytime, so its component composition changes throughout the day (Mith et al., 2016).

An innovative method for the therapeutic use of this plant species is the green synthesis of silver nanoparticles from cornflowers as an alternative to chemical methods. This approach is safe for the environment (Jayaseelan and Rahuman, 2012).

Phytochemical analysis of *Ocimum canum* plants revealed high concentrations of flavonoids (10.0%), saponins, tannins, and low levels of phenolic compounds and alkaloids. The leaves contained carbohydrates (639.6 $g.kg^{-1}$), ash, and a low protein content. Of the elements, there is a high content of calcium (50.72 $g.kg^{-1}$), potassium, sodium, phosphorus, and magnesium. The plant is a good source of iron, zinc, and manganese. In addition, the concentrations of cadmium (0.01 $g.kg^{-1}$) and lead (0.02 $g.kg^{-1}$), which are toxic metals, are very low. A high content of vitamin C (0.05 $g.kg^{-1}$) was also found (Aluko et al., 2012; Silva et al., 2018). According to the obtained results, the content and composition of *Ocimum* plant essential oil depend on the genotype, developmental phase, and soil-climatic cultivation conditions. Thus, in Benin (West Africa), essential oil was obtained from the aerial parts of the species: *Ocimum basilicum*, *Ocimum gratissimum*, and *Ocimum canum*. It was found that the main components in *O. basilicum* oil are estragole (43.0–44.7%) and linalool (24.6–29.8%), *O. gratissimum* oil are thymol (28.3–37.7%) and γ-terpinene (12.5–19.3%), and *O. canum* oil carvacrol (12.0–30.8%) and *p*-cymene (19.5–26.2%) (Mith et al., 2016).

From plants of *Ocimum canum*, grown in India, 2 ml of essential oil was obtained by hydrodistillation from 800 g of raw material. The researchers found 36 compounds in the essential oil, of which the main component was camphor (39.77%), limonene (8.67%), naphthalene (7.37%), valentene (5.80%), caryophyllene (5.60%), α-pinene (5.59%), camphene (5.20%) and myrtenyl acetate (2.74%). In their opinion, there are chemotypes of *Ocimum canum* with a wide range of main compounds (Selvi et al., 2015). The content and component composition of the essential oil of plants of two species of the genus *O. selloi* and *O. canum*, grown in northeastern Brazil, were studied. The mass fraction of essential oil in summer was 0.12% in *O. canum* and 0.16% in *O. selloi* Benth., the same parameter in winter was 0.06 and 0.18%, respectively. Thirty components were identified in the essential oil of *O. selloi*, while 31 components were identified in *O. canum*. It was observed that the oil content of *O. canum* varied during the season, while that of *O. selloi* was stable. Methylhavicol and linalool were the main components found in the entire aerial mass and separately in the leaves of *O. canum*, which allows for characterization of this sample as a new chemotype of *O. canum* (Nascimento et al., 2011).

Comparative characterization of samples of two species of cornflowers grown in Cameroon (Central Africa) showed that the essential oil content of *O. canum* leaves was four times higher (0.44%) than that of *O. basilicum* (0.11%). Oxygenated monoterpenes predominate in *O. canum* oil (63.3%), while monoterpene hydrocarbons predominate in the essential oil of *O. basilicum* (56.2%). The main components of the essential oil of *O. canum* are linalool (53.8%) and limonene (22.2%). The essential oil of *O. basilicum* is characterized by the predominance of compounds such as linalool (18.9%), limonene (30.9%), and β-phellandrene (15.3%) (Belong et al., 2013). The chemical composition of essential oils of *O. canum* of different origins has been the subject of many studies. The literature reports the existence of several physiological forms or chemotypes of *O. canum*. Thus, the analysis of the essential oil of two chemotypes grown in Cameroon was carried out: *O. canum* essential oil type I is characterized by a high percentage of monoterpene alcohols (total 91.9%), especially represented by linalool (44.9%) and geraniol (38.2%). *O. canum* essential oil type II contains less oxygenated monoterpenes (total about 25.0% and 30.9%) and more monoterpene hydrocarbons (total about 61.3–24.1%) and sesquiterpene derivatives (total about 13.2–44.0%) with the main components limonene (41.5 and 5.7%), 1,8-cineole (10.1 and 18.5%), δ-cadinene (4.0% and 18.0%), α-pinene (4.7 and 10.2%) and α-terpineol (6.9 and 6.4%) (Ngassoum et al., 2004).

In the component composition of the essential oil of cornflowers grown in Rwanda, it was found that in most samples, a high percentage belongs to linalool, however, there are a small number of samples in which other components predominate (Ntezurubanza et al., 1985).

In Côte d'Ivoire, scientists found three new chemotypes of *O. canum*, which were grown in different regions of the country. In the chemotype grown in the central region, the essential oils obtained from the leaves and inflorescences were rich in 1,8-cineole (28.7–34%) and camphor (15.7–18%). In the chemotypes from the south, the main components were 1,8-cineole (13.1%), bornyl acetate (10.2%), (E)-β-farnesene (9.5%), and (Z,E)-α-farnesene (15.1%). And in plants grown in the west of the country, the predominant components of the essential oil were linalool (38.4%) and eugenol (13.3%) (Tonzibo et al., 2008). In northwestern Africa (the Republic of Mali), scientists studied 16 samples of *Ocimum canum*. Fifty-three components were identified in the oils obtained from the plants. Two new chemotypes were identified, one of which is rich in α-terpineol (48–64%), and the other in methylchavicol-α-terpineol (44–52% and 20–27%). Scientists note that until now, α-terpineol was registered only in trace amounts in *O. canum* oil (Chalchat et al., 1999).

In the conditions of the Ternopil region, a sample of *O. sanctum* revealed 50 components (identified 35). The main components of the essential oil of *O. canum* are linalool (46.76%), methylchavicol (16.99%), and *epi*-γ-cadinol (5.016%) (Shanaida, 2019).

Our research aims to study the content and composition of the essential oil of *O. canum* in the conditions of the Ukrainian Southern Steppe.

## Material and Methodology

### Conditions of Plant Growth

The research was conducted in 2021–2022 in the conditions of the Kherson region based on the State Enterprise "Research Farm "Novokakhovske" of the Institute of Climate-Oriented Agriculture. The research farm "Novokakhovske" is located in the first, northern agro-climatic region of the Kherson region, which is characterized by a temperate continental climate with a short spring, a relatively long hot and dry summer, and a mild winter with frequent thaws. The site where the experiments were carried out is located on chernozem light loamy soils with a humus layer thickness of 76 cm and a humus content in the arable layer of 1.33% (Svydenko and Kremenchuk, 2015).

### Plant Material

The *Ocimum canum* Sims f. 17–31, which was previously introduced to the Research Farm "Novokakhovske" from the Nikitsky Botanical Garden, was used in this research. The plants were investigated in the flowering stage.

### Morphometrical Measurings

The following morphometric measures were conducted: plant height (cm), plant diameter (cm), shoot number of first order, shoot number of second order, leaf length (cm), leaf width (cm), inflorescence length (cm), and inflorescence number. The mass of one plant was determined. It was described the colour of shoots, lamina, and flowers, and lamina was described.

### Mass Fraction and Component Determination of Essential Oil

The mass fraction of essential oil in the samples was determined by the Ginsberg method on the Clevenger apparatus (Elyemni et al., 2019). A portion of freshly cut *O. canum* herb (100 g) is crushed, placed in a flask, and filled with 300–400 ml of water. The flask is closed with a cork stopper, into which the lower end of the refrigerator is inserted. The flask is heated. The mixture of vapors of essential oil and water rises to the refrigerator, condenses and flows through the receiver, where the essential oil is collected in a graduated tube, floating above the water, the excess of which is drained through the side tube. Distillation of the oil is completed in approximately 1 hour.

The study of the component composition of the essential oil was carried out by high-performance gas-liquid chromatography on an Agilent Technology 6890N chromatograph (Svydenko et al., 2024). Chromatographic column quartz, capillary HP 5MS, Evaporator temperature 250 °C. Carrier gas is helium. Carrier gas speed 1 $mL.min^{-1}$. Sample introduction with flow separation 1/50. Thermos temperature 50 °C. With programming 3 $°C.min^{-1}$ to 220 °C. Detector and evaporator temperatures are 250 °C. The components of essential oils were identified by searching for mass spectra of chemicals included in the studied mixtures obtained during chromatography with data from the NIST02 mass spectrum library. The component retention indices were calculated based on the results of control analyses of essential oils with a set of normal alkanes (Jennings et al., 1980).

### Statistical Analysis

The results are expressed as mean values of three replications ±standard deviation(SD). Data were analysed with the ANOVA test, and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

## Results and Discussion

The *Ocimum* spp. plants‘ morphological parameters, yield (Patel et al., 2015), phenological stages (Kholia et al., 2022), and essential oil composition depended on growth conditions (Burducea et al., 2016; Saha et al., 2016a; Saha et al., 2016b).

The sample of *O. canum* f. 17–31 was sown in the conditions of the Southern Steppe after the 20th of April, and after 6 days, shoots were obtained. Two months after the emergence of shoots, the beginning of flowering was noted. The studied sample is similar in its morphological features to *O. basilicum*, but has some differences. It does not exceed 60 cm in height. It has a medium-compact bush shape. Shoots are pubescent. Leaves are only green in colour, and petiolate. The edge of the leaf blade is toothed. There is no blistering of the leaves. The morphometric parameters of *O. canum* are represented in Figure 1. The yield of the aboveground mass during the flowering period varies from 200 to 340 g per plant, with an average of 280.67 g.

The study of *O. basilicum* showed 5.44 cm of leaf length and 2.89 cm of leaf width (Oliveira et al., 2017), whereas *O. canum* in our study had 6.98 and 4.1 cm, respectively. Parida et al. (2020) represented the morphological description of *O. canum*: leaf ovate, obovate, hairy,

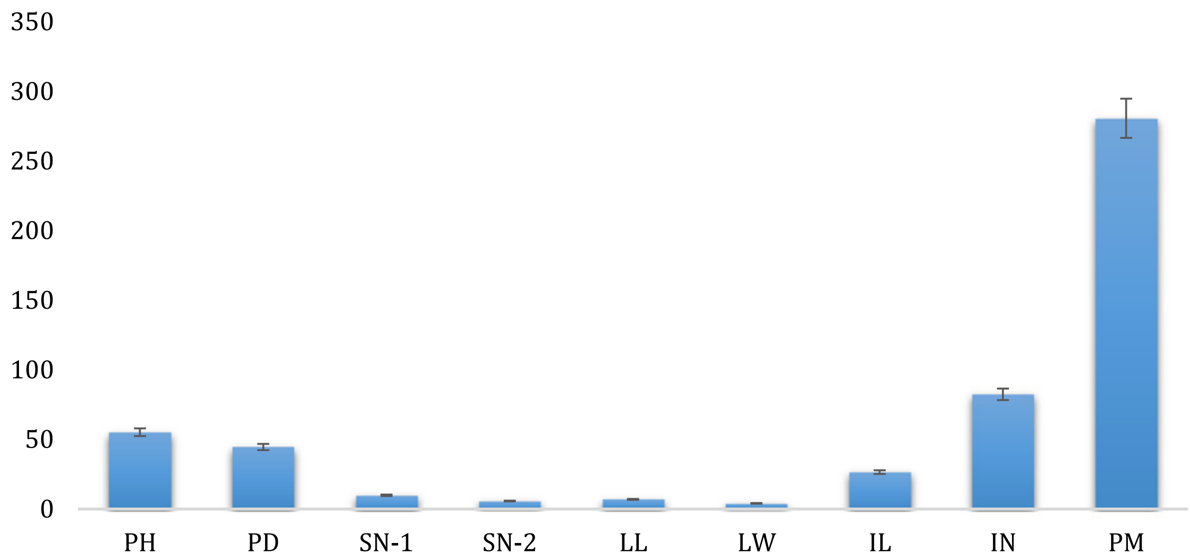


**Figure 1** The morphometrical parameters of *Ocimum canum* Sims plants in the flowering stage
PH – plant height, cm; PD – plant diameter, cm; SN-1 – shoot number of first order, pcs; SN2 – shoot number of second order, pcs; LL – leaf length, cm; LW – leaf width, cm; IL – inflorencense's length, cm; IN – inflorescence number, psc per plant; PM – mass of one plant, g

**Table 1** *Ocimum canum* Sims. f. 17–31 shape and colour morphological characteristics

| Parameter | Description |
|---|---|
| **Shoot colour** | light green |
| **Lamina shape** | broadly ovoid |
| **Lamina colour** | green |
| **Flower colour** | white |

inflorescence greenish, flower white to whitish grey, calyx green, etc. As reported Zuškevičienė et al. (2022), the productivity and morphometric parameters of *O. basilicum* and their genotypes depended on growth and meteorological conditions. Moreover, this study demonstrated 120–620 g per plant of green mass in the field conditions and 130–790 g in greenhouse conditions. According to Branca et al. (2024), the plant height of *O. basilicum* varied from 19.12 to 44.08 cm, lamina length from 2.25 to 8.80 cm, and lamina width from 1.04 to 7.83 cm, depending on genotype.

*O. canum* essential oil demonstrated numerous biological activities such as antioxidant, antimicrobial (Pandey et al., 2014; Selvi et al., 2015; Morsy and Hammad, 2021). The essential oil is an extract obtained from various plant parts, with the main bioactive compounds such as terpenes and terpenoids, whose composition depends on plant species. The essential oil has many therapeutic and biological activities, such as antioxidant, antimicrobial, antiviral, anti-inflammatory, etc. (Pezantes-Orellana et al., 2024). An important use of essential oil is clinical aromatherapy for a wide range of ailments, however, it requires deep investigations (Vora et al., 2024). The widest studied essential oil of *Ocimum* species is *O. basilicum* and its genotypes according to morphological and growth peculiarities (Marotti et al., 1996), fertilization (Dzida, 2010). It was highlighted that the "linalool", "linalool and methylchavicol", and "linalool eugenol" chemotypes with own morphological characters (Marotti et al., 1996).

The content of essential oil in the aboveground mass in the conditions of the Southern Steppe gradually increases according to the phases of growth, reaching a maximum in the phase of full flowering. In this phase, the mass fraction of essential oil is 0.09% of the fresh mass or 0.35% of the absolutely dry mass (Figure 2). The total yield of essential oil from one plant is 0.3 g.

The essential oil yield of *Ocimum* spp. depended on harvesting time, while some species had minimal oil content at 10 hours (morning). However, *O. canum* essential oil yield was stable (1.69–1.78%) (Mith et al., 2016). Many authors claim that there are several chemotypes of *O. canum*, in the essential oil of which certain components predominate, and their use depends on the percentage of the main components. In the essential oil of the studied sample, we identified

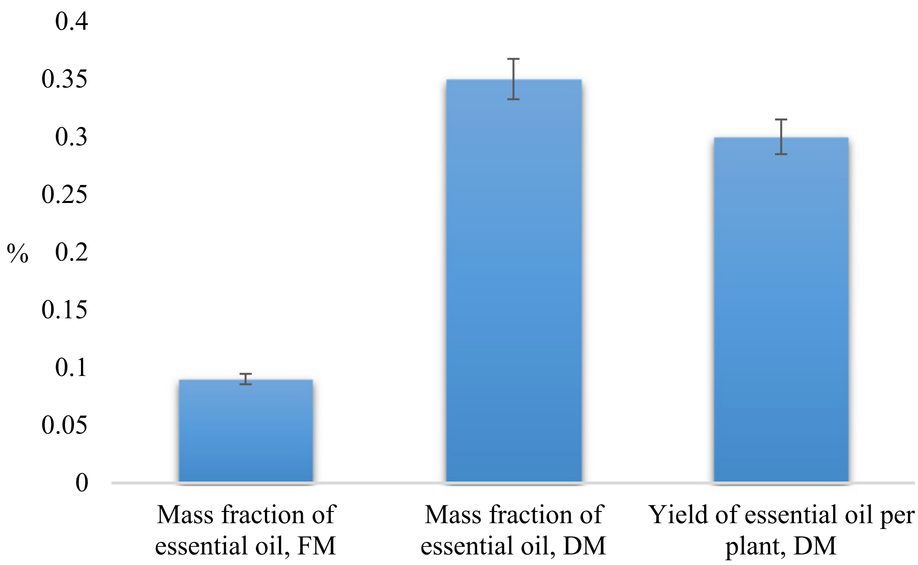


**Figure 2** Yield of essential oil of *Ocimum canum* Sims (g)
FM – fresh mass; DM – dry mas

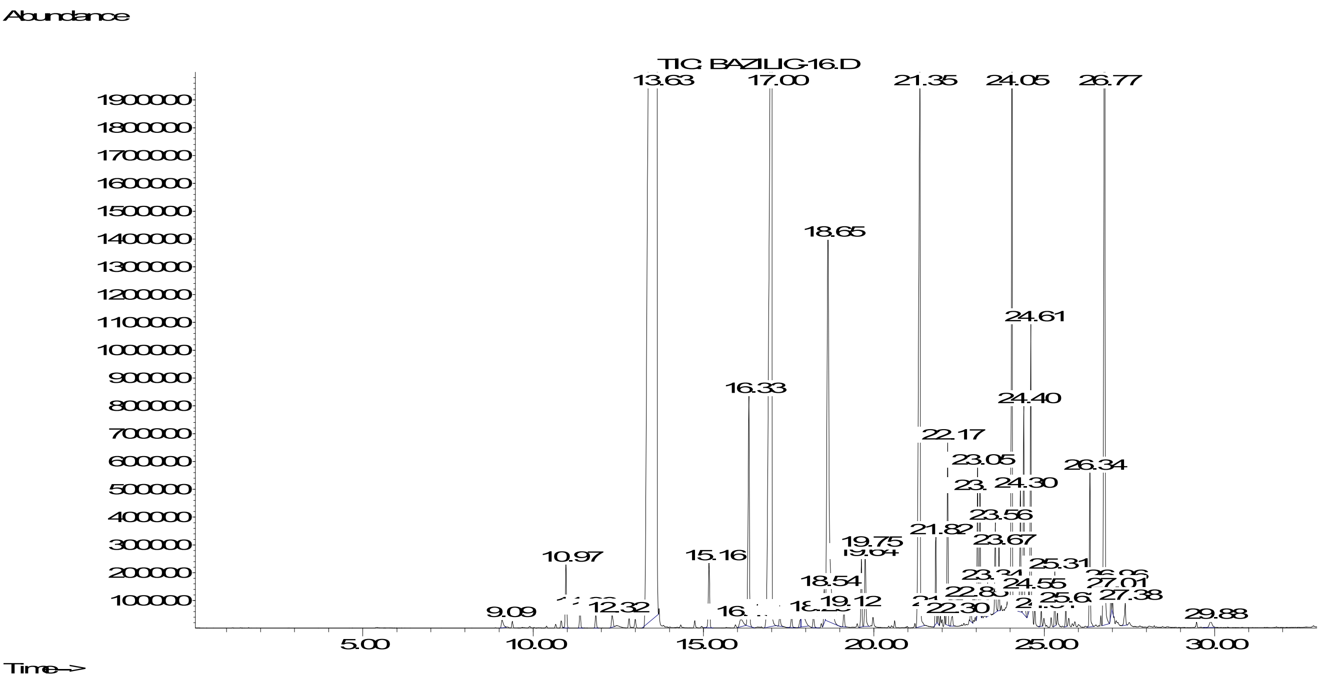


**Figure 3** The essential oil chromatogram of *Ocimum americanum* L. (f. 17–31) in the Ukrainian Southern Steppe conditions

**Table 2** Component composition of *Ocimum canum* Sims essential oil

| Component | Yield (%) | Component | Yield (%) |
|---|---|---|---|
| **1-octen-3-Ol** | 0.077 | **β-elemene** | 0.947 |
| **1,8-cineol** | 0.400 | **Methyl eugenol** | 0.080 |
| ***Cis*-ocimene** | 0.109 | **β-caryophyllene** | 0.108 |
| **γ-terpinene** | 0.083 | ***Trans*-α-bergamotene** | 0.658 |
| ***Trans*-sabinene hydrate** | 0.091 | **α-guaiene** | 0.518 |
| **Linalool** | 58.985 | **Unknown** | 0.192 |
| **Camphor** | 0.438 | **Humulene** | 0.442 |
| **Borneol** | 0.131 | ***Epi*-bicyclosesquiphellandrene** | 0.287 |
| **Terpinene-4-ol** | 1.654 | **Germacrene D** | 3.077 |
| **Methyl chavicol** | 10.667 | **Bicyclohermacrene** | 0.681 |
| **Octyl acetate** | 0.075 | **α-bulnesene** | 1.122 |
| **Fenchyl acetate** | 0.081 | **Germakren A** | 0.081 |
| **Nerol** | 0.059 | **γ-cadinene** | 1.252 |
| **Citronellol** | 0.227 | **Unknown** | 0.077 |
| **Neral** | 0.085 | **Nerolidol** | 0.247 |
| **Linalyl acetate** | 0.170 | **Ledol** | 0.102 |
| **Geraniol** | 4.346 | **Copaenol(?)** | 0.755 |
| **Geranial** | 0.100 | ***Epi*-α-cadinol** | 5.143 |
| **Bornyl acetate** | 0.375 | ***Epi*-α-muurool** | 0.120 |
| **Eugenol** | 4.711 | **β-eudesmol** | 0.076 |
| **Geranyl acetate** | 0.398 | **α-bisabolol** | 0.147 |
| **Copaen** | 0.072 | | |

43 components, 41 of which were identified, and two could not be established (Figure 3).

The *O. canum* essential oil has an antibacterial effect against food-borne pathogens (Wouatsa et al., 2014).

The essential oil composition of aromatic plants varied depending on plant species (Svydenko et al., 2022; Svydenko et al., 2024). The main components of the *O. canum* f. 17–35 essential oil of this sample are linalool (58.99%), methyl chavicol (10.66%), *epi*-α-cadinol (5.14%), eugenol (4.71%), and geraniol (4.35%) (Table 2).

The obtained results are consistent with the data of Ntezurubanza et al. (1985) concerning the linalool content (60–90%) as the main essential component. The monoterpenoid linalool accounts for about 60% of the essential oil content. It has a sedative and antiviral effect. In slightly smaller percentages, compounds such as eugenol and geraniol are present. It is known that the biological activity of the essential oil of certain medicinal plants is not simply the sum of the activities of its constituent components, but a new quality that manifests itself in their joint effect (Shanaida, 2019).

According to Félix Zanahi et al. (2008), 1,8-cineole was identified in the *O. canum* leaves of plants from West Africa, ranging from 13.1 to 34% depending on the region of growth. Also, this research demonstrates that in some areas the *O. canum* essential oil contained 15.7–18% of camphor, which was 35–41 times less in our study, 10.2% of bornyl acetate, which was 27 times less in our study, 15.1% of α-farnesene, and 9.5% of (E)-β-farnesene.

In the aerial parts of plants from West Bengal, camphor (33.2%), β-caryophyllene (5.7%), limonene (5.6%), and α-pinene (3.0%) were determined as major compounds of the essential oil. According to this study, the comparison of essential oil composition showed that the investigated plants had 1,8-cineol 4 times higher than from West Bengal (0.1%), whereas borneol was 5.3 times lower (0.7%), β-elemene 1.2 times lower (1.2%), etc. (Pragadheesh et al., 2013). Selvi et al. (2015) reported that major compounds in the leaf essential oil was camphor (39.77%), limonene (8.67%), naphthalene (7.37%), valencene (5.80%), caryophyllene (5.60%), α-pinene (5.59%), camphene (5.20%), and myrtenyl acetate (2.74%). In the study of Abdoul-Latif et al. (2022), the major compounds of *Ocimum* spp. essential oil were linalool (41.2%), carotanacetol (38.4%), estragol (27.5–30.1%).

## Conclusion

Thus, *O. canum* f. 17–35 investigated in the Kherson Oblast of Ukraine can be recommended for cultivation as aromatic and essential oil plants and replenish the assortment of cultivated *Ocimum* species. The data obtained showed that *O. canum* has a mass fraction of 0.35% (DM), and 43 components of essential oil were found, 41 of which were detected. These plants relate to linalool (59%) and methylchavicol (10.7%) chemotypes. These results can be useful for further investigation in pharmacology, plant biochemistry, food production, and breeding work.

### Conflict of Interest

The authors declare no conflict of interest.

### Ethical Statement

This article does not contain any studies that would require an ethical statement.

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