Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: http://www.tandfonline.com/loi/teop20

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To cite this article: Fethi Ahmet Ozdemir, Omer Kilic & Sinasi Yildirimli (2017) Essential Oil Composition and Antimicrobial Activity of Endemic Phlomis sieheana Rech. From Bingol (Turkey), Journal of Essential Oil Bearing Plants, 20:2, 516-523, DOI: 10.1080/0972060X.2017.1304833

To link to this article: http://dx.doi.org/10.1080/0972060X.2017.1304833

Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Essential Oil Composition and Antimicrobial Activity of Endemic *Phlomis sieheana* Rech. From Bingol (Turkey)

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Received 02 December 2016; accepted in revised form 08 March 2017

Abstract: *Phlomis sieheana* Rech. is a native plant belongs to Lamiaceae family; which can use in modern medicine and in different industries for its essential oils. The chemical composition essential oils of *Phlomis sieheana* was analyzed by GC-MS. Eventually fifty six components, representing 89.6 % of the total oil were identified. The main compounds of *P. sieheana* were determined as β-caryophyllene (10.8 %), germacrene D (15.6 %) and α-pinene (7.8 %). The chemical distribution of the essential oil compounds in the genus pattern discussed in means of natural products. Furthermore, the antimicrobial activity of the oil was evaluated against seven Gram-positive bacteria (*Bacillus subtilis* ATCC 6337, *Brevibacillus brevis*, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IM 622, *Bacillus cereus* EMC 19, *Staphylococcus aureus* 6538 P, *Listeria monocytogenes* NCTC 5348) and the nine Gram negative bacteria (*Salmonella typhimurium* NRRLE 4413, *Pseudomonas fluorescens*, *Enterobacter aerogenes* CCM 2531, *Klabsiella pneumoniae* EMCS, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC II, *Pseudomonas aeruginosa* DSM 50070, *Proteus vulgaris*, *Salmonella enterica* ATCC 13311) using the disc diffusion method. It was found that the oil exhibited strong antimicrobial activity against all of the tested microorganisms.

Key words: *Phlomis sieheana*, essential oil, antimicrobial activity

Introduction

Lamiaceae has many economical, medicinal and aromatic plant taxa. Genus *Phlomis* L. is in the Lamiaceae family and consist of more than 100 taxa spreaded in many part of the world [1]. In Turkey the *Phlomis* is represented about fifty two taxa, of which thirty four are endemic [2]. Endemic species *Phlomis sieheana* generally grows in steps, rocky igneous slopes and has a habitat from Central to East Anatolia. Aerial parts of aromatic *Phlomis* taxa are used as carminative, heart enhancer, antiseptic, anticancer, boil cure and abdominal pain in folk medicine in Turkey [3-5]. Some *Phlomis* taxa have also been investigated for their antiinflammatory, antimutagenic, anti-nociceptive, antifibriel, free radical scavenging, anti-allergic, anti-malarial, antimicrobial effects and essential oil compositions [6-12]. In the essential oil of *Phlomis fruticosa*; β-caryophyllene, (E)-methyl-isoeugenol and α-asarone were detected as major constituents [13]; the antimutagenic activity of the essential oil and crude extract of this species was evaluated by the same research group [14]. In another research, the flowers of *P. fruticosa* ess-

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ential oil was found to be rich in germacrene D, γ-bisabolene, α-pinene and β-caryophyllene [15].

Medicinal and aromatic plants like some *Phlomis* taxa are considered essential raw material source for the discovery of new molecules necessary for the development of new natural pruducts. Medicinal and aromatic plants are an important source of immense variety of bioactive molecules; so these plants contain rich essential oils that stimulates interests for use in the food industry, in ethnobotany, in cosmetics, in pharmacy and play a very important role against cancer, cardiovascular disease and can be used for their antimicrobial effects [16]. In order to discover some medicinal properties of genus *Phlomis* and *Stachys* L. the antimicrobial activity of the methanolic extracts of *Phlomis bruguieri*, *P. herba-venti*, *P. olivieri* and some *Stachys* genuses are investigated [16]. The methanolic extracts of these plants were more active against gram positive microorganisms. Antibacterial activities of *Phlomis caucasica* from Iran were evaluated against a range of gram positive and negative bacterial strains [17]. The antibacterial activity of aerial part of *Phlomis pungens* var. *hirta* was evaluated by disc diffusion method. The hexane, acetone and methanol extracts were tested against nine bacteria [18]. There have been no previous essential oil composition and antimicrobial activity studies on endemic *P. sieheana*.

In this study we aimed to determine essential oil composition and antimicrobial activity of the essential oil of endemic *P. sieheana*.

Materials and methods

Plant materials

Plant samples was collected in natural habitats from vicinity of Hazersah village (Solhan-Bingöl), Aksakal Ggl position, steppe, on 30.06.2016, at an altitude of 1600-1700 m., by O.Kilic 5349. Plant sample was identified by Kilic with Flora of Turkey and East Aegean Islands [19]. The voucher specimens have been deposited at the Technical Vocational College, Department of Park and Garden Plants, Bingol University and in Herbarium of Yildirimli from Ankara (Turkey).

Isolation of the essential oil

Aerial parts of the dried *P. sieheana* (200 g) were exposed to hydrodistillation using a Clevenger apparatus for four hour.

Gas chromatography - Mass spectrometry

The essential oil of plant samples were analyzed with 60-m long column packed with CP-Wax 52 CB 0.25 mm i.d. in Bingol University. The column and analyzes circumstances were the same as in GC-MS. The percentage of the essential oil composition was calculated from GC-FID peak areas without correction factors. A Varian 3800 gas chromatograph, exactly interfaced with a Varian 2000 ion trap mass spectrometer, was used with a splitless injection mode and an injector temperature of 260°C. The oven temperature was 45°C held for 5 min, then increased to 80°C at a rate of 10°C min^{-1}, and to 240°C at a rate of 2°C min^{-1}. Helium was the carrier gas which used at a stable pressure of 10 psi; the transfer line temperature was 250°C; with an electron impact ionisation mode an acquisition range of 40 to 200 m z^{-1} and a scan rate of 1 us^{-1}. Alkanes were used as reference points in the calculation of relative retention indices (RRI).

Supple-mantary identification was determined using Wiley and Nist libraries, mass spectral library and verified by the retention indices which were calculated as described by Van den Dool and Kratz [20, 21]. The relative amounts were calculated on the basis of peak-area ratios. The essential oils composition of studied sample is showed in Table 1.

Antimicrobial assay

The antimicrobial activity of the essential oil *Phlomis sieheana* was tested against selected seven Gram-positive bacteria (*Bacillus subtilis* ATCC 6337, *Brevibacillus brevis*, *Bacillus megaterium* DSM 32, *Bacillus subtilis* IM 622, *Bacillus cereus* EMC 19, *Staphylococcus aureus* 6538 P, *Listeria monocytogenes* NCTC 5348) and the nine Gram negative bacteria (*Salmonella typhimurium* NRRLE 4413, *Pseudomonas fluorescens*, *Enterobacter aerogenes* CCM 2531, *Klabsiella pneumoniae* EMCS, *Escherichia coli* ATCC 25922, *Proteus vulgaris* FMC II, *Pseudomonas aeruginosa* DSM 50070, *Proteus vulgaris*, *Salmonella enterica* ATCC 13311) using the disc diffusion method. The essential oil was dissolved in dimethyl sulphoxide containing 12.0 mg/l. Briefly, suspension in Nutrient broth of the tested microorganism (100 µl of 10^6 cell/ml) was spread on the solid Mueller Hinton Agar media plates. Paper discs (6 mm in diam., Bioanalyse) were impregnated with 20 µl of the essential oil and placed on the inoculated plates. Plates were placed at 4°"C for two hours, then were incubated at 37°C for twenty four hours. The diameter of the inhibition zones were measured in millimeters. Control disks with 20 µl DMSO showed no inhibition zone. All the tests were repeated triplicate.

Results and discussion

Dried aerial parts of *Phlomis sieheana* was analyzed by GC-MS. The essential oil composition of studied plant is shown in (Table 1). 56 components representing 89.6% of the total oil were identified. Germacrene D (15.6 %), β-caryophyllene (10.8 %), and α-pinene (7.8 %) were identified as the main constituents of this native plant. In this research, Germacrene D (15.6 %) was found to be one of the predominant compounds. Germacrene D was also identified as the main constituent of *P. lunariifolia* (7.7 %), *P. sieheana* (16.6 %) and *P. armeniaca* (23.4 %) [22]. In the essential oil composition of *P. olivieri* from Iran sesquiterpenes (germacrene D and β-caryophyllene) were among the major components [23]. α-Pinene (25.4 %), limonene (15.7 %) and *trans*-caryophyllene (8.8 %) were found as major components of *Phlomis lanata* essential oil [13]. In this research Germacrene D (15.6 %), β-caryophyllene (10.8 %) and α-pinene (7.8 %) were also detected as the major compounds

Essential oils are highly concentrated, volatile, hydrophobic mixtures of chemicals extracted from different parts of plants. The name essential derives from the very aromatic nature of the oils and essential oils are most commonly extracted by steam distillation, while organic solvent extraction is also sometimes used. Lamiaceae family is rich in respect to essential oils. Essential oils have characteristic flavor and fragrance properties and many of them also possess antimicrobial activities. In addition, essential oils are used in many industries and they are widely used for aromatherapy and in other alternative healthcare products.

In the essential oil of *P. lanceolata*, germacrene D (47.0 %), (E)-β-farnesene (10.5 %) and α-pinene (8.7 %), were the main compounds. In *P. anisodonta* germacrene D (65.0 %), β-caryophyllene (11.0 %); and in *P. bruguieri* germacrene D (60.5 %), γ-elemene (16.5 %) and germacrene

Table 1. Essential oil composition of *P. sieheana*

No.	Compounds	RRI	% Percentage
1	α-Pinene	1032	7.8
2	α-Thujene	1037	0.2
3	β-Pinene	1120	0.1
4	β-Mrycene	1170	0.1
5	Limonene	1195	0.3
6	Nonanal	1395	0.1
7	α-Cubebene	1465	2.3
8	α-Ylangene	1490	0.3
9	Bicycloelemene	1495	0.4
10	α-Copaene	1498	1.8
11	Decanal	1505	0.2
12	β-Bourbenene	1530	1.2
13	β-Cubebene	1545	0.5
14	Linalool	1550	0.8
15	Isocaryophyllene	1585	0.3

table 1. (continued).

No.	Compounds	RRI	% Percentage
16	β-Copaene	1598	0.6
17	β-Elemene	1605	1.1
18	β-Caryophyllene	1610	10.8
19	Aromadendrene	1625	0.3
20	β-Farnesene	1662	5.9
21	Ledene	1705	0.2
22	Germacrene D	1725	15.6
23	α-Muurolene	1735	1.3
24	β-Selinene	1745	0.6
25	Bicyclogermacrene	1755	4.8
26	α-Cadinene	1770	0.8
27	γ-Cadinene	1775	0.2
28	α-Selinene	1782	0.5
29	Decadienal	1822	1.2
30	Calamenene	1845	0.3
31	Germacrene-B	1852	0.7
32	Geranyl acetone	1865	0.4
33	1,5-Epoxy-salvial(4)14-ene	1935	0.9
34	Cubebol	1945	0.2
35	Isocaryophyllene oxide	1995	0.3
36	Caryophyllene oxide	2005	2.3
37	Salvial-4(14)-en-1-one	2035	0.5
38	(E)-Nerolidol	2045	0.3
39	Humulene epoxide	2070	0.7
40	1-*epi*-Cubenol	2085	0.3
41	Globulol	2092	1.6
42	Viridiflorol	2100	0.8
43	Hexahydrofarnesyl acetone	2125	1.2
44	Spathulenol	2140	1.8
45	α-Muurolol	2200	0.9
46	Carvacrol	2236	2.5
47	*trans*-α-Bergamotol	2240	0.6
48	α-Cadinol	2250	1.7
49	Decanoic acid	2290	0.2
50	Tricosane	2300	1.2
51	Tetracosane	2390	0.8
52	Pentacosane	2450	1.8
53	Dodecanoic acid	2530	0.9
54	Tetradecanoic acid	2670	0.3
55	Heptacosane	2700	2.1
56	Hexadecanoic acid	2920	3.9
	Total		89.6

RRI: Relative Retention Indices

B (7.1 %) were found to be as dominant constituents [22]. α-Pinene has been identified in *P. lanata* (25.4 %), *P. olivieri* (11.7 %) and *P. fruti-cosa* flowers (8.9 %) as the main components [23]. In this study α-pinene was also (7.8 %) detected to be as the main compound (Table 1), whereas in other reported analyses on *Phlomis* spp. oils the amount of α-pinene was in low amounts [24,25]. In *P. leucophracta* α-pinene (19.2 %) and limonene (11.0 %); in *P. chimerae*, α-pinene (11.0 %), limonene (5.5 %), linalool (4.7 %) were the principal monoterpenes. In *P. grandiflora* var. *grandiflora*, monoterpenes were little represented (7.4 %), with α-pinene (2.4 %) and limonene (2.7 %) as main constituents. In the essential oil of these three species of *Phlomis*, the main monoterpenes were α-pinene and limonene and the main sesquiterpenes were β-caryophyllene and germacrene D. In this study also α-pinene was among the main monoterpenes, and the main sesquiterpenes were β-caryophyllene and germacrene D (Table 1).

The evaluation of the antimicrobial activity of the essential oil tested against selected bacterial species (Table 2). Previous antimicrobial activity studies on various *Phlomis* taxa from different localities showed inhibitory activity against a wide spectrum of microorganisms [26-30]. However, this is the first study reporting on the antimicrobial activity of endemic *P. sieheana* essential oil. The essential oil aerial part of *P. sieheana* exhibited moderate to strong antimicrobial avtivity against the tested microorganisms. The inhibitory effects were compared with DMSO. The essential oil of *P. sieheana* has shown larger growth inhibition zone diameters (24 and 26 mm) against the gram positive tasted bacterial strains as compared with gram negative bacteria (10 and 15 mm). Different studies showed that essential oils of *Phlomis* taxa were found more active against Gr (+) bacteria when compared to Gr (-) [31,32] which support our findings. Low antibacterial activity against Gram-negative bacteria was ascribed to the presence of an outer membrane, which possessed hydrophilic polysaccharide chains as a barrier to hydrophobic essential oil and phospholipid, and more pores in cell envelope [33]. In a study, *Salmonella typhimurium* was one of the most resistant Enterobacteriaceae to plant extracts [34]. Therefore, we believe that *P. sieheana* essential oil

Table 2. Antimicrobial activity of the essential oil of *P. sieheana*

Microorganisms	Gram	Inhibition zone diameter (mm)
Bacillus subtilis ATCC 6337	Positive	24
Brevibacillus brevis	Positive	21
Bacillus megaterium DSM 32	Positive	23
Bacillus subtilis IM 622	Positive	22
Bacillus cereus EMC 19	Positive	20
Staphylococcus aureus 6538 P	Positive	23
Listeria monocytogenes NCTC 5348	Positive	26
Salmonella typhimurium NRRLE 4413	Negative	12
Pseudomonas fluorescens	Negative	11
Enterobacter aerogenes CCM 2531	Negative	13
Klabsiella pneumoniae EMCS	Negative	14
Escherichia coli ATCC 25922	Negative	15
Proteus vulgaris FMC II	Negative	10
Pseudomonas aeruginosa DSM 50070	Negative	12
Proteus vulgaris	Negative	10
Salmonella enterica ATCC 13311	Negative	11
DMSO	Control	0

would be of interest as far as it was active toward this strain. Among the tested bacteria in the present study, *Listeria monocytogenes* was the more sensitive to the essential oil, while *Proteus vulgaris* appeared to be the most resistant.

Generally four factors are paticularly important when testing essential oils for antimicrobial activity: the growth medium, the assay method, the microorganism and the essential oil; because of the high viscosity of essential oils, a diffusion of higher concentrations through the agar medium takes place with difficulty. A reason for the phenomenon observed may be that not only the antimicrobial activity but also the physicochemical features governing the transport rate of the constituents are important for the inhibition of a microorganism in an agar overlay assay [35]. Testing and evaluation of the antimicrobial activity of essential oils is difficult because of their volatility, their water (in-) solubility, and their complexity; however, from the given results it may be concluded that essential oils of *Phlomis sieheana* possess antimicrobial activity. Further researches could comprise bioassay-guided fractionation to characterize the active constituents and their use in pharmacy and medicine.

The presented study support to an important degree the traditional medicinal uses and antimicrobial effect of the *P. sieheana* and reinforce the concept that the ethnobotanical approach to screening plants as potential sources of bioactive substances could be successful. Furthermore, in the present study the essential oils showed a potentially antimicrobial effect against both Gram-positive and Gram-negative bacteria.

Essential oils generally show selective toxicity towards various pathogens and are relatively safe both to animals and humans. In complex mixtures like essential oils, synergism of individual components is also expected so that microorganisms hardly can develop resistance towards essential oils. According to studied results in the field of identifying the chemical composition in different species essential oil *Phlomis* genus, probably germacrene D can be introduced as the indicate components of this genus. Endemic *P. sieheana* essential oils evaluated in this study showed varying inhibitory activity on all tested microorganisms. It is worthwhile also to test other fractions for their antimicrobial activity potential.

Acknowledgements

The authors acknowledge the Scientific and Research Council of Bingol University (BAP - TBMYO.2016.00.001) for support this study.

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