Yield, Composition and Chemotypes of Essential Oils from Origanum vulgare L. Aerial Parts Cultivated in Different Euro-Pean Countries

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Article

Yield, Composition and Chemotypes of Essential Oils from *Origanum vulgare* L. Aerial Parts Cultivated in Different European Countries

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Abstract: *Origanum vulgare* L. (Lamiaceae) is widely used in the food, pharmaceutical, perfumery and cosmetic industries for the needs for which this raw material is cultivated. *O. vulgare* is a polymorphic species with subspecies, which differ in the structure of their reproductive organs, the location of essential oil (EO) trichomes, and the composition of EO. Therefore, it is very important to identify the most valuable chemotypes of the species and cultivate them more widely. The aim of the research was to study the chemical composition of *O. vulgare* EO from aerial parts (n=17) of cultivated plants (leaves, leaves and flowers, flowering tops) from different European countries (n=5), to determine the dynamics of EO yield and its components accumulation depending on vegetation phases, and to establish chemotypes of *O. vulgare*, which are the most promising for cultivation. EOs from the raw materials were obtained by hydrodistillation according to the European Pharmacopoeia method; their analysis was carried out by GC-MS. 17 studied samples of *O. vulgare* aerial parts from Estonia, Turkey, Scotland, Moldova and Italy contained 1.9 - 11.0 mL/kg of EO. The highest yields of the EO have been found in the samples from Moldova (11.0 mL/kg) and Italy (9.3 mL/kg). In total, 89 substances were identified in the studied EOs, and the ratios of terpene groups and correlations between the content of individual components were established. The highest content of EO was noted in the phase of full flowering (5.1 mL/kg), and in the phase of the end of blooming, the EO oil content slightly decreased (4.8 mL/kg). The six chemotypes of *O. vulgare* rich in 1) caryophyllene oxide; 2) sabinene; 3) caryophyllene oxide - *(E)*-β-caryophyllene; 4) *(E)*-β-caryophyllene; 5) carvacrol, and 6) thymol - carvacrol were found. In terms of quantitative EO content of *O. vulgare*, none of the studied samples (n=17) and in terms of total carvacrol and thymol, most of the samples (n=15) did not meet the minimum standards of the European Pharmacopoeia. When cultivating *O. vulgare* for the pharmaceutical industry, it is necessary to proceed from plant propagation material rich in EO and chemotypes rich in carvacrol and thymol.

Keywords: *Origanum vulgare*; essential oil; terpenoids; chemotype; cultivation

1. Introduction

The genus *Origanum* belongs to the family Lamiaceae and includes 66 species. The name "oregano" comes from the Greek words (*oreos* - mountains/hills and *ganeos* - decorations/joys/beauty), which translates as "mountain decoration" [1]. More than half of the *Origanum* species grow wild in Turkey, so the country accounts for more than 60% of the world's oregano trade and exports the plant as a spice and herbal tea [2].

O. vulgare is the most common and widely used species. Its native range is Macaronesia, Europe, from the Middle East to Central China (Figure 1) [3,4]. *O. vulgare* is a polymorphic species, distinguished by subspecies *Origanum vulgare* subsp. *glandulosum* (Desf.) Ietsw., *Origanum vulgare* subsp. *gracile* (K.Koch) Ietsw. *Origanum vulgare* subsp. *hirtum* (Link) A.Terracc., *Origanum vulgare*

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subsp. *virens* (Hoffmanns. & Link) Bonnier & Layens, *Origanum vulgare* subsp. *viridulum* (Martrin-Donos) Nyman, *Origanum vulgare* subsp. *vulgare* [3]. The subspecies differ by the structure of their reproductive organs, location of the essential oil (EO) trichomes, and composition of the respective EO [5]. Thus, it should be taken into account in this plant cultivation.

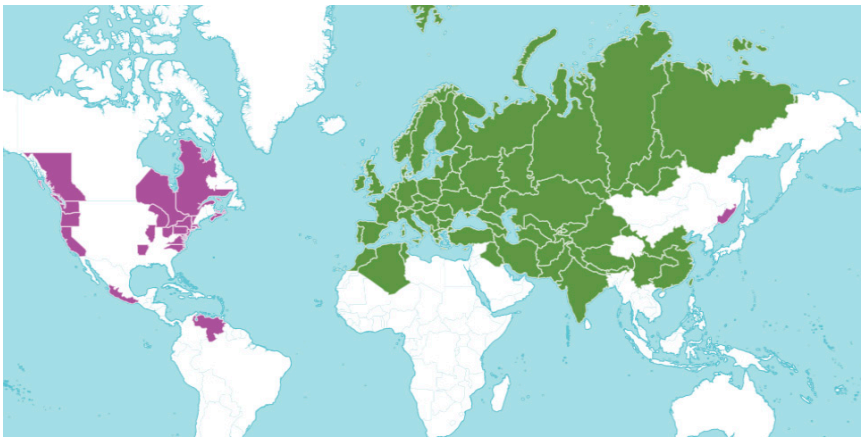

Figure 1. Natural habitats and introduction of Origanum vulgare [3]: - native; - introduced.

Among the Baltic countries, *O. vulgare* is the most widespread in the western and northern parts of Estonia [6]. In Latvia, due to intensive use, the species has significantly reduced its distribution area, and in Lithuania, it is listed as an endangered species. Oregano is more often collected from the wild, resulting in heterogeneous crops. The natural variability of *O. vulgare* will provide a wide range of biotypes for selection, subsequent breeding programs and rearing [7]. Intensive harvesting of *Origanum* from wild populations has led to overexploitation of genetic resources, so many countries have begun cultivating oregano [4].

O. vulgare is leading in the food, pharmaceutical, perfume and cosmetic industries [4,8]. *O. vulgare* is widely used in cooking to improve the taste and aroma of food products (meat, sausages, canned food, sauces and soups, salads), and in alcoholic beverages as a preservative. Its EOs exhibit antioxidant and antibacterial properties; therefore, they are used in the meat, baking and cheese industries as a valuable preservative for food preservation [9–12]. Oregano EO is used as one of the major dietary supplements in the European Union to improve health [13]. Micro- and nanoencapsulation systems are currently being developed that allow the use of oregano EO in biotechnological and biomedical applications, increasing its stability in aqueous environments, thereby improving its bioavailability and reducing its toxic effects, as well as providing controlled release and masking strong aroma [14,15].

The medicinal properties of *O. vulgare* have long been known. The main effect of *Origanum* preparations is to increase the outflow of mucus from the bronchi, so a water infusion is used for coughs, bronchitis and colds [16]. They also relieve inflammation of the mucous membrane of the mouth and throat, stimulate appetite and regulate digestion, reduce spastic pain in the gastrointestinal tract, and improve bile secretion [2]. A water infusion of *Origanum* is indicated for lethargy and inflammation of the intestines, gastritis with low acidity, constipation, and flatulence [17]. Traditionally, the dried herb, leaves, and EO of oregano are used to treat various respiratory conditions, rheumatoid arthritis, gastrointestinal disorders, and urinary tract infections [18]. Antioxidant, antimicrobial, antibacterial, anti-inflammatory, hypotensive and calming effects are exerted by phenolic compounds, especially flavonoids (naringenin, rutin, luteolin, etc.), caffeic acid derivatives (chlorogenic and rosmarinic acids), as well as EO components [19–22]. According to various authors, the main components of *O. vulgare* EO are γ-muurolene, linalool, carvacrol [23] and thymol [24]. *p*-Cymene, spathulenol, γ-terpinene, β-fenchyl alcohol, caryophyllene, germacrene D and δ-terpineol were also identified [25]. Such terpenes as α-terpineol, β-caryophyllene, and γ-terpinene induce analgesia in a murine model of neuropathic pain [26]. The dominance of carvacrol/thymol in the EO of *O. vulgare* (especially subspecies *hirtum*) determines high antimicrobial

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activity [27,28]. Although there is evidence that *O. vulgare* subsp. *vulgare*, which contained small amounts of these phenolic monoterpenes, accumulated sabinene to a greater extent and exhibited significant antimicrobial activity [29]. The studied oregano EO revealed an antibacterial effect on phytopathogenic bacteria. Thus, *Pseudomonas syringae* pv. *Phaseolicola* was completely inhibited by all doses of oregano EO tested. *P. savastanoi* and *Xanthomonas campestris* were only inhibited at the highest dose tested (10,000 ppm) [30]. In the pharmaceutical industry, herbal medicines are made from EOs, and the pure phenolic compound thymol is also isolated. Thanks to its thymol content, EO is used to relieve toothache. In addition, EO is used in the perfumery and cosmetics industry, as well as in the production of cologne and soap [2,31].

In many earlier studies [4–7,9,11,12,14,15,18–20,22–25,28,30–33], the focus has been on EO composition and chemotypes rather than on the quantitative content of EO, which is of significant importance in the cultivation and practical use of the drug. The accumulation of EO and its composition in plants is influenced by climatic conditions (place of growth, temperature fluctuations, soil composition, precipitation, light, etc.), geographical location, as well as the chemotype of the plant, phenophase of development, and plant organ [32–35]. The EO concentrations vary with geographic location and other factors such as climate, soil, and altitude [32,36]. In our previous study [6], we analyzed the yield of EO from 7 samples (5 from Estonia) of *O. vulgare*. The results obtained were remarkable (1.7-3.6 mL/kg), as none of them met the European Pharmacopoeia standard (not less than 25 mL/kg) [37]. Thus, while cultivating *O. vulgare*, these factors should also be considered. The current study helps to draw attention to an important aspect of the cultivation of *O. vulgare*.

The research aimed to study the content and chemical composition of *O. vulgare* EOs from aerial parts (leaves, leaves and flowers, flowering tops) of cultivated plants from different European countries, to determine the dynamics of EO and its components accumulation depending on vegetation phases, and to establish chemotypes of *O. vulgare*, which are the most promising for cultivation.

2. Materials and Methods

Data on the collection of the plant's raw materials and places of origin are given in Table 1. The cultivated samples 1–4 were collected in the municipality of Kehtna vald (Estonia) at different phases from the same plants during one growing season. The cultivated samples 5–9 were collected in different municipalities of Estonia during the mass flowering of plants; the samples 10–17 were commercial ones purchased from pharmaceutical farms or pharmacies in different countries. *O. vulgare* raw materials were collected at the flowering stage (early August) in the morning, after the dew had dried. To dry, the plants were tied with string and hung in a room with good air circulation. The drying temperature of the collected preparations did not exceed 30-40 °C. Leaves and flowers were separated from dried plants. Commercial samples were purchased from herbal farms or pharmacies.

Table 1. Characteristics of the studied samples of *O. vulgare.*

Sample	Type of raw material	Area of cultivation	Method of collection
1	Leaves (vegetative phase)	Kehtna municipality, Estonia	Collected
2	Leaves and flower buds (budding phase)	Kehtna municipality, Estonia	Collected
3	Leaves and blooming flowers (flowering phase)	Kehtna municipality, Estonia	Collected
4	Leaves and flowers (end of flowering phase)	Kehtna municipality, Estonia	Collected
5	Leaves and flowers	Varbla municipality, Estonia	Collected
6	Leaves and flowers	Rapla municipality, Estonia	Collected
7	Leaves and flowers	Sangaste vald, Estonia	Collected
8	Leaves and flowers	Märjamaa municipality, Estonia	Collected
9	Leaves and flowers	Padise municipality, Estonia	Collected

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10	Leaves and flowers	Energia talu, Estonia	Commercial sample from herb farm
11	Leaves and flowers	Vadi firma, Estonia	Commercial sample from herb farm
12	Leaves and flowers	Kesklinna Pharmacy, Tartu, Estonia	Commercial sample from pharmacy
13	Leaves and flowers	Kubja herb farm, Estonia	Commercial sample from herb farm
14	Leaves and flowers	Turkey	Commercial sample from pharmacy
15	Leaves and flowers	Scotland	Commercial sample from pharmacy
16	Leaves and flowers	Moldova	Commercial sample from pharmacy
17	Leaves and flowers	Italy	Commercial sample from pharmacy

Taxonomic identification of the plants was carried out by Prof. A. Raal. The herbariums are stored at the Institute of Pharmacy, University of Tartu (Tartu, Estonia). The EOs hydrodistilled from the dried herbs of *O. vulgare* using the method described in the European Pharmacopoeia [37]. The air-dried plant materials (30 g) with 400 mL of purified water were hydrodistilled in a 1000 mL round-bottom flask for 2 hours (2-3 mL/min). Hexane (0.5 mL) was added to a graduated tube to remove the distilled oil.

The gas chromatographic determination was run on Chrom-5 and Varian CP-3800 (FID) instruments using two fused silica capillary columns (50 m x 0.2 mm) with bonded stationary phases OV-101 (film thickness 0.5 μm) and PEG 20M (film thickness 0.25 μm). Carrier gas was helium with a split ratio of about 1:150, a flow rate of about 1.3 mL/min for OV-101 and 1.5 mL/min for PEG 20M. The oven temperature was programmed from 50°-250°C (OV-101) and from 70°- 250°C (PEG 20M) at a rate of 2°C/min. The injector temperature was about 160°C. The mass spectra of the compounds were recorded at 70 eV on Varian Saturn 3 GC/MS instrument, the mass range 30-350 amu. The fused silica capillary column with chemically bonded phase used in the GC/MS analysis was OV 1701 (50 m x 0.2 mm). The oven temperature program was 5 min at 40 °C, then from 40°- 270 °C at 2 °C/min, then 3 min to 270 °C. The injector temperature was 160 °C. The identification of the oil components was carried out by comparison of retention indices and mass spectra with those of authentic samples and literature data. Components were quantified as FID area percentages of total oil using the OV-101 column without response factors [35,38].

3. Results

Totally 89 components were identified in the studied samples of EOs (Appendix A, Table A), which were represented by aliphatic, aromatic, monocyclic, bicyclic monoterpenoids; aliphatic, aromatic, monocyclic, bicyclic, tricyclic sesquiterpenoids; and aliphatic bicarbonates (Table 2, Figure 2). In various Estonian samples the content of EOs ranged from 1.9 to 7.9 mL/kg. The commercial samples 10, 11 and 13 from Estonia contained minor amounts of EO (1.9 mL/kg, 1.3 mL/kg and 1.1 mL/kg, respectively). The commercial samples 16 from Moldova and 17 from Italy had the highest amount of EO (11.0 mL/kg and 9.3 mL/kg, respectively). The sample 15 from Scotland contained 4.3 mL/kg of EO, and from Turkey 1.0 mL/kg. Thus, none of the *O. vulgaris* samples (n=17) from different countries met the European Pharmacopoeia minimum requirement of 25 ml/kg in terms of EO content [37].

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Table 2. Analysis of component groups in the EOs of *O. vulgare*.

Groups of components (number of substances)	Average content (values of component groups, %)
Monoterpenoids	
Aliphatic (8)	10.3 (0.7-28.5)
Aromatic (4)	15.3 (1.1-73.5)
Monocyclic (11)	11.4 (1.3-57.7)
Bicyclic (16)	11.69 (0.6-24.6)
Total (39)	**48.57**
Sesquiterpenoids	
Aliphatic (3)	0.8 (0-6.8)
Aromatic 2	0.6 (0-1.9)
Monocyclic 5	2.5 (0-13)
Bicyclic 29	36.3 (2.7-68.9)
Tricyclic 2	0.3 (0-0.5)
Total (41)	**40.48**
Other substances not classified as mono- and sesquiterpenoids	
Aliphatic hydrocarbons (9)	2.4 (0-5.3)

As a result of the analysis of correlations between groups of substances, a positive stable correlation was established between the groups of bicyclic sesquiterpenes and tricyclic sesquiterpenes (r = 0.75), monocyclic sesquiterpenes and bicyclic sesquiterpenes (r = 0.66), weaker between bicyclic sesquiterpenes and aliphatic sesquiterpenes (r =0.55). There is an inverse correlation between the groups of aromatic monoterpenes to bicyclic monoterpenes (r=-0.56) and monocyclic monoterpenes to bicyclic sesquiterpenes (r=-0.50).

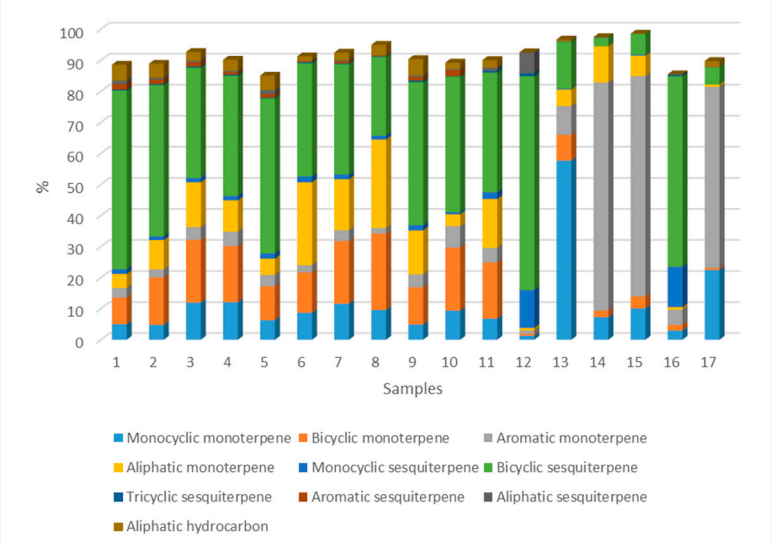

Figure 2. Average content of component groups in the EOs of *O. vulgare*.

p-Cymene, thymol, carvacrol, and 2,6-dimethyl-*p*-cymene have been identified among the aromatic monoterpenoids. According to the European Pharmacopoeia monography "Origanum herba", the minimum content of EO should be 25 mL/kg, and the sum of carvacrol and thymol should be a minimum of 60% in the EO [37]. Thymol was contained in all the samples, and carvacrol was only in thirteen (Table 3). Of all the samples examined (n=17), only two had a total content of carvacrol and thymol above 60%. Thus, most of the examined samples do not meet the corresponding requirement of the European Pharmacopoeia.

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Table 3. Content of thymol and carvacrol in the *O. vulgare* EOs.

Compound	Sample *													
	3	5	6	7	8	9	10	11	12	13	14	15	16	17
Thymol, %	0.3	0.5	0.2	0.7	0.3	0.6	1.6	1	0.5	1.2	1.9	2.1	0.8	26
Carvacrol, %	0.2	0.5	0.1	0.1	nd	0.1	0.8	1.3	0.5	7.4	68.5	58.1	2.9	22.6
The sum of substances, %	0.5	1	0.3	0.8	0.3	0.7	2.4	1.4	1	8.6	70.4	60.2	3.7	48.6

Note * - samples collected during the flowering phase were used for analysis; "nd" - not detected.

A selection of components whose concentration in at least two samples exceeded 2% were made. Totally 20 components out of the 89 identified ones were included. In Table 4 these components, their belonging to the groups by structural characteristics, as well as the average content in the studied EO samples are presented.

Table 4. Average content of components with a concentration of more than 2% in the studied samples of *O. vulgare.*

Compound	Group/structural characteristics	Number of samples	Average content
Sabinene	Bicyclic monoterpene	11	5.5
1-Okten-3-ol	Aliphatic hydrocarbon	8	0.9
β-Myrcene	Aliphatic monoterpene	11	1.7
p-Cymene	Aromatic monoterpene	14	3.2
1,8- Cineole	Bicyclic monoterpene	13	2.4
(Z)-β-Ocimene	Aliphatic monoterpene	11	3.9
(E)-β- Ocimene	Aliphatic monoterpene	10	2.3
γ-Terpinen	Monocyclic monoterpene	12	1.8
Linalool	Aliphatic monoterpene	13	2.1
Terpinene-4-ol	Monocyclic monoterpene	13	2.0
α-Terpineol	Monocyclic monoterpene	14	1.1
Carvone methyl ester	Monocyclic monoterpene	4	4.5
Carvacrol	Aromatic monoterpene	13	12.5
(E)-β-Caryophyllene	Bicyclic sesquiterpene	14	6.4
Germacrene D	Bicyclic sesquiterpene	12	4.2
Spathulenol	Bicyclic sesquiterpene	13	1.9
Caryophyllene oxide	Bicyclic sesquiterpene	14	0.8
Humulene oxide	Monocyclic sesquiterpene	10	0.8
Caryophyllene epoxide	Bicyclic sesquiterpene	7	0.8
α-Eudesmol	Bicyclic sesquiterpene	7	1.2

The dynamics of EO accumulation in the plant were also studied. Samples 1-4 were collected from one site in one area in Kehtna Vald, Estonia, at different phases of plant growth. The content of EO in the samples varied from 1.4 to 5.1 mL/kg (Table 5). During the leaf formation phase, the EO

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accumulated in the smallest amount (1.0 mL/kg). During the budding phase, the oil content increased slightly (1.9 mL/kg). The highest EO content was observed in the phase of the full flowering of oregano (5.1 mL/kg), and at the end of the flowering phase, the oil content decreased slightly (4.8 mL/kg).

Table 5. Dynamics of essential oil accumulation in *O. vulgare* herb.

Sample	Plant phenophase	Essential oil content, mL/kg	Total content of identified components in EO, %	Thymol in EO, %	Carvacrol in EO, %
1	Vegetation	1.0	88.6	0.6	0.9
2	Budding	1.9	88.9	0.5	0.2
3	Mass flowering	5.1	92.7	0.8	0.1
4	End of flowering	4.8	90.2	0.3	0.2

The change in the content of the main components in EO during the growing season of *O. vulgare* is presented in Table 6.

Table 6. Content of main components in oregano EO in the samples collected during the growing season.

Compound	Group	Content in EO (%)			
		Vegetation	Budding	Mass flowering	End of flowering
Sabinene	Bicyclic monoterpene	2.8	8.6	10	5.8
(E)-Sabinene hydrate	Bicyclic monoterpene	0.3	0.3	0.2	2
1,8- Cineole	Bicyclic monoterpene	3	3.3	5.8	5.3
p-Cymene	Aromatic monoterpene	1.6	1.9	3.6	3.7
(Z)-β-Ocimene	Aliphatic monoterpene	0.6	4.2	4.6	2.6
(E)-β- Ocimene	Aliphatic monoterpene	0.1	1.3	3.5	1.9
Linalool	Aliphatic monoterpene	2.1	1.5	4.1	4.2
Terpinene-4-ol	Monocyclic monoterpene	1.3	1.1	5.8	5.6
α-Terpineol	Monocyclic monoterpene	1.3	1.1	3	3.5
(E)-β-Caryophyllene	Bicyclic sesquiterpene	4	5.8	9	6.2
Germacrene D	Bicyclic sesquiterpene	2.4	2.1	4.6	2.4
Spathulenol	Bicyclic sesquiterpene	12.1	8.7	4.3	3.1
Caryophyllene oxide	Bicyclic sesquiterpene	17.3	16.7	4.3	11.9
Caryophyllene epoxide	Bicyclic sesquiterpene	2.6	1.9	1	2
α-Eudesmol	Bicyclic sesquiterpene	3.6	2	3.1	1.7
δ- Cadinol	Bicyclic sesquiterpene	2.3	1.4	0.5	1.3
Eudesma-4(15),7-dien-1-β-ol	Bicyclic sesquiterpene	3.6	1.8	0.6	0.8

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Total amount, %	61	63.7	68	64

Chemotypes

Analysis of samples 3-17, collected during the flowering phase, made it possible to identify the following chemotypes of *O. vulgare* (Figure 3).

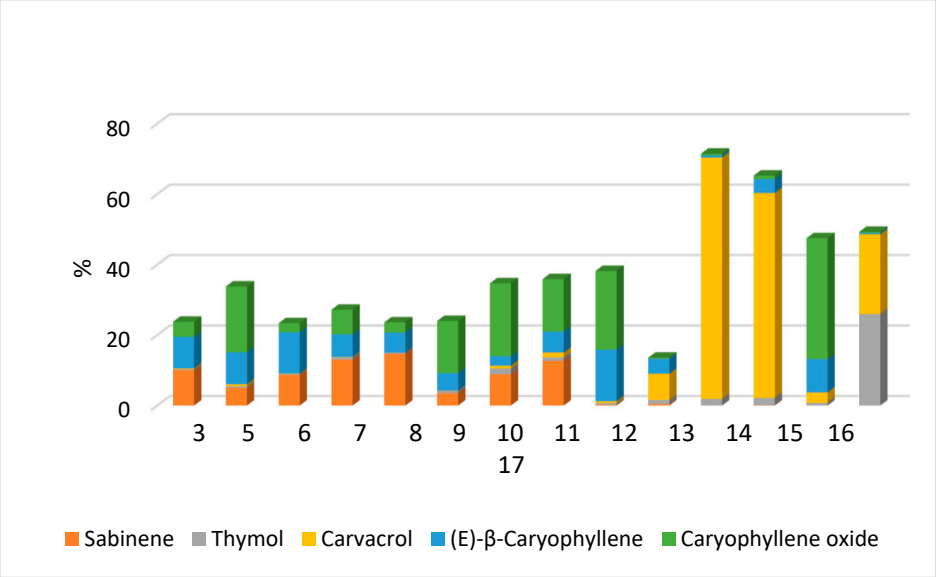

Figure 3. Chemotypes of *O. vulgare.*

Our studies identified the following six chemotypes: caryophyllene oxide, sabinene, caryophyllene oxide and *(E)*-β-caryophyllene, *(E)*- β-caryophyllene, carvacrol, and thymol-carvacrol.

4. Discussion

The studied samples of EOs from areal parts of *O. vulgare* contained 89 identified components. According to Table 2, a total of 48.6% monoterpenoids accumulated in the EO of *O. vulgare*, most of which were represented by bicyclic (16 components) and monocyclic (11 components) ones, which in percentage terms amounted to 11.4% and 11.7%, respectively. There were only 4 aromatic monoterpenoid components, but their percentage was the highest (15.3%). Aliphatic monoterpenoids were represented by 8 substances, their content was 10.3%. The total content of sesquiterpenoids in the EO of *O. vulgare* was 40.5%, most of which are represented by bicyclic sesquiterpenoids - 29 substances (36.3%). Monocyclic (2.5%), aliphatic (0.8%), aromatic (0.6%), and tricyclic (0.3%) sesquiterpenoids accumulated in minimal quantities. Substances not related to mono- and sesquiterpenoids are represented by aliphatic hydrocarbons, their content was 2.4%.

O. vulgare is a plant with an extremely variable chemical composition of EO, which largely depends on growing conditions and genetic predisposition. Aliphatic monoterpenes are represented by β-myrcene, *(Z)*-β-ocimene, *(E)*-β- ocimene, *cis*-linalool oxide, linalool, geranial, geranyl acetate, geranyl isovalerate. β-Myrcene was detected in twelve samples. The content of β-myrcene in samples 6 and 8 was 9.3% and 5.3%, respectively; in another sample, this substance accumulated in insignificant quantities. The content of (Z)-β-ocimene was higher in samples 8, 6 and 11 (12.7%, 10.2% and 9%, respectively), and less in samples 9, 7 and 3 (7.7%, 5.8 % and 4.6%, respectively). *(E)*-β-Ocimene was identified in ten samples. In samples 7 and 8, its amount was almost the same (7.9% and 7.3%, respectively); in samples 6, it was 5.9%; and in samples 11 and 3, it was 3.9% and 3.5%, respectively. Linalool was identified in thirteen samples and was accumulated in large quantities in samples 14 and 15 (7.1% and 5.6%, respectively). Geranial was detected in three samples, geranyl acetate was only in one, and geranyl isovaleriate was found in seven samples in minimal quantities.

According to the European Pharmacopoeia monography “Origanum herba”, the sum of carvacrol and thymol should be at least 60% in the EO [39]. Thymol and carvacrol accumulated in

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minimal amounts in samples 6, 8, 3, 9, 7, 5, 12, 11, 10, 16, in significant quantities only in samples 17, 15 and 14. Only samples 14 and 15 accumulated thymol and carvacrol in total, more than 60%. Also, none of the studied samples (n=17) met the pharmacopoeial requirements regarding EO quantitative content. This may be due to the subspecies of *O. vulgare*, the area where these plants grow, and the chemotype. Therefore, when planning the cultivation of *O. vulgare* for industrial purposes, especially for the pharmaceutical industry, EO-rich plant propagation material and optimal chemotypes should be preferred.

It should be noted that *p*-cymene was identified in all the samples and accumulated in the greatest quantities in the sample 15 (10.7%), less in the samples 17 (6.2%), 10 (4.5%), in almost equal quantities in the samples 3, 9, 14 (3.6%-3.1%). Among monocyclic monoterpenoids, only α-terpineol was detected in all samples. In sample 5, its content was 2.4%, and in samples 6-9, it was less than two per cent (1.1%-1.8%). In other samples, it was in minor quantities. The limonene content in sample 13 was 3.9%, in samples 6-8 (1.8%-1.5%), and in another eight samples – in minimal quantities.

γ-Terpinene was found in twelve samples. This component accumulates in large quantities in the samples 15 (5.4%), 14 (4.3%), 17 (3.5%) and 11 (2.7%). Terpinene-4-ol was found in all the samples except sample 17. In samples 3 and 7, it accumulated in large quantities (5.8% and 4.4%, respectively) and almost equal quantities in samples 5, 8, and 10 (2. 3%-2.6%). Carvone methyl ester was found in only four samples, and in sample 13, its content was 52% and 10-17% in others.

Among the identified bicyclic monoterpenoids, sabinene and 1,8-cineole accumulated in large quantities. The sabinene content was the highest in the samples 8 (14.7%), 7 (13%), 11 (12.7%), and the lowest in the sample 3 (10%). Almost equal quantities of sabinene were contained in the samples 6 and 10 (8.5% and 8.95%, respectively). 1,8-Cineole was detected in large quantities in the sample 10 (7.4%), almost identical in the samples 3 and 8 (5.8% and 5.2%). 1,8-Cineole accumulated in smaller amounts in the samples 9 and 5 (4.7% and 3%), 7 and 11 (2.3% and 2.1%).

Sabinene synthesis occurs by deprotonation of an olefin [31]. The cyclization involves the enzyme sabinene synthase (SS), which initiates the reaction through Mg-dependent ionization of geranyl diphosphate. The bound 3R-linalyl diphosphate in the *cis* conformation isomerizes (thus overcoming the geometric barrier to cyclization of the geranyl precursor) and re-ionizes to cyclize the 4R-α-terpinyl cation. Next, a 1,2-hydride shift of the α-terpinyl cation occurs, formation of a secondary cyclopropane ring and deprotonation of the methyl group, giving (+)-sabinene. In each reaction cascade, intermediates bind to enzymes [39]. Sabinene has anti-inflammatory, antifungal and antioxidant effects. Sabinene can be used for dermatophytosis and inflammatory diseases [40].

Among the sesquiterpenoids, bicyclic ones accumulated in the greatest quantities. *(E)*-β-Caryophyllene was detected in all the samples. Large amounts of *(E)*-β-caryophyllene accumulated in samples 12 (14.7%) and 6 (11.8%), and almost equal amounts in the samples 16 and 9 (9.5% and 9.1%, respectively) and 7 and 11 (6.4% and 6%, respectively), 8 and 9 (5.7% and 5%, respectively). This substance was detected in smaller quantities in samples 15 (%) and 10 (2.7%). Only the samples 14 and 17 contained less than one percent *(E)*-β-caryophyllene. Caryophyllene oxide was also identified in all samples. It accumulated in maximum quantities in the samples 12 (33.2%), 10 (20.7%), and 5 (18.7%). The substance was contained in equal quantities in samples 11 and 7 (14.9% each), less in samples 4 (11.9%), 7 (7%) and 3 (4.3%). Previous studies have shown that oregano grown in Estonia contains *(E)*-β-caryophyllene, caryophyllene oxide and sabinene as its main components [6,7,31].

During the biosynthesis of (E)-β-caryophyllene and caryophyllene oxide, a series of enzymatic reactions occur, leading to cyclization, elimination, and the formation of subsequent structures. Various sesquiterpenoids, such as germacrene D and β-Caryophyllene, are formed from intermediates such as germacrene and humulyl cations. From the latter, β-caryophyllene oxide is obtained by cyclization and epoxylation after autoxidation and decomposition [41]. β-Caryophyllene has been found to have anti-inflammatory, antitumor and cytotoxic activities and can auto-oxidize to β-caryophyllene oxide when exposed to air. Caryophyllene oxide has many pharmacological effects: antibacterial, antifungal, anti-inflammatory, antirheumatic. In addition, it has cytotoxic, antimalarial, anticancer and mosquito-repellent effects and is also considered an immunomodulator [42]. The FDA has approved synthetic β-caryophyllene and caryophyllene oxide as a preservative, adjuvant, and

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flavoring agent in the food and cosmetic industries [43]. As an antitumor agent, caryophyllene oxide reduces the activity of genes whose products have anti-apoptotic, cellular, inflammatory and metastasis-promoting effects. Caryophyllene oxide has been found to inhibit the proliferation of many tumour cells, especially multiple myeloma, prostate and breast cancer, and liver and lung adenocarcinoma, and may therefore act as a potential anticancer compound. Due to its antiproliferative, proapoptotic, and anti-invasive properties, the suppression of NF-κB and NF-κB-regulated gene products may be mediated. Thus, caryophyllene oxide can treat myeloid leukemia and other cancers that do not respond to chemotherapy or radiation therapy [44]. These studies are carried out at the cellular level and require further animal studies.

Germacrene D was detected in twelve samples. Its content was highest in the samples 12 (13.5%) and 16 (8.7%). In samples 13, 11, and 6, the germacrene D content ranged from 6.3% to 5.7%), and in samples 7 and 3, it was the same (4.7% each). Spathulenol was accumulated in thirteen samples. This substance accumulated in large quantities in the samples 9 (5.9%) and 3 (4.3%). In samples 7, 10, and 5, the spathulenol content ranged from 3.4% to 3.1%. α-Eudesmol was detected in only eight samples. The content of α-eudesmol in samples 5 (4.4%) and 3 (3.1%) was the highest, and in samples 6, 7, 9 the component accumulated in equal amounts (2.5%). Eudesma-4(15),7-dien-1-β-ol content was higher than two per cent in the samples 8 (3%) and 9 (2.7%). Only the samples 12 and 16 contained all five identified monocyclic sesquiterpenoids. The content of α-humulene was in the samples 12 (2.5%) and 16 (1.9%), β-bisabolene 12 (5%) and 16 (3.4%), humulene oxide 12 (2.7%) and 16 (4.7%).

Among the substances whose concentration in at least two samples exceeded 2%, only (E)-β-caryophyllene was present in all the samples, the average content was 6.4%, and carvacrol was detected in 13 samples, and the average content was 12.5%. More than 2% of the components included 6 bicyclic sesquiterpenes, 4 aliphatic, 4 monocyclic, 2 bicyclic and 2 aromatic monoterpenes, 1 aliphatic hydrocarbon and 1 monocyclic sesquiterpene. According to Table 4, thymol and carvacrol were not included in the top 20 components. It is also clear from other studies that *O. vulgare* with high levels of caryophyllene, or caryophyllene oxide, has low levels of thymol and carvacrol or only one of them, and vice versa [45–47]. As a result of the analysis of the research data, a strong correlation was established for such pairs of compounds as terpinene-4-ol - α-terpineol (r = 0.81), sabinene - *(E)*-β-ocimene (r = 0.84), *(E)*-β-caryophyllene – germacrene D (r = 0.75), β-myrcene – (Z)-β-ocimene (r = 0.72). For pairs of compounds linalool – carvacrol (r = 0.69), β-myrcene – (E)-β-ocimene (r = 0.64), 1,8-cineole – α-terpineol (r = 0.63), 1,8-cineole – terpinene-4-ol (r = 0.61), sabinene –1,8-cineole (r = 0.59) the correlation was insignificant, and for the pair sabinene – terpinene-4-ol (r = 0.50) weak. Also, for pairs of substances sabinene – carvacrol (r = -0.52) and 1,8-cineole – carvacrol (r = -0.50), a weak negative correlation was noted. The literature describes the following pattern: in the case of high content of carvacrol and/or thymol and their precursors - γ-terpinene and *p*-cymene in the EO, there is little linalool, sabinene, borneol and its derivatives and is often accompanied by high amounts of sesquiterpenes [48–51]. A wealth of evidence indicates that the biological activity of an EO may depend not only on the ratio of components in which the major active compounds are present but also on the interactions between them and minor components in the oil [52].

Dynamics of EO accumulation in *O. vulgare* showed that the highest EO content was observed in the phase of full flowering (Table 5). The content of thymol and carvacrol was high during the growing season, then decreased during the budding phase. During the phase of mass flowering, the content of thymol increased, while the content of carvacrol was minimal and increased slightly at the end of flowering.

The analyses of changes in the content of the main EO components (Table 6) demonstrated that the content of sesquiterpenoids was higher than that of monoterpenoids. The total content of substances increased during the vegetative development of the plant and was maximum during the mass flowering phase. Among the presented groups of compounds, bicyclic sesquiterpene accumulated the most during all phases of the growing season. The maximum amount of these substances accumulated during the growing season of *O. vulgare* (47.9%) and budding (40.4%), and during the mass flowering phase it decreased by half (27.4%) and increased slightly at the end of flowering. The content of bicyclic monoterpene and aliphatic monoterpene gradually increased and

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reached maximum values in the phase of mass flowering of the plant (16% and 12.2%, respectively). The content of monocyclic monoterpene and aromatic monoterpene also increased during the mass flowering phase (8.8% and 3.6%, respectively) but reached a maximum at the end of the flowering phase (9.1% and 3.7%, respectively). Table 6 shows that during the growing season, the content of the components sabinene, *(Z)*-β-ocimene, *(E)*-β-ocimene, terpinene-4-ol, *(E)*-β-caryophyllene, germacrene D in the EO of *O. vulgare* clearly increased from the leaf formation phase to full flowering and decreased at the end of flowering. Changes in the chemical composition of EO in *O. vulgare* at different phases of development are also confirmed by different authors [53]. So, in *O. vulgare* subsp. *hirtum* in the spring, *p*-cymene predominated over carvacrol, and by the end of the growing season, the ratio changed. This pattern is observed within one plant, where young leaves contained more *p*-cymene than old ones. It was shown that the content of EO (γ-terpinene, *p*-cymene, thymol and carvacrol) changes during the season: in autumn, there were more phenols in plants compared to mid-summer [54,55].

Differences in the composition of EOs may be due to different geographical locations, different climate zones and different environmental variables [48,56]. Although the genetic and chemical diversity profiles of EOs have been widely studied worldwide, more efforts are needed to study the phenotypes of the genus *Origanum* [57,58]. Many studies have examined intraspecific variation in the chemical composition of EOs from *O. vulgare* species, while significant variation has also been observed within each subspecies, with marked differences in the chemical profile and possibly associated biological properties [28,52,59].

In this study, it was found that four samples (5, 9, 10, 11) from different regions of Estonia and sample 16 from Moldova contained caryophyllene oxide in larger quantities. Three oregano samples from Estonia (3, 7, 8) were assigned to the sabinene chemotype. Samples 6 and 12 from Estonia were assigned to the (E)-β-caryophyllene chemotype. This chemotype was previously described [6]. The samples 13 from Estonia, 14 from Turkey, and 15 from Scotland are assigned to the carvacrol chemotype, and 17 from Italy to the thymol chemotype. There is also evidence that the genus *Origanum* exhibits two chemotypes with different concentrations of monoterpenes, such as terpinen-4-ol, *cis*- and *trans*-sabinene hydrate, carvacrol and thymol [31,51,60]. Chemotypes of *O. vulgare* (E)-β-caryophyllene and sabinene, caryophyllene oxide, have been described in the literature [7,30,61].

The EO of different subspecies of *O. vulgare* differs significantly in chemical composition [62]. It was found that the EO fractions of cultivated and wild *O. vulgare* contained carvacrol (52.99–91.18%), β-caryophyllene (0.04–1.87%), terpinen-4-ol (0.02–0.32%), limonene (0.03–0.19%), thymoquinone (0.02–0.19%), and *(Z)*-β-ocimene (0.12–0.18%), and *p*-cymene, were absent in cultivated samples but present in wild samples [63].

It studied the chemotypes of the Baneh, Rasht, Gilan, Kaleybar and Ardabil populations of *O. vulgare* grown in Iran. The main components of the EO were carvacrol (0.3–46.8%), linalyl acetate (0.2–44.3%), *(Z)*-α-bisabolene (0.0–40.3%), *(E)*-β-caryophyllene (0.0–24.0%) and caryophyllene oxide (0.1–21.3%). According to cluster analysis and principal component analysis, populations were grouped into four main chemotypes: carvacrol chemotype, (Z)-α-bisabolene chemotype, linalyl acetate chemotype, caryophyllene oxide/germacrene chemotype D/(E)-β-caryophyllene [32,36].

Wild *O. vulgare* L. in Ukraine was assigned to the monoterpene chemotype [53]. Monoterpene hydrocarbons α-terpinene and α-terpineol together accounted for 29–33%, acyclic monoterpenes - β-myrcene - 7%, linalool - 4%, while the share of the compound p-cymene accounted for 15%. The cultivated plants in Slovakia were assigned to the carvacrol chemotype, the main ingredients of which were carvacrol and thymol (together 71%) and isopropyltoluene (4.0%).

Wild *O. vulgare* subsp. *hirtum* from Montenegro, oxygenated monoterpenes predominated (76.6%), including carvacrol (74.3%) [64]. And in *O. vulgare* subsp. *vulgare*, oxygen-containing monoterpenes (17.4 - 54.2%) and sesquiterpene hydrocarbons (32.7-59.1%) accumulated significantly. The dominant oxygen-containing monoterpenes were α-terpineol (4.8-17.8%), linalyl acetate (0.5-9.7%), linalool (3.0-8.8%), thymol (0.2-8. 3), terpinene 4-ol (1.5-8.3%), and sesquiterpene hydrocarbons - germacrene D (15.4-27.9%) and β-caryophyllene (7.7-14.6%). Populations rich in linalool and/or sesquiterpenes (e.g. β-caryophyllene) may be of interest from a practical point of view. Due to the

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pleasant aroma of these substances, linalool and/or sesquiterpene-rich chemotypes of *O. vulgare* subsp. *vulgare* can be used in the cosmetic industry. Linalool is often an essential ingredient in perfumes and household detergents. It is used in food flavours and industry; linalool exhibits analgesic, anxiolytic, sedative, anti-inflammatory, antitumor and antibacterial effects [65].

Thus, according to European Pharmacopoeia standards, it is considered valuable to cultivate the *O. vulgare* chemotype rich in thymol and carvacrol. The pharmaceutical perspective of other culturable chemotypes should also be considered.

5. Conclusions

The quantitative content and qualitative composition of 17 samples of EO from *O. vulgare* aerial parts cultivated in Estonia, Turkey, Scotland, Moldova and Italy were determined. The EO content was in the range of 1.9-11.0 mL/kg. The highest content of EO was noted in the phase of full flowering (5.1 mL/kg), and in the phase of the end of blooming, the EO content slightly decreased (4.8 mL/kg).

In total, 89 substances were identified in the studied EOs, which are represented by monocyclic, bicyclic, aromatic and aliphatic monoterpenes, monocyclic, bicyclic, tricyclic, aromatic and aliphatic sesquiterpenes, and aliphatic hydrocarbons. Among all the substances, only *(E)*-β-caryophyllene (6.37% average content) was present in all the samples, and carvacrol (12.5% average content) was detected in 13 samples. During the growing season, the content of such components as sabinene, *(Z)*-β-ocimene, (E)-β-ocimene, terpinene-4-ol, *(E)*-β-caryophyllene, germacrene D in the EOs increased from the leaf formation phase to full bloom. Some stable correlations between the content of substance groups and individual compounds were established. Our studies identified the following six chemotypes: 1) caryophyllene oxide; 2) sabinene; 3) caryophyllene oxide - *(E)*-β-caryophyllene; 4) *(E)*-β-caryophyllene; 5) carvacrol, and 6) thymol - carvacrol.

In terms of the quantitative content of *O. vulgare* EO, none of the studied samples (n=17) met the requirements of the European Pharmacopoeia; in terms of the sum of carvacrol and thymol, only two samples met the minimum requirement. According to European Pharmacopoeia standards, it is considered valuable to cultivate the *O. vulgare* chemotype rich in EO, as well as in thymol and carvacrol. The pharmaceutical perspective of other culturable chemotypes should also be considered.

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Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A. Component composition of essential oils of *O. vulgare*.

№	Compound	Group	RI		Concentration, %																
			SPB-5	SW-10	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
The content of EO, mL/kg					1.03	1.9	5.1	4.8	2.6	6.1	6.0	7.9	4.5	1.9	1.3	1.6	1.1	1.0	4.3	11.0	9.3
1.	α-Thujene	Bicyclic monoterpene	924	1024	nd	0.2	0.2	0.1	0.1	0.4	0.2	0.4	0.1	0.3	0.4	nd	nd	0.4	0.2	nd	0.1
2.	α-Pinene	Bicyclic monoterpene	927	1021	0.1	0.4	0.6	0.2	0.2	0.6	0.5	0.6	0.4	0.5	0.5	nd	0.5	0.5	0.3	nd	0.3
3.	Camfene	Bicyclic monoterpene	940	1062	nd	nd	nd	nd	0.1	nd	nd	nd	0.1	0.1	nd	nd	nd	0.2	0.1	nd	0.3
4.	Sabinene	Bicyclic monoterpene	966	1120	2.8	8.6	10.0	5.8	5.0	8.7	13.0	14.7	3.5	8.9	12.7	0.2	0.4	nd	0.1	nd	nd
5.	β-Pinene	Bicyclic monoterpene	968	1107	0.4	0.7	0.8	0.6	0.5	0.9	0.6	0.8	0.7	0.9	0.6		3.7	0.1	0.3	nd	nd
6.	1-Okten-3-ol	Aliphatic hydrocarbon	980	1448	1.4	1.7	1.4	2.0	1.2	0.3	1.3	2.1	3.3	0.9	2.1	nd	nd	nd	nd	nd	nd
7.	2-Octanone	Aliphatic hydrocarbon	984	1254	0.6	0.6	0.4	0.4	0.3	0.1	0.2	0.3	nd	0.5	0.2	nd	nd	nd	nd	nd	nd
8.	β-Myrcene	Aliphatic monoterpene	987	1160	0.9	1.7	1.4	0.8	0.9	9.3	1.2	5.3	1.1	0.4	1.4	nd	1.4	1.0	0.7	nd	0.3
9.	3-Oktanol	Aliphatic hydrocarbon	994	1380	0.5	0.3	0.1	0.4	0.2	0.2	0.1	0.3	0.4	0.3	0.2	nd	nd	nd	nd	nd	nd
10.	α- Terpinene	Monocyclic monoterpene	1012	1172	nd	0.1	0.4	0.1	nd	0.5	0.7	0.8	0.1	0.8	0.7	nd	nd	1.0	0.9	nd	0.4
11.	p-Cymene	Aromatic monoterpene	1019	1270	1.6	1.9	3.6	3.7	2.7	2.0	2.7	1.5	3.5	4.5	2.4	0.1	0.1	3.1	10.7	1.2	6.2
12.	Limonene	Monocyclic monoterpene	1022	1200	0.5	0.9	0.7	1.1	0.7	1.8	1.5	1.5	0.6	0.9	0.6	nd	3.9	0.3	0.4	nd	0.1

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13.	1,8- Cineole	Bicyclic monoterpene	1025	1206	3.0	3.3	5.8	5.3	3.0	1.2	2.3	5.2	4.7	7.4	2.1	0.2	1.4	0.1	0.3	0.3	nd
14.	*(Z)*-β-Ocimene	Aliphatic monoterpene	1034	1234	0.6	4.2	4.6	2.6	1.2	10.2	5.8	12.7	7.7	1.5	9.0	0.3	0.9	nd	0.1	nd	nd
15.	*(E)*-β- Ocimene	Aliphatic monoterpene	1044	1251	0.1	1.3	3.5	1.9	0.3	5.9	7.9	7.3	2.3	0.7	3.9	0.2	0.3	nd	nd	nd	nd
16.	γ-Terpinen	Monocyclic monoterpene	1052	1241	0.1	0.1	1.2	0.5	0.1	1.7	1.8	1.6	0.2	2.4	2.7	0.1	nd	4.3	5.4	nd	3.5
17.	*(Z)*-Sabinene hydrate	Bicyclic monoterpene	1063	1460	0.4	0.6	0.9	1.6	0.2	0.2	0.4	0.5	0.6	0.5	0.4	nd	nd	0.1	nd	nd	nd
18.	*(Z)*-Linalool oxide	Aliphatic monoterpene	1066	1425	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.1	0.2	nd	nd	0.1	0.4
19.	Terpinolene	Monocyclic monoterpene	1085	1274	0.1	0.1	0.4	0.3	0.1	0.3	0.4	0.4	0.2	0.5	0.3	nd	0.1	0.2	0.2	nd	nd
20.	(E)-Sabinene hydrate	Bicyclic monoterpene	1096	1543	0.3	0.3	0.2	2.0	0.2	0.3	1.5	1.2	0.4	0.3	0.4	nd	0.9	nd	nd	nd	nd
21.	Linalool	Aliphatic monoterpene	1100	1545	2.1	1.5	4.1	4.2	1.9	0.8	1.1	3.1	2.2	0.4	1.2	0.2	0.8	7.1	5.6	0.7	
22.	n- Nonanal	Aliphatic hydrocarbon	1102	1400	0.1	0.2	0.2	0.1	0.2	0.1	0.1	0.3	0.2	0.3	nd	nd	nd	nd	nd	nd	nd
23.	1,3,8-Menthatriene	Monocyclic monoterpene	1111	1139	0.6	0.7	nd	0.3	0.2	nd	nd	nd	0.1	0.1	nd	nd	nd	nd	nd	nd	nd
24.	β- Thujone	Bicyclic monoterpene	1118	1438	0.2	0.1	0.4	0.4	0.1	0.2	0.3	0.3	0.1	0.2	nd	nd	nd	nd	nd	nd	nd
25.	Camphor	Bicyclic monoterpene	1140	1502	0.3	0.2	0.3	0.4	0.2	0.1	0.3	0.2	0.4	0.2	nd	nd	nd	nd	0.2	nd	nd
26.	Isoborneol	Bicyclic monoterpene	1154	1663	0.2	0.2	0.1	0.4	0.1	0.1	0.2	0.3	0.1	0.3	nd	nd	0.3	nd	0.1	nd	nd
27.	Borneol	Bicyclic monoterpene	1162	1702	0.3	0.1	0.3	0.4	0.2	0.1	0.2	nd	0.2	0.2	nd	nd	nd	0.7	1.1	nd	nd
28.	Terpinene-4-ol	Monocyclic monoterpene	1172	1595	1.3	1.1	5.8	5.6	2.3	1.7	4.4	2.6	1.3	2.5	1.2	0.5	1.2	1.1	1.9	1.2	nd

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29.	Myrtenal	Bicyclic monoterpene	1186	1642	0.3	0.2	0.2	0.3	0.5	0.1	0.3	0.1	0.3	0.2	0.2	nd	1.1	nd	nd	nd	nd
30.	α-Terpineol	Monocyclic monoterpene	1189	1690	1.3	1.1	3.0	3.5	2.4	1.1	1.6	1.5	1.8	0.9	0.2	0.6	0.3	0.2	0.4	0.6	0.6
31.	Myrtenol	Bicyclic monoterpene	1192	1762	0.2	0.3	0.2	0.5	0.3	nd	0.3	0.2	0.2	0.2	nd	nd	nd	nd	nd	nd	nd
32.	n-Dekanaal	Aliphatic hydrocarbon	1209	1500	0.4	0.4	0.4	0.2	0.2	0.3	0.5	0.2	0.3	tr	nd	nd	nd	nd	nd	nd	nd
33.	Pulegone	Monocyclic monoterpene	1234	1630	0.3	0.2	0.1	0.3	0.1	0.1	0.2	0.1	0.1	0.6	nd	nd	nd	nd	nd	nd	nd
34.	Carvone	Monocyclic monoterpene	1238	1733	0.2	0.1	nd	0.1	0.1	tr	0.2	tr	0.2	0.4	0.7	0.1		0.1	0.9	1.2	7.9
35.	Carvone methyl ester	Monocyclic monoterpene	1245	1630	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.4	nd	52.0	0.1	nd	nd	10.0
36.	Geranial	Aliphatic monoterpene	1265	1720	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.2	nd	nd	3.5	0.2	nd	nd
37.	Bornyl acetate	Bicyclic monoterpene	1273	1575	0.1	0.1	0.2	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.8	0.1	nd	nd	nd	nd	nd
38.	Isobornyl acetate	Bicyclic monoterpene	1283	1820	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.1	nd	nd	1.2	1.5	nd
39.	Thymol	Aromatic monoterpene	1289	2174	0.6	0.5	0.3	0.8	0.5	0.2	0.7	0.3	0.6	1.6	1.0	0.5	1.2	1.9	2.1	0.8	26.0
40.	Carvacrol	Aromatic monoterpene	1302	2213	0.9	0.2	0.2	0.1	0.5	0.1	0.1	nd	0.1	0.8	1.3	0.5	7.4	68.5	58.1	2.9	22.6
41.	α-Cubebene	Bicyclic sesquiterpene	1336	1450	nd	nd	nd	nd	nd	nd	nd	nd	0.1	0.3	nd	nd	nd	nd	nd	nd	nd
42.	α-Ylangene	Bicyclic sesquiterpene	1367	1480	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	nd	nd	nd	nd	nd	nd	nd
43.	α-Copaene	Bicyclic sesquiterpene	1374	1478	1.4	1.0	0.6	1.8	0.9	0.5	0.3	0.3	0.7	0.6	0.7	0.1	0.1	0.2	0.1	nd	nd
44.	γ-Elemene	Monocyclic sesquiterpene	1384	1575	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.8	nd	nd	nd	1.2	nd

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45.	Geranyl acetate	Aliphatic monoterpene	1383	1758	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.7	nd	nd	nd	nd
46.	β-Bourbonene	Tricyclic sesquiterpene	1384	1519	0.3	0.3	0.3	0.3	0.2	0.3	0.2	0.2	0.5	0.1	0.2	0.5	0.1	nd	nd	0.3	nd
47.	(E)-β-Caryophyllene	Bicyclic sesquiterpene	1409	1580	4.0	5.8	9.0	6.2	9.1	11.8	6.4	5.7	5.0	2.7	6.0	14.7	4.3	0.6	4.0	9.5	0.4
48.	n-Dodecanale	Aliphatic hydrocarbon	1417	1690	0.2	0.2	0.1	0.3	0.2	0.2	0.1	0.1	0.2	nd	nd	nd	nd	nd	nd	nd	nd
49.	2,6-Dimethyl-p- cymene	Aromatic monoterpene	1419	1697	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.6	nd	nd	nd	3.5
50.	α-Humulene	Monocyclic sesquiterpene	1441	1644	0.8	0.8	1.1	1.0	1.3	1.7	1.2	1.0	1.0	0.5	0.9	2.5	0.2	nd	0.2	1.9	nd
51.	Alloaromadendrene	Bicyclic sesquiterpene	1450	1625	1.2	1.0	0.7	0.7	1.3	0.7	0.9	0.6	1.1	0.4	0.6	0.9	0.1	0.1		0.6	nd
52.	γ-Muurolene	Bicyclic sesquiterpene	1468	1688	nd	nd	0.3	nd	0.3	nd	nd	nd	nd	nd	nd	0.1	nd	nd	nd	0.2	nd
53.	Germacrene D	Bicyclic sesquiterpene	1470	1690	2.4	2.1	4.6	2.4	0.5	5.7	4.7	3.8	1.7	2.8	6.0	13.5	6.3	nd	0.1	8.7	nd
54.	ar-Curcumene	Aromatic sesquiterpene	1479	1790	0.5	0.3	1.0	0.2	0.1	0.1	0.2	nd	0.2	0.5	nd	nd	nd	nd	nd	nd	nd
55.	β- Ionone	Monocyclic monoterpene	1486	1922	0.6	0.4	0.4	0.3	0.3	1.5	0.8	1.1	0.3	0.3	nd	nd	0.2	nd	nd	nd	nd
56.	α-Muurolene	Bicyclic sesquiterpene	1491	1730	0.5	0.3	0.5	0.3	0.5	0.5	0.4	0.3	0.3	nd	0.4	0.1	1.4	0.1	0.2	0.9	nd
57.	Bicyclogermacrene	Bicyclic sesquiterpene	1500	1712	0.5	0.6	0.2	0.5	0.1	1.4	0.6	0.5	0.4	nd	1.6	3.6	0.9	0.1	nd	nd	nd
58.	α-Selinene	Bicyclic sesquiterpene	1503	1707	0.3	0.3	0.9	0.9	1.0	1.4	1.4	1.0	0.4	1.6	nd	0.3	nd	nd	nd	nd	nd
59.	γ-Cadinene	Bicyclic sesquiterpene	1504	1752	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.3	0.4	0.1	0.2	nd	0.4	3.3
60.	n- Tridecanal	Aliphatic hydrocarbon	1508	1795	0.2	0.1	nd	nd	0.4	0.1	nd	0.1	0.1	nd	nd	nd	nd	nd	nd	nd	nd

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61.	β-Bisabolene	Monocyclic sesquiterpene	1508	1736	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	5.0	nd	nd	0.1	3.4	nd
62.	δ- Cadinene	Bicyclic sesquiterpene	1516	1740	0.9	1.1	1.3	0.9	1.0	1.7	1.6	1.2	1.0	0.6	1.2	2.6	0.3	nd	0.2	0.8	1.4
63.	Cadina-1,4-dieen	Bicyclic sesquiterpene	1527	1800	0.2	0.1	0.2	tr	0.1	0.1	0.1	0.1	0.1	0.4	nd	nd	0.1	nd	nd	nd	nd
64.	α- Cadinene	Bicyclic sesquiterpene	1538	1738	nd	nd	0.3	0.6	nd	1.2	0.7	0.2	nd	nd	nd	0.4	nd	nd	nd	0.4	nd
65.	α-Calacorene	Aromatic sesquiterpene	1540	1896	1.3	1.1	0.3	0.6	1.0	0.2	0.4	0.2	0.9	1.4	nd	nd	nd	nd	nd	nd	nd
66.	Hedycariol	Monocyclic sesquiterpene	1548	2077	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.2	nd	nd	nd	1.8	nd
67.	(E)-Calamenene	Bicyclic sesquiterpene	1551	1850	0.7	0.6	0.3	0.5	0.6	0.2	0.3	0.1	0.8	0.7	0.7	nd	nd	nd	nd	nd	nd
68.	(E)-Nerolidol	Aliphatic sesquiterpene	1565	2055	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3	0.5	nd	nd	nd	0.3	nd
69.	Spathulenol	Bicyclic sesquiterpene	1568	2115	12.1	8.7	4.3	3.1	2.3	1.4	3.4	1.9	5.9	3.2	nd	0.2	0.6	0.3	1.2	1.2	0.2
70.	Caryophyllene oxide	Bicyclic sesquiterpene	1572	1960	17.3	16.7	4.3	11.9	18.7	2.6	7.0	2.9	14.8	20.7	14.9	22.3	0.3	0.5	1.0	34.3	0.3
71.	Germacrene D-4-ol	Bicyclic sesquiterpene	1583	2018	1.4	0.8	0.5	0.7	0.9	0.4	0.6	0.2	1.2	1.1	0.7	1.0	nd	nd	nd	0.9	nd
72.	Globulol	Bicyclic sesquiterpene	1588	2050	0.1	0.1	nd	0.2	0.2	nd	0.2	nd	0.3	nd	nd	nd	nd	nd	nd	nd	nd
73.	Humulene oxide	Monocyclic sesquiterpene	1592	2032	0.7	0.4	0.3	0.4	0.4	0.2	0.3	0.2	0.7	0.2	1.3	2.7	nd	nd	nd	4.7	nd
74.	Ledol	Tricyclic sesquiterpene	1594	2022	nd										0.3	0.4	nd	nd	nd	nd	nd
75.	Caryophyllene epoxide	Bicyclic sesquiterpene	1594	1990	2.6	1.9	1.0	2.0	2.2	1.0	1.2	0.9	2.4	3.0	nd	nd	nd	nd	nd	nd	nd
76.	Viridiflorol	Bicyclic sesquiterpene	1602	2074	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.7	0.2	nd	nd	0.2	nd

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77.	Geranylisovaleriate	Aliphatic monoterpene	1604	1885	0.8	0.7	0.8	0.6	0.9	0.5	0.4	0.1	0.8	0.8	nd	nd	nd	nd	nd	nd	nd
78.	Cubenol	Bicyclic sesquiterpene	1626	2055	nd	nd	nd	0.5	0.4	0.1	0.2	nd	nd	0.5	nd	nd	nd	nd	nd	nd	nd
79.	τ-Cadinol	Bicyclic sesquiterpene	1635	2167	0.7	1.8	1.7	0.8	2.1	1.5	1.4	0.8	2.1	0.4	1.1	nd	0.2	nd	nd	nd	nd
80.	Epicubenol	Bicyclic sesquiterpene	1638	2087	1.4	0.4	0.4	0.6	0.8	0.4	0.3	0.2	0.8	0.6	nd	nd	nd	nd	nd	nd	nd
81.	T-Murolol	Bicyclic sesquiterpene	1644	2193	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.3	3.3	0.3	nd	nd	1.6	nd
82.	α-Eudesmol	Bicyclic sesquiterpene	1649	2216	3.6	2.0	3.1	1.7	4.4	2.5	2.5	1.6	2.5	0.8	nd	nd	nd	nd	nd	nd	nd
83.	α- Cadinol	Bicyclic sesquiterpene	1659	2218	0.2	0.2	0.2	0.3	0.4	0.1	nd	nd	0.4	nd	2.0	4.7	nd	0.2	nd	1.7	nd
84.	δ- Cadinol	Bicyclic sesquiterpene	1664	2150	2.3	1.4	0.5	1.3	0.9	0.6	0.5	nd	1.3	1.8	1.1	nd	nd	0.4	nd	nd	nd
85.	Eudesma-4(15),7-dien-1-β-ol	Bicyclic sesquiterpene	1677	2364	3.6	1.8	0.6	0.8	1.1	0.6	0.8	3.0	2.7	1.4	nd	nd	nd	nd	nd	nd	nd
86.	n-Heptadecane	Aliphatic hydrocarbon	1700	1700	0.5	0.3	0.2	0.2	0.3	0.2	0.1	nd	0.4	0.2	nd	nd	0.3	nd	nd	nd	1.0
87.	Farnesol	Aliphatic sesquiterpene	1752	2330	1.0	0.5	0.4	0.3	0.6	nd	0.3	0.1	0.4	0.2	0.7	6.3	nd	nd	nd	nd	0.1
88.	Hexahydrofarnesyl acetone	Aliphatic sesquiterpene	1842	2069	0.1	0.1	0.1	0.1	0.7	0.1	0.1	nd	0.1	0.1	nd	nd	nd	0.2	nd	nd	nd
89.	Palmitic acid	Aliphatic hydrocarbon	1985	2930	1.2	0.7	0.1	nd	1.7	nd	0.1	nd	0.4	nd	nd	nd	0.3	0.1	nd	0.8	0.9
Unidentified compounds,%					11.4	11.1	7.3	9.8	14.9	8.7	7.5	5	9.6	10.7	9.9	7.4	3.3	2.5	1.4	13.7	10.2
Identified compounds, %					88.6	88.9	92.7	90.2	85.1	91.3	92.5	95	90.4	89.3	90.1	92.6	96.7	97.5	98.6	86.3	89.8

Note. "nd" component not detected.

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