Molecules **2007**, *12*, 772-781

molecules

ISSN 1420-3049

www.mdpi.org/molecules

Full Paper

Chemical Composition and Antimicrobial Activity of the Essential Oil of Algerian *Phlomis bovei* De Noé subsp. *bovei*

Christos Liolios [1], Hocine Laouer [2], Nacira Boulaacheb [2], Olga Gortzi [3] and Ioanna Chinou [1,*]

[1] Department of Pharmacy, Division of Pharmacognosy, Chemistry of Natural Products, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece; E-mail: liolios@pharm.uoa.gr; Fax: (+30) 2107274115.

[2] Department of Biology, Faculty of Sciences, University Ferhat Abbas, Setif, Algeria; E-mails: hocine_laouer@yahoo.fr; boulaacheb1bv@yahoo.fr; Fax: (+213) 36 92 51 22

[3] Department of Food Technology, Technological Educational Institution (T.E.I.) of Larissa, Terma Temponera str., Karditsa, Greece; E-mail: ogkortzi@teiath.gr; Fax: (+30) 2441072070

[*] Author to whom correspondence should be addressed; e-mail: ichinou@pharm.uoa.gr, Fax: (+30) 2107274115

Received: 27 February 2007; in revised form: 27 March 2007 / Accepted: 3 April 2007 / Published: 12 April 2007

Abstract: The chemical composition of essential oil obtained by steam distillation of dried aerial parts of *Phlomis bovei* De Noé subsp. *bovei* collected from Algeria, was analyzed by GC and GC/MS. Seventy five constituents (corresponding to 86.37% of the total weight) were identified. The main components were: germacrene D, *β*-caryophyllene, *β*-bournonene, thymol and hexahydrofarnesyl acetone. Furthermore, the antimicrobial activity of the oil was evaluated against six Gram (+/-) bacteria and three pathogenic fungi, using the agar dilution technique. It was found that the oil exhibited strong antimicrobial activity against most of the tested microorganisms.

Keywords: *Phlomis bovei* De Noé; chemical composition; essential oil; antimicrobial activity.

Introduction

The plants of the genus *Phlomis* are native to Turkey, North Africa, Europe and Asia. *Phlomis bovei* De Noé, syn. *Phlomis samia* Desfontaines (Lamiaceae) is a rare Algerian endemic plant, commonly known as *Kayat El Adjarah* [1] in the Algerian dialect or variously named Farseouan, Tarseouan, Iniji, R'ilef and Azaref throughout the North of Africa [2]. It is one among the nine endemic plants recorded in the 'Rapport National sur la Diversité Biologique' [1]. *P. bovei* is a herbaceous perennial plant, which grows up to 0.8 m. and often develops a stout woody base. All parts are sticky, because of its dendroid stellate glandular hairs. Its basal leaves are green, heart-shaped, with scalloped margins, 6.5-25 x 4.5-20 cm and it has a petiole of between 4-18 cm in length. To date two subspecies have been recorded for *P. bovei De Noé*: *P. bovei De Noé* subsp. *bovei* and *P. bovei* De Noé subsp. *maroccana* Maire. The present study refers to the former, which to our knowledge has never been studied phytochemically before, whereas previous studies on the essentials oils of *Phlomis* species from around the Mediterranean have included: *Phlomis fruticosa*, *P. cretica*, *P. samia*, *P. lanata*, *P. linearis*, *P. leucophracta*, *P. chimerae* and *P. grandiflora* var. *grandiflora*.

Results and Discussion

The essential oil obtained by hydrodistillation of aerial parts of *Phlomis bovei* De Noé subsp. *bovei* was light yellow in color and possessed a distinct sharp odor. The yields were 0.22 % w/w. The analysis of the volatile constituents was carried out using two different GC-MS systems, equipped with two columns of different polarities (HP-5 and Aquawax, respectively). The chemical compositions are summarized in Tables 1 and 2. The identified components represented 86.37% of all the components found in the oil samples. These percentages were based on normalization of peak areas without application of the response correction factor. The major components included: germacrene D (21.45%), thymol (8.43%), *β*-caryophyllene (7.05%) and hexahydrofarnesyl acetone (5.84%). We should also note the presence in the essential oil of a total 6.03 % of normal saturated hydrocarbons (see Table 2). Although most of the identified constituents occurred in both methods of analysis, it was also noted that some chemical constituents occurring in appreciable amounts in HP-5 were absent in Aquawax and *viceversa*. This was due to the differences between the GC-MS instruments, the two columns and the absence of reference retention indexes for the second column. Thus the identification of the components for the second column was based on their mass spectra and by comparison of their retention times with those of authentic samples.

Table 1. Main components of the essential oil from the aerial parts of *P. bovei* De Noé.

	Compounds*	RI[§]		% in	Method of
		HP-5	Aquawax	Essent. oil	identification
1.	1-Octen-3-ol	977	1505	1.08	a, b, d,
2.	3-Octanol	993	1394	0.07	a, b, d
3.	*n*-Octanal	1004	1289	0.08	a, b, d
4.	*E,E*-2,4-Heptadienal	1012	-	0.03	a, b, d
5.	*p*-Cymene	1023	1270	0.04	a, b, c, d

Table 1. Cont.

6.	Limonene	1024	-	0.05	a, b, c,d
7.	Phenylacetaldehyde	1046	-	0.02	a, b, d
8.	*γ*-Terpinene	1058	1246	0.06	a, b, d
9.	*n*-Octanol	1071	1574	0.13	a, b, d
10.	Linalool	1104	1570	0.43	a, b, d
11.	Nonanal	1107	-	0.8	a, b, d
12.	Benzeneethanol	1115	1896	0.06	a, b, d
13.	*trans*-2-Nonenal	1160	1555	0.29	a, b, d
14.	Terpine-4-ol	1175	1593	0.21	a, b, d
15.	*α*- Terpineol	1187	-	0.24	a, b, c, d
16.	Caprylic acid [Octanoic acid]	1189	2021	0.12	a, b, d
17.	Methyl salicylate	1191	1749	0.05	a, b, d
18.	*n*-Decanal	1203	-	0.15	a, b, d
19.	*β*-Cyclocitral	1215	-	0.04	a, b, d
20.	Thymol methyl ether	1225	-	0.04	a, b, d
21.	Carvacrol methyl ether	1230	-	0.04	a, b, d
22.	*trans*-2-Decenal	1269	1630	0.32	a, b, d
23.	Thymol	1296	2065	8.43	a, b, d, c
24.	Carvacrol	1303	-	1.03	a, b, d, c
25.	*trans,trans*-2,4-Decadienal	1328	1783	0.15	a, b, d
26.	Thymol methyl ester	1331	-	0.05	a, b, d
27.	*α*-Cubebene	1342	-	0.17	a, b, d
28.	2-Undecenal	1359	-	0.54	a, b, d
29.	*α*-Copaene	1364	1523	0.73	a, b, d
30.	*β*-Bourbonene	1376	1540	2.96	a, b, d, c
31.	*trans*-*β*-Damascenone	1387	1789	0.68	a, b, d
32.	*β*-Elemene	1389	-	1.42	a, b, d
33.	Dodecanal	1399	-	0.31	a, b, d
34.	*β*-Caryophyllene	1418	1586	7.05	a, b, d, c
35.	*trans* -*β*-Copaene	1423	-	0.66	a, b, d
36.	*β*-Gurjunene	1432	-	0.41	a, b, d
37.	*α*-Humulene	1443	1646	1.45	a, b, d
38.	*trans*-*β*-Farnesene	1462	1664	1.49	a, b, d,
39.	Germacrene D	1475	1689	21.45	a, b, d, c
40.	*α*-Selinene	1499	-	0.83	a, b, d,
41.	*α*-Muurolene	1503	1763	0.38	a, b, d
42.	Germacrene A	1509	-	0.27	a, b, d
43.	*β*-Bisabolene	1510	1713	1.08	a, b, d
44.	Butylated hydroxytoluene [Ional]	1515	1905	0.88	a, b, d
45.	*epi*-Bicyclosesquiphellandrene	1517	1582	0.03	a, b, d
46.	*δ*-Cadinene	1525	1736	2.16	a, b, d, c

Table 1. Cont.

47.	Cadina-1(2),4-dien	1532	-	0.34	a, b, d
48.	*α*- Cadinene	1535	-	0.22	a, b, d
49.	*α*- Calacorene	1540	-	0.16	a, b, d
50.	Nerolidol	1561	2014	0.36	a, b, c, d
51.	Spathulenol	1577	2037	0.79	a, b, d
52.	Caryophyllene Oxide	1584	1947	2.41	a, b, d, c
53.	Copaen-4-A-Ol	1585	-	0.43	a, b, d
54.	*nor*-Copaenone	1627	-	0.11	a, b, d
55.	Cadina-1,4-Dien-3-Ol	1628	-	0.19	a, b, d
56.	*epi*-α-Muurolol	1643	-	0.97	a, b, d
57.	*α*-Muurolol [Torreyol]	1649	-	0.7	a, b, d
58.	Amylcinnamalaldehyde	1662	2081	0.54	a, b, d
59.	*α*-Cadinol	1664	-	2.38	a, b, d
60.	Eudesmadienol derivative	1685	-	2.56	a, b, d
61.	Hexahydrofarnesyl acetone (6,10,14-Trimethyl-2-pentadecanone)	1851	2043	5.84	a, b, d
62.	Nonadecane	1900	1900	0.19	a, b, d
63.	Farnesyl acetone B	1925	-	0.24	a, b, d
64.	Hexadecanoic acid methyl ester	1926	-	0.14	a, b, d
65.	Eicosane	2000	2000	0.19	a, b, d
66.	Heneicosane	2100	2100	0.21	a, b, d
67.	Docosane	2200	2200	0.18	a, b, d
68.	Tricosane	2300	2300	1.4	a, b, d
69.	Tetracosane	2400	2400	0.27	a, b, d
70.	Pentacosane	2500	2500	1.21	a, b, d
71.	Hexacosane	2600	2600	0.06	a, c, d
72.	Heptacosane	2700	2700	1.19	a, c, d
73.	Octacosane	2800	2800	0.13	a, c, d
74.	Triacontane	3000	3000	0.66	a, c, d
75.	Hentriacontane	3100	3100	0.34	a, c, d
			Total:	86.37	

*Compounds listed in order of elution from a HP-5 MS column.

§Retention indices (KI) on HP-5 MS capillary column.

a= Retention time; b = Retention Index; c = Peak enrichment; d = mass spectra.

Table 2. Composition of *P. bovei* De Noé subsp. *bovei* essential oil by substance class.

Compounds	% in essential oil
Monoterpenes	0.15
Sesquiterpenes	43.26
Saturated	6.03
Hydrocarbons total :	***49.44***
Alcohols	18.38
Aldehydes	3.27
Ketones, Ethers, Acids, Esters, Oxides	12.28
Oxygenated compounds total:	***36.93***
Total compounds:	**86.37**

For the essential oil obtained from the leaves of *P. fruticosa* collected in Montenegro (Table 3) the main constituents were: *β*-caryophyllene (12.0%), (E)-methyl-isoeugenol (15.3%), *α*-asarone (10.9%), caryophyllene oxide (8.1%) and *α*-pinene (6.6%)[3]. The antimutagenic activity of the essential oil and of the crude extract was evaluated by the same research group [4]. Studies on the same plant, from the same region, have been conducted considering the antimicrobial and the antifungal activity of its essential oil, as well as its methanolic extract, with moderate results [5]. Traditionally the infusion of *P. fruticosa* leaves is used in Greece as a tonic drink, whereas in Italy the dried leaves are used as a poultice on wounds [6].

The flowers of *P. fruticosa* collected in Greece (Table 3) yielded an essential oil rich in germacrene D (17.8%), *γ*-bisabolene (12.6%), *α*-pinene (8.9%) and *β*-caryophyllene (8.7%) [7]. In another study on the essential oil from the aerial parts of *P. fruticosa* collected in central-East Peloponnesus, the main constituents were: germacrene D (21.4%), Z-*γ*-bisabolene (7.1%), *α*-pinene (12.6%) and *β*-caryophyllene (12.6%) and linalool (8.0%) [8]. In the same study, the volatile constituents of two other Greek *Phlomis* species - *P. cretica* and *P. samia* - were studied. For *P. cretica* the major compounds were: *α*-pinene (9.4%), limonene (7.1%), *cis*-*β*-ocimene (5.4%), linalool (7.5%), *β*-caryophyllene (17.3%) and germacrene D (20.1%). *P. samia* also exhibited large amounts of *β*-caryophyllene (5.8%), germacrene D (6.3%) and linalool (2.3%) but its major compound was (E)-*β*-farnesene (20.7%). The essential oils were tested against Gram (±) bacteria and fungi, showing moderate activity [8].

The main chemicals identified in the essential oil of the aerial parts of *P. lanata*, another *Phlomis* growing in Greece (Table 3) were: *α*-pinene (25.41%), limonene (15.67%), *β*-caryophyllene (8.76%), isocomene (4.91%) and *γ*-muurolene (4.53%). The essential oil of the plant was tested against Gram (±) bacteria and fungi. Like the previous study, it showed moderate antimicrobial activity, with the exception of *E. coli* and *P.aeruginosa*, towards which it exhibited stronger activity [9].

P. linearis Boiss. & Bal., growing in central East and Southeast Anatolia, an endemic *Phlomis* of Turkey, was characterized by the predominance of: *β*-caryophyllene (24.2%), germacrene D (22.3%) and caryophyllene oxide (9.2%) [10].

Table 3. Main components of the essential oils from different Mediterranean *Phlomis* species.

Components	***Phlomis bovei De Noé subsp. bovei***	***P.cretica* [8]**	***P.fruticosa* [3]**	***P.fruticosa* [7]**	***P.fruticosa* [8]**	***P.samia* [8]**	***P.linearis* [10]**	***P.lanata* [9]**	***P.leucophracta* [11]**	***P.cimereae* [11]**	***P.grandiflora* var.*grandiflora* [11]**
Hexahydro-Farnesyl Acetone	5.84	-	-	-	-	-	-	-	-	0.40	-
Spathulenol	0.79	0.10	0.50	-	-	3.70	-	-	0.30	-	0.40
α -Pinene	-	9.40	6.60	8.90	12.60	0.80	-	25.41	19.20	11.00	2.40
Limonene	0.05	7.10	0.50	0.40	0.90	0.10	-	15.67	11.00	5.50	2.70
cis-*β*-Ocimene	-	5.40	-	-	0.50	-	-	2.89	-	0.40	0.60
δ-Cadinene	2.16	1.20	0.90	1.80	1.00	2.40	1.00	1.51	0.40	5.00	1.30
(E)-Methyl-Isoeugenol	-	-	15.30	-	-	-	-	-	-	-	-
γ-Bisabolene	1.08	-	1.40	12.60	7.10	-	-	-	-	0.20	2.50
α-Asarone	-	-	10.90	-	-	-	-	-	-	-	-
Thymol	8.43	-	0.20	-	-	-	0.50	-	-	-	-
Germacrene D	21.45	20.10	2.30	17.80	21.40	6.30	22.30	-	4.50	6.10	45.40
β-Caryophyllene	7.05	17.30	12.00	8.70	12.60	5.80	24.20	8.76	20.20	31.60	22.80
γ-Muurolene	-	-	-	-	-	-	0.40	4.53	tr	-	tr
Linalool	-	7.50	0.60	0.70	8.00	2.30	0.60	0.78	-	4.70	0.60
E-*β*-Farnesene	1.49	-			0.60	20.70	-	-	1.10	0.50	1.00
Caryophyllene Oxide	2.41	0.60	8.10	1.90	0.80	3.20	9.20	2.86	1.70	4.80	0.40
Bicyclogermacrene	-	-	-	-	-	-	1.10	-	0.80	-	4.90

The essential oils of three other Turkish *Phlomis* species (Table 3) have also been studied previously [11]. The essential oil of *P. leucophracta* consisted mainly of *β*-caryophyllene (20.2%), *α*-pinene (19.2%) and limonene (11.0%), while in *P. chimerae* the principal compounds were *β*-caryophyllene (31.6%), *α*-pinene (11.0%), germacrene D (6.1%), limonene (5.5%) and linalool (4.7%), and in *P. grandiflora* var. *grandiflora*: germacrene D (45.4%), *β*-caryophyllene (22.8%) and bicyclogermacrene (4.9%) have been identified among the most abundant constituents [11]. The oils of *P. bovei De Noé* and of the other Mediterranean species: *P. grandiflora* var. *grandiflora* [11], *P. cretica* [8], *P. fruticosa* [3, 7, 8], *P. samia* [8], *P. linearis* [10], *P. lanata* [9], *P. leucophracta* [11] and *P. cimereae* [11], presented great amounts of the sesquiterpenoids germacrene D, *E*-*β*-farnesene and *β*-caryophyllene. In accordance to these results, in our study besides

the presence of germacrene D (21.45%) and *β*-caryophyllene (7.05%), hexahydrofarnesyl acetone (5.84%) has been also identified among the most abundant compounds, which could be considered as the biosynthetic predecessor of the above referred sesquiterpenoids, from the well known mevalonic acid pathway [12].

The essential oil of *Phlomis bovei* De Noé subsp. *bovei* exhibited a wide profile of antimicrobial activity against most of the tested microorganisms, in comparison with the tested antibiotics and the standards *β*-caryophyllene and thymol (Table 4), while only *K. pneumoniae* appeared to be a microorganism displaying significant resistence. Considering the fact that *β*-caryophyllene possesses in general moderate antimicrobial activity, we conclude that the antimicrobial activity of the essential oil from *P. bovei* can be attributed, to a considerable degree, to the presence of germacrene D and thymol, which are well known to posses strong antimicrobial activity [13-15].

Table 4. Antimicrobial activities (MIC mg/mL) of the studied *Phlomis* essential oils and its main components.

Species-Essential Oils	***S. aureus***	***S. epidermidis***	***P. aeruginosa***	***E. cloacae***	***K. pneumoniae***	***E. coli***	***C. albicans***	***C. tropicalis***	***C. glabrata***
P. bovei	0.98 ±0.004	0.85 ±0.007	1.00 ±0.011	1.37 ±0.018	4.75 ±0.035	1.12 ±0.018	1.35 ±0.008	0.95 ±0.015	0.89 ±0.013
β-Caryophyllene	>20	>20	>20	>20	>20	>20	-	-	-
Thymol	1.25 ±0.010	1.38 ±0.008	2.45 ±0.022	2.00 ±0.005	2.88 ±0.027	1.70 ±0.023	1.50 ±0.013	1.34 ±0.020	1.18 ±0.018
Itraconazole	-	-	-	-	-	-	$1x10^{-3}$	$0.1x10^{-3}$	$1x10^{-3}$
5-Flucytocine	-	-	-	-	-	-	$0.1x10^{-3}$	$1x10^{-3}$	$10x10^{-3}$
Amphotericin B	-	-	-	-	-	-	$1x10^{-3}$	$0.5x10^{-3}$	$0.4x10^{-3}$
Netilmicin	$4x10^{-3}$	$4x10^{-3}$	$8.8x10^{-3}$	$8x10^{-3}$	$8x10^{-3}$	$10x10^{-3}$	-	-	-
Amoxicillin	$2x10^{-3}$	$2x10^{-3}$	$2.4x10^{-3}$	$2.8x10^{-3}$	$2.2x10^{-3}$	$2x10^{-3}$	-	-	-
Clavulanic acid	$0.5x10^{-3}$	$0.5x10^{-3}$	$1x10^{-3}$	$1.6x10^{-3}$	$1x10^{-3}$	$1.2x10^{-3}$	-	-	-

- = not active

Conclusions

Our GC and GC/MS study of the essential oil from Algerian *Phlomis bovei* De Noé led to the identification of 75 constituents (corresponding to 86.37% of the total weight) among which germacrene D, *β*-caryophyllene, *β*-bournonene, thymol, and hexahydrofarnesyl acetone were the main ones. The oil exhibited a broad spectrum of strong antimicrobial activities and it possessed a much better antimicrobial activity in comparison with all previously tested and assayed samples from Greek *Phlomis* species [8], showing that this plant oil could have a commercial potential as an antiseptic agent, however, further investigation should be carried out against new series of pathogenic microorganisms.

Experimental

Plant material and essential oil isolation

Aerial parts of *Phlomis bovei* De Noé were collected from the wild in July 2004 at ca. 1,550 m of altitude on Megriss Mountain (Eastern Algeria). The plants were authenticated by the staff of the Laboratory of Natural Resource Valorization by comparison with herbarium specimens. Voucher specimens are deposited in the Herbarium of the Institute of Biology, University of Setif, Algeria. The material was air-dried indoors prior to isolation of the essential oil. The dried aerial parts were subjected to hydro-distillation in 0.4 L of water in a Clevenger-type apparatus for 4 hrs, using a water-cooled oil receiver to reduce formation of potential artifacts due to overheating during the hydro-distillation process [16]. The essential oil was collected over water, dried over anhydrous sodium sulfate (Panreac Quimica S.A. Barcelona, Spain) and stored at 4^{o}–6 ^{o}C until it was analyzed.

Essential oil analysis

The oil was analysed by GC on a Perkin-Elmer 8500 gas chromatograph equipped with a FID, fitted with a Supelcowax-10 fused silica capillary column (30 m x 0.32 mm; film thickness, 0.25 μm). The column temperature was programmed from 75 ^{o}C to 200 ^{o}C at a rate of 2.5 ^{o}C/min. The injector and detector temperatures were programmed at 230 ^{o}C and 300 ^{o}C, respectively. Helium was used as carrier gas at flow rate of 0.6 mL/min. The GC-MS analysis was carried out using two different GC-MS systems. The first was a Hewlett Packard 5973-6890 GC-MS operating on EI mode (equipped with a HP 5MS 30 m x 0.25 mm x 0.25 μm film thickness capillary column). Helium (1 mL/min) was used as carrier gas. Temperature program: initial temperature of the column was 60 °C (for 5 min), then raised to 280 ^{o}C at 3 °C/min, and held there for 30 min (total time: 93.33 min). The compounds were identified by comparison of their retention indexes (RI) [17], retention times (RT) and mass spectra with those of authentic samples and/or the NIST/NBS, NIST02, Wiley 575 libraries spectra and the literature [18]. The percentage composition of the essential oil is based on peak areas obtained without FID factor corrections. The second GC-MS system analysis was a Finnigan Trace GC Ultra system, operating on EI mode and equipped with AT™ Aquawax 30 m x 0.32 mm x 0.25 μm film thickness capillary column. Helium was used as the carrier gas, at a flow rate of 1.5 mL/min (constant flow) and a 1:10 split ratio. Temperature program: initial temperature of the column 60 °C (for 5 min), then raised to 235 ^{o}C at 3°C/min, and held there for 30 min (total time: 93.33 min). The MS parameters were as follows: source temperature, 200 °C; ionization energy, 70 eV; emission, 200 μA; mass range, 35-650 Da; scan time,1.25 s., scan rate (amu/s) 500.0; scans per second, 0.7974.

Antimicrobial activity

Antimicrobial activity of the essential oils against bacteria and fungi was determined by using the agar dilution technique. The microorganisms included two Gram-positive bacteria: *Staphylococcus aureus* (ATCC 25923) and *Staphylococcus epidermidis* (ATCC 12228); four Gram-negative bacteria: *Escherichia coli* (ATCC 25922), *Enterobacter cloacae* (ATCC 13047), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 227853); and the pathogenic fungi *Candida albicans*

(10231), *C. tropicalis* (13801) and *C. glabrata* (28838). Standard antibiotics (netilmicin and amoxicillin with clavulanic acid) were used as controls for the sensitivity of the tested bacteria and 5-flucytocine, amphotericin B and itraconazole were used as controls for the tested fungi. The technical details have been described previously [19]. Minimum inhibitory concentrations (MICs) were determined for oil samples and the standard pure compounds *β*-caryophyllene and thymol (Extrasynthese SAS, France), under identical conditions, for comparison purposes. Statistical analysis: data are expressed as means $\pm$ S.D.

References

1. Quezel, P.; Santa, S. In *Nouvelle flore de l'Algérie et des régions désertiques méridionales*, TII, CNRS: Paris, **1963**; p. 812.
2. Trabut L. In *Répertoire des noms indigènes des plantes spontanées, cultivées et utilisées dans le Nord de l'Afrique*. *Collection du centenaire de l'Algérie.* Imprimeries "La Typo-Litho" et Jules Carbonnel Réunies: Alger, **1935**; p. 190**.**
3. Sokovic, M.D.; Marin, P.D.; Janackovic, P.; Vajs, V.; Milosavljevic, S.; Dokovic, D.; Tesevic, V.; Petrovic, S. Composition of the essential oils of *Phlomis fruticosa* L. (Lamiaceae). *J. Essent. Oil Res.* **2002**, *14*, 167-168.
4. Sokovic, M.D.; Marin, P.D.; Simic, D.; Knezevic-Vukcevic, J.; Vajs, V.; Petrovic, S. Antimutagenic activity of essential oil and crude extract of *Phlomis fruticosa*. *Pharm. Biol.* **2002**, *40*, 311-314.
5. Ristic, M.D.; Duletic-Lausevic, S.; Knezevic-Vukcevic, J.; Marin, P.D.; Simic, D.; Vukojevic, J.; Janackovic, P.; Vajs, V. Antimicrobial activity of essential oils and ethanol extract of *Phlomis fruticosa* L. (Lamiaceae). *Phytother. Res.* **2000**, *14*, 267-271.
6. Tammaro, F., Xepapadakis, G. Plants used in phytotherapy, cosmetics and dyeing in the Pramanda district (Epirus, northwest Greece). *J Ethnopharmacol.* **1986**, *16*, 167-74.
7. Tsitsimi, E.; Loukis, A.; Verykokidou, E. Composition of the essential oil of the flowers o*f Phlomis fruticosa* L. from Greece. *J. Essent. Oil Res.* **2000**, *12*, 355-356.
8. Aligiannis, N.; Kalpoutzakis, E.; Kyriakopoulou, I.; Mitaku, S.; Chinou, I.B. Essential oils of *Phlomis* species growing in Greece chemical composition and antimicrobial activity. *Flav. Frag. J.* **2004**, *19*, 320-324.
9. Couladis, M.; Tanimanidis, A.; Tzakou, O.; Chinou, I.B.; Harvala, C. Essential oil of *Phlomis lanata* growing in Greece: chemical composition and antimicrobial activity. *Planta Med.* **2000**, *66*, 670-672.
10. Demirci, B.; Dadandi, M.Y.; Paper, D.H.; Franz, G.; Baser, K.H. Chemical composition of the essential oil of *Phlomis linearis* Boiss. & Bal., and biological effects on the CAM-assay: a safety evaluation. *Z. Naturforsch. C* **2003**, *58*, 826-829.
11. Celik, S.; Gokturk, R.S.; Flamini, G.; Cioni, P.L.; Morelli, I. Essential oils of *Phlomis leucophracta, Ph. chimerae* and *Ph.grandiflora* var. *grandiflora* from Turkey. *Biochem. Syst. Ecol*. **2005**, *33*, 617-623.
12. Umlauf, D.; Zapp, J.; Becker, H.; Adam, K.P. Biosynthesis of the irregular monoterpene artemisia ketone, the sesquiterpene germacrene D and other isoprenoids in *Tanacetum vulgare* L. (Asteraceae). *Phytochemistry* **2004**, *65*, 2463–2470.

13. Juteau, F.; Masotti, V.; Bessière, J.M.; Dherbomez, M.; Viano, J. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia* **2002**, *73*, 532-535.
14. Ettayebi, K.; El Yamani, J.; Rossi-Hassani, B.D. Synergistic effects of nisin and thymol on antimicrobial activities in *Listeria monocytogenes* and *Bacillus subtilis*. *FEMS Microbiol. Letts.* **2000**, *183*, 191-195.
15. Olasupo, N.A.; Fitzgerald, D.J.; Gasson, M.J.; Narbad, A. Activity of natural antimicrobial compounds against *Escherichia coli* and *Salmonella enterica* serovar *Typhimurium Lett. Appl. Microbiol*. **2003**, *37*, 448-451.
16. *British Pharmacopoeia 1993, Vol. I, International Ed*.; HMSO: London, **1993**.
17. Massada, Y. *Analysis of Essential Oil by Gas Chromatography and Spectrometry*; John Wiley & Sons: New York, **1976**.
18. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*; Allured: Carol Stream, IL, USA, **2001**.
19. Ngassapa, O.; Runyoro, D.K.B.; Harvala, E.; Chinou, I.B. Composition and Antimicrobial Activity of the Essential Oils of two different populations of *Lippia javanica* growing in Tanzania. *Flav. Frag. J.* **2003**, *18*, 221-224.

Sample availability: Samples of the essential oils are available from the authors.