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The essential oil compositions of *Rosmarinus officinalis* L. leaves growing in Mersin, Turkey

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ABSTRACT

This study reports on the chemical compositions of the essential oil of *Rosmarinus officinalis* L. (Rosemary) grown in Mersin, Turkey. The essential oil of rosemary was obtained by hydrodistillation method, and the yield of rosemary oil was found to be about 1.2 % (*v*:*w*). The hydrodistilled volatile oil was analyzed by gas chromatography and mass spectrometry techniques. Forty-five components were identified in the essential oil of *R. officinalis*, which represented 100% of the total essential oils. The oxygenated monoterpenes content possessed the highest value, 64.78% of the oil, among which eucalyptol (33.15%) and camphor (10.31%) were the most abundant components. In addition, the oil contained mainly monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, and diterpenes. The least amount of diterpenes were found in the content of the oil. Isopimara-9 (11),15-diene (0.14%) and α-springene (0.06%) were two compounds determined as diterpene compounds.

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1. Introduction

Essential oils obtained from plants are called aromatic or etheric oils, and these liquids are often rich in aroma. Essential oils are extracted from plants by dry distillation, water vapor distillation, or mechanical extraction methods. Consumers' positive perception of being environmentally friendly and natural instead of synthetic additives has increased their interest in essential oils and their applications in recent years [1].

Commonly known as rosemary, *Rosmarinus officinalis* L., belonging to the Lamiaceae family, is a pleasant smelling woody aromatic perennial shrub that grows in Mediterranean areas and is widely distributed in many parts of the world [2-5]. Forms range from upright to trailing; the upright forms can reach 1.5 m, rarely 2 m. The leaves are 10-25 × 1-2(-4) mm, dark green, rugulose, and pilose above, white tomentose beneath. In addition, there are glandular hairs on the upper and lower surfaces of the leaf. The essential oil is stored in the secretion hairs on the epidermis [6]. It is known as biberiye, pürem, akpüren, hasalban, kuşdili, and urum çiçeği in Turkish [7,8]. In Turkey, *R. officinalis* is found growing on scrub areas, hillsides, and dry rocky slopes, in pine forests and particularly in the Mediterranean region, from just above sea level to 1.000 m [9].

Rosemary leaves are commonly used for flavoring foods, also this plant has also been widely used for different ethno-botanical and medicinal purposes. In traditional medicine, rosemary has been used as a stimulant and mild analgesic, and it has been considered as one of the most effective herbs for treating headaches, inflammatory diseases, poor circulation, and physical and mental fatigue. Rosemary has also been used empirically as a choleretic and hepatoprotective agent in folk medicine [5, 10,11].

Essential oil of *R. officinalis* is important for its medicinal uses and its powerful antibacterial, antioxidant, and antiproliferative properties [12]. Likewise, the oil was characterized by its biological activities like antimicrobial [7,13], insecticidal [14,15], antioxidant [16], and anticancer properties [17].

R. officinalis essential oil is a colorless or pale-yellow liquid with the characteristic odor of the plant. Many studies on rosemary essential oil compositions have been reported.

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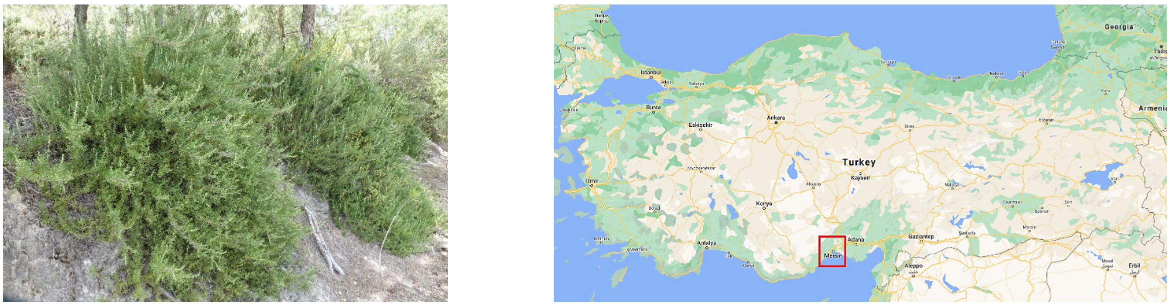

(**a**) (**b**)

Figure 1. (**a**) Habitus and (**b**) map of the distribution of *R. officinalis.*

The compositions of rosemary essential oil from different studies revealed the occurrence of the consists mostly of monoterpenes such as 1,8-cineole, α-pinene, camphor, borneol, camphene, α-terpineol, linalool, verbenane and bornyl acetate [5,18-34].

In previous studies, rosemary essential oil content and rates have varied. These variations were mostly associated with differences in the chemical composition of oils based on their distribution areas, environmental and agronomic conditions, collecting time, development stages of plants, and extraction method [22,35-38].

This study aims to determine the essential oils of *R. officinalis* wild in Mersin (Turkey) in order to compare the results with previously studies on the same plants.

2. Experimental

2.1. Chemicals

All chemical solvents and reagents used were of analytical reagent grade and purchased from Merck, Darmstadt, Germany.

2.2. Plant materials

The *R. officinalis* was collected by Dr. Riza Binzet from Mersin (Location: C5 Mersin, Mersin University Campus, damp slopes and scrub area, 14 May 2019, 36° 47' 53" N 34° 21' 47" E, 110 m, Binzet 201944 (Figure 1). The plant was identified by Dr. Riza Binzet. The voucher specimens are deposited in the Mersin University Research Herbarium (MERA), Mersin, Turkey.

2.3. Isolation of the essential oil

Fresh leaves were harvested from wild plants of *R. officinalis* from the distribution area. The collected leaves were air-dried in the shade at room temperature (25 °C) for two months. Then, the dried leaves were powdered using a grinder (Blender 8011ES Model HGB2WTS3 400 W).

In order to extract the essential oils, 50 g of the leaf powder was placed in a 1-liter round-bottomed flask with distilled deionized water (500 mL) and connected to the Clevenger apparatus. The steams in combination with the essential oils were distilled in to a graduated cylinder for 5 hours. The essential oil was separated from water, dried over anhydrous sodium sulphate, and stored prior to analysis in a sealed vial at +4 °C. They were analyzed in three weeks.

2.4. Analysis of essential oil

2.4.1. GC analysis

The GC analysis was done using an Agilent Technologies 7890B GC. The carrier gas was helium at a flow of 3 mL/min. The column temperature was kept at 50 °C for 1 min and then heated to 250 °C with a 2 °C/min rate and kept constant at 280 °C for 10 min. The split ratio was 75:1 and the injector temperature was set at 280 °C. The purity of helium gas was 99.999 %. Essential oil samples (1 µL) were injected manually.

2.4.2. GC-MS analysis

The GC-MS analysis was carried out with an Agilent 5977A GC-MSD system. The system was equipped with an automatic liquid sampler (Agilent 7693). HP-5MS 5% capillary column (coated with methyl silicone) (30 m × 250 µm × I.D., 0.25 µm) was used as the stationary phase. The temperature was programmed from 60 to 325 °C at a rate of 1°C/minute. The injector and interface temperatures were maintained at 325 and 350 °C, respectively. Mass spectra were taken at 70 eV. Mass range was from *m/z* 10 to 600.

2.5. Identification of components

The qualitative identification of different compounds of the essential oil was performed on the basis of retention indices (RI) determined with reference to a homologous series of *n*-alkanes, under identical experimental conditions, co-injection with standards. Kovats index values were calculated according to the retention times and comparison of Kovats retention indices with literature values was carried out. The mass spectra were compared with those reported in the NIST14.L and W10N14.L computer libraries and those published in literature to date [39-53]. The minimum matching factor for deconvolution in The Automatic Mass Spectral Deconvolution and Identification System (AMDIS) is generally set above 90.

3. Results and discussion

In this study, pale yellow coloured liquid essential oil of *R. officinalis* was obtained with the Clevenger apparatus with 1.20 % (*v*:*w*, volume:dry weight) yield. In many studies on rosemary, the essential oil has been reported by different researchers that it represents approximately 1-2.5% of the total weight and chemical composition of the plant [8,20,25,26,37,54-60]. The rate of essential oil obtained in this study and the rates of essential oil obtained in the above studies were found to be similar.

The oil compounds identified by GC-MS analysis of *R. officinalis* qualitative and quantitative analyses results of essential oil were showed in Table 1 along with their Kovats indices and percentage composition. The GC-MS analysis of the essential oil of *R. officinalis* is shown in Figure 2. Representative mass spectra of essential oils extracted from *R. officinalis* are also given in Figure 3. The compounds are organized in order to their elution on HP-5MS column. The volatile compounds were identificated by comparing their mass spectra with Wiley library as well as with authentic compounds.

Table 1. Chemical compositions of the essential oil extracted from *R. officinalis* as identified by GC-MS.

No	**Compounds**	**Rt** ^{1}	**KI$_{exp}$** ^{2}	**KI$_{lit}$** ^{3}	**Percentage**	**References**
1	Tricyclene	6.959	914	919	0.20	[39]
2	3-Thujene	7.136	919	923	0.23	[40]
3	α-Pinene	7.409	927	933	8.11	[41]
4	Camphene	7.785	937	952	4.22	[40]
5	Sabinene	8.592	960	973	0.10	[41]
6	β-Pinene	8.727	964	964	3.60	[42]
7	β-Myrcene	9.315	981	991	1.48	[43]
8	S-2-Carene	9.729	993	993	0.07	[75]
9	*o*-Cymene	10.343	1010	1011	1.04	[44]
10	Eucalyptol (1,8-Cineole)	10.894	1026	1030	33.15	[45]
11	γ-Terpinene	11.906	1055	1059	0.69	[41]
12	α-Terpinolene	13.122	1090	1088	0.27	[43]
13	Linalool	13.548	1102	1098	0.75	[43]
14	Camphor	14.867	1140	1143	10.31	[43]
15	Borneol	16.161	1176	1165	7.16	[43]
16	Terpinen-4-ol	16.690	1192	1179	1.95	[40]
17	α-Terpineol	17.265	1208	1207	4.92	[40]
18	Geraniol	18.904	1255	1255	0.08	[43]
19	Bornyl acetate	21.576	1331	1302	5.66	[46]
20	Thymol	21.697	1334	1308	0.18	[46]
21	Carvacrol	21.923	1341	1314	0.13	[46]
22	Methyl eugenol	24.240	1321	1401	0.49	[43]
23	Isocaryophyllene	25.114	1432	1413	7.02	[41]
24	α-Caryophyllene	25.740	1450	1454	2.23	[43]
25	γ-Muurolene	26.182	1463	1477	0.25	[43]
26	β-Bisabolene	26.915	1483	1509	0.09	[43]
27	γ-Cadinene	26.962	1485	1512	0.24	[47]
28	*trans*-Calamenene	27.039	1487	1510	0.11	[48]
29	δ-Cadinene	27.182	1491	1524	0.41	[43]
30	Caryophyllene oxide	28.419	1526	1573	2.04	[41]
31	1,2-Epoxide-humulene	29.011	1543	1593	0.30	[48]
32	Methyl jasmonate	29.488	1557	1647	0.15	[43]
33	Alloaromadendrene	29.611	1560	1478	0.22	[49]
34	Caryophylla-4(12),8(13)-dien-5α-ol	29.708	1563	1602	0.32	[50]
35	Isoaromadendrene epoxide	30.290	1580	1579	0.80	[51]
36	Longiborneol	30.383	1583	1583	0.09	[76]
37	β-Caryophyllene	30.496	1586	1594	0.12	[52]
38	β-Caryophyllene oxide	30.631	1589	1581	0.20	[43]
39	α-Bisabolol	31.072	1602	1683	0.10	[43]
40	Cadalene	33.624	1676	1674	0.07	[43]
41	Germacrene B alcohol	34.319	1695	1694	0.09	[77]
42	α-Springene	37.392	1783	1781	0.06	[78]
43	Epi-Cryptomeridiol	37.719	1793	1790	0.08	[79]
44	Isopimara-9(11),15-diene	38.696	1820	1898	0.14	[53]
45	1S4R6R-g-Himachalen-4-yl acetate	39.216	1835	1830	0.08	[80]

[1] Rt: Retention time.
[2] KI$_{Lit}$: Published Kovats retention indices.
[3] KI$_{exp}$: Kovats index determined experimentally relative to C8-C28 *n*-alkanes.

In addition, the retention indices were calculated against *n*-alkane standards as reference. This was confirmed by comparison of their retention indices with those of authentic compounds as well as with data published in the literature [39-53]. The percentage composition amounts were calculated from total ion chromatograms (TIC) by the computer.

The GC-MS analyses of the obtained oil indicated the presence of forty-five volatile compounds, which included 100% of the total oil composition. The major compound of pale-yellow oil was found to be 1,8-cineole with 33.15 %. 1,8-Cineole is a natural organic monoterpenoid, also known as eucalyptol [61]. The name "Eucalyptol" is due to the fact that the major component of Eucalyptus oil is 1,8-cineole. The content of 1,8-cineole in eucalyptus oil varies from species to species, for example, it has been determined to be in high concentrations in *Eucalyptus nicholii* [62]. However, it is component of the essential oils of many plants. Eucalyptol is often used in food, fragrances, and cosmetics because of its fresh mint-like fragrance, spicy aroma, and taste [63]. Because of these properties, like many aromatic oils, 1,8-cineole is used in traditional medicine as a cough suppressor in bronchitis. Many researchers have studied the bioactive effect of rosemary essential oil, the main component of which is 1,8-cineole [61]. In our study, the other components in essential oil are camphor (10.31%), α-pinene (8.11%), isocaryophylene (7.02%), bornyl acetate (5.66%), α-terpineol (4.92%), camphene (4.22%), β-pinene (3.60%), α-caryophylene (2.23%), caryophyllene oxide (2.04%), terpinen-4-ol (1.95%), β-myrcene (1.48 %) and *o*-cymene (1.04%).

In this study, the chemical profiles were marked by the presence of high amounts of oxygenated monoterpenes (64.78%) followed by monoterpene hydrocarbons (20.01%) (Table 2). The most abundant oxygenated monoterpene compounds identified in the oil were 1,8-cineole (33.15%) and camphor (10.31%). The sesquiterpene fraction (15.01%) was mainly composed of sesquiterpene hydrocarbons (10.54%), with isocaryophyllene (7.02%) being the main compound. Caryophyllene oxide (2.04%) was the most abundant of the six oxygenated sesquiterpenes identified. Isopimara-9 (11), 15-diene (0.14%), and α-springene (0.06%) were determined as diterpene compounds.

In recent years, the chemical content of rosemary from different Mediterranean regions were examined by many researchers [8,17,25,27,35,36,37,57,60]. The chemical contents of essential oils of rosemary samples collected from different regions of Mersin province were investigated by a few researchers [7,8,36,63-65]. Essential oil components and their relative proportions were found to be different in this region. Generally, in Mersin, 1,8-cineole compound was determined as the main component. However, the 1,8-cineole percentage rates defined in essential oils were found to be different from each other [36,63-65].

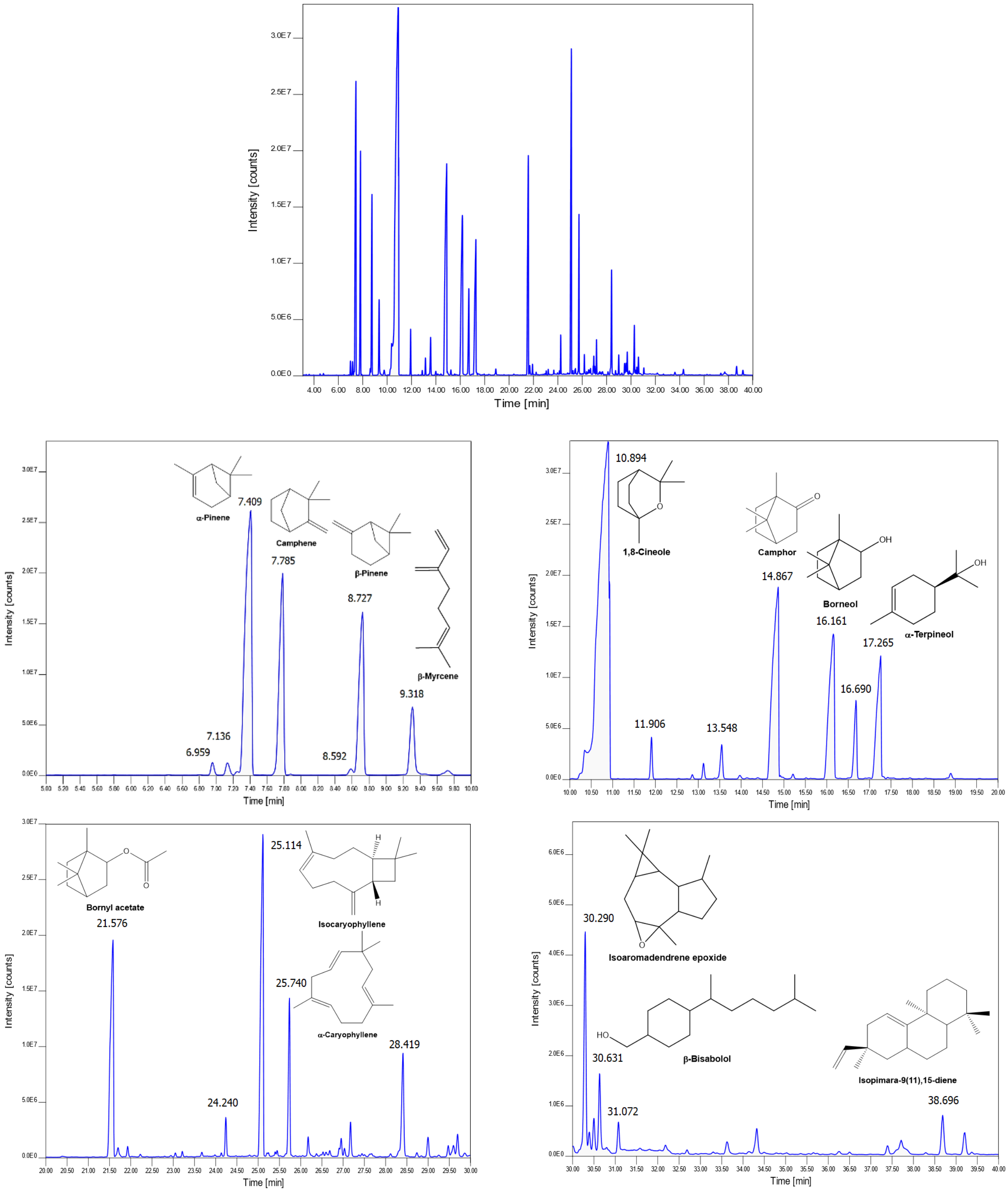

Figure 2. Profiles of GC-MS analysis for essential oil extracted from *R. officinalis*.

Celiktas *et al.* [36], the percentage of 1,8-cineole was found highest with a rate of 50.7-61.4%. Similarly, in our study, 1,8-cineole was found as the highest main component with a value of 33.15%. On the other hand, Bagci *et al.* [8] in their study, camphor was identified as the highest main component with 14.48%, while Ozcan *et al.* [7] determined *p*-cymene as the main component with 44.02%. In our study, camphor was determined as the second main component with 10.31%, while *p*-cymene was not found. In the analysis of essential oil compounds made with rosemary plants in Mersin, the 1,8-cineole, camphor, α-pinene and β-pinene compounds were defined as common compounds. According to the literature survey, the quantitative composition and the relative propotions of the oil's components are widely influenced by environmental factors, soil characteristics, growing conditions and altitude [7,8,36,63-65].

In the study conducted by Celiktas *et al.* [36], the *R. officinalis* was collected from three different regions (Mersin, Canakkale, and Izmir) at four different time (December, March, June, and September) intervals of the year. In their study, they determined the variation in the essential oil composition of rosemary seasonally and regionally.

Table 2. Main classes and subclasses of compounds of the essential oil extracted from *R. officinalis*.

Classes of compounds		Percentage
Monoterpenes		84.79
Monoterpene hydrocarbons	*20.01*	
Oxygenated monoterpenes	*64.78*	
Sesquiterpenes		15.21
Sesquiterpene hydrocarbons	*10.54*	
Oxygenated sesquiterpenes	*4.47*	
Diterpenes		0.20
Total		100.00

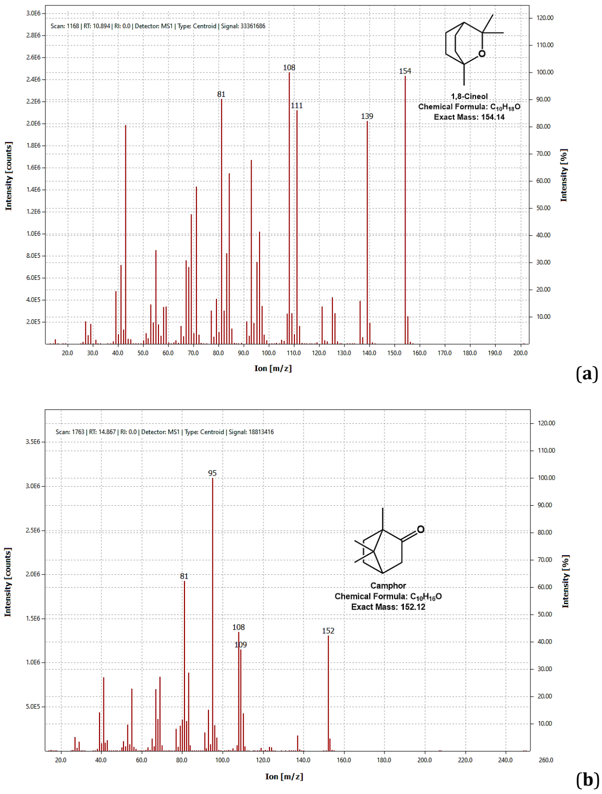

Figure 3. Mass spectra of (**a**) 1,8-Cineol (RT: 10.894) and (**b**) Camphor (RT: 14.867) in essential oils extracted from *R. officinalis.*

The seasons affected the main components amount, but it was not seasonal correlation was determined in essential oil compositions. It was determined that the components and their percentage values of essential oil of rosemary in our study differ from the components and their percentage values of essential oil of rosemary collected by Celiktas *et al.* [36] from Mersin in March-June, 2019. According to the results of both studies, no regular increase or decrease in essential oil compositions was determined seasonally. Similarly, in Yildirim [66] (2018), the seasonal variation of the essential oil compositions of the rosemary plant was examined in Kahramanmaraş and could not detect any correlation between the seasons. However, α-fenchene, γ-3-carene, dehydro-1,8-cineole, limonene, β-ocimene, 5-methyl-3-heptanone, 3-hexenol, 1-octan-3-ol, α-camppholene aldehyde, phenylacetaldehyde and humulene compounds were determined in the study by Celiktas *et al.* while these compounds were not detected in our study. This showed us that the chemical composition of rosemary oil varies according to the geographical region where it was collected. In addition, it has been determined that the main components of essential oil change in studies conducted in different regions [36,66]. Camphor was found as the main component in the analysis of essential oil components of rosemary plants, which spread in Izmir, Aydin, Antalya, Adana, and Hatay provinces (growing in Ankara ecologic conditions) by Gurbuz *et al.* [63]. According to this study, the amount of camphor in essential oil increases in cold climate conditions. In the study at Balıkesir [67], Fethiye [68], and Kahramanmaraş [66], in the essential oil compositions of rosemary were determinated limonene, sabinene, and ocimene, while they were not found in our study. Essential oil of *R. officinalis* from Tunisia [56,60,68], Pakistan [11], Lebanon [57], and Belgrade [69,70], shows a high content of 1,8-cineole, while the essential oil of *R. officinalis* from Iran [58,71], Spain [72], Italy [20,73], Morocco [59] shows low content of this molecule, and yields a high concentration of α-pinene instead; essential oil of *R. officinalis* from Brazil [54], in its turn, has a high concentration of camphor. These data indicated that the chemical composition variation due to the geographical area, edaphic features, and altitude where the plant is collected.

The essential oil composition shows phytochemical variations according to the different parts of the plant used. Yosr *et al.* [74] noticed that the essential oil of *R. officinalis* obtained from leaves had 1,8-cineole (35.8%) as the major compound, while caryophyllene (16.7%) was the main compound in stem-extracted oil. However, in the essential oil extracted from flowers, the predominant component was caryophyllene oxide (11.9%). Similarly, in our study, essential oil was obtained from the leaves of rosemary and 1,8-cineole was determined as the main component. Our findings are consistent with the results of the study conducted by Yosr *et al.* [74].

The major compound of the essential oil of rosemary obtained in this study was 1,8-cineole; this compound is widely used in the food and pharmaceutical industries. Thus, it is predicted that increasing the essential oil rate in rosemary can provide an important economic income source potential for our region.

4. Conclusion

The present study is outlined to probe the chemical compositions of the essential oil of *R. officinalis* collected from Mersin province in Turkey. The hydrodistilled volatile oil was analyzed by GC-MS technique. Forty-five volatile compounds were determined according to NIST14.L and W10N14.L and literatures. The major compound in the essential oil was found to be 1,8-cineole with 33.15 %. The other components in volatile oil are camphor (10.31%), α-pinene (8.11%), isocaryophylene (7.02%), bornyl acetate (5.66%), α-terpineol (4.92%), terpinen-4-ol (1.95%), camphene (4.22%), β-pinene (3.60%), α-caryophylene (2.23 %), caryophyllene oxide (2.04 %), β-myrcene (1.48 %) and *o*-cymene (1.04%), respectively. The results of this study and other literature results showed that there is no seasonal correlation between essential oil components and their quantities.

Disclosure statement

Conflict of interest: The authors declare that they have no conflict of interest.
Author contributions: All authors contributed equally to this work.
Ethical approval: All ethical guidelines have been adhered.
Sample availability: Samples of the compounds are available from the author.

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