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Short communication

Comparative analysis of essential oil components of three *Phlomis* species in Qinling Mountains of China

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Abstract

The essential oils of three wild-growing *Phlomis* species (*Phlomis umbrosa* Turcz., *Phlomis megalantha* Diels and *Phlomis szechuanensis* C.Y. Wu), collected from Qinling Mountains of China during the bloom stage, were obtained by hydrodistillation and analyzed by gas chromatography (GC) and gas chromatography/mass spectrometry (GC–MS). Under the optimum extraction and analysis conditions, 22, 26 and 19 constituents (mainly aliphatic compounds) were identified in *P. umbrosa*, *P. megalantha* and *P. szechuanensis* which represented 92.5%, 96.3% and 93.1% of the oils, respectively. The main constituents were hexadecanoic acid (7.1–52.1%), *trans*-phytol (5.7–50.8%) and 9,12,15-octadecatrien-1-ol (2.2–24.8%). Fatty acids and aliphatic esters were the major groups of *P. umbrosa* and *P. megalantha*, but *P. szechuanensis* showed higher content of alcohols. *P. megalantha* has relatively higher amounts of oxygenated monoterpenes and oxygenated sesquiterpenes than the others. The comparison of essential oil components of *Phlomis* species between the present and previous work indicated that the composition of oils vary greatly with respect to the geographical environment, mainly for the proportion of aliphatic compounds and terpenoids. This study is the first report on the chemical composition of essential oils of the three wild-growing herbs mentioned above.

Keywords: *Phlomis umbrosa* Turcz.; *Phlomis megalantha* Diels; *Phlomis szechuanensis* C.Y. Wu; Essential oils; Chemical composition; GC–MS

1. Introduction

The genus *Phlomis* as perennial herbs of Lamiaceae consists of more than 100 species distributed in Africa, Asia and Europe. In China, 43 species have already been recorded, particularly in Sichuan and Yunnan [1]. Many members of the genus *Phlomis* have aromatic and medicinal characteristics. 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 [2,3,6]. Furthermore, there is evidence indicating various activities such as anti-inflammatory, immunosuppressive [4], free radical scavenging [5] and antimicrobial [6] for some species of this plant [4–6].

Phlomis species are one of the most popular herbs in China owing to their aromatic and medicinal properties. It is believed that a part of these activities is due to the volatile constituents. Therefore, the composition analysis of essential oils of *Phlomis* species has been the subject of many publications [7–24]. Comparing the chemical composition of the essential oils within *Phlomis* species, many similarities are obvious. Generally, all taxa are characterized by the presence of terpenoids and aliphatics. However, considerable differences can be found among the diverse *Phlomis* species, especially in the composition and contents of the essential oils. Since the components of more than 70 *Phlomis* species are still unclear, more investigations are needed to search the existence of a chemical polymorphism and potential biochemical activities.

To the best of our knowledge, no research has been conducted on the chemical composition of the essential oils of *Phlomis umbrosa Turcz., Phlomis megalantha Diels and Phlomis szechuanensis* C.Y. Wu. As part of our studies on essential oil-bearing herbs from Qinling Mountains of China, the

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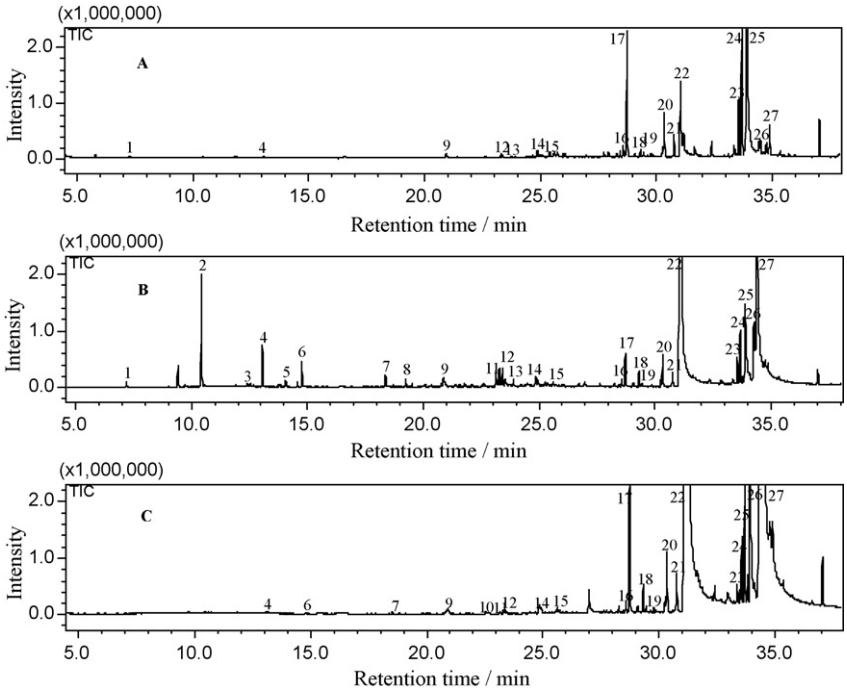

Fig. 1. GC–MS total ion chromatograms (TICs) of the essential oils from three *Phlomis* species: (A) *P. szechuanensis*; (B) *P. megalantha*; and (C) *P. umbrosa.* For peak numbering see Table 1. For chromatographic conditions see Section 2.

objective of this study was to determine the composition of essential oils of three wild-growing *Phlomis* and compare with other species of this plant.

2. Materials and methods

2.1. Chemicals and plant materials

Anhydrous sodium sulfate (analytical grade) was purchased from Tianjin Chemical Reagent Co. Ltd. (Tianjin, China). HPLC grade acetone was obtained from Xi'an Chemical Reagent Company (Xi'an, China). Sample solutions of the essential oils were prepared by dissolving the essential oil in acetone (1/100, v/v). *n*-Alkane standard solutions of C_{10}–C_{18}, C_{20}, C_{24}, C_{28}, C_{32}, C_{36}, C_{40} and C_{44} (Catalog no. 48179) were purchased from Supelco (Bellefonte, PA, USA).

Leaves of *P. umbrosa* (voucher no. MPR 609), *P. megalantha* (voucher no. MPR 610) and *P. szechuanensis* (voucher no. MPR 608) were collected from north slope of Taibai Mountain (the peak of Qinling Mountains), Shaanxi Province of China, when flowering (July–August, 2006), respectively. Voucher specimens were determined by Dr. Yi Ren and deposited in the Ministry of Education, Key Laboratory for Medicinal Plant Resource (MPR) and Natural Pharmaceutical Chemistry, Shaanxi Normal University, Xi'an, Shaanxi, P.R. China.

2.2. Optimization of the extraction method

One hundred grams of the air-dried plant materials from each taxon were ground to fine powder, and then put into a 2000 ml distillation flask. One thousand milliliter of distilled water was added and the essential oil distillation apparatus was set according to the Chinese pharmacopoeia [25]. The mixture was distilled and the essential oils were collected from the condenser, dried over anhydrous sodium sulfate, and subsequently stored at +4 °C in the dark until analyzed by GC and GC–MS. In order to determine the optimum extraction time, different times (2, 4, 6, and 8 h) were tested by comparing the yields of the essential oils at different extraction times. This increased until 6 h. Further extraction up to 8 h did not show great increase. For subsequent studies, the extraction time was set at 6 h.

2.3. Analysis of the essential oils

The essential oil extracts were analyzed using gas chromatography with flame ionization detection (GC–FID) and gas chromatography–mass spectrometry (GC–MS). The GC–FID analysis was performed on an Agilent Technology (Palo Alto, CA, USA) model 6890N gas chromatograph equipped with a FID detector. A fused silica capillary Agilent Technology HP-5ms (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used for the separation. Injector and detector temperatures were set at 250 °C and 200 °C, respectively. Oven temperature was raised from 50 °C to 240 °C by a rate of 5 °C/min. Flow rate of nitrogen was 1.5 ml/min. Diluted samples of 1.0 μl were injected manually and in the splitless mode. For semi-quantification purposes, the percentage composition of the oils was computed by the normalization method from the FID areas, which were calculated as mean values of three injections of each oil sample, without using response factors. The GC conditions were optimized on the basis of the property of the essential oils. Good resolution and sharp peak

Table 1
Composition of the essential oils from three *Phlomis* species

No.	R.I.[a]	Compound[b]	*Phlomis umbrosa* (%)[c]	*Phlomis megalantha* (%)[c]	*Phlomis szechuanensis* (%)[c]
1	982	1-Octen-3-ol	tr	0.1	0.1
2	1102	β-Linalool	tr	3.8	tr
3	1177	Menthol	–	0.1	–
4	1196	α–Terpineol	0.1	1.5	0.1
5	1251	Carvone	–	0.2	–
6	1258	Lemonol	0.2	1.0	–
7	1394	α-Bourbonene	0.1	0.4	–
8	1429	β-Caryophyllene	tr	0.3	–
9	1494	Germacrene D	0.3	0.3	0.4
10	1564	Nonanoic acid	0.1	tr	tr
11	1577	Spathulenol	0.1	0.6	–
12	1596	Caryophyllene oxide	0.2	0.8	0.3
13	1602	α-Chamigrene	tr	0.8	0.2
14	1666	τ-Muurolol	0.3	0.4	0.8
15	1699	Myristic acid	0.1	0.1	0.4
16	1840	3-Eicosyne	0.2	0.3	0.8
17	1847	Hexahydrofarnesyl acetone	1.8	1.2	8.5
18	1876	Isobutyl phthalate	0.4	0.6	0.5
19	1883	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	0.1	0.2	0.4
20	1928	Hexadecanoic acid, methyl ester	0.6	1.2	2.9
21	1950	Isophytol	0.4	0.5	1.6
22	1968	Hexadecanoic acid	52.1	46.0	7.1
23	2105	9,12-Octadecadienoic acid, methyl ester	0.6	1.2	3.9
24	2113	9,12,15-Octadecatrienoic acid, methyl ester	1.8	2.3	11.0
25	2125	*trans*-Phytol	5.7	6.2	50.8
26	2147	9,12-Octadecadienoic acid	2.5	3.6	1.1
27	2155	9,12,15-Octadecatrien-1-ol	24.8	22.6	2.2
Total identified			92.5	96.3	93.1
Aliphatics					
Alkanes, alkenes			0.2	0.3	0.8
Alcohols			31.0	29.6	55.1
Aldehydes			–	–	–
Ketones			1.8	1.2	8.5
Fatty acids and aliphatic esters			58.1	55.0	26.9
Terpenoids					
Monoterpene hydrocarbons			–	–	–
Oxygenated monoterpenes			0.3	6.6	0.1
Sesquiterpene hydrocarbons			0.5	1.8	0.6
Oxygenated sesquiterpenes			0.6	1.8	1.1

[a] Retention indices (R.I.) on HP-5ms capillary column.
[b] Compounds listed in order of elution from a HP-5ms column.
[c] Mean value of three determinations (three replicates) calculated from FID areas, relative standard deviation (R.S.D.) values = 0.8–2.4%; tr (trace): relative content < 0.1%; (–) not detected.

shape were obtained by the developed simple and linear GC temperature program.

GC–MS was performed with a SHIMADZU QP2010 instrument and SHIMADZU ChemStation software (SHIMADZU corporation analytical and measuring instruments division, Kyoto, Japan). A fused silica capillary SHIMADZU RTX-5ms (5% phenyl methyl siloxane) column (30 m × 0.25 mm i.d., film thickness 0.25 μm) was used for the separation. Injector and ion source temperature were set at 250 °C and 200 °C, respectively. A 2 μl aliquot of oil was injected into the column using a 20:1 split injection. The operating conditions were the same as described above. Helium was used as a carrier gas at a flow rate of 1.30 ml/min. The mass spectrometer was operated in electron-impact ionization (EI) mode with 70 eV energy. The scan range was 40–600 amu and the scan rate was 0.2 s per scan.

2.4. Identification of components

Linear retention indices for all the compounds were determined by co-injection of the sample with the homologous series of C_{10}–C_{18}, C_{20}, C_{24}, C_{28}, C_{32}, C_{36}, C_{40} and C_{44} *n*-alkanes. Individual identification of components was based on matching their recorded mass spectra with those of NIST05.LIB and

NIST05s.LIB (National Institute of Standards and Technology) libraries data provided by the software of GC–MS system. The results were also confirmed by the comparison of their retention indices relative to *n*-alkanes reported in the literature [26–29].

3. Results and discussion

The distilled essential oils of *P. umbrosa*, *P. megalantha* and *P. szechuanensis* gave yellowish wax, brown liquid and yellow wax oils in yields of $0.05 \pm 0.01\%$ (w/w), $0.11 \pm 0.01\%$ (w/w) and $0.04 \pm 0.01\%$ (w/w), on dry weight basis, respectively. The odor of all investigated oils was spicy except *P. szechuanensis* which smelled very mild.

Fig. 1 shows the total ion chromatograms (TICs) of the essential oils. The GC and GC–MS analysis of these three oils (*P. umbrosa*, *P. megalantha* and *P. szechuanensis*) resulted in the identification of 22, 26 and 19 constituents, representing 92.5%, 96.3% and 93.1% of the oils, respectively (Table 1). The main constituents of oils are as follows: in *P. umbrosa*: hexadecanoic acid (52.1%), 9,12,15-octadecatrien-1-ol (24.8%), *trans*-phytol (5.7%), 9,12-octadecadienoic acid (2.5%), 9,12,15-octadecatrienoic acid methyl ester (1.8%) and hexahydrofarnesyl acetone (1.8%); in *P. megalantha*: hexadecanoic acid (46.0%), 9,12,15-octadecatrien-1-ol (24.8%), *trans*-phytol (5.7%), 9,12-octadecadienoic acid (2.5%), β-linalool (3.8%), 9,12,15-octadecatrienoic acid methyl ester (1.8%) and α-terpineol (1.5%); in *P. szechuanensis*: *trans*-phytol (50.8%), 9,12,15-octadecatrienoic acid methyl ester (11.0%), hexahydrofarnesyl acetone (8.5%), hexadecanoic acid (7.1%), 9,12-octadecadienoic acid methyl ester (3.9%), hexadecanoic acid methyl ester (2.9%), 9,12,15-octadecatrien-1-ol (2.2%) and isophytol (1.6%). Generally, all the essential oils investigated showed a similar trend, containing a large amount of aliphatic compounds. Fatty acids and aliphatic esters were the main group of constituents of all taxa, except *P. szechuanensis*, which had a higher content of alcohols. *P. megalantha* has relatively higher amounts of oxygenated monoterpenes and oxygenated sesquiterpenes than others.

Numerous studies have reported that the essential oils of *Phlomis* species are among the most potent essential oils owing to their medicinal characteristics [7–24]. Comparing the essential oil composition of *Phlomis* species many similarities are obvious. Germacrene D, the main compound of the essential oils of *Phlomis* species, was detected in almost all the *Phlomis* species studied, although in some species only in small amounts [7–24]. The oils of other species (*P. anisodonta*, *P. bruguieri*, *P. bruguieri*, *P. cancellata*, *P. chimerae*, *P. chorassanica*, *P. cretica*, *P. cretica*, *P. ferruginea*, *P. fruticosa*, *P. grandiflora* var. *grandiflora*, *P. herba-venti*, *P. lanata*, *P. lanceolata*, *P. leucophracta*, *P. linearis*, *P. nissolii*, *P. olivieri*, *P. olivieri*, *P. olivieri*, *P. persica*, *P. persica*, *P. pungens*, *P. rigida*, *P. samia* and *P. samia*) from 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 (>10%) [7–14,16–23], while samples collected from Yugoslavia were found to contain a low percentage of germacrene D, but to be rich in β-caryophyllene, (*E*)-methyl isoeugenol and α-asarone [15]. In contrast to the reports above, the essential oil of *P. younghunsbandii* from Tibet showed different qualitative and quantitative profiles, in which eugenol, hexadecanoic acid and 9, 12-octadecadienoic acid methyl ester were the major components [24].

Our oil seems to be much closer to the last group (*P. younghunsbandii* from Tibet), but is characterized by the presence of hexadecanoic acid (7.1–52.1%), *trans*-phytol (5.7–50.8%) and 9,12,15-octadecatrien-1-ol (2.2–24.8%). It is possible that the chemical differentiation is correlated to the existence of a new chemotype, provoked either by different climatic factors in China, or may originate as a result of pollination caused by genetic differentiations (infraspecific or intrapopulation crosspollination). The considerable differences among *Phlomis* species may depend on the season, the stage of development and the distinct habitat in which the plant has been collected. It can be concluded that the composition of oils vary greatly with respect to the geographical proximity (different species collected in the same region have similar composition), mainly for the proportion of aliphatic compounds and terpenoids. All these differences suggest further investigations on other species of *Phlomis* that could represent a biodiversity wealth.

This study can be considered as the first information on the composition of essential oils of three wild-growing *Phlomis* species (*P. umbrosa*, *P. megalantha* and *P. szechuanensis*) from Qinling Mountains of China, indicating the existence of a chemical polymorphism in the genus *Phlomis*. Further investigations are needed to reflect taxonomic relationships and biochemical activities in *Phlomis*, since its more than 100 species remain to be investigated.

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References

[1] X.W. Li, I.C. Hedge, in: Z.Y. Wu, Q.H. Raven (Eds.), Flora of China, Science Press, Beijing, PR China, 1994, p. 143.
[2] I. Saracoglu, K. Kojima, U.S. Harput, Y. Ogihara, Chem. Pharm. Bull. 46 (1998) 726–727.
[3] H. Kirmizibekmez, P. Montoro, S. Piacente, C. Pizza, A. Doenmez, I. Calis, Phytochem. Anal. 16 (2005) 1–6.
[4] I. Saracoglu, M. Inoue, I. Calis, Y. Ogihara, Biol. Pharm. Bull. 18 (1995) 1396–1400.
[5] I. Kyriakopoulo, P. Magiatis, A. Skaltounis, N. Aligiannis, C. Harvala, R. Samioside, J. Nat. Prod. 64 (2001) 1095–1097.
[6] M. Couladis, A. Tanimanidis, O. Tzakou, I.B. Chinou, C. Harvala, Planta Med. 669 (2000) 670–672.
[7] P. Sarkhail, G. Amin, M.H.S. Surmaghi, A. Shafiee, Flavour Frag. J. 20 (2005) 327–329.
[8] K. Morteza-Semnani, M. Saeedi, Flavour Frag. J. 20 (2005) 344–346.

[9] K. Morteza-Semnani, K. Moshiri, M. Akbarzadeh, J. Essent. Oil Res. 18 (2006) 672–673.
[10] S. Celik, R.S. Gokturk, G. Flamini, P.L. Cioni, I. Morelli, Biochem. Syst. Ecol. 33 (2005) 617–623.
[11] P. Sarkhail, G. Amin, A. Shafiee, Flavour Frag. J. 19 (2004) 538–540.
[12] N. Aligiannis, E. Kalpoutzakis, I. Kyriakopoulou, S. Mitaku, I.B. Chinou, Flavour Frag. J. 19 (2004) 320–324.
[13] A. Basta, O. Tzakou, M. Couladis, Flavour Frag. J. 21 (2006) 795–797.
[14] C. Formisano, F. Senatore, M. Bruno, G. Bellone, Flavour Frag. J. 21 (2006) 848–851.
[15] M.D. Sokovic, P.D. Marin, P. Janackovic, V. Vajs, S. Milosavljevic, D. Dokovic, V. Tesevic, S. Petrovic, J. Essent. Oil Res. 14 (2002) 167–168.
[16] K. Morteza-Semnani, M. Azadbakht, A. Goodarzi, Flavour Frag. J. 19 (2004) 29–31.
[17] B. Demirci, M.Y. Dadandi, D.H. Paper, G. Franz, K.H.C. Baser, Z. Naturforsch. C 58 (2003) 826–829.
[18] N. Kirimer, K.H.C. Baser, M. Kürkcüoglu, J. Essent. Oil Res. 18 (2006) 600–601.
[19] M. Mirza, Z.B. Nik, Flavour Frag. J. 18 (2003) 131–132.
[20] M.A. Khalilzadeh, A. Rustaiyan, S. Masoudi, M. Tajbakhsh, J. Essent. Oil Res. 17 (2005) 624–625.
[21] P. Sarkhail, G. Amin, A. Shafiee, DARU 14 (2006) 71–74.
[22] S. Masoudi, A. Rustaiyan, K. Larijani, J. Essent. Oil Res. 18 (2006) 16–18.
[23] B. Demirci, K.H.C. Baser, M.Y. Dadandi, J. Essent. Oil Res. 18 (2006) 328–331.
[24] C.R. Bian-ba, M. Wang, F. Wei, S.L. Ge-sang, Z.J. Zhang, R.C. Lin, Zhongguo Yaoxue Zazhi 37 (2002) 904–905.
[25] Pharmacopoeia Committee of the People's Republic of China, Pharmacopoeia of the People's Republic of China, Chemical Industry Press, Beijing, PR China, 2005 (appendix 57).
[26] R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, Allured Publishing Co., Illinois, IL, 1995, pp. 10–24.
[27] Q.B. Wang, Y. Yang, X.M. Zhao, B. Zhu, P. Nan, J.Y. Zhao, L. Wang, F. Chen, Z.J. Liu, Y. Zhong, Food Chem. 98 (2006) 52–58.
[28] G. Flamini, P.L. Cioni, S. Maccioni, R. Baldini, Food Chem. 103 (2007) 1237–1240.
[29] G. Flamini, P.L. Cioni, I. Morelli, A. Bader, Food Chem. 100 (2007) 732–735.