THE COMPOSITION OF ESSENTIAL OIL FROM THREE ECOTYPES OF *ORIGANUM VULGARE* L. SSP. *VULGARE* CULTIVATED IN POLAND

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Abstract

The chemical composition of oregano essential oil of different origin extracted from oregano plants grown in south-eastern Poland was analysed using the gas chromatography - mass spectrometry (GC-MS) and gas chromatography - flame ionization detector (GC-FID). The dominant compound was sabinene; its percentage proportion ranged from 10.85% to 25.46%, depending on the ecotype studied. Moreover, the oregano volatile oil was characterized by a significant percentage of the following compounds: germacrene D (9.36-15.34%), *Z*-(*β*)-ocimene (9.10-16.33%), and *E*-caryophyllene (9.38-12.87%). The investigated oil did not contain phenolic compounds.

Rezumat

A fost analizată compoziţia chimică a uleiurilor esenţiale provenite de la trei specii (ecotipuri) de *Origanum*, cultivate în Polonia. Studiile au fost efectuate prin GC-MS (gaz-cromatografie cuplată cu spectrometrie de masă) şi GC-FID (gaz cromatografie cu detector de ionizare în flacără). Compusul identificat în cantitatea cea mai mare a fost sabinelolul (10,85-25,46%). Rezultatele obţinute au demonstrat de asemenea prezenţa următoarelor principii active: germacren D (9,36-15,34%), Z-(*β*)-ocimen (9,10-16,33%) şi *E*-carioflen (9,38-12,87%).

Keywords: oregano, volatile oil content, sabinene, gas chromatography - mass spectrometry (GC-MS), gas chromatography - flame ionization detector (GC-FID).

Introduction

Oregano belonging to the family *Lamiaceae* is found in natural conditions in Europe and Central Asia as well as in North America. In Poland this is a wild growing species as well as it is cultivated for seasoning, medicinal, and decorative purposes. Oregano is a durable, aromatic, abundantly and long flowering, melliferous plant. Raw material and oil obtained from this plant were known and used already in very ancient times. Oregano, which was valued as a medicine and a ritual plant in ancient Egypt and Greece, is appreciated until today as an aromatic spice and medicine used in disorders of the digestive system and in respiratory system diseases. Extracts from the oregano herb have antioxidative [1,2], anti-inflammatory [3], and antibacterial activity [4].

The therapeutic properties of *Origanum herba* are associated with the content of essential oil, sesquiterpenes, phenolic acids, including mainly rosmarinic acid, and flavonoids. Biosynthesis of secondary metabolites, in particular essential oil, even though determined genetically, is strongly affected by ontogenetic and environmental factors [5-8]. The composition of the essential oil extracted from the oregano herb is characterized by large variations, which is attributable, among others, to the great morphological and chemical diversity within the genus *Origanum vulgare* L. [9]. The dominant constituents of oregano volatile oil are as follows: sabinene, linalool, terpinen-4-ol, *β*-caryophyllene, caryophyllene oxide, germacrene D, *β*-ocimene, thymol, and carvacrol, and the proportions of the above-mentioned components depend on the form of oregano and plant growth conditions [10-13].

The present study informs about the chemical composition of oregano essential oil of different origin extracted from oregano plants grown in south-eastern Poland. Volatile oil extracted from the herb of *Origanum vulgare* L. was analysed using the gas chromatography - mass spectrometry (GC-MS) and gas chromatography - flame ionization detector (GC-FID).

Materials and Methods

Plant material

The herb of *Origanum vulgare* L. originated from experimental cultivation of Department of Vegetable Crops and Medicinal Plants, University of Life Sciences in Lublin (51º23`N, 22º56`E). The type of soil was grey-brown podzolic derived from medium loam. Climatic conditions during the vegetation period were shown in table I. The seeds of oregano plants were obtained from Botanical Garden of Maria Curie-Skłodowska University in Lublin (UMCS) where the a voucher specimen has been deposited. Oregano ecotypes were designated as: 1 (plants originated from Dobre; 51º17`N, 21º53`E), 2 (plants originated from Botanical Garden of UMCS; 51º14`N, 22º34`E) and 3 (plants originated from Kazimierz Dolny; 51º19`N, 21º58`E). The raw material harvested on the blossom stage (July 2008) was naturally dried in the shadow. The essential oil was isolated by hydrodeistillation, using a Clevenger-type glass apparatus. The oil obtained was collected in dark glass vessels, dried using dehydrated sodium sulphate and stored at below -10ºC until chromatographic determination.

Table I

Mean air temperature (ºC) and total rainfall (mm) for May-July in 2008 and the long term average

Month	Air temperature			Mean air temperature	1951-2005
	1st decade	2nd decade	3rd decade		
May	11.3	13.3	13.6	12.8	13.0
June	18.0	16.4	18.8	17.7	16.2
July	17.1	18.9	18.9	18.3	17.8
	Total rainfall			Sum of rainfall	1951-1995
May	57.1	34.7	9.8	101.6	57.7
June	0.0	19.6	6.3	25.9	65.7
July	39.6	19.3	18.2	77.1	83.5

Qualitative and Quantitative analysis, GC-MS

The GC-MS Instrument ITMS Varian 4000 GC-MC/MS (Varian, USA) was used, equipped with a CP-8410 auto-injector and a 30m x 0.25nm i.d. VF-5ms column (Varian, USA), film thickness 0.25µm; carrier gas, helium at a rate of 0.5ml/min; injector and detector temperature, 220ºC and 200ºC, respectively; split ratio, 1:100; injector volume, 1µl. A temperature gradient was applied (50º C for 1min, then incremented by 4ºC/min to 250ºC and held at this temperature for 10 min.); ionization energy, 70eV; mass range, 40-1000Da; scan time, 0.8s. The linear retention indices from temperature-programming, using definition of Van den Dool and Kratz [14], were determined for series of n-alkanes C_6-C_{40}.

GC-FID

A Varian 3800 Series (Varian, USA) instrument with a DB-5 column (J&W, USA) was used, operated under the same conditions as GC-MS; FID, 260ºC, split ratio, 1:50.

Qualitative Analysis

The qualitative analysis was carried on the basis of MS spectra, which were compared with the spectra of the NIST library [15] and with data available in the literature [16]. The identity of the compounds was confirmed by their retention indices, taken from the literature [16, 17] and our own data. The quantity composition of the volatile oil was determined by GC (FID) by assuming that a total area percents of all the particular oil constituted 100%.

Results and Discussion

The investigated oregano plants were characterized by a high concentration of essential oil in air-dried herb and it was, respectively: 0.73%, 0.66%, and 0.86% (Table II); this concentration was much higher than the results obtained by some other authors [13, 18, 19] and comparable to the results of our previous research [20]. The highest amount of essential oil was found in the herb from the population of oregano plants originating from the town of Kazimierz Dolny, while its lowest amount was found in the herb obtained from the population growing in the Botanical Garden. In comparing these results, it can be noticed that wild growing plants of the oregano population were characterized by a higher concentration of essential oil than the population propagated and grown in the Botanical Garden. Opposite relationships were shown by Ivask et al. [18] who investigated the composition of volatile oil extracted from oregano plants grown in Estonia, which can be associated with the great diversity within the genus *Origanum* as well as with environmental variability modifying the chemical composition of herbal plants [7, 8]. The climatic conditions prevailing during the growing period of the oregano plants under study (Table I) promoted the biosynthesis of essential oil. Temperature was in an optimal range for this species, while a short-term water deficit could have even promoted oil biosynthesis [19].

Table II

Chemical composition of essential oil from three samples of *Origanum vulgare* L. ssp. *vulgare*

No	Compound	RT	RI	Concentration (%)		
				1	2	3
1	α-thujene	8.112	933	0.35±0.01	0.25±0.36	tr.
2	α-pinene	8.352	939	0.94±0.01	1.16±0.00	1.08±0.02
3	camphene	8.924	956	tr.	1.14±0.02	Tr.
4	sabinene	9.658	977	25.46±0.47	10.85±0.09	16.35±0.12
5	β-pinene	9.848	982	1.42±0.19	1.65±0.02	2.21±0.12
6	1-octen-3-ol	10.017	987	tr.	tr.	-
7	myrcene	10.184	991	2.13±0.02	1.74±0.04	1.75±0.05
8	α-phellandrene	10.834	1009	tr.	tr.	-
9	α-terpinene	11.192	1019	0.78±0.02	2.56±0.03	0.68±0.03
10	*p*-cymene	11.502	1027	0.64±0.01	tr.	5.29±0.09
11	limonene*	11.632	1031	1.59±0.04	3.34±0.03	1.59±0.16
12	β-phellandrene*	11.710	1033			-
13	*Z*-(β)-ocimene	11.807	1035	12.07±0.18	9.10±0.03	16.33±0.28
14	*E*-(β)-ocimene	12.198	1046	4.42±0.01	2.12±0.03	2.76±0.33
15	γ-terpinene	12.672	1059	2.12±0.06	5.46±0.04	3.52±0.07
16	*cis*-sabinene hydrate	13.239	1074	tr.	1.36±0.01	tr.
17	terpinolene	13.688	1086	tr.	1.08±0.00	tr.
18	*trans*-sabinene hydrate	14.325	1103	2.18±0.14	20.70±0.04	tr.

No	Compound	RT	RI	Concentration (%)		
				1	2	3
19	*cis-p*-menth-2-en-1-ol	15.141	1127	tr.	tr.	-
20	*trans-p*-menth-2-en-1-ol	15.890	1149	tr.	tr.	-
21	borneol	16.950	1180	tr.	2.87±0.03	tr.
22	terpinen-4-ol	17.245	1189	1.03±0.04	5.86±0.00	tr.
23	*α*-terpineol	17.859	1207	tr.	1.13±0.00	-
24	unknown	20.911	1297	tr.	tr.	tr.
25	*δ*-elemene	22.287	1337	tr.	tr.	tr.
26	*α*-cubebene	22.811	1353	tr.	tr.	tr.
27	*α*-copaene	23.765	1380	0.54±0.02	tr.	tr.
28	*β*-bourbonene	24.046	1389	1.17±0.01	tr.	1.35±0.03
29	*β*-elemene	24.196	1393	0.63±0.01	tr.	tr.
30	*E*-caryophyllene	25.228	1427	9.38±0.02	10.12±0.17	12.87±0.35
31	*β*-copaene	25.552	1439	tr.	tr.	tr.
32	*β*-gurjunene	25.687	1443	tr.	tr.	tr.
33	*cis*-muurola-3,5-diene	26.016	1455	tr.	tr.	tr.
34	*α*-humulene	26.376	1467	1.62±0.04	1.10±0.02	2.29±0.16
35	allo-aromadendrene	26.513	1472	0.62±0.04	0.56±0.04	tr.
36	*cis*-muurola-4(14),5-diene	26.573	1474	tr.	tr.	tr.
37	*trans*-cadina-1(6),4-diene	26.784	1481	tr.	tr.	tr.
38	*γ*-muurolene	26.938	1487	tr.	tr.	tr.
39	germacrene D	27.163	1494	15.34±0.01	9.36±0.12	14.16±0.18
40	viridoflorene	27.352	1501	tr.	tr.	tr.
41	bicyclogermacrene	27.617	1509	6.12±0.52	1.62±0.05	3.31±0.17
42	(*E*,*E*)-*α*-farnesene	27.760	1514	1.00±0.19	-	tr.
43	*β*-bisbolene	27.907	1518	tr.	tr.	tr.
44	*γ*-cadinene	28.146	1526	0.53±0.05	0.63±0.03	tr.
45	*δ*-amorphene	28.254	1529	1.57±0.03	1.26±0.06	0.78±0.09
46	*α*-cadinene	28.854	1548	tr.	tr.	tr.
47	spathulenol	30.090	1587	2.49±0.21	1.12±0.05	1.68±0.15
48	caryophyllene oxide	30.292	1593	1.97±0.06	tr.	9.95±0.03
49	globulol	30.390	1596	tr.	tr.	tr.
50	viridiflorol	30.668	1606	tr.	tr.	tr.
51	humulene epoxide II	31.138	1623	tr.	tr.	0.84±0.04
52	*epi-α*-muurolol	31.840	1650	tr.	tr.	tr.
53	*α*-muurolol	32.094	1659	0.71±0.03	0.74±0.01	tr.
54	*α*-cadinol	32.398	1671	1.19±0.11	1.10±0.07	tr.
	total			100%	100%	100%
	Essential oil content (%)			0.73	0.66	0.86

RT- retention time on a VF-5ms column (DB-5 equivalent)
RI - retention index on a VF-5ms column (DB-5 equivalent) for the alkane series C_6-C_{40}, following Van Den Dool and Kratz (1963)
* - compounds are not separated in GC-FID analysis
tr. – content below 0.05%

This study found the presence of 54 compounds in oregano essential oil, including one unidentified compound. The dominant compound was sabinene; its percentage proportion ranged from 10.85 to 25.46%, depending on the ecotype studied. This compound, with a characteristic scent of forest as well as citrus and tropical fruits and hence used in the perfume and cosmetics industries, appears to be one of more important components of the volatile oil extracted from the herb of *Origanum vulgare* L. ssp. *vulgare*, which is confirmed by other research reports [5,12,13,18,20,21].

Moreover, the volatile oil from the oregano ecotypes under investigation was characterized by a significant percentage of the following compounds: germacrene D (9.36-15.34%), *Z*-(*β*)-ocimene (9.10-16.33%), and *E*-caryophyllene (9.38-12.87%). The chemotype growing wild in Lithuania, which was described as *β*-ocimene-based, as well as other chemotypes collected from the district of Vilnius were characterized by a similar composition of essential oil [12, 21]. Furthermore, sabinene, *Z*-(*β*)-ocimene, *β*-caryophyllene, germacrene D, and bicyclogermacrene are predominant in the composition of the oil obtained from *Origanum vulgare* L. ssp. v*ulgare* oregano cultivated in Estonia [18]. Among the other compounds that are found in larger amounts in the investigated oregano oil, the following should be mentioned: *E*-(*β*)-ocimene (2.12-4.42%), *γ*-terpinene (2.12-5.46%), and bicyclogermacrene (1.62-6.12%). Attention should be drawn to the absence of phenolic compounds, thymol, and carvacrol in the oregano oil under investigation, confirmed in other studies [5, 11, 12]. The presence of phenolic compounds in the composition of volatile oil from *Origanum vulgare* L. can be determined genetically, which is indicated by the composition of essential oil obtained from other forms of this species [6, 13, 19, 22]. In addition, the inversely proportional ratio of thymol to *γ*-terpinene in oregano volatile oil [10] can explain the absence of the former compound with, at the same time, a noticeable proportion of the latter one.

Conclusions

Plants of the studied ecotypes of *Origanum vulgare* L. ssp. *vulgare* are characterized by a high content of essential oil in the herb, distinguished by a rich chemical composition. Sabinene, germacrene D, *Z*-(*β*)-ocimene, and *E*-caryophyllene were the dominant compounds in the oil. The investigated oil did not contain phenolic compounds. The wild growing plants were characterized by a higher concentration of essential oil and higher proportions of sabinene, *Z*-(*β*)-ocimene, and germacrene D than the plants propagated and cultivated in the Botanical Garden for many years.

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