Composition and Antimicrobial Activity of the Essential Oils of Two *Origanum* Species

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The essential oils obtained from the aerial parts of *Origanum scabrum* and *Origaum microphyllum*, both endemic species in Greece, were analyzed by means of GC and GC-MS. Forty-eight constituents were identified, representing 98.59 and 98.66% of the oils, respectively. Carvacrol, terpinen-4-ol, linalool, sabinene, α-terpinene, and γ-terpinene were found as the major components. Furthermore, both samples exhibited a very interesting antimicrobial profile after they were tested against six Gram-negative and -positive bacteria and three pathogenic fungi.

Keywords: *Origanum essential oils; O. scabrum; O. microphyllum; antimicrobial activities; GC-MS; carvacrol; terpinen-4-ol; linalool; sabinene;* α*-terpinene*

INTRODUCTION

The *Origanum* (Lamiaceae family) genus consists of 38 species widespread in the Mediterranean region, although 75% of them are restricted to the eastern Mediterranean area. Eleven species occur in Greece, five of which are found in Crete (*1*). Members of the genus are widely used in the flavoring of food products and alcoholic beverages (*2*, *3*). Many *Origanum* plants are characterized by a wide range of volatile secondary metabolites and by the existence of chemical differences with respect to both essential oil content and composition. In our continuing research on the essential oils of Greek aromatic and edible plants, we examined the essential oils of the fresh aerial parts of *Origanum scabrum* Boiss. & Heldr. In Boiss. and *Origanum microphyllum* Vogel as well as their antimicrobial activities against several pathogenic bacteria and fungi.

O. microphyllum is a dwarf shrub endemic to Crete (Lefka Ori and Dhikti Mountains), and *O. scabrum*, which is endemic to the mountains of southern Greece (Parnon, Taygetos, and Dirfis as well Sterrea Ellas), is a rhizomatous perennial plant (*4*).

In the literature, there are several studies on the essential oil composition of *O. vulgare* (*5*, *6*), and only one paper on the volatile composition of *O. microphyllum* (*7*, *8*), whereas the oil of *O. scabrum* has never been studied before.

MATERIALS AND METHODS

Plant Material. The aerial parts of *O. scabrum* were collected during the flowering stage in July 1999 on Mount Taygetos in South Peloponissos, where it is endemic; *O. microphyllum* was collected during the same period on Mount Dhikti on the island of Crete. Both collection locations were at an altitude of 1200 m. Voucher specimens are kept at the Herbarium of the Pharmacognosy Laboratory, University of Athens.

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Isolation of the Essential Oils. The fresh aerial parts of these plants were steam distilled for 3 h according to the method found in ref *9*, and the resulting oils collected were dried over anhydrous sodium sulfate, preserved in sealed flasks, and stored at 4−6 °C until the moment of analysis.

Gas Chromatography—Mass Spectrometry (GC-MS). The chemical composition of the essential oils was analyzed using GC and GC-MS techniques. The identification of the components was based on the comparison of their mass spectra with those of Wiley275, NBS (*10*), and NST Libraries and those described by Adams (*11*), as well as by comparison of their retention indices with literature values (*11*). The mass spectrometer employed for GC-MS analysis was an HP 5973 mass selective detector in the electron impact (EI) ionization mode (70 eV). A Hewlett-Packard 6890 gas chromatograph was employed under the following conditions: capillary column, HP-5 MS (30 m × 0.25 mm; film thickness = 0.25 μm); temperature program, 60 °C (held for 5 min) raised to 280 °C at a rate of 3 °C/min; injector temperature, 200 °C; carrier gas, helium, at flow rate of 0.6 mL/min. Retention indices (RI) have been obtained according to the method of Van den Dool (*12*).

Antimicrobial Strains and Media. The antibacterial activity of the essential oils against the two Gram-positive bacteria *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228) and the four Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883), and *Pseudomonas aeruginosa* (ATCC 227853) and the antifungal activities against the pathogenic fungi *Candida albicans*, *Candida tropicalis*, and *Torulopsis glabrata* were determined, using the dilution technique (*13*). The culture medium used for bacteria was Müller−Hinton agar, whereas Sabouraud agar was used for growing the fungi. The incubation conditions used were 24 h at 37 °C for the bacteria and 48 h at 28 °C for the fungi. These particular strains were standard reference ones (of the American Type Culture Collection) that are routinely used for the evaluation of antimicrobial compounds.

Antimicrobial Assay. The minimum inhibitory concentrations (MICs) were measured as described previously (*14*) for the oils, carvacrol, γ-terpinene, and *p*-cymene (Table 2). Initial emulsions of oils were prepared at 10 mg/mL in sterile distilled water with 10% Tween 80. Serial dilutions of the stock solutions in broth medium (100 μL of Müller−Hinton broth or on Sabouraud broth) were prepared in a microtiter plate (96 wells). Then 1 μL of the microbial suspension (in sterile distilled water) was added to each well. For each strain, the growth conditions and the sterility of the medium were

10.1021/jf001494m CCC: $20.00
Published on Web 08/25/2001

Table 1. Chemical Constituents of the Essential Oils of *O. scabrum* and *O. microphyllum*

	compound[a]	KI	GC area % *O. scabrum*	GC area % *O. microphyllum*
1	α-thujene	929	0.66	2.25
2	α-pinene	936	0.31	1.91
3	camphene	950		1.09
4	sabinene	975	0.09	7.70
5	β-pinene	978	0.09	
6	octen-3-ol	980	0.83	0.26
7	3-octanone	988	0.21	
8	myrcene	993	1.10	1.75
9	3-octanol	996	0.25	0.04
10	β-phellandrene	1005	0.17	0.73
11	δ-3-carene	1009	0.08	0.05
12	α-terpinene	1017	0.79	9.86
13	*p*-cymene	1026	5.41	1.36
14	β-phellandrene	1030	0.30	2.34
15	*cis*-ocimene	1040		0.09
16	phenylacetaldehyde	1044		0.06
17	*trans*-ocimene	1050		0.09
18	γ-terpinene	1061	4.66	13.83
19	*cis*-sabinene hydrate	1069	0.24	0.66
20	terpinolene	1089	0.09	3.51
21	linalool	1098	0.25	10.81
22	octen-3-yl acetate	1111		0.70
23	*p*-menth-2-en-1-ol	1119		1.27
24	*cis*-pinene hydrate	1123		0.08
25	terpin-1-ol	1136		0.94
26	borneol	1166		0.68
27	terpin-4-ol	1178	0.89	24.86
28	α-terpineol	1190	0.23	2.38
29	estragol	1195		0.08
30	*trans*-dihydrocarvone	1199	0.11	
31	*trans*-piperitol	1205		0.20
32	octanol acetate	1211		0.11
33	thymol, methyl ether	1233		0.77
34	carvacrol, methyl ether	1243		0.17
35	linalool acetate	1257		0.25
36	bornyl acetate	1286		1.26
37	thymol	1292	4.51	0.17
38	carvacrol	1300	74.86	
39	neryl acetate	1364		0.05
40	geranyl acetate	1383		0.05
41	decanol acetate	1408		0.07
42	β-caryophyllene	1417	1.32	5.55
43	α-humulene	1451		0.30
44	bicyclogermacrene	1492	0.32	0.04
45	β-bisabolene	1508	0.25	
46	γ-bisabolene	1535	0.23	
47	spathulenol	1575	0.13	
48	caryophyllene oxide	1579	0.21	0.29
	total		98.59	98.66

[a] Compounds listed in order of elution from a DB-5 column.

checked, and the plates were incubated as described above. MICs were determined as the lowest concentrations preventing visible growth. Standard antibiotics (netilmicin, amoxicillin, and clavulanic acid) were used to control the sensitivity of the tested bacteria, whereas 5-flucytocine, amphotericin B, and intraconazole were used as controls against the tested fungi.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oils. The fresh aerial parts of the plants were subjected to steam distillation for 3 h, using a modified Clevenger-type apparatus to yield 0.60 and 0.65% of yellowish oils for *O. scabrum* and *O. microphyllum*, respectively. The oils after preparation were submitted to GC and GC-MS analyses. The physical properties for the oils of *O. scabrum* and *O. microphyllum* were $[\alpha]_D^{20} = 0.8$ (in $CHCl_3$, *c* 0.5) and $[\alpha]_D^{20} = 10.8$ (in $CHCl_3$, *c* 0.5), respectively.

The chemical composition of the essential oils was analyzed using a GC-MS technique. Qualitative and quantitative analytical results are shown in Table 1. Forty-eight components were determined and identified by GC and combined GC-MS, representing about 98.59 and 98.66% of the oils of *O. scabrum* and *O. microphyllum*, respectively.

Twenty-eight constituents were identified in *O. scabrum*, representing 98.59% of the oil (see Table 1). Carvacrol (74.86%), *p*-cymene (5.41%), γ-terpinene (4.66%), and thymol (4.51%) were found as the major compounds.

Forty-one constituents were determined in the present study, in the essential oil of *O. microphyllum*, representing 98.66% of the oil (see Table 1). The oil was characterized by the presence of terpin-4-ol (24.86%), γ-terpinene (13.83%), linalool (10.81%), α-terpinene (9.86%), sabinene (7.70%), β-caryophyllene (5.55%), and terpinolene (3.51%). On the other hand, sabinene (14.24−24.23%), *cis*-sabinene hydrate (22.45−31.09%), *trans*-sabinene hydrate (12.42−26.34%), and linalool (9.37−14.16%) were found as the main volatile constituents of *O. microphyllum*, from CH_2Cl_2 leaf extract and from the leaves−flowers (separately) using the headspace method, as reported by Scoula et al. (*7*). It is noteworthy that according to Scoula et al. (*7*) the taxon is almost dominated by sabinyl compounds, whereas the studied essential oil of *O. microphyllum* was shown to contain mainly terpin-4-ol, γ-terpinene, α-terpinene, terpinolene, and either *cis*- or *trans*-sabinene hydrates, probably depending on the different analytical method as well as on the different plant material investigated. In our study, we used as plant material fresh aerial parts of *O. microphyllum*, whereas Scoula et al. used a CH_2Cl_2 extract of dried leaves as well as dried leaves and dried flowers separately.

Antimicrobial Activity. The results of the bioassays showed that the oil of *O. scabrum* (containing mainly carvacrol 74.86%) exhibited an extremely strong activity against all of the tested microorganisms, especially against the tested bacteria (MIC values = 0.28−1.27 mg/mL) as well as against the pathogenic fungi (MIC values = 0.65−1.27 mg/mL). On the other hand, the oil of *O.*

Table 2. Antimicrobial Activities (MIC, Milligrams per Milliliter) of the Essential Oils of *Origanum* Species and Their Main Components

essential oil	*S. aureus*	*S. epidermidis*	*P. aeruginosa*	*E. cloacae*	*K. pneumoniae*	*E. coli*	*C. albicans*	*C. tropicalis*	*T. glabrata*
O. scabrum	0.35	0.38	1.27	1.12	0.72	0.28	1.27	1.23	0.65
O. microphyllum	6.21	5.32		8.85		3.35	3.23	2.89	1.81
γ-terpinene									
p-cymene									
carvacrol	0.1	0.10	1	0.75	0.50	0.1	1	1	0.35
intraconazole							1×10^{-3}	0.1×10^{-3}	1×10^{-3}
5-flucytocine							0.1×10^{-3}	1×10^{-3}	10×10^{-3}
amphotericin B							1×10^{-3}	0.5×10^{-3}	0.4×10^{-3}
netilmicin	4×10^{-3}	4×10^{-3}	8.8×10^{-3}	8×10^{-3}	8×10^{-3}	10×10^{-3}			
amoxycillin	2×10^{-3}	2×10^{-3}	2.4×10^{-3}	2.8×10^{-3}	2.2×10^{-3}	2×10^{-3}			
clavulanic acid	0.5×10^{-3}	0.5×10^{-3}	1×10^{-3}	1.6×10^{-3}	1×10^{-3}	1.2×10^{-3}			

microphyllum showed, in general, weaker activities (MIC values = 1.81−8.85 mg/mL), whereas against *P. aeruginosa* and *K. pneumoniae* it appeared to be completely inactive. In the antimicrobial screening, standards of the pure carvacrol, γ-terpinene, and *p*-cymene were tested on the same cultures under identical conditions to compare their activities with those of the investigated oils. The results suggest that the activity of the oils can be attributed, to a considerable degree, to the existence mostly of carvacrol, which appears to possess similar activities against all of the tested microorganisms. Essential oils rich in phenolic compounds such as carvacrol are reported to possess high levels of antimicrobial activity (*5*, *15*). Similarly, essential oils from other *Origanum* species have been shown to possess high levels of antimicrobial activity (*2*, *5*). Of the main compounds tested γ-terpinene and *p*-cymene did not show any activity against the bacterial strains tested, whereas carvacrol exhibited high levels of antimicrobial activity against all of the tested strains with the only exception being *P. aeruginosa*, against which it showed a lower activity, as this bacterium exhibits resistance to many antimicrobial agents.

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Received for review December 19, 2000. Revised manuscript received June 15, 2001. Accepted June 17, 2001.

JF001494M