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CHEMICAL COMPOSITION OF ESSENTIAL OILS OF FOUR *PHLOMIS* SPECIES FROM TURKEY: A CHEMOTAXONOMIC APPROACH

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Abstract

The chemical composition of the essential oils of dried aerial parts of four *Phlomis* species; *P. rigida*, *P. oppositiflora*, *P. linearis* and *P. kurdica* analyzed by GC and GC-MS. Forty, 32, 42 and 41 components were identified representing 94.5, 95.1, 92.8 and 92.9% of the oils, respectively. The main compounds were determined as epizonaren (14.3%), δ-cadinene (11.0%), spathulenol (10.7%), α-copaene (7.5%) and β-bourbonene (5.4%) in *P. rigida*; germacrene D (22.7%), germacrene B (15.0%), bicyclogermacrene (9.0%), camphor (5.9%) and caryophyllene oxide (5.4%) in *P. oppositiflora*; germacrene D (17.3%), chrysanthenyl acetate (5.9%), *trans*-chrysanthenol (5.8%) and 2-pentadecanone (5.2%) in *P. linearis* and germacrene D (36.5%), β-farnesene (14.5%), β-pinene (10.5%) and bicyclogermacrene (7.2%) in *P. kurdica*. The results were discussed in view of natural products and chemotaxonomy.

Introduction

Lamiaceae is an important economic plant source of essential oils and the genus *Phlomis* L. (Lamiaceae) consists of about 100 species distributed in Euro-Asia and North-Africa (Kyriakopoulo *et al*. 2001, Albaladejo *et al.* 2004, Azizian and Moore 1982). It is recently documented that 52 taxa including 6 varieties, 12 natural hybrids and 34 endemic taxa naturally grow in Turkey (Demirci *et al*. 2006). The genus *Phlomis* was revised by Huber-Morath (1982) for the Flora of Turkey. Baytop (1999) reported that Turkish *Phlomis* species are used as herbal teas (Dağ çayı), tonic, carminative, appetizer and stimulants in the folk medicine and recognized by local names as ‘‘Ballıkotu, Calba, Çalba or Şalba” (Baytop 1999).

According to their morphological characteristics, the genus is divided into 3 groups and the differentiation of the groups was done according to the corolla colors, plant habits (herb or shrub) and the number of bracteoles. The species *P. rigida* Labill. was represented in group A, and *P. oppositiflora* Boiss. & Hausskn, *P. linearis* Boiss. & Bal. and *P. kurdica* Boiss. & Bal. were represented in group C in Flora of Turkey. *Phlomis oppositiflora* is reported as no near allies and *Phlomis linearis* is related to the *P. brunneogaleata* and both the species are endemic to Anatolia, Turkey. *P. kurdica* is near to *P. polioxantha* Rech. fil. from Iran and Iraq and *P. olivieri* Bentham from Iran (Davis 1982).

Many members of the genus *Phlomis* have aromatic and medicinal characteristics. They have various uses that differ from one country to another. Their flower parts are generally used in gastrointestinal troubles and to promote good health by protecting the liver, kidney, bone and cardiovascular system. In addition, some *Phlomis* species have culinary uses (Amor *et al*. 2009). The aerial parts of some species have distinctive tastes and can be used for herbal tea in traditional medicine as stimulants, tonics, diuretics and are claimed to exhibit biological properties for the treatment of ulcers and hemorrhoids (Saracoglu *et al.* 1998, Couladis *et al.* 2000, Kirmizibekmez *et al*. 2005). Furthermore, there is evidence indicating various activities such as anti-inflammatory, immuno-suppressive (Saracoglu *et al.* 1995), free radical scavenging (Kyriakopoulo *et al*. 2001)

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and antimicrobial (Couladis *et al.* 2000) for some species of these plants (Saracoglu *et al.* 1998, Couladis *et al.* 2000, Kyriakopoulo *et al*. 2001). Numerous studies have reported that the essential oils of *Phlomis* L. species are among the most potent essential oils owing to their medicinal characteristics (Table 2).

The aim of this study was to determine the essential oil composition of the four *Phlomis* species from Turkey, to examine their potential chemotaxonomical significance and contributions on the systematics of the genus and renewable resources with essential oils compositions.

Materials and Methods

Phlomis rigida Labill. specimens were collected from natural habitats in Baskil, Hacı Mustafa village, Elazig; in 2008; *P. oppositiflora* Boiss. & Hausskn specimens were collected from Gözeli between Sivrice 14. km, Elazig, in 2009; *P. linearis* Boiss. & Bal. samples were collected from Haroglu Mountain, Elazig, in 2010 and *P. kurdica* Boiss. & Bal. specimens were collected from Harput, Elazig, 2009. Four voucher specimens were deposited in Fırat University Herbarium (FUH) under registration numbers 7512, 7885, 7987 and 8111, respectively.

The essential oil was extracted by hydrodistillation using a modified Clevenger apparatus coupled to a 2 litre round-bottom flask. A total of 100 g of fresh plant material (aerial parts) and 1 L of water were used for the extraction. The extraction was performed for over 3 hrs. Subsequently, the hydrolate was collected and centrifuged at 10,000 rpm for 10 minutes. The organic phase was removed with the aid of a Pasteur pipette, and subsequently transferred to black coloured vial, wrapped in parafilm and aluminum foil and 4^{o}C under refrigeration until analysis. The yield of oils were calculated on the basis of the dry mass.

The essential oil was analysed using HP 6890 GC equipped with FID detector and HP- 5 MS (30 m × 0.25 mm i.d., film tickness 0.25 μm) on a capillary column was used. The column and analysis conditions were the same as in GC-MS expressed below. The percentage composition of the essential oils was computed from GC-FID peak areas without correction factors. GC-MS analyses of the oils were performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with Hewlett Packard 5973 mass spectrometer system equipped with a HP 5-MS capillary column (30 m × 0.25 mm id, film thickness 0.25 μm). The oven temperature was programmed from 70 - 240ºC at the rate of 5^{o}C/ min. The ion source was set at 240ºC and electron ionization at 70 eV. Helium was used as the carrier gas at a flow rate of 1 ml/min. Scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 μl) was injected into the GC-MS.

The identification of constituents was performed on the basis of retention indices (RI) determined by co-injection with reference to a homologous series of *n*-alkanes, under identical experimental conditions. Further identification was performed by comparison of their mass spectra with those from NIST 98 Libraries (on Chem Station HP) and Wiley 7th Version. The relative amount of individual components was calculated based on the GC (HP-5MS column) peak area (FID response) without using correction factors. The identified constituents of the essential oils are listed in Table 1.

Results and Discussion

In the essential oil analysis of the four *Phlomis* species, qualitative and quantitative differences were found. Forty, 32, 42 and 41 compounds were identified in *P. rigida*, *P. oppositiflora*, *P. linearis* and *P. kurdica*, respectively, accounting for94.5, 95.1, 92.8 and 92.9% of the whole essential oils. The yields of essential oils also were 0.4, 0.3, 0.5 and 0.4% (v/w), respectively. The compositions of four *Phlomis* essential oils are listed in Table 1.

Table 1. Chemical composition of *P. kurdica* (Pk), *P. oppositiflora* (Po), *P. linearis* (Pl) and *P. rigida* (Pr) essential oil.

Constituent classes	Constituents	RI	Pk (%)	Po (%)	Pl (%)	Pr (%)
o	Benzene, ethyl	969	0.8	–	–	–
o	Benzene, 1,3-dimethyl	975	0.3	–	–	–
m	α-pinene	1022	1.5	–	0.1	–
m	β-pinene	1056	**10.5**	0.7	–	0.1
o	2-pentyl furan	1063	0.1	–	–	0.1
m	3-octanol	1069	0.2	–	–	–
m	α-terpinene	1085	–	–	–	0.2
m	p-cymene	1091	0.1	0.3	–	0.1
m	Limonene	1095	0.3	–	–	–
m	1,8-cineole	1097	–	**3.8**	0.5	0.1
m	γ-terpinene	1117	0.1	–	–	0.3
m	*cis*-linalool oxide (furanoid)	1125	–	–	**4.5**	0.2
m	Bicyclo [4.1.0]-hept-2-ene	1137	0.1	–	–	0.1
m	Linalool	1147	0.8	0.8	0.6	2.5
o	Nonanal	1150	0.5	–	**3.0**	–
m	*trans*-chrysanthenol	1158	–	–	**5.8**	–
m	*trans*-pinocarveol	1176	–	1.2	–	–
m	*trans*-verbenol	1180	0.3	–	–	–
m	Camphor	1182	–	**5.9**	2.9	0.1
m	Pinocarvone	1192	–	0.9	0.4	–
m	Borneol	1198	–	1.5	–	–
m	3-cyclohexen-1-ol	1201	0.4	–	–	–
m	Terpinen-4-ol	1204	–	–	–	0.9
m	α-terpinolene	1213	0.3	**3.3**	0.3	0.2
m	Chrysanthenyl acetate	1237	–	0.5	**5.9**	–
m	Pulegone	1243	0.3	–	–	–
m	Geraniol	1251	0.2	–	0.2	0.1
m	Endobornyl acetate	1283	–	0.7	–	–
m	Thymol	1285	–	–	–	0.8
o	Cyclohexasiloxane	1295	0.3	–	0.2	–
s	Bicycloelemene	1322	0.2	0.2	–	–
s	α-cubebene	1335	–	–	–	**3.9**
s	(+)-cyclosativene	1353	0.3	–	–	2.7
s	α-copaene	1358	1.1	–	0.5	**7.5**
s	β-bourbonene	1366	0.9	0.8	**3.5**	**5.4**
s	β-elemene	1369	1.1	0.8	0.8	–
s	1H-cycloprop[e]azulene	1382	0.4	–	–	–
s	β-caryophyllene	1393	0.7	**4.4**	**3.2**	1.5
s	β-cubebene	1399	0.7	–	0.5	0.1

(Contd.)

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Constituent classes	Constituents	RI	Pk (%)	Po (%)	Pl (%)	Pr (%)
m	Geranyl acetate	1410	–	–	–	0.5
s	β-farnesene	1415	**14.5**	**3.5**	0.8	0.2
s	α-humulene	1417	–	0.8	0.2	0.1
s	Alloaromadendrene	1419	–	–	2.1	0.5
s	α-amorphene	1430	–	–	0.4	–
s	Germacrene D	1435	**36.5**	**22.7**	**17.3**	2.0
s	α-selinene	1439	–	–	2.1	–
s	Valencene	1440	2.0	–	0.7	0.9
s	Bicyclogermacrene	1443	**7.2**	**9.0**	**3.6**	2.7
s	β-bisabolene	1450	–	–	2.0	–
s	δ-cadinene	1457	2.2	1.0	**4.9**	**11.0**
s	α-calacorene	1471	–	–	–	2.9
s	Germacrene B	1482	2.5	**15.0**	2.6	–
s	1,5-epoxysalvial-4(14)-ene	1488	–	–	–	2.8
m	3-hexen-1-ol, benzoate	1490	–	–	2.0	–
s	Spathulenol	1495	1.3	**3.6**	2.8	**10.7**
s	Caryophyllene oxide	1497	–	**5.4**	2.5	**4.9**
s	Salvial-4[14]en-1-one	1503	0.8	1.0	–	–
s	γ-gurjunene	1509	–	1.1	–	–
s	Epizonaren	1512	–	–	–	**14.3**
s	İsospathulenol	1524	–	0.7	–	–
s	τ-muurolol	1530	–	–	0.8	–
s	α-cadinol	1537	1.0	2.0	1.7	**4.2**
s	β-selinene	1546	–	0.8	–	**3.7**
o	Cyclotetradecane	1548	0.9	–	–	–
o	Tetradecanal	1568	0.1	0.7	–	0.9
o	Cyclononasiloxane	1600	0.3	–	–	–
o	2-pentadecanone	1630	0.9	1.0	**5.2**	**3.9**
o	Nonadecane	1657	–	–	0.8	–
m	δ-fenchane	1661	–	–	0.6	–
o	Cyclodecasiloxane	1669	–	–	0.4	–
o	Tridecanoic acid	1688	–	0.2	–	–
o	Hexadecanoic acid	1691	0.1	–	0.2	0.2
o	Heneicosane	1786	–	–	0.4	–
o	Tricosane	1900	–	–	1.5	0.8
o	Tetracosane	1946	–	–	0.4	–
o	Eicosane	1987	0.1	0.8	**3.9**	0.4
Total	**92.9**		**95.1**	**92.8**	**94.5**	
Monoterpenes	15.1		19.6	23.8	6.2	
Sesquiterpenes	73.4		72.8	53.0	82.0	
Others	4.4		2.7	16.0	6.3	

In *P. kurdica,* 41 compounds were also identified representing 92.9% of the oil. Germacrene D was also found to be present at a high percentage (36.5%). The presence of β-farnesene (14.5%), β-pinene (10.5%) and bicyclogermacrene (7.2%) were also important for the oil profile. In the case of *P. oppositiflora*, 32 components were identified representing 95.1% of the oil. Germacrene D was the predominant compound (22.7%) followed by germacrene B (15.0%), bicyclogermacrene (9.0%), camphor (5.9%) and caryophyllene oxide (5.4%).

In the essential oil composition of the *P. linearis*, 42 components were identified representing 92.8% of the oil (Table 1). Germacrene D was the predominant compound (17.3%) followed by chrysanthenyl acetate (5.9%), *trans*-chrysanthenol (5.8%) and 2-pentadecanone (5.2%). In case of *P. rigida*, 40 compounds were identified representing 94.5% of the oil. Epizonaren was found to be present at a high percentage (14.3). The presence of δ-cadinene (11.0%), spathulenol (10.7%), α-copaene (7.5%) and β-bourbonene (5.4%) were also important for the oil profile.

The essential oil of *Phlomis* is composed of four chemotypes dominated by monoterpenes (α–pinene, limonene and linalool), sesquiterpenes (germacrene D and β-caryophyllene), aliphatic compounds (9,12,15-octadecatrienoic acid methyl ester), fatty acids (hexadecanoic acid) and other components (trans-phytol, 9,12,15-octadecatrien-1-ol) (Amor *et al.* 2009). The main essential oil constituents of some *Phlomis* species reported from different studies are shown in Table 2.

Comparing the essential oil composition of *Phlomis* L. species many similarities are obvious. Germacrene D, was the main compound of the essential oils of three *Phlomis* species (36.5, 22.7, 17.3%) and it was detected in most of the *Phlomis* species studied and reported, although in some species only in small amount (2.0%) as in the *P. rigida* studied in here. The oils of some other *Phlomis* species (*P. anisodonta*, *P. bruguieri*, *P. cancellata*, *P. chimerae*, *P. chorrasanica*, *P. cretica*, *P. ferruginea*, *P. fruticosa*, *P. grandiflora* var. *grandiflora*, *P. herba-venti*, *P. lanata*, *P. lanceolata*, *P. leucophracta*, *P. nissolii*, *P. olivieri*, *P. persica*, *P. pungens* and *P. samia*) from localities belonging to different countries (Iran, Turkey, Greece and Italy) are reported to be rich in germacrene D, β-caryophyllene, γ-elemene, β-farnesene, limonene, bicyclogermacrene, β-selinene and hexadecanoic acid (Demirci *et al*. 2003, Aligiannis *et al*. 2004, Morteza-Semnani *et al.* 2004, Celik *et al*. 2005, Khalilzadeh *et al*. 2005, Sarkhail *et al*. 2005, Morteza-Semnani *et al*. 2006, Basta *et al*. 2006, Formisano *et al*. 2006, Kirimer *et al*. 2006, Masoudi *et al*. 2006, Demirci *et al.* 2006, Mirza and Nik 2007). While samples (*P. fruticosa*) collected from Yugoslavia were found to contain a low percentage of germacrene D, but rich in β-caryophyllene, (*E*)-methyl isoeugenol and α-asarone (Sokovic *et al.* 2002). In contrast to the reports above, the essential oil of *P. younghunsbandii* from Tibet showed different qualitative and quantitative oil profiles, with eugenol as the major component (Bian-ba *et al*. 2002).

The essential oils of *P. herba-venti* and *P. olivieri* endemic species in Iran, germacrene D was also the main constituent of the leaf and aerial parts oils (33.9 and 28.1%, respectively) (Morteza-Semnani *et al.* 2004, Mirza and Nik 2007). The essential oil of *P. fruticosa* of Greek origin was characterized by the abundance of the sesquiterpenes, germacrene D (17.8%), γ-bisabolene (12.6) and (*E*)-caryophyllene (8.7%) (Tsitsimi *et al.* 2000) whereas in the essential oil of the endemic *P. lanata* growing in Greek α-pinene (25.41%) was the dominant component followed by limonene (15.67%) and (*E*)-caryophyllene (8.76%) (Couladis *et al*. 2000). Kirimer *et al.* reported that germacrene D (33.9%), bicyclogermacrene (15.3%) and (*Z*)-β-farnesene (10.7%) were the main components of *P. nissolii* (Kirimer *et al*. 2006).

Earlier studies of the *Phlomis* genus patterns showed that the oils of the genus are distributed among four chemotype groups. These are germacrene D, spathulenol, β-caryophyllene and α-pinene. Germacrene D is dominated in *P. grandiflora* var. *grandiflora* (Celik *et al.* 2005) and

Table 2. Comparison of the main essential oil constituents of some *Phlomis* species.

Species	Major constituents	References
Phlomis linearis	β-caryophyllene (24.2%) Germacrene D (22.3%) Caryophyllene oxide (9.2%)	Demirci *et al.* (2009)
P. viscosa	Germacrene D (33.9%) Bicyclogermacrene (15.3%) (Z)-p-Farnesene (10.7%)	Kırımer *et al*. (2006)
P. olivieri	Germacrene D (28.1%) β-caryophyllene (16.1%) β-selinene (10.2%) bicyclogermacrene (7.4%) α-selinene (4.1%) δ-cadinene (3.6%) γ-elemene (3.5%) β-bourbonene (3.4%)	Mirza and Nik (2007)
P. bruguieri	α-pinene (6.8%) Germacrene D (23.6%) β-caryophyllene (6.7%)	Morteza-Semnani (2005)
	β-caryophyllene (9%) (Z)-β-farnesene (6.5%)	Demirci *et al.* (2008)
Phlomis lunariifolia	Germacrene D (7.7%) Spathulenol (3.9%) Hexadecanoic acid (9.7%)	
P. amaniaca	(Z)-β-farnesene (8.3%) Germacrene D (14.7%) Bicyclogermacrene (10.7%) Spathulenol (6.3%) α-cadinol (1.4%) 15-ısopimaradien-11-α−ol (22.8%)	Demirci *et al.* (2008)
P. monocephala	α-pinene (4.9%) Limonene (3.9%), β-caryophyllene (5.1%) (Z)-β-farnesene (3.1%) Germacrene D (6%) Spathulenol (3.8%) Manoyl oxide (6.1%) 15-ısopimaradien-11-α-ol (12.7%)	Demirci *et al.* (2008)
P. sieheana	(Z)-β-farnesene (11.7%) Germacrene D (16.6%) β-selinene (6.7%) Spathulenol (3%)	Demirci *et al.* (2008)
P. armeniaca	(Z)-β-farnesene (6.2%) Germacrene D (23.4%) Hexadecanoic acid (4.9%)	Demirci *et al.* (2008)

P. fruticosa (Tsitsimi *et al*. 2000). Spathulenol is dominated in *P. olivieri* (Ghassemi *et al*. 2001). β-caryophyllene is dominated in *P. chimerae* and *P. leucophracta* (Celik *et al.* 2005), while α-pinene is dominated in *P. lanata* (Couladis *et al.* 2000).

Demirci *et al.* (2009), studied the essential oils of *P. lunariifolia*, *P. amanica*, *P. monocephala*, *P. sieheana, P. armeniaca*. Essential oil of *P. lunariifolia* was characterized by a high content of hexadecanoic acid (9.7%), β-caryophyllene (9.0%), germacrene D (7.7%), and (Z)-β-farnesene (6.5%). Overall, the oil was found to be rich in sesquiterpene hydrocarbons. Germacrene D (14.7%), bicyclogermacrene (10.7%), (Z)-β-farnesene (8.3%) and spathulenol (6.3%) were the major constituents of the essential oils of *P. amanica*. Germacrene D (16.6 and 23.4%) and (Z)-β-farnesene (11.7 and 6.2%) were identified as the major constituents of *P. sieheana* and *P. armeniaca* essential oils, respectively (Demirci *et al.* 2009).

The results and the chemical data gave more clues on the chemotaxonomy of genus *Phlomis*. In this study, germacrene D dominated group were *P. oppositiflora*, *P. linearis* and *P. kurdica* whereas epizonaren dominated group was *P. rigida*. The essential oil composition of three *Phlomis* species (*P. kurdica, P. linearis, P. oppositiflora*) were very similar to each other except *P. rigida* (Table 1). Essential oil analysis performed here showed that the whole *Phlomis* species is rich sesquiterpenes and low in monoterpene compounds. Table 1 shows the main terpene groups in four *Phlomis* species studied here.

It is possible to say that, *P. rigida* showed epizonaren/δ-cadinene type, *P. oppositiflora* showed germacrene D/germacrene B, *P. linearis* showed germacrene D/chrysanthenyl acetate, and *P. kurdica* also comprised of germacrene D/β-farnesene type essential oils. In conclusion, this study demonstrates the occurrence of epizonaren chemotype in *P. rigida* and germacrene D chemotype in *P. oppositiflora*, *P. linearis* and *P. kurdica* in eastern Anatolian region of Turkey. Finally, the chemical findings supported the morphological group separation in the Flora of Turkey (*P. rigida* in group A; *P. oppositiflora*, *P. linearis* and *P. kurdica* in group C).

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