

GLC-MS profiling of non-polar extracts from *Phlomis bucharica* and *P. salicifolia* and their cytotoxicity

[Perfiles GLC-MS de extractos no polares de *Phlomis bucharica* y *P. salicifolia* y su citotoxicidad]

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Abstract: *Phlomis* species (*Phlomis bucharica* Regel and *P. salicifolia* Regel) have been traditionally used by Uzbek people as stimulant, tonic, diuretic, and in the treatment of ulcers, hemorrhoids, wounds and gynecological problems. In the present study, we characterized the chemical composition of non-polar extracts from *P. bucharica* and *P. salicifolia* by high resolution GLC-MS and evaluated their cytotoxicity. Concentrations of hexadecanoic acid in hexane and chloroform extracts were higher in *P. bucharica* than in *P. salicifolia*. 1,8-Cineol, camphor, borneol, α -terpinol, thymol, and isobornyl acetate were detected in *P. bucharica* but not in *P. salicifolia*. About 45 components were identified in *P. bucharica* and 40 in *P. salicifolia*. The chloroform extract from *P. bucharica* showed cytotoxicity in HeLa and HL-60 cells, with IC₅₀ values of 26.07 and 29.42 μ g/ml, respectively.

Keywords: *Phlomis bucharica*, *P. salicifolia*, GLC-MS, Volatiles, Essential oil, Cytotoxicity

Resumen: Las especies *Phlomis* (*Phlomis bucharica* Regel y *P. salicifolia* Regel) se han utilizado tradicionalmente por la gente de Uzbekistán como estimulante, tónico, diurético, y en el tratamiento de las úlceras, hemorroides, heridas y problemas ginecológicos. En el presente estudio, hemos caracterizado la composición química de los extractos no polares de *P. bucharica* y *P. salicifolia* por GLC-MS de alta resolución y se evaluó su citotoxicidad. Las concentraciones de ácido hexadecanoico en extractos de hexano y cloroformo fueron mayores en *P. bucharica* que en *P. salicifolia*. 1,8-cineol, alcanfor, borneol, se detectaron α -terpinol, timol, y acetato de isobornilo en *P. bucharica* pero no en *P. salicifolia*. Cerca de 45 componentes fueron identificados en *P. bucharica* y 40 en *P. salicifolia*. El extracto de cloroformo a partir de *P. bucharica* mostró citotoxicidad en células HL-60 y HeLa, con valores de CI 50 de 26,07 y 29,42 μ g/ml, respectivamente.

Palabras clave: *Phlomis bucharica*, *P. salicifolia*, GLC-MS, Compuestos volátiles, Aceite esencial, Citotoxicidad.

Recibido | Received: August 18, 2014

Aceptado | Accepted: December 31, 2014

Aceptado en versión corregida | Accepted in revised form: September 15, 2015

Publicado en línea | Published online: November 30, 2015

Declaración de intereses | Declaration of interests: Financial support from UNESCO-L'ORÉAL and DAAD foundation for a research fellowship to N.Z. Mamadalieva is gratefully acknowledged.

Este artículo puede ser citado como / This article must be cited as: NZ Mamadalieva, V Vinciguerra, M Sobeh, E Ovidi, ML Ashour, M Wink, A Tiezzi. 2015. GLC-MS profiling of non-polar extracts from *Phlomis bucharica* and *P. salicifolia* and their cytotoxicity. *Bol Latinoam Caribe Plant Med Aromat* 14 (6): 442 – 448.

INTRODUCTION

Plants have been used in many countries for centuries as an important source for biologically active secondary metabolites that can be used in the treatment of many health disorders. Since only 10–20% of all flowering plant species in the world flora have been explored, phytochemical and pharmacological investigations are still needed for many plants (Van Wyk & Wink, 2004).

Phlomis is a large genus of the family Lamiaceae distributed particularly in Asia, Africa and Europe with more than 75 species (Mabberley, 2008; Mathiesen *et al.*, 2011). Phytochemical studies of the genus revealed the presence of various phenolics and terpenoids, such as flavonoids, phenylethanoids, lignans, iridoids and essential oils (El-Negoumy *et al.*, 1986; Kamel *et al.*, 2000; Kyriakopoulou *et al.*, 2001; Aligiannis *et al.*, 2004; Kırmızıbekmez *et al.*, 2005; Delazar *et al.*, 2008; Zhang & Wang, 2008). *Phlomis* species have been employed widely for medicinal purposes in the form of herbal tea with many biological activities, namely, antidiabetic, anti-inflammatory and anti-allergic properties. Furthermore, some *Phlomis* species have recently attracted attention in modern medicine as potential anticancer agents (Gürbüz *et al.*, 2003; Sarkhail *et al.*, 2003; Shin & Lee, 2003; Kırmızıbekmez *et al.*, 2004; Mohajer *et al.*, 2005; Kim, 2006; Sarkhail *et al.*, 2007).

To our knowledge, the chemical composition and the biological properties of *P. bucharica* and *P. salicifolia* from Uzbekistan have not been investigated. In this study, we determined both the chemical composition of non-polar extracts from *P. bucharica* and *P. salicifolia* and their cytotoxic activities against HeLa and HL-60 cancer cell models.

MATERIALS AND METHODS

Plant material

Aerial parts of the *Phlomis bucharica* and *P. salicifolia* were collected in the Surkhan-Darya and Tashkent regions of Uzbekistan in the summer of 2010. The plants were identified at the Department of Herbal Plants, Institute of the Chemistry of Plant Substances (ICPS), Uzbekistan by Dr. Nigmatullaev O.A. The voucher specimens of *P. bucharica*, and *P. salicifolia*, and *P. salicifolia* (accession number N 20101022 and N 201010112) have been deposited at the Department of Herbal Plants (ICPS, Uzbekistan).

Preparation of samples

The plant material (aerial parts or roots from flowering plants) was air-dried at room temperature before grinding it to a fine powder with a Waring blender. About 100 g of the powdered plant material was extracted with 500 ml of the following solvents (methanol, hexane, chloroform and water, respectively). Extraction with each solvent was carried out for one day. The solvents were evaporated in a rotary vacuum evaporator at 40° C. Yields of methanol, hexane, chloroform and water extracts from *P. bucharica* were 7.23%, 1.95%, 2.17%, 13.73% and from *P. salicifolia* 12.0%, 0.74%, 2.0%, 10.4%, respectively. The extracts were kept in a refrigerator until further use.

GLC/MS analysis

Gas-liquid chromatography–mass spectrometry was carried out on a Hewlett-Packard 5890 gas chromatograph equipped with a DB-5MS fused silica column (5% diphenyl/95% dimethyl arylenepoly-siloxane 60 m × 0.32 mm, film thickness 0.25 mm, Agilent Technologies), interfaced with a Hewlett-Packard mass selective detector 5971 (Agilent Technologies) operated by HP Enhanced ChemStation software, version A.03.00. Interface temperature: 280° C; MS source temperature: 180° C; ionization energy: -70 eV; scan range: 35–500 atomic mass units; scans per second: 1.65. GLC conditions: cold on-column injection (oven-track temperature); oven temperature was kept at 85° C for 2 min, then programmed to 150° C at a rate of 50° C/min and held at 150° C for 2 min; finally increased to 275° C at a rate of 30° C/min and held at 275° C for 15 min. The carrier gas was helium at a flow rate of 1.33 mL/min (constant flow conditions). Diluted samples were injected with split mode (split ratio, 1:15).

Qualitative and quantitative analyses

Components of the non-polar extracts were identified using: (i) their mass spectra by matching with reference spectra from Wiley/NIST database; (ii) and literature data) (Adams, 2007; Maurer *et al.*, 2007). The quantification of the individual components was based on GLC/MS raw data of percent areas under the curve from three independent runs using the normalization method.

Cell cultures

Cytotoxic activities of the samples were investigated against HeLa (human cervix adenocarcinoma), and

HL-60 (leukemia cancer cell lines). HeLa cells were maintained in DMEM complete media (L-glutamine supplemented with 10% heat-inactivated fetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin). HL-60 cells were grown in RPMI 1640 media which were supplemented with 10% heat inactivated fetal bovine serum, 4 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Cells were grown at 37° C in a humidified atmosphere of 5% CO₂ (Mamadalieva *et al.*, 2011).

MTT assay

Cytotoxicity of the samples was determined in triplicate using the MTT cell viability assay (Mosmann, 1983). The samples were dissolved in dimethylsulfoxide (DMSO) and further serially diluted with the media in two-fold fashion into six different concentrations in order to attain final

concentrations ranging from 6.25 to 200 µg/ml for extracts in 96-well plates. 100 µl media which contains the sample was dispensed into each well. The concentration of the solvent DMSO did not exceed 0.05% in the media for the highest concentration in samples. Cells (2×10^4 cells/well of exponentially growing HeLa cells and 1×10^4 cells/well for HL-60 cells) were seeded in a 96-well plate (Greiner Labortechnik), the cells were cultivated for 24 h and then incubated with various concentrations of the serially diluted tested samples at 37° C for 24 h and then with 0.5 mg/mL MTT for 4 h. The formed crystals (blue color) were dissolved by the addition of the 100 µl DMSO in each well. The absorbance was measured at 595 nm with a Tecan Sunrise Reader (Tecan Group Ltd., Switzerland).

The cell viability (%) of three independent experiments was calculated by the following formula:

$$\text{Cell viability (\%)} = (\text{OD of treated cells}) / (\text{OD of control cells}) \times 100\%$$

The IC₅₀ was determined as the drug concentration which resulted in a 50% reduction in cell viability or inhibition of the biological activity. IC₅₀ values were calculated using a four-parameter logistic curve (SigmaPlotR 11.0). Doxorubicin was used as positive control (Mamadalieva *et al.*, 2011).

Statistical analysis

All experiments were carried out three times unless indicated. Continuous variables were presented as mean ± SD of three individual experiments. All data were statistically evaluated using Student's t-test and/or the Kruskal-Wallis test (GraphPad PrismR 5.01; GraphPad Software, Inc., La Jolla, CA, USA) followed by Dunn's post-hoc multiple comparison test when the significance value was < 0.05 using the same significance level. The criterion for statistical significance was P < 0.05.

RESULTS AND DISCUSSION

The chemical composition of both hexane and chloroform extracts of *P. bucharica* and *P. salicifolia* was investigated using high-resolution gas-liquid chromatography-mass spectrometry as indicated in Table 1. In the hexane extract of *P. bucharica*, 35 compounds were identified accounting for about

98.9% of the total peak area. Hexadecanoic acid was the most abundant compound accounting for 21.6% followed by linoleic acid (17.9%), nonacosane (15.7%) and octadecanoic acid (11.2%), respectively. While 38 similar compounds were identified in the chloroform extract representing 99.0% of the peak area (Table 1). Hexadecanoic acid has been found in other *Phlomis* species (*Phlomis megalantha* (Zhang & Wang, 2008), *Phlomis venti* (Morteza-Semnani *et al.*, 2004)) where it was more abundant than in *P. bucharica*, but less abundant in *P. lunariifolia* (Demirci *et al.*, 2003). Linoleic acid content was higher in *P. bucharica* than in *Phlomis elliptica* from Iran (Javidnia *et al.*, 2010).

In the hexane extract of *P. salicifolia* 32 compounds were identified (98.0 % of the peak area). The major identified compounds were nonacosane (13.2%), linoleic acid (13.1%), hexacosane (11.3%), octacosane (11.2%) and hexadecanoic acid (9.6%). In the chloroform extract; the most abundant compounds were nonacosane (17%) followed by hexadecanoic acid (12.8%). Furthermore, the essential fatty acid linoleic acid (11.6%) was found in the hexane extract, whereas linolenic acid (4.7%) was identified only in the chloroform extract.

Table 1The chemical composition of hexane and chloroform extracts from *P. bucharica* and *P. salicifolia*.

Compound name	Calculated Kovat's index (RI)	Relative abundance (%)			
		<i>P. bucharica</i>		<i>P. salicifolia</i>	
		Hexane	CHCl ₃	Hexane	CHCl ₃
1,8-Cineol	1038	0.2	–	–	–
Camphor	1153	0.5	0.5	–	–
Borneol	1177	0.8	0.6	–	–
α -Terpinol	1196	0.1	0.2	–	–
Isobornyl acetate	1283	0.1	0.8	–	–
Thymol	1290	1.0	0.1	–	–
α -Terpinyl acetate	1345	0.2	0.3	–	–
α -Copaene	1382	–	–	0.1	–
β -Bourbonene	1390	–	–	0.1	–
(E)-Caryophyllene	1420	–	0.9	–	–
(E)- β -Farnesene	1452	–	0.2	0.1	–
α -Humulene	1458	–	0.5	–	–
Dihydroactinidiolide	1532	–	0.3	0.2	0.4
Caryophyllene oxide	1584	2.1	1.0	0.8	0.5
Humulene epoxide II	1608	0.8	0.6	–	–
β -Biotol	1618	–	–	0.1	–
Eremoligenol	1631	–	0.6	–	–
Caryophylla-4(12),8(13)-dien-5a-ol	1640	0.9	0.4	–	–
α -Eudesmol	1653	–	1.2	–	–
α -Cadinol	1658	1.1	–	–	–
3-Tujopsanone	1658	–	–	0.1	–
14-Hydroxy-(Z)-caryophyllene	1667	–	0.6	–	–
(Z)- α -Santalol	1672	0.5	–	–	–
Amorpha-4,9-dien-2-ol	1693	–	–	0.7	1.0
Heptadecane	1700	–	0.3	–	–
Tetradecanoic acid	1762	0.4	0.3	0.4	0.6
(-)-Loliolide	1783	–	–	–	0.3
6,10,14-Trimethyl-2-pentadecanone	1833	2.0	1.2	0.9	1.0
Pentadecanoic acid	1863	–	–	–	0.3
Methyl hexadecanoate	1914	1.7	0.3	2.6	1.5
Hexadecanoic (palmitic) acid	1968	21.6	25.0	9.6	12.8
Isopropyl hexadecanoate	2012	0.1	0.3	0.6	0.3
Heptadecanoic acid	2059	0.4	0.3	tr	0.3
Methyl linoleate	2080	0.6	0.1	1.3	1.0
Methyl linolenate	2087	2.1	0.3	1.3	–
Methyl oleate	2090	–	–	–	2.1
Phytol isomer	2098	0.2	0.5	1.0	1.1
Methyl octadecanoate	2113	0.7	–	0.7	1.2
Linoleic acid	2139	17.9	17.5	13.1	11.6
Linolenic acid	2146	–	11.8	–	4.7
Octadecanoic acid	2170	11.2	8.5	2.2	4.0
Docosane	2200	0.7	–	0.5	–
Tricosane	2300	1.1	0.3	3.0	0.8
Hydrocarbon	2323	–	–	3.8	–
Hydrocarbon	2339	–	–	2.1	0.9
Eicosanoic acid	2359	–	2.4	–	1.2

Tetracosane	2400	0.8	0.2	2.2	0.5
Polyisoprene	2435	–	–	7.3	–
Pentacosane	2500	1.4	0.5	2.2	2.0
Hexacosane	2600	1.5	0.4	11.3	0.6
Heptacosane	2700	4.8	2.8	5.1	5.5
Methyl tetracosanoate	2712	–	–	–	1.3
β -Sitosterol	2763	–	–	–	3.0
Octacosane	2800	1.7	3.7	11.2	10.5
Polyisoprene	2805	1.2	–	–	8.9
Nonacosane	2900	15.7	11.2	13.2	17.0
Triacontane	3000	2.8	2.3	0.2	1.9
Alkanes, alkenes and hydrocarbons		30.5	21.7	54.8	39.7
Ketones, alcohols and aldehydes		2.0	1.2	0.9	1.0
Fatty acids and aliphatic esters		56.7	66.8	31.8	42.9
Terpenes and other compounds		9.7	8.8	3.2	15.2
Total identified		98.9	98.5	98.0	98.8

Compounds are listed in order of their retention on DB-5MS column

** The abundance is calculated as average of three analyses; total peak area = 100%. The identification is based on MS libraries, RI and co-elution with available authentic compounds. Buthylhexadecanoate, thymol acetate, α -tocopherol and tetradecanal were detected as traces.*

- = not detected

The phytochemical profile of *P. bucharica* and *P. salicifolia* were quite similar (Table 1). However, the hexadecanoic acid content was higher in *P. bucharica* than in *P. salicifolia*. 1,8-Cineol, camphor, borneol, α -terpinol, thymol, and isobornyl acetate could not be detected in *P. salicifolia* despite their detection in *P. bucharica*.

The anti-proliferative activity of water, methanol, chloroform and hexane extracts from *P. bucharica* and *P. salicifolia* extracts was assessed against two different cancer cell lines using the MTT assay. The IC₅₀ values are presented in Table 2. The chloroform and hexane extracts of *P. bucharica* showed a higher cytotoxicity (IC₅₀ values between 26.07 μ g/ml for HeLa and 29.42 μ g/ml HL-60 cells) in comparison with water and methanol extracts of the same species and in comparison with the other *Phlomis* species. Our results against both cell lines showed better activity than those reported previously about other *Phlomis* species (Thoppil *et al.*, 2013; Soltani-Nasab *et al.*, 2014). Aqueous extracts of *Phlomis platystegia* exhibit weak toxicity towards

HepG-2 cell proliferation. IC₅₀ values of different extracts from *Phlomis lanceolata* against HT29, Caco2, T47D and NIH3T3 cell lines were higher than 200 μ g/ml. The reported cytotoxic activity is usually attributed to the phenylethanoid, phenylpropanoids, verbascosides and caffeic acid contents (Li *et al.*, 2010; Limem-Ben Amor *et al.*, 2009). The potent cytotoxic activity may be attributed to the high content of free hexadecanoic acid (= palmitic acid) and other lipophilic constituents that already showed a high selective cytotoxic activity against human leukemia cells MOLT-4 and induced apoptosis at 50 μ g/ml without affecting the topoisomerase II enzyme (Harada *et al.*, 2002). However, the other lipophilic compounds which can interact with membrane permeability and protein conformation could also be relevant in this context (Wink, 2008). More studies are needed on individual compounds and of polar extracts of *Phlomis* to understand their traditional applications and potential future exploitation in phytomedicine.

Table 2
***In vitro* cytotoxic activity of *P. bucharica* and *P. salicifolia* extracts tested against cancer cell lines HeLa and HL-60 after exposure for 24 h (MTT test).**

Name of plant	Extract	IC ₅₀ (µg/ml)	
		HeLa	HL-60
<i>P. bucharica</i>	Chloroform	26.07 ± 1.30	29.42 ± 1.76
	Methanol	>100	>100
	Water	>100	>100
	Hexane	>100	30.83 ± 2.16
<i>P. salicifolia</i>	Chloroform	>100	53.96 ± 2.16
	Methanol	>100	>100
	Water	>100	>100
	Hexane	>100	47.73 ± 2.39
Doxorubicin (µM) (positive control)		1.84 ± 0.19	0.02

CONCLUSION

In this study, we report the chemical composition of both the hexane and chloroform extracts of the aerial parts of *Phlomis bucharica* and *P. salicifolia* collected in the Surkhan-Darya and Tashkent regions of Uzbekistan. Altogether, 57 compounds were identified by GLC/MS in both extracts representing not less than 98% of the total detected compounds. The cytotoxicity of the extracts were assessed against HL-60 and HeLa cell lines. The antiproliferative activity of the extracts depends largely upon the concentration of extracts. The chloroform extract of *P. bucharica* showed highest cytotoxic activity against HL-60 and HeLa cells, while other extracts showed the lowest activity. This warrants further investigations regarding the cytotoxic properties and other constituents of the chloroform extract of *P. bucharica*.

ACKNOWLEDGEMENTS

Financial support from UNESCO-L'ORÉAL and DAAD foundation for a research fellowship to N.Z. Mamadaliyeva is gratefully acknowledged.

REFERENCE

- Adams RP. 2007. **Identification of essential oil components by gas chromatography/quadrupole mass spectroscopy**. 4th ed., Allured Publishing Co., Illinois, USA.
- Aliigiannis N, Kalpoutzakis E, Kyriakopoulou I, Mitaku S, Chinou IB. 2004. Essential oils of *Phlomis* species growing in Greece: chemical composition and antimicrobial activity. **Flavour Fragr J** 19: 320 - 324.
- Delazar A, Sabzevari A, Mojarrab M, Nazemiyeh H, Esnaashari S, Nahar L, Razavi S, Sarker S. 2008. Free-radical-scavenging principles from *Phlomis caucasica*. **J Nat Med** 62: 464 - 466.
- Demirci B, Dadandi MY, Paper DH, Franz G, Başer KH. 2003. Chemical composition of the essential oil of *Phlomis linearis* Boiss. & Bal., and biological effects on the CAM-assay: a safety evaluation. **Z Naturforsch C** 58: 826 - 829.
- El-Negoumy SI, Abdalla MF, Saleh NA. 1986. Flavonoids of *Phlomis aurea* and *P. floccosa*. **Phytochemistry** 25: 772 - 774.
- Gürbüz İ, Üstün O, Yesilada E, Sezik E, Kutsal O. 2003. Anti-ulcerogenic activity of some plants used as folk remedy in Turkey. **J Ethnopharmacol** 88: 93 - 97.
- Harada H, Yamashita U, Kurihara H, Fukushi E, Kawabata J, Kamei Y. 2002. Antitumor activity of palmitic acid found as a selective cytotoxic substance in a marine red alga. **Anticancer Res** 22: 2587 - 2590.
- Javidnia K, Miri R, Soltani M, Khosravi AR. 2010. Essential oil composition of two species of *Phlomis* L. (*Phlomis aucheri* Boiss. and *Phlomis elliptica* Benth.) (Lamiaceae) from Iran. **J Essent Oil Res** 22: 314 - 317.
- Kamel MS, Mohamed KM, Hassanean HA, Ohtani K, Kasai R, Yamasaki K. 2000. Iridoid and megastigmane glycosides from *Phlomis aurea*. **Phytochemistry** 55: 353 - 357.
- Kim J-S. 2006. **Compositions for inducing secretion of insulin-like growth factor-1**, US 6984405 B1.
- Kirmizibekmez H, Calis I, Perozzo R, Brun R, Donmez AA, Linden A, Ruedi P, Tasdemir D. 2004. 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** 70: 711 - 717.
- Kırmızıbekmez H, Montoro P, Piacente S, Pizza C, Dönmez A, Çalış İ. 2005. Identification by HPLC-PAD-MS and quantification by HPLC-PAD of phenylethanoid glycosides of five *Phlomis* species. **Phytochem Anal** 16: 1 - 6.
- Kyriakopoulou I, Magiatis P, Skaltsounis A-L, Aligiannis N, Harvala C. 2001. Samioside, a new phenylethanoid glycoside with free-radical scavenging and antimicrobial activities from *Phlomis samia*. **J Nat Prod** 64: 1095 - 1097.
- Li MX, Shang XF, Jia ZP, Zhang RX. 2010. Phytochemical and biological studies of plants from the genus *Phlomis*. **Chem Biodivers** 7: 283 - 301.
- Limem-Ben Amor I, Boubaker J, Ben Sgaier M, Skandrani I, Bhourri W, Neffati A, Kilani S, Bouhlel I, Ghedira K, Chekir-Ghedira L. 2009. Phytochemistry and biological activities of *Phlomis* species. **J Ethnopharmacol** 125: 183 - 202.
- Mabberley D. 2008. **Mabberley's Plant-book: A Portable Dictionary of Plants, their Classifications, and Uses**. Cambridge University Press. Cambridge, New York, USA.
- Mamadaliyeva NZ, Herrmann F, El-Readi MZ, Tahrani A, Hamoud R, Egamberdieva DR, Azimova SS, Wink M. 2011. Flavonoids in *Scutellaria immaculata* and *S. ramosissima* (Lamiaceae) and their biological activity. **J Pharm Pharmacol** 63: 1346 - 1357.
- Mathiesen C, Scheen AC, Lindqvist C. 2011. Phylogeny and biogeography of the lamioid genus *Phlomis* (Lamiaceae). **Kew Bull** 66: 83 - 99.
- Maurer HH, Pflieger K, Weber AA. 2007. **Mass spectral and GC data of drugs, poisons, pesticides, pollutants and their metabolites.**, 3rd edition, Wiley-VCH Verlag, Weinheim, Germany.
- Mohajer M, Sarkhail P, Hajarolasvadi N, Zamani M, Khorasani R, Shafiee A, Amin G, Abdollahi M. 2005. Antiinflammatory and analgesic effects of *Phlomis lanceolata* Boiss and Hohen. extracts and examination of their components. **Int J Pharmacol** 2: 50 - 54.
- Morteza-Semnani K, Azadbakht M, Goodarzi A. 2004. The essential oils composition of *Phlomis herba-venti* L. leaves and flowers of Iranian origin. **Flavour Fragr J** 19: 29 - 31.
- Mosmann T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. **J Immunol Methods** 65: 55 - 63.
- Sarkhail P, Abdollahi M, Shafiee A. 2003. Antinociceptive effect of *Phlomis olivieri* Benth., *Phlomis anisodonta* Boiss. and *Phlomis persica* Boiss. total extracts. **Pharmacol Res** 48: 263 - 266.
- Sarkhail P, Rahmanipour S, Fadyevatan S, Mohammadirad A, Dehghan G, Amin G, Shafiee A, Abdollahi M. 2007. Antidiabetic effect of *Phlomis anisodonta*: Effects on hepatic cells lipid peroxidation and antioxidant enzymes in experimental diabetes. **Pharmacol Res** 56: 261 - 266.
- Shin TY, Lee JK. 2003. Effect of *Phlomis umbrosa* root on mast cell-dependent immediate-type allergic reactions by anal therapy. **Immunopharmacol Immunotoxicol** 25: 73 - 85.
- Soltani-Nasab F, Asgarpanah J, Majdzadeh M, Ostad SN. 2014. Investigating the effect of *Phlomis lanceolata* Boiss and Hohen on cancer cell lines. **Pharmacol Res** 52: 333 - 336.
- Thoppil RJ, Harlev E, Mandal A, Nevo E, Bishayee A. 2013. Antitumor activities of extracts from selected desert plants against HepG2 human hepatocellular carcinoma cells. **Pharm Biol** 51: 668 - 674.
- Van Wyk BE, Wink M. 2004. **Medicinal plants of the world: an illustrated scientific guide to important medicinal plants and their uses**. Timber Press, Portland, USA.
- Wink M. 2008. Evolutionary advantage and molecular modes of action of multi-component mixtures used in phytomedicine. **Curr Drug Metab** 9: 996 - 1009.
- Zhang Y, Wang ZZ. 2008. Comparative analysis of essential oil components of three *Phlomis* species in Qinling Mountains of China. **J Pharm Biomed Anal** 47: 213 - 217.