



Determination of the compositions of *Origanum minutiflorum* over three different years

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ARTICLE INFO		Abstract
Article Type	: Research Article	Medicinal and aromatic plants are medicinally crucial because of their biological activities, including antioxidant, antibacterial, antifungal, and antimutagenic. The biological activities of the plants have been attributed to their bioactive compounds, mostly their secondary metabolites. Therefore, determining the chemical composition and revealing the conditions that change these plants' chemical composition are of great importance to researchers. In this study, the chemical composition of the essential oil of <i>Origanum minutiflorum</i> grown in Diyarbakir province of Türkiye in three years (2020, 2021, and 2022) was determined by GC-MS analysis. Consequently, some compounds, including carvacrol, gamma-terpinene, terpinene-4-ol, carvacrol methyl ether, cymene, etc., were present in all the essential oils. However, significant differences were observed in the proportions of these components in the essential oils obtained in different years. This situation was compared with the meteorological data of the region.
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1. Introduction

Medicinal and aromatic plants have been used by humans since prehistoric times. They were used in their mystical rituals to connect with supernatural beings, and then their flavoring and healing potentials were discovered. Besides, medicinal and aromatic plants play pivotal roles in human life, serving as sources of modern pharmaceuticals for treating medical conditions, as spices, and in many other applications ranging from cosmetics to textiles (Inoue & Craker, 2014). It is known that over 85% of the population in places such as the Middle East, Latin America, Africa, and Asia rely mainly on herbal medicine for their healthcare requirements. According to forecasts, the global herbal supplements market reached approximately US\$115 billion in 2020, with Europe being the largest market (Shetty & Rinaldi, 2015). After revealing the potentially harmful effects of synthetic drugs, people have been increasingly demanding plant-derived remedies day by day. As a result, investing in facilitating higher quality and yield of agricultural production has become crucial (Jamshidi-Kia *et al.*, 2017). One of the most valuable products derived from

aromatic plants is essential oils, which are obtained through various extraction and distillation methods (Veličković *et al.*, 2012; Ullah *et al.*, 2019). Essential oils are complex mixtures of various chemicals belonging to terpenes and terpenoids (e.g., limonene, menthol), phenols (e.g., thymol, eugenol), aromatic compounds (e.g., cinnamaldehyde), aliphatic alcohols (e.g., linalool, geraniol), aliphatic ketones (e.g., camphor), esters of aliphatic acids (acetates, propionates) (Miguel, 2010; Dhifi *et al.*, 2016). Typically, a few compounds comprise approximately 20-70% of the oil, while the remaining compounds are in trace amounts. The diversity and quantity of compounds are responsible for the essential oil's bioactivities, such as antibacterial, antifungal, antiviral, anti-inflammatory, sedative, and immunomodulatory effects (Dhifi *et al.*, 2016; Żukowska & Durczyńska, 2024). Since the chemical content of oils and the effectiveness of their biological activities are crucial, it is essential to investigate the factors that influence the composition of oils. The compositions of these oils can be varied because of multiple factors such as soil structure, climate, latitude, and altitude where the plant grows, the stress factors affecting

the plants, and their extraction methods (Giacometti *et al.*, 2018).

Origanum minutiflorum (Genus: *Origanum*, Family: Lamiaceae) is an endemic plant grown in Türkiye and has various valuable chemical compounds (Güllü *et al.*, 2023). In this study, we aimed to search the chemical composition and quantity of compounds of essential oil of this species grown in Diyarbakir province in three different years (2020, 2021, and 2022). The chemical compositions of the essential oils were analyzed using GC-MS analysis and compared to each other. Some meteorological parameters, which are one of the reasons for the difference in content, were examined.

2. Materials and methods

2.1. Plant materials and harvesting

Plant seeds were supplied by GAP International Agricultural Research and Training Center Directorate. Planting was done in pre-prepared 1x1 meter trial plots each year in April. The harvest was carried out in October. After removing the edge effect of fresh herb yield in each plot, the remaining plants were mowed at a soil level of 10-15 cm using garden shears. The harvested fresh herb was then dried in the shade, and the resulting dry weight was recorded. The dried herb was pounded with a stick to create a blended mixture, which was subsequently passed through 4 mm sieves to separate the stem and leaf parts. The stems were discarded, and the remaining material, considered the drug part, was used to extract essential oil.

2.2. Extraction of essential oils

The herba was subjected to hydro-distillation using the Neo-Clevenger device in the Analysis Laboratory of GAP International Agricultural Research and Training Center Directorate, Field Crops Department, Medicinal and Aromatic Plants Unit. 50 g of dry herb was transferred to a 1000 ml glass flask, and 750 ml of water was added. After the water started to boil, the distillation time was kept for 2 hours. The obtained oil was stored at +4°C for further studies.

2.3. GC Analyses of the Essential Oils

Analysis of the essential oils was performed by GC-MS (Agilent brand 7890A Model GC-FID and Agilent brand 5977B model mass spectrometer). The oil was analyzed using an HP-5MS column (30m x 0.25 mm x 0.25 µm film thickness) with helium gas at a 1.0 mL/min flow rate. The GC oven temperature was held at 50°C for 5 min, increased to 220°C at a speed rate of 5°C/min, and had constant at this temperature (250°C) for 5 min. The Mass spectrometer (EI/MS) was adjusted to 70eV ionization energy, and the injection volume was 1.0 µL. The relative percentage

amount of the components of *O. minutiflorum* essential oil given in Table 1 was calculated from the data collected in the GC-FID analysis (Akdeniz *et al.*, 2022).

2.1. Meteorological data

Meteorological data such as Monthly Total Rainfall, Monthly Average Temperature and Monthly Average Relative Humidity were obtained from the General Directorate of Meteorology of the Ministry of Environment, Urbanization and Climate Change.

3. Results and discussion

The *Origanum* genus belonging to the Lamiaceae grows abundantly across Türkiye and includes many species (Karaoglan *et al.*, 2020). Commonly referred to as "kekik", locals have traditionally used *Origanum* species to produce aromatic water and essential oils (Baser, 2002). *Origanum* species have been utilized as digestives, expectorants, stimulants, anti-diabetics, diuretics, menstrual regulators, tonics, sedatives, carminatives, analgesics, antihelminthics, and antiparasitics. They are employed for treating colds, gastrointestinal complaints, and asthma (Nakiboglu *et al.*, 2007; Loizzo *et al.*, 2009; Chishti *et al.*, 2013; Karaboduk *et al.*, 2014). It is known that this genus has antimicrobial, antioxidant, antimutagenic, antifungal, insecticidal, anticarcinogenic, antispasmodic, and antiviral activities (Chishti *et al.*, 2013; Karaboduk *et al.*, 2014; Sarikurkcu *et al.*, 2015). *Origanum* species have been reported to contain essential oils, flavonoids, phenolic compounds, and triterpenes. As the chemical compounds affect the biological activities of the plant, it is crucial to reveal the composition of the products derived from the plants (Karaoglan *et al.*, 2020).

In this study, *Origanum minutiflorum* grown in Diyarbakir province was collected before flowering period over three years (2020, 2021, and 2022), and the essential oils of the samples were obtained by hydrodistillation. After obtaining the essential oils, they were analyzed and compared to each other. The chemical compounds and their percentages were given in Table 1).

According to the GC-MS analysis results, several compounds were consistently present across all samples. The main compounds identified in these essential oils were carvacrol, gamma-terpinene, terpinen-4-ol, carvacrol methyl ether, and cymene.

Carvacrol was found in concentrations of 51.17 % in essential oil from 2020, 25.58 % in 2021, and 44.85 % in 2022. Gamma-terpinene was present at 9.53 % in 2020, 12.86 % in 2021, and 12.64 % in 2022. Terpinen-4-ol was

Table 1. Essential oil content of the *Origanum munitiflorum* collected from same area in three different years

2020			2021			2022		
RT (min)	Compounds	Area (%)	RT (min)	Compounds	Area (%)	RT (min)	Compounds	Area (%)
10,515	α -Pinene	0,69	10,515	α -Pinene	1,40	10,514	α -Pinene	0,96
10,637	α -Thujene	1,03	10,637	α -Thujene	1,81	10,637	α -Thujene	1,35
15,998	β -Myrcene	0,85	13,666	β -Pinene	1,01	13,665	β -Pinene	0,69
16,748	α -Terpinene	2,53	14,232	Sabinene	0,88	14,233	Sabinene	0,62
19,065	β -Ocimene	1,53	15,998	β -Myrcene	1,35	15,998	β -Myrcene	1,08
19,568	γ -Terpinene	9,01	16,747	α -Terpinene	5,32	16,749	α -Terpinene	3,79
20,639	Cymene	3,69	18,059	β -Phellandrene	0,61	19,064	β -Ocimene	2,33
21,11	α -Terpinolene	0,68	19,063	β -Ocimene	2,86	19,572	γ -Terpinene	12,64
28,124	trans-Sabinene hydrate	5,41	19,573	γ -Terpinene	12,86	20,637	Cymene	3,90
30,686	Linalool	0,46	20,64	Cymene	12,74	21,108	α -Terpinolene	1,11
30,964	cis-Sabinene hydrate	0,75	21,107	α -Terpinolene	1,64	28,123	trans-Sabinene hydrate	5,32
32,602	Carvacrol methyl ether	3,84	27,382	1-Octen-3-ol	0,70	30,962	cis-Sabinene hydrate	1,07
32,707	Terpinen-4-ol	8,05	31,417	cis-Sabinene hydrate	0,72	31,417	cis- <i>p</i> -Menth-2-en-1-ol	0,65
35,49	α -Terpineol	0,39	32,608	Carvacrol methyl ether	6,41	32,602	Carvacrol methyl ether	4,62
36,044	Germacrene	0,47	32,716	Terpinen-4-ol	21,64	32,708	Terpinen-4-ol	12,21
36,707	Bicyclogermacrene	0,49	33,415	1-Terpineol	0,60	35,489	α -Terpineol	0,92
37,552	cis- α -Bisabolene	1,10	35,488	α -Terpineol	1,30	36,042	Germacrene	0,51
46,691	Spathulenol	0,45	37,552	cis- α -Bisabolene	0,58	36,707	Bicyclogermacrene	0,36
47,557	Thymol	7,44	48,266	Carvacrol	25,58	37,551	cis- α -Bisabolene	1,05
48,269	Carvacrol	51,17				48,267	Carvacrol	44,85

detected at 8.05 % in 2020, 21.64 % in 2021, and 12.21 % in 2022. Carvacrol methyl ether was found at 3.84 % in 2020, 6.42 % in 2021, and 4.64 % in 2022. Cymene was present at 3.69 % in 2020, 12.74 % in 2021, and 12.64 % in 2022. The minor compounds found in all the essential oils from *Origanum munitiflorum* collected over three years were determined as follows: alpha-terpinene: 2.53 % in 2020, 5.32 % in 2021, and 3.79 % in 2022; beta-ocimene: 1.53 % in 2020, 2.86 % in 2021, and 2.33 % in 2022; cis-alpha-bisabolene: 1.10 % in 2020, 0.58 % in 2021, and 1.05 % in 2022; alpha-thujene: 1.03 % in 2020, 1.81 % in 2021, and 1.35 % in 2022; beta-myrcene: 0.85 % in 2020, 1.35 % in 2021, and 1.08 % in 2022; cis-sabinene hydrate: 0.75 % in 2020, 0.72 % in 2021, and 1.07 % in 2022; alpha-terpinolene: 0.68 % in 2020, 1.64 % in 2021, and 1.11 % in 2022; alpha-terpineol: 0.39 % in 2020, 1.30 % in 2021, and 0.92 % in 2022.

Considering the compounds found in essential oils from *Origanum munitiflorum* collected over three years, it was seen that most of the compounds (gamma-terpinene, terpinen-4-ol, carvacrol, cymene, alpha-terpinene, beta-ocimene, alpha-thujene, beta-myrcene, alpha-terpinolene, and alpha-terpineol) were present higher concentrations in 2021 compared to 2020 and 2022. Only carvacrol, a characteristic compound for *Origanum* species, was significantly higher in 2020 (51.17 %) than in 2021 (25.58 %) and slightly higher than in 2022 (44.85%). Besides, cis-alpha-bisabolene was found higher in 2020 (1.10 %) than in 2021

(0.58 %) and almost equal in 2022 (1.05 %). Cis-sabinene hydrate was almost the same in all the years.

Interestingly, thymol, a characteristic compound of *Origanum* species, was found in the plant only during the first year, with a concentration of 7.44 %. The other compounds found in the first year's product were linalool, with a concentration of 0.45 %, and spathulenol, with a concentration of 0.45 %.

The compounds found in the products of the first year (2020) and third year (2022) were trans-sabinene, germacrene, and bicyclogermacrene, and were determined in approximately as same quantities. In addition, the compounds found in the oils in the second (2021) and third year (2022) were beta-pinene and sabinene in approximately the same quantity.

According to the results, it can be concluded that the conditions affecting the plants collected in 2020 and 2022 were more similar than in 2021. Besides, the conditions were more similar in 2021 and 2022 than in 2020. The compositions of essential oils are known to depend on the factors that affect the plants from which they are extracted. As the area where the plants collected was the same, it can be concluded that the climatic factors. Besides, the presence and concentration of the compounds might vary significantly with the plant's age (Miguel, 2010; Toker *et al.*, 2017). According to the literature, the compounds revealed in essential oil of *Origanum minutiflorum* were alpha-

pinene, alpha-thujene, camphene, beta-pinene, myrcene, alpha-phellandrene, alpha-terpinene, limonene, 1,8-cineole, gamma-terpinene, cymene, terpinolene, 3-octanol, 1-octen-3-ol, sabinene hydrate, linalool, terpinen-4-ol, beta-caryophyllene, alpha-terpineol, borneol, caryophyllene oxide, spathulenol, eugenol, thymol, and carvacrol (Toker et

Table 2. Meteorological data (Monthly Total Rainfall, Monthly Average Temperature and Monthly Average Relative Humidity) of three harvest period

Monthly Total Rainfall (mm=kg/m ²)												
Year/Month	1	2	3	4	5	6	7	8	9	10	11	12
2020	77.9	57.9	189.6	111.6	74.3	0.0	2.9	0.0	0.0	0.0	54.7	30.8
2021	46.0	32.9	57.9	7.3	3.6	0.0	1.0	8.2	0.0	24.2	21.2	22.4
2022	45.4	36.9	76.9	5.4	97.4	13.2	0.0	0.0	0.7	28.0	96.4	3.3
Monthly Average Temperature (°C)												
Year/Month	1	2	3	4	5	6	7	8	9	10	11	12
2020	4.7	4.5	11.5	14.7	20.9	27.4	32.4	31.3	28.8	20.5	11.6	5.7
2021	5.2	8.3	9.5	17.2	24.7	28.6	32.7	30.9	25.6	18.8	11.9	4.4
2022	2.5	7.8	6.2	17.9	19.4	28.1	32.1	31.8	27.0	19.7	11.0	7.3
Monthly Average Relative Humidity (%)												
Year/Month	1	2	3	4	5	6	7	8	9	10	11	12
2020	77.6	79.2	75.9	73.3	54.4	30.0	22.8	20.9	23.5	30.5	65.8	83.5
2021	71.8	63.1	63.5	52.1	30.7	25.5	24.9	29.1	31.6	38.7	70.7	78.8
2022	75.0	64.5	71.9	43.2	53.7	32.9	23.0	28.0	27.6	46.1	77.9	82.1

It has been observed that in May, which is important for plant germination, there was almost no rainfall in 2021, while in other years there was at least 25 times as much rainfall. In the 8th month, which is the flowering and harvesting time, 8 kg of rain fell per square meter in 2021, while this untimely rainfall was not observed in other years.

4. Conclusions

Consequently, the results of the study exhibited that different conditions could cause different compositional essential oils. As a result, it can be said that the amount of precipitation and humidity affects the oil yield and content of the plant.

Authorship Contribution Statement

AAK: Investigation, data curation, formal analysis. **FT:** Methodology, project administration, writing – original draft preparation. **DE:** Conceptualization, supervision, writing – review & Editing.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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al., 2017). While it is observed from meteorological data that temperature differences are close to each other every year, it was observed that precipitation and humidity rates showed significant changes in some months, especially in 2021 compared to other years (Table 2).

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