BIOLOGIJA. 2005. Nr. 4. P. 53–58

Chemical composition of essential oil and antimicrobial activity of *Origanum vulgare*

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Essential oils of *Origanum vulgare* field accessions were analyzed by GC and GC/MS. Seventy five compounds representing 82.2–98.4% of total oil were identified in inflorescences and leaves. Mono- and sesqiuterpene hydrocarbons were dominant compounds accounting for 49.8–76.8% of total essential oil in inflorescences and for 41.9–71.4% in leaves. The content of phenols (thymol and carvacrol) was up to 5%. Essential oils were tested for their effectiveness against fungil, yeasts and yeast-like fungal microorganism species. Antimicrobial assay results showed that leaf oils were more active than inflorescence oils against all microorganisms. The inhibitory effect was best at 0.5% oil concentration. The essential oils highly differed in their composition and antimicrobial activity.

Key words: *Origanum vulgare*, essential oils, GC and GC/MS, antimicrobial activity

INTRODUCTION

The *Origanum* L. species plays a primary role among culinary herbs in world trade (Oliver, 1997). The increasingly growing popularity of oregano is a result of scientific research. Recent findings report the antimicrobial, fungicidal and antioxidant properties of oregano [1, 2]. The use of *O. vulgare* L. as a medicinal plant is attributed to the biological properties of p-cymene and carvacrol. Bernãth [3] has noted that there are intraspecific taxa of oregano that exhibit no “oregano” character based on the presence of carvacrol. Most of commercial oregano comes from wild populations of Turkey and Greece [4, 5]. Despite its economic importance, its genetic resources and variability, potential for utilization have not yet been fully explored [6]. A number of studies have shown that variation within the species may occur in its morphological and chemical features [7–10]. Moreover, the research on germplasm conservation is very sparing outside the Mediterranean region. On the other hand, there is a need to have knowledge on the whole genetic diversity of the genus and not just of what might be of interest to the industrial sector.

Populations of *O. vulgare* in Lithuania are characterized by a limited distribution and low resources of raw material. The field collection of oregano at the Institute of Botany holds 23 accessions, which have been gathered from indigenous populations. The botanical identification of the plants was based on the description of subspecies given by Ietswaart [11]. On the basis of discriminative morphologic characters (bracts, calyces, corollas), the oregano growing in Lithuania was attributed to *O. vulgare* L. ssp. *vulgare* [12].

The results of the qualitative and quantitative analyses of essential oil isolated from different accessions and their antifungal activity are presented in the work.

MATERIALS AND METHODS

Plant material

The plant material (inflorescences and leaves) of 21 accessions was collected during the flowering period (July 2001) from the field collection of the Medicinal and Aromatic Plants of the Institute of Botany, Vilnius, Lithuania. Voucher specimens of each field accession were deposited in the Herbarium of the Institute of Botany (BILAS, Vilnius, Lithuania).

Essential oil analysis

Essential oils were obtained from the air-dried inflorescences and leaves by hydrodistillation for two

hours. Analyses of essential oils were carried out by GC and GC/MS. An HP 5890II chromatograph equipped with FID and an HP-FFAP capillary column (30 m × 0.25 mm i. d., film thickness 0.25 μm) was used for quantitative analysis. The GC oven temperature was set at 70 °C for 10 min and then programmed from 70 to 210 °C at the rate of 3 °C / min using He as the carrier gas (0.7 ml/min). The injector and detector temperatures were 200 and 250 °C, respectively. Analyses by GC/MS were performed using a chromatograph interfaced to an HP 5971 mass spectrometer (ionization voltage 70 eV) and equipped with a CP-Sil 8 CB capillary column (50 m × 0.32 mm i. d., film thickness 0.25 μm). The oven temperature was held at 60 °C for 2 min, programmed 60–160 °C at the rate of 5 °C/min held for 1 min, then programmed 160–250 °C at the rate of 10 °C/min and isothermal at 250 °C for 3 min using He as the carrier gas (2.0 ml/min). The injector and detector temperature was 250 °C.

The percentage composition of oils was computed from GC peak areas without correction factors. Qualitative analysis was based on a comparison of retention times and indices on both columns and mass spectra with corresponding data in the literature [13] and computer mass spectra libraries (Wiley and NBS 54K).

Antimicrobial assay

Microorganisms were obtained from the culture collection at the Institute of Botany (Vilnius, Lithuania). All microorganisms were isolated from food or fruits and vegetables during their storage. The fungal species used in the experiments were: *Acremonium strictum* W. Gams, *Aspergillus niger* Tiegh, *Aspergillus ochraceus* K. Wilh., *Fusarium avenaceum* (Fr.) Sacc., *Paecilomyces variotii* Bainier, *Paecilomyces farinosus* (Holmsk. Et Gray), *Penicillium expansum* Link, *Penicillium brevicompactum* Dierckx, *Penicillium verrucosum* Dierckx and *Rhizopus stolonifer* (Ehrenb.) Vuill. var *stolonifer*; the yeast-like fungi used were: *Aureobasidium pullulans* (de bary) G. Arnaud var. *pullulans* and *Geotrichum candidum* Link.; the yeasts used were: *Candida glabrata* (H. W. Anderson) S. A. Mey & Yarrow, *Rhodotorula rubra* (Schimon) F. C. Harrison and *Saccharomyces cerevisiae* Meyen ex E. C. Hansen.

Evaluation of the effectiveness of seven essential oils against microorganisms was carried out *in vitro* by the plate diffusion procedure using wells in dishes [14]. To this end, 1 ml of microorganism suspension (approximately 10^6 spores or cells) prepared with sodium chloride solution containing 0.5% Tween 80 was uniformly spread on sterile MEA medium in Petri dishes. After inoculums absorption by the MEA medium, wells were made with the help of sterile metallic borer (diameter 7 mm) and filled with 100 μl of solutions of an essential oil. Two concentration solutions of essential oil (0.2 and 0.5%) were used to assay antimicrobial activity. Control was carried out with Tween 80 mixture without essential oil.

Data analysis

The definition of the oil profiles was based on hierarchical cluster analysis using the Person correlation and average linkage method. The linkage between the groups was measured using the Euclidean distances. Statistical analysis was performed using XLSTAT–Pro version 6.1 (Addinsolf SARL, France).

RESULTS AND DISCUSSION

The identified components of the essential oils of *O. vulgare* and their range percentages are listed in the order of their RI in Table 1. Essential oils were a complex mixture of 75 compounds, amounting to a total percentage of 82.2–98.4%. Mono- and sesquiterpene hydrocarbons were the dominant compounds accounting for 49.8–76.8% in inflorescences and for 41.9–71.4% in leaves of total essential oil. They were represented mainly by β-caryophyllene (4.7–25.0% in inflorescences and 5.4–24.5% in leaves), *cis*- and *trans*-β-ocimene (0.9–18.1% / inflorescences and 1.1–22.6% / leaves), sabinene (0.3–25.1% / inflorescences and 0.9–18.3 / leaves) and germacrene-D (2.1–20.1% in inflorescences and 1.5–13.2% in leaves). The percentage

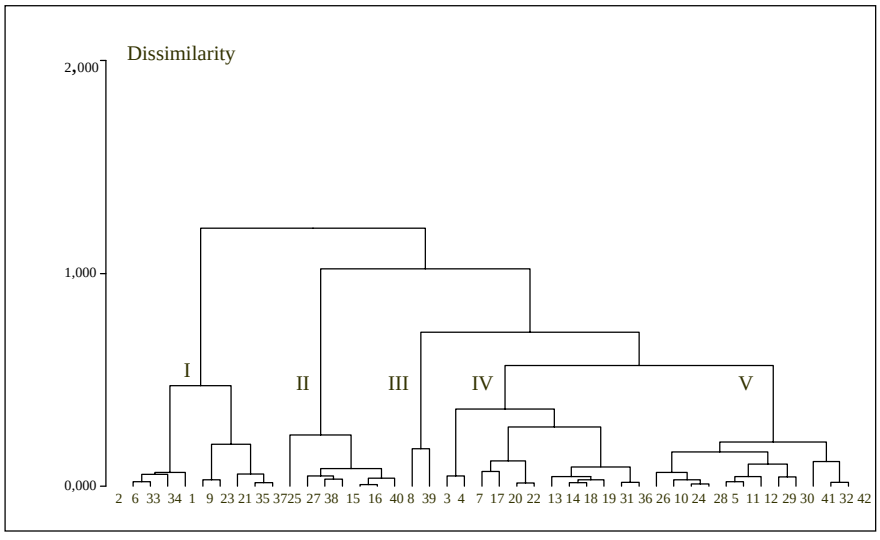

Figure. Dendrogram obtained by hierarchical cluster analysis of the percentage composition of essential oils from *Origanum vulgare*, clustering method based on squared Euclidean distances

Table 1. **Composition of essential oils of *Origanum vulgare* ssp. *vulgare* inflorescences and leaves of field accessions**

No.	Compound	RI	Range	
			Inflorescences	Leaves
1	α-Thujene	931	0–2.5	t–0.9
2	α-Pinene	939	0.2–3.4	t–1.3
3	Camphene	954	0–2.8	0–0.1
4	**Sabinene**	976	**0.3–25.1**	**0.9–18.3**
5	β-Pinene	980	t–3.4	t–2.6
6	Octen-3-ol	1979	0–2.2	0–3.1
7	Myrcene	991	t–2.3	t–4.6
8	α-Terpinene	1018	t–2.4	t–2.4
9	*p*-Cymene	1026	t–8.2	0–6.1
10	**1,8-Cineole**	1033	**t–14.2**	**t–5.5**
11	***(Z)*-β-Ocimene**	1040	**0.9–7.1**	**0.3–12.5**
12	***(E)*-β-Ocimene**	1050	**0–11.0**	**0.8–10.1**
13	γ-Terpinene	1061	0–4.1	t–4.5
14	*cis*-Sabinene hydrate	1070	t–2.9	0–1.7
15	Terpinolene	1088	0–1.5	0–2.7
16	*cis-p*-Mentha-2,4(8)-diene	1088	0–0.5	T
17	*trans*-Sabinene hydrate	1098	t–5.0	0–1.4
18	Linalool	1099	0–5.0	0–3.5
19	*cis-p*-Menth-2-en-1-ol	1122	0–1.1	0–0.6
20	Sabinol	1142	0–0.3	0–0.3
21	*trans*-p-Menth-2-en-1-ol	1141	0.4	0–0.4
22	*allo-neo*-Ocimene	1144	0.2–2.3	t–4.5
23	Camphor	1146	0.0.5	0
24	*iso*-Menthone	1163	0	0–1.5
25	Borneol	1169	0.4.7	0–2.7
26	*p*-Mentha-1,5-dien-8-ol	1170	0–0.9	0–1.3
27	Menthol	1172	0–1.0	0–3.1
28	**Terpinen-4-ol**	1177	**0.5–17.9**	**t–8.6**
29	α-Terpineol	1189	0.2–3.6	0–2.4
30	*cis*-Piperitol	1196	0–0.7	0–0.5
31	*trans*-Piperitol	1208	0–1.0	0–1.1
32	*trans*-Carveol	1217	0–0.1	T
33	Carvone	1243	0–0.2	0
34	Bornyl acetate	1285	0–0.7	0
35	Thymol	1290	0–2.9	0–1.5
36	Carvacrol	1298	0–1.2	0–1.4
37	σ-Elemene	1338	0–1.1	0–0.8
38	α-Terpinyl acetate	1349	0–2.7	0–1.1
39	α-Copaene?	1377	0–0.7	0–0.6
40	β-Bourbonene	1387	t–0.8	t–3.0
41	β-Elemene	1391	t–1.0	0–1.4
42	**β-Caryophyllene**	1418	**4.7–25.0**	**5.4–24.5**
43	β-Gurjunene	1432	0.2–1.1	t–1.1
44	Aromadendrene	1441	0–0.5	0–1.6
45	α-Humulene	1454	0.6–5.7	t–3.6
46	*allo*-Aromadendrene	1461	0–1.7	0.3–2.0
47	γ-Muurolene	1480	0–2.2	0–2.0
48	**Germacrene D**	1485	**2.1–20.1**	**1.5–13.2**
49	β-Ionone	1489	T	0–0.4
50	*trans*-Muurola-4(14), 5-diene	1494	0–0.2	0–0.6
51	Bicyclogermacrene	1500	t–5.9	t–2.6
52	α-Muurolene	1500	0–2.1	0–1.8
53	α-Farnesene	1508	0–4.0	0.29

Table 1 continued

No.	Compound	RI	Range	
			Inflorescences	Leaves
54	β-Bisabolene	1509	0–5.4	0–2.4
55	γ-Cadinene	1514	0–4.5	0–1.5
56	*endo*-1-Bourbonanol	1520	0–1.9	0–1.8
57	σ-Cadinene	1524	0–7.5	0–7.2
58	Elemol	1550	0–1.2	0–1.2
59	α-Cadinene	1539	0–0.2	0–4.6
60	α-Calacorene	1546	t	0–0.3
61	*trans*-Cadina1(2)-4-diene	1574	0–0.6	0–0.9
62	Germacrene D-4-ol	1577	0–4.5	0–5.6
63	Spathulenol	1578	0.3–6.1	1.9–9.8
64	**Caryophyllene oxide**	1581	**0.3–18.8**	**0.7–24.4**
65	Viridiflorol	1593	0–0.9	0–8.7
66	Salvial-4(14)-en-1-one	1595	0–1.1	0–1.7
67	Humulene epoxide II	1608	0–0.9	0–2.6
68	α-Acorenol?	1633	0–0.7	0–1.3
69	*epi* -β-Cadinol	1640	0–3.7	0–4.2
70	*epi* -α- Muurolol	1642	0–4.6	0–2.9
71	α-Muurolol	1646	0–3.5	0–7.2
72	**α-Cadinol**	1653	**0.5–9.6**	**0.6–9.8**
73	Calamenen-10-ol	1661	0–0.2	0–0.3
74	*epi*-E-Caryophyllene-14-hydro-9	1670	0–0.6	0–1.0
75	β-Eudesma-4(15),7-dien-1-ol	1688	t–4.3	t–1.3
	Total, %		87.3–98.4	82.2–96.0
	Monoterpene hydrocarbons		4.4–39.3	5.1–43.4
	Oxygenated monoterpenes		4.5–31.8	4.7–16.6
	Sesquiterpene hydrocarbons		18.3–62.9	16.5–46.0
	Oxygenated sesquiterpenes		6.2–26.9	9.6–48.6

RI – Retention index on the CP-Sil8CB column; t – traces ≤ 0.05%.

Table 2. Range of zones of microorganism growth inhibition (mm) by leaf and inflorescence essential oils from different accessions of *Origanum vulgare*

Microorganism group	Species	Inflorescences		Leaves	
		Range			
Yeasts		0.2%	0.5%	0.2%	0.5%
	Candida glabrata	0–3.5	1.3–7.9	0.8–8.5	3.1–18.9
	Rhodotorula rubra	0–10.7	0.9–22.2	0.8–11.6	2.5–20.7
	Saccharomyces cerevisiae	0–11.5	1.2–21.7	1.2–16.8	3.1–33.4
Yeasts-like fungi					
	Geotrichum candidum	0–1.5	0–4.6	0.5–6.1	1.8–15.0
	Aureobasidium pullulans	0–2.9	0–7.7	1.9–6.8	6.3–18.3
Fungi					
	Aspergillus niger	0–2.4	1.7–7.6	2.7–12.4	12.8–25.7
	Acremonium furcatum	0–1.3	0–6.9	2.3–6.4	5.3–11.8
	Fusarium avenaceum	1.5–5.8	3.8–19.5	4.2–11.6	9.9–22
	Paecilomyces variotii	0–13.2	1.5–18.9	2.7–22.7	5.1–33.8
	Penicillium verrucosum	0–12.1	1.5–19.1	2.0–14.8	5.0–28.1
	Rhizopus stolonifer	0–12.5	6.8–19.4	5.3–20.1	15.9–35.7
	Scopulariopsis brevicaulis	2.1–15.6	6.5–20.3	8.7–19.8	12.4–31.8

of oxygenated components exhibited a high variation in total oil. Oxygenated monoterpenes accounted for 4.7–16.6% in leaves and for 4.5–31.8% in inflorescences. The main oxygenated monoterpenes were 1,8-cineole (t–14.2% / inflorescences and t–5.5% / leaves) and terpinen-4-ol (0.5–17.9% / inflorescences and t–8.6% / leaves). Oxygenated sesquiterpenes made up 9.6–48.6% and 6.2–26.9% of total essential oil in inflorescences and leaves, respectively, and were represented by major constituents: caryophyllene oxide (0.3–18.8% / inflorescences and 0.7–24.4% / leaves), terpinen-4-ol (0.5–17.9% / inflorescences and t–8.6 / leaves), and α-cadinol (0.5–9.6% in inflorescences and 0.6–9.8% in leaves). The content of phenols (thymol and carvacrol) was up to 5%.

The application of hierarchal cluster analysis using percentage of principal components grouped all oils into five main clusters which corresponded to five chemical profiles:

I – *cis*- and *trans*-β-ocimene/germacrene D + sabinene/1,8-cineole + β-caryophyllene

II – *cis*- and *trans*-β-ocimene/β-caryophyllene/terpinen-4-ol + sabinene/germacrene D;

III – caryophyllene oxide + sabinene/spathulenol;

IV – β-caryophyllene/sabinene + *cis*- and *trans*-β-ocimene/caryophyllene oxide/germacrene D;

V – β-caryophyllene/*cis*- and *trans*-β-ocimene/germacrene D + caryophyllene oxide/germacrene D/sabinene.

A *cis*- and *trans*-β-ocimene and β-caryophyllene were the major constituents in four chemical profiles of essential oils. The principal constituents that determined the profiles of oils were sabinene, germacrene-D, 1, 8-cineole, cryophyllene oxide, terpinen-4-α-ol, and spathulenol. The corresponding reported data have revealed carvacrol, p-cymene, γ-terpinene, sabinene, *cis*-ocimene [15, 16], or germacrene, terpinen-4-ol, β-bisabolene [17] as principal constituents in essential oil of *O. vulgare* ssp. *vulgare*. In our study, carvacrol and β-bisabolene were detected only in minor quantities. The accessions investigated in our study could be attributed to the kind of plants that did not contain carvacrol.

Remarkable differences were seen between the composition of oils obtained from our plant material and those recorded for previously investigated populations from wild habitats [18]. Essential oil composition of field accessions appears to differ from that of wild plants by a higher variability of chemotypes. Previous results represented a volatile composition of *O. vulgare* only from Vilnius district where one chemotype (β-ocimene/germacrene-D/β-caryophyllene) of essential oil was distinguished, which corresponded to the chemical profile of oils from the first cluster. Our results represent the chemical variability of all regional populations of *O. vulgare*, which exposed a high variation in the chemical composition of essential oil.

The results of antifungal activity of *O. vulgare* oils, which was determined by measuring the inhibition zone diameter of the test microorganisms, are given in Table 2. Leaf oils were more active against all test microorganisms than oils from inflorescences. Significant differences were observed in the action of 0.2 and 0.5% concentrations of oils. Oregano oils both from leaves and inflorescences at a concentration of 0.5% were effective against all microorganisms tested, while oils at a concentration of 0.2% showed activity against fungi and were less effective against yeasts and yeast-like fungi. Inhibition zones were 2–3 times larger at an oil concentration of 0.5% than at 0.2%. The antifungal activity of oils was strongest against *Fusarium avenaceum*, *Paecilomyces variotii*, *Rhizopus stolonifer* and *Scopulariopsis brevicaulis.* The growth inhibition zone varied from 9.9 to 22 mm, from 5.1 to 33.8 mm, from 15.9 to 35.7 mm and from 12.4 to 31.8 mm, respectively (Table 2). The microorganisms most resistant to the test oils were yeasts (*Candida glabrata*, *Saccharomyces cerevisiae*); yeast-like fungi (*Geotrichum candidum*, *Aureobasidium pullulans*), and the fungus *Acremonium furcatum*.

Previous investigations showed that oregano oil, which is used as a food flavoring agent, possesses a broad spectrum of *in vitro* antimicrobial activities attributed to the phenolic derivatives such as carvacrol and thymol [18–23]. The essential oils in our study contained very low amounts of carvacrol and thymol. The antimicrobal activity of the oils could be caused by other compounds.

The results showed differences among the accessions of oregano both in essential oil composition and antimicrobial activity. The investigations indicated the existence of infraspecific variations and chemical polymorphism of oregano, which exhibited different effects on the test microorganisms.

ACKNOWLEDGEMENTS

The study was conducted within the framework of the Plant Genetic Resources Conservation Programme supported by Ministry of Education and Science of Lithuania.

Received 16 October 2005
Accepted 24 November 2005

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Origanum vulgare ETERINIO ALIEJAUS SUDËTIS BEI ANTIMIKROBINIS AKTYVUMAS

S a n t r a u k a

Tirta paprastojo raudonëlio 21 kolekcinio pavyzdþio þiedynø bei lapø eterinio aliejaus kiekybinë bei kokybinë sudëtis. Analizë atlikta dujø chromatografijos bei masiø spektrofotometrijos metodais. Raudonëlio eteriniame aliejuje identifikuoti 75 komponentai, sudarantys 82,2–98,4% viso aliejaus tûrio. Eteriniame aliejuje vyrauja mono- bei seskviterpenø hidrokarbonai, kurie þiedynuose sudaro 49,8–76,8%, o lapuose – 41,9–71,4% jo tûrio. Palyginus su kituose kraðtuose auganèiu raudonëliu fenoliniø junginiø kiekis mûsø tirtuose augaluose labai maþas. Buvo ávertintas eteriniø aliejø antimikrobinis aktyvumas prieð kai kurias mikromicetø, mieliagrybiø bei mieliø rûðis. Labiausiai jautrios aliejø poveikiui buvo *Fusarium avenaceum*, *Paecilomyces variotii*, *Rhizopus stolonifer* ir *Scopulariopsis brevicaulis*, o atsparios – *Candida glabrata*, *Saccharomyces cerevisiae*, *Geotrichum candidum*, *Aureobasidium pullulans* ir *Acremonium furcatum* rûðys. Lapø eteriniai aliejai buvo veiksmingesni prieð tirtus mikroorganizmus. Aliejaus koncentracija, slopinusi visø mikroorganizmø vystymàsi, buvo 0,5%. Tyrimas parodë, kad paprastojo raudonëlio eteriniø aliejø sudëtis bei jø antimikrobinis aktyvumas labai ávairuoja.