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Original article

Essential oils of four *Phlomis* species growing in Iran: chemical composition, antimicrobial and antifungal activity

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Summary. The essential oils of four *Phlomis* species (*Phlomis olivieri*, *Phlomis bruguieri*, *Phlomis rigida* and *Phlomis kurdica*), collected from kordestan province of Iran during the flowering stage, were hydrodistillated and analyzed by gas chromatography/mass spectrometry (GC/MS). Totally, 20 compounds in the oil of *P. olivieri* (representing 86.3%), 17 compounds in the oil of *P. bruguieri* (representing 80.5%), 17 compounds in the oil of *P. rigida* (representing 93.6%) and 26 compounds in the oil of *P. kurdica* (representing 92.1%) were identified. The major components of all examined oils were germacrene D, trans-caryophyllene and caryophyllene oxide except for *P. rigida* which was rich in tetradecanoic acid (29.6%). The antimicrobial activities of the oils were tested against a gram-positive, two gram-negative bacteria and a pathogenic fungus. The essential oils of *P. kurdica* and *P. oliveri* showed moderate antifungal activity (MICs: 20 and 25 mg/ml, respectively) against *C. albicans*. The essential oil of *P. rigida* was found to be the most active oil against *Staphylococcus aureus* and *Candida albicans* comparing to other oils (MICs: 6 and 3 mg/ml, respectively) that possibly attributed to the high amount of tetradecanoic acid.

Key words: *Phlomis*, germacrene D, trans-caryophyllene, *Staphylococcus aureus*

Introduction

The genus *Phlomis* (Lamiaceae) comprises 100 species, which are native to the Mediterranean region across central Asia to China. This genus represented by 17 species, 10 of which are endemic such as *P. olivieri*, *P. bruguieri*, *P. rigida*, and *P. kurdica* in Iran (1, 2). Several species of *Phlomis* are used in folk medicine as tonics, stimulants, diuretics and for the treatment of haemorrhoid and respiratory tract diseases (3-6). In addition, there are several reports indicating various biological and pharmacological activities for some plants of this genus such as anti-malarial (7), antimicrobial (8-10), anti-allergic (11), antifibriel effects (12), immunosuppressive and free radical scavenging properties (13). Different classes of secondary metabolites comprising diterpenoids, iridoids, phenylpropanoids, phenylethanoids and flavonoids have been previously identified in *Phlomis* genus (14-18).

The emergence of antibiotic resistance in microorganisms makes it necessary to continue the search for new antimicrobial active substances. Natural products for isolation of antibacterial agents have attracted scientist's interest in previous investigations (19-21). The aim of this study was to investigate the chemical compositions and antimicrobial activities of essential oils of four species of *Phlomis* genus.

Material and Methods

Plant material

Arial parts of *P. olivieri*, *P. bruguieri*, *P. rigida* and *P. kurdica* was collected during the flowering period from Kurdistan province in Iran and deposited with voucher specimens of ACECR-1612, ACECR-1581, ACECR-1557 and ACECR-1588, respectively at the Herbarium of Complex of Academic Center for Educational and Cultural Research.

Isolation of the essential oil

The fresh aerial parts of the plants (200 g) were submitted to hydrodistillation using Clevenger type apparatus for 4 h (22). The oils were dried over anhydrous sodium sulphate (Merck, Darmstadt, Germany) and stored in sealed dark glass vials at 4°C for further investigations.

GC/MS analysis

The GC/MS analysis was carried out on a HP-5973 mass selective detector in the electron impact (EI) ionization mode (70 eV); Hewlett-Packard 6840 gas chromatograph; capillary column HP-5 MS (30 m × 0.25 mm; film thickness: 0.25 μm), which was scheduled as follows: 60°C for 3 min, rising to 230°C at 6°C/min. The carrier gas was helium at a flow rate of 2 ml/min. Retention indices were calculated by using retention times of n-alkanes that were injected after the oil at the same chromatographic conditions. The compounds were identified by comparison of retention indices (RRI) with those reported in the literature and by comparison of their mass spectra with the Wiley library (23) or with the published mass spectra (24).

Antimicrobial and antifungal Activity

Antimicrobial activities of the oils were evaluated using agar dilution method and the minimum inhibitory concentrations (MICs) regarding each oils of the plants were assessed (25, 26). Microorganism including *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853) and *Candida albicans* (ATCC 10231) were obtained from the Department of Microbiology, Faculty of Medical Sciences, Tehran University of Medical Sciences. The Mueller-Hinton broth was used for bacteria, while Sabouraud dextrose broth was used for growing the *C. albicans*. The incubation conditions used were 24 h at 37°C for the bacteria and 48 h at 28°C for the *C. albicans*. All samples was resuspended in DMSO, ranging from 90 mg/ml to 0.75 mg/ml. Serial dilutions of the samples solutions in broth medium (100 μl Muller-Hinton broth or on Sabouraud Dextrose Broth) were prepared in a microplate (96 wells). Then 50 μl microbial suspension of the tested microorganism (of 10^5 cells ml in sterile distilled water) was added to each well. Broth was used as negative control and standard antibiotics (Erythromycin, Gentamicin, and Imipenem) were used in order to control the sensitivity of the tested bacteria.

Results and Discussion

The GC/MS analysis of the essential oil of *P. olivieri*, *P. bruguieri*, *P. rigida* and *P. kurdica* is reported in Table 1 that yielded 0.1%, 0.98%, 0.90% and 0.97% w/w of fresh weight materials, respectively. The essential oils were light yellow with a distinct sharp odor. Twenty constituents were identified from the oil of *P. olivieri*, representing 86.3% of the total oil. germacrene D (15.1%), trans-caryophyllene (12.6%), tetradecanoic acid (9.4%), and dodecanoic acid (8.7%) were found its major compounds. seventeen constituents were identified from the oil of *P. bruguieri*, representing 80.5% of the total oil. Caryophyllene oxide (16.3%), γ-Muurolene (15.5%), α-Selinene (7.1%), and cis-Calamene (6.7%) were identified the main compounds of the plant oil. Seventeen components were characterized from the oil of *P. rigida*, representing 93.6% of the total oil. The major constituents of the oil were tetradecanoic acid (29.6%), trans-caryophyllene (17%), germacrene D (13.4%), and α-Selinene (7.3%). Twenty six constituents were identified from the oil of *P. kurdica*, representing 92.1% of the total oil. Dodecanoic acid (10%), germacrene D (9.9%), trans-caryophyllene (8.7%), α-Selinene (6.3%) were composed the main constituents of the oil. The main sesquiterpenes in the essential oil of these four species of *Phlomis* were identified trans-caryophyllene and germacrene D. Germacrene D have been identified in other species of *Phlomis* as the main components (27, 28).

Regarding the main compounds of the oils, the examined species could be considered chemically similar in composition of their essential oils. However, many differences can be noted in the percentage distribution of monoterpenes and sesquiterpenes. All the tested oils were rich in sesquiterpene hydrocarbons as well as oxygenated sesquiterpenes. The total amounts of the sesquiterpene fractions in the oils of *P. olivieri*, *P. bruguieri*, *P. rigida* and *P. kurdica* were 63.9%, 62.6%, 60.5% and 67%, respectively. Although, the tested specious contained scarce amounts of monoterpene hy-

Table 1. Chemical constituents and percentage of the essential oils of *P. olivieri*, *P. bruguieri*, *P. rigida* and *P. kurdica* based on GC/MS analysis.

NO.	Compounds	Percent (%)				
		P. olivieri	*P. bruguieri*	*P. rigida*	*P. kurdica*	RRI[a]
1	α-Pinene	-	5.5	-	3.2	939
2	1-Octen-3-ol	-	-	-	0.6	979
3	β-Pinene	-	-	-	0.7	981
4	Limonene	-	-	-	1	1029
5	Terpinolene	0.9	0.7	-	-	1089
6	n-Undecane	-	-	-	0.8	1100
7	n-Nonanal	1	0.9	0.4	0.4	1101
8	Camphor	0.9	-	-	-	1146
9	α-Terpineol	0.9	-	-	0.8	1189
10	n-Decanal	-	-	0.7	0.3	1202
11	Geraniol	-	0.7	-	0.8	1253
12	Nonanoic acid	0.6	-	0.8	1	1271
13	α-Cubebene	-	-	-	5.9	1351
14	α-Copaene	-	1	-	3.6	1377
15	β-Damascenone	-	4	1.6	-	1385
16	β-Bourbonene	2.5	-	1.4	5.2	1388
17	α-Gurjunene	-	4	-	1.3	1410
18	trans-Caryophyllene	12.6	1	17	8.7	1419
19	β-Farnesene	-	-	1.5	-	1443
20	α-Caryophyllene	1.6	-	-	-	1454
21	α-Humulene	-	1.6	1	5.6	1455
22	γ-Muurolene	1.2	15.5	-	-	1480
23	Germacrene D	15.1	3.6	13.4	9.9	1485
24	β-Selinene	8.4	3.2	5.4	4.7	1490
25	α-Selinene	5.6	7.1	7.3	6.3	1495
26	Bicyclogermacrene	1.8	2.6	1.5	2.6	1500
27	γ-Cadinene	0.9	-	-	-	1514
28	δ-Cadinene	1.6	-	0.8	3.1	1523
29	trans-Nerolidol	-	-	1.8	-	1533
30	cis-Calamene	-	6.7	-	0.8	1540
31	Dodecanoic acid	8.7	6.1	-	10	1567
32	Spathulenol	2.2		3.5	3.7	1578
33	Caryophyllene oxide	7.5	16.3	5.9	5.6	1583
34	Viridiflorol	-	-	-	-	1593
35	α-Cadinol	2.9	-	-	-	1654
35	Tetradecanoic acid	9.4	-	29.6	5.5	-
	Monoterpene Hydrocarbon	0.9	6.2	-	4.9	
	Oxigenated Monoterpene	1.8	4.7	2.3	1.9	
	Sesquieterpene Hydrocarbon	51.3	46.3	49.3	57.7	
	Oxigenated Sesquieterpene	12.6	16.3	11.2	9.3	
	None Terpene	19.7	7	30.8	18.3	
	Total identified	86.3	80.5	93.6	92.1	
	Yield (% w/w)	0.1	0.98	0.90	0.97	

[a] *RRI: relative retention indices as determined on a HP-5 MS column using the homologous series of n- alkanes.*

drocarbons, these compounds were absent in the oil of *P. rigida*.

The antimicrobial activities of the oils were tested against a Gram-positive, two Gram-negative bacteria and a pathogenic fungus (Table 2). This essential oils displayed varied antibacterial and antifungal activities against the studied pathogens. Among tested bacteria, *S. aureus* was more susceptible to the oils comparing to the

Table 2. Minimum inhibitory concentration (MIC) of *P. olivieri*, *P. bruguieri*, *P. rigida*, and *P. kurdica*.

Samples	MIC (mg/ml)			
	S. aureus	***E. coli***	***P. aeruginosa***	***C. albicans***
P. olivieri	50	-	50	25
P. bruguieri	11.2	-	45	45
P. rigida	6	-	12	3
P. kurdica	40	-	80	20
Gentamicin	1.0×10^{-3}	1.0×10^{-3}	2.0×10^{-3}	-
Nystatin	-	-	-	125×10^{-3}

All determinations were done in triplicate; Gentamicin was used as positive control.

other bacteria. The samples of *P. rigida* exhibited higher MIC activity against all the tested microorganisms and especially against *C. albicans* (MIC: 3 mg/ml). The essential oils of *P. kurdica* and *P. oliveri* showed moderate antifungal activity (MICs: 20 and 25 mg/ml, respectively) against *C. albicans*. To the best of our knowledge, the present study is the first report on the antimicrobial properties of *P. kurdica* essential oil. Investigation of the antibacterial activity of essential oils of *P. rigida* and *P. bruguieri* showed better activity (MICs: 6 and 11.2 mg/ml) against *S. aureus*. *Escherichia coli* was resistant in the presence of all the essential oils.

Essential oils from medicinal plants and herbs have been shown to possess antimicrobial activities and could serve as a source of antimicrobial agents against food pathogens (29, 30). For instance, the ethanol extract of *Phlomis fruticosa* showed antibacterial activity against *S. aureus* and *Bacillus subtilis* and antifungal activity against *Aspergillus niger* (31). The results of previous study revealed that essential oil of *Phlomis fruticosa* exhibited moderate antibacterial activity against eight bacteria, mostly against Gram-positive bacteria. The main components of the *P. bacteria* oil were characterized as β-Caryophyllene, hexadecanoic acid, germacrene D and caryophyllene oxide (32). In the present study all the tested oils were not active against *E. coli*. However, the oil of *P. rigida* was rich in tetradecanoic acid (29.6%), which has known potential antibacterial and antifungal activities (33). Therefore, stronger activity of the plant oil could be attributed to the presence of high amount tetradecanoic acid which make it suitable candidate to inhibit growth of the tested bacteria and fungus.

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Refrences

1. Mozaffarian V. A Dictionary of Iranian Plant Names: Latin - English - Persian: Farhang Mo'aser; 1966.
2. Rechinger K. Flora Iranica Graz-Austeria, Akademische Druck-U. Verlagsantalt. 1982: 439-40.
3. Saracoglu I, Kojima K, Harput US, Ogihara Y. A new phenylethanoid glycoside from Phlomis pungens Willd. var. pungens. Chem Pharm Bull (Tokyo). 1998; 46(4): 726-7.
4. Sarkhail P, Abdollahi M, Shafiee A. Antinociceptive effect of Phlomis olivieri Benth., Phlomis anisodonta Boiss. and Phlomis persica Boiss. total extracts. Pharmacol Res. 2003; 48(3): 263-6.
5. Kirmizibekmez H, Montoro P, Piacente S, Pizza C, Donmez A, Calis I. Identification by HPLC-PAD-MS and quantification by HPLC-PAD of phenylethanoid glycosides of five Phlomis species. Phytochem Anal. 2005; 16(1): 1-6.
6. Couladis M, Tanimanidis A, Tzakou O, Chinou IB, Harvala C. Essential oil of Phlomis lanata growing in Greece: chemical composition and antimicrobial activity. Planta Med. 2000; 66(7): 670-2.
7. Kirmizibekmez H, Calis I, Perozzo R, Brun R, Donmez AA, Linden A, et al. Inhibiting activities of the secondary metabolites of Phlomis brunneogaleata against parasitic protozoa and plasmodial enoyl-ACP Reductase, a crucial enzyme in fatty acid biosynthesis. Planta Med. 2004; 70(8): 711-7.
8. Toroğlu S, Çenet M. Comparison of antimicrobial activities of essential oil and solvent extracts of endemic Phlomis oppositiflora Boiss. & Hausskn. from Turkey. Pakistan J Zool. 2013; 45(2): 475-82.
9. Kamel MS, Mohamed KM, Hassanean HA, Ohtani K, Ka-

sai R, Yamasaki K. Iridoid and megastigmane glycosides from Phlomis aurea. Phytochemistry. 2000; 55(4): 353-7.
10. Kyriakopoulou I, Magiatis P, Skaltsounis A-L, Aligiannis N, Harvala C. Samioside, a New Phenylethanoid Glycoside with Free-Radical Scavenging and Antimicrobial Activities from Phlomis samia. Journal of natural products. 2001; 64(8): 1095-7.
11. Shin T-Y, Lee J-K. Effect of Phlomis umbrosa root on mast cell-dependent immediate-type allergic reactions by anal therapy. Immunopharmacology and immunotoxicology. 2003; 25(1): 73-85.
12. Katagiri M, Ohtani K, Kasai R, Yamasaki K, Yang C-R, Tanaka O. Diterpenoid glycosyl esters from Phlomis younghusbandii and P. medicinalis roots. Phytochemistry. 1994; 35(2): 439-42.
13. Çalış Ih, Kırmızıbekmez H. Glycosides from Phlomis lunariifolia. Phytochemistry. 2004; 65(18): 2619-25.
14. Calis I, Basaran AA, Saracoglu I, Sticher O, Ruedi P. Phlinosides D and E, phenylpropanoid glycosides, and iridoids from Phlomis linearis. Phytochemistry. 1991; 30(9): 3073-5.
15. Takeda Y, Kinugawa M, Masuda T, Honda G, Otsuka H, Sezik E, et al. Phlomisethanoside, a phenylethanoid glycoside from Phlomis grandiflora var. grandiflora. Phytochemistry. 1999; 51(2): 323-5.
16. Sarkhail P, Amin G, Surmaghi MHS, Shafiee A. Composition of the volatile oils of Phlomis lanceolata Boiss. & Hohen., Phlomis anisodonta Boiss. and Phlomis bruguieri Desf. from Iran. Flavour and fragrance journal. 2005; 20(3): 327-9.
17. Mirza M, Nik ZB. Volatile constituents of Phlomis olivieri Benth. from Iran. Flavour and fragrance journal. 2003; 18(2): 131-2.
18. Ismailoglu U, Saracoglu I, Harput U, Sahin-Erdemli I. Effects of phenylpropanoid and iridoid glycosides on free radical-induced impairment of endothelium-dependent relaxation in rat aortic rings. Journal of ethnopharmacology. 2002; 79(2): 193-7.
19. Vazirian M, Faramarzi MA, Ebrahimi SES, Esfahani HRM, Samadi N, Hosseini SA, et al. Antimicrobial effect of the Lingzhi or Reishi medicinal mushroom, Ganoderma lucidum (higher Basidiomycetes) and its main compounds. International journal of medicinal mushrooms. 2014; 16(1).
20. Sabri N, Yassa N, Fazeli MR, Alavi SHR, Fouladi F, Salimi L, et al. Antibacterial activity of peucedanum ruthenicum, johreniopsis seseloides and cervaria cervariifolia extracts. Iranian Journal of Pharmaceutical Sciences. 2009; 5(1): 37-42.
21. Esfandyari-Manesh M, Ghaedi Z, Asemi M, Khanavi M, Manayi A, Jamalifar H, et al. Study of antimicrobial activity of anethole and carvone loaded PLGA nanoparticles. Journal of Pharmacy Research. 2013; 7(4): 290-5.
22. Manayi A, Kurepaz-Mahmoodabadi M, Gohari AR, Ajani Y, Saeidnia S. Presence of phthalate derivatives in the essential oils of a medicinal plant Achillea tenuifolia. Daru: journal of Faculty of Pharmacy, Tehran University of Medical Sciences. 2014; 22(1): 78-.
23. Massada Y. Analysis of essential oil by gas chromatography and spectrometry. Analysis of essential oil by gas chromatography and spectrometry. 1976.
24. Adam R. Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy. Allured: Carol Stream, IL. 2001.
25. Baron E, Finegold S. Streptococci and related genera. Bailey and Scott's Diagnostic Microbiology. 1990; 8: 333.
26. Ali NA, Jülich W-D, Kusnick C, Lindequist U. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. Journal of Ethnopharmacology. 2001; 74(2): 173-9.
27. Aligiannis N, Kalpoutzakis E, Kyriakopoulou I, Mitaku S, Chinou IB. Essential oils of Phlomis species growing in Greece: chemical composition and antimicrobial activity. Flavour and Fragrance Journal. 2004; 19(4): 320-4.
28. Sarkhail P, Amin G, Shafiee A. Composition of the essential oil of Phlomis persica Boiss and Phlomis chorassanica Bunge from Iran. Flavour and Fragrance Journal. 2004; 19(6): 538-40.
29. Deans S, Ritchie G. Antibacterial properties of plant essential oils. International journal of food microbiology. 1987; 5(2): 165-80.
30. Kim J, Marshall MR, Wei C-i. Antibacterial activity of some essential oil components against five foodborne pathogens. Journal of Agricultural and Food Chemistry. 1995; 43(11): 2839-45.
31. Ristib M, Duletib-Laurevib S, Kneyevib-Vukcevib. Phytother Res. 2000; 14: 267.
32. Formisano C, Senatore F, Bruno M, Bellone G. Chemical composition and antimicrobial activity of the essential oil of Phlomis ferruginea Ten.(Lamiaceae) growing wild in Southern Italy. Flavour and fragrance journal. 2006; 21(5): 848-51.
33. Agoramoorthy G, Chandrasekaran M, Venkatesalu V, Hsu MJ. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. Brazilian Journal of Microbiology. 2007; 38: 739-42.

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