Pharmaceutical sciences, 2014, 20(1), 22-28

http://pharm-sci.tbzmed.ac.ir

Essential Oil Composition, Antioxidant Activity and Total Phenolic Content of Some Lamiaceae Taxa Growing in Northwest of Iran

Mohammad-Reza Delnavazi[1], Farzin Baba-ali[2], Sepideh Soufiabadi[1], Mona Sherafatmand[1], Fariba Ghahremani[1], Saeed Tavakoli[1], Narguess Yassa[1]*

[1]*Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.*

[2]*Science and Research Campus, Islamic Azad University, Tehran, Iran.*

ARTICLEINFO

Article Type:
Original Research

Article History:
Received: 28 June 2014
Accepted: 2 July 2014

Keywords:
Ziziphora tenuior
Scutellaria orientalis
Eremostachys laciniata
Phlomis herba-venti
DPPH
Folin-Ciocalteu

ABSTRACT

Background: Lamiaceae family is one of the main sources of aromatic and medicinal plants. In the present study essential oil constituents, free radical-scavenging activity and total phenolic content of the aerial parts of four Lamiaceae taxa, *Ziziphora tenuior* (ZT), *Scutellaria orientalis* subsp. *virens* (SO), *Eremostachys laciniata* subsp. *iberica* (EL) and *Phlomis herba-venti* subsp. *pungens* (PH), collected from "Mishu-Dagh" region (Northwest of Iran) were investigated. ***Methods:*** GC and GC-MS were applied for the analysis of plants oils. Free radical-scavenging activity and total phenolic content of the plants hydroalcoholic extracts and their polar and non-polar fractions were also evaluated using DPPH and Folin-Ciocalteu methods, respectively. ***Results:*** A total of 58 compounds were identified in essential oils, among them 1,8-cineol (19.6%), germacrene D (16.5%), linalool (10.2%) and germacrene D (11.7%) were characterized as main compounds in ZT, SO, EL and PH oils, respectively. In DPPH assay, polar fractions of EL and ZT exhibited considerable free radical-scavenging activity (IC_{50}; 11.0 ± 2.3 and 12.7 ± 2.7 µg ml^{-1}, respectively) in comparison with BHT (IC_{50}; 10.8 ± 2.1 µg ml^{-1}). The former fractions were also found to contain the highest total phenolic content (231.9 ± 9.3 and 214.1 ± 11.3 mg EGA/g, respectively). ***Conclusion:*** The present study introduces these four taxa as the plants with terpene rich oils and suggests them as potential sources of free radical-scavenging compounds.

Introduction

Lamiaceae (*alt.* Labiatae) family with a wide distribution all over the world is one of the main sources of aromatic and medicinal plants.[1] In Iran, this family is represented by 46 genera and 410 species/subspecies, of which 74 species have been mentioned as medicinal plants in the ancient Iranian medicinal literature.[1]

"Mishu-Dagh" mountains located in East-Azerbaijan province (Northwest of Iran), is an important vegetation ecotone within the Irano-Turanian phytogeographical region.[2] About 390 species in 69 families have been described in the flora of Mishu-Dagh.[2] In the present study four Lamiaceae taxa which were collected from this region, namely *Ziziphora tenuior* L., *Scutellaria orientalis* subsp. *virens* (Boiss. & Kotschy) J.R.Edm., *Eremostachys laciniata* subsp. *iberica* (Vis.) Popov and *Phlomis herba-venti* subsp. *pungens* (Willd.) Maire ex De Filipps, were investigated for their essential oil constituents, free radical-scavenging capacities and total phenolic contents.

Ziziphora tenuior ("Kakuti" in Persian) is an aromatic herbaceous plant, distributed throughout Iran.[3] In folk medicine of Iran, infusion of this plant aerial parts is used in treatment of fever, dysentery, coughing, painful menstruation and diarrhea, and also as carminative, expectorant and emmenagogue.[1] While pharmacological studies have shown antibacterial[4], sedative[5], analgesic[6] and immunostimulant[7] activities of *Z. tenuior*, the previous phytochemical investigations have established the presence of six flavonoid derivatives, luteolin, apigenin, 5-O-methyl-apigenin, apigenin-7-O-glucoside and ziziphorins A & B and some triterpenoid derivatives in the plant extract[8] as well as high amounts of pulegone (71-87%) in its essential oil.[9,10]

Scutellaria orientalis is a polymorphic species with many infraspecific taxa growing in south-western

***Corresponding Author:** Narguess Yassa, Department of Pharmacognosy, Faculty of pharmacy, Tehran University of Medical Sciences, Tehran, Iran. Tel/Fax: +98 21 66959101, E-mail: yasa@sina.tums.ac.ir

Asia.[11] Various subspecies of this plant are used as tonic, astringent and hemostatic in folk medicine of Turkey.[12] It has also been reported that the tincture of *S. orientalis* aerial parts possesses hypotensive and sedative effects and its plaster is useful in treatment of tumors.[13] *S. orientalis* subsp. *virens* is a perennial plant, native to Iran and Turkey.[3] In flora of Iran this subspecies has been regarded as an independent species as *S. virens* Boiss. & Kotschy.[3] To date, phytochemical constituents of this subspecies has not been investigated. However, some flavonoids, phenethyl-alcohol glycosides and neo-clerodane diterpenoids have been reported from other subspecies of *S. orientalis* (e.g. subsp. *sintenisii*, subsp. *pinnatifida*, subsp. *porphyrostegia*).[13,17]

Eremostachys laciniata with two subspecies (subsp. *laciniata* and subsp. *iberica*) is one of the seventeen *Eremostachys* species from the flora of Iran.[3] In Iranian traditional medicine this species is known as "Chelle-Daghi" and its rhizomes are used as emollient to relieve rheumatoid arthritis pains.[18] Moreover, decoction of the rhizomes and flowers of *E. laciniata* are traditionally used to treat allergies, headache and liver diseases.[19] Some pharmacological properties such as antioxidant[20], antibacterial[21], antidepressant[22], anti-inflammatory[23] and analgesic[24] effects have been documented for *E. laciniata*. Previous phytochemical investigations have also reported the isolation of iridoid, phenylethanoid and flavonoid derivatives from the aerial parts[25] and some iridoid and furanolabdane diterpene glycosides from the rhizomes of this medicinal species.[21,26]

Phlomis herba-venti subsp. *pungens* (Syn.: *Phlomis pungens* subsp. *pungens*) with 25-60 cm in height is a perennial plant, distributed in the north, north-west and center of Iran.[3] Antioxidant, antibacterial and antifungal activity of *P. herba-venti* have been reported during previous surveys.[27,28] Three phenylethanoid glycosides, namely forsythoside B, alyssonoside and leucosceptoside B, together with an iridoid glycoside, lamiide, have also been isolated from the aerial parts of subspecies *pungens*.[29] Although there is no report on ethnobotanical indications of this subspecies, the calyxes of *P. herba-venti* have been reported that are used in Jaén region (Spain) as veterinary antidiarrheic and for soothe muscle pains.[30]

According to our literature survey, this is the first report on essential oil composition, free radical-scavenging activity and total phenolic content of these four taxa from East-Azerbaijan, northwest of Iran.

Material and Methods

Plant materials

The aerial parts of the plants were collected from southern slopes of "Mishu-Dagh" mountains (Shanjan region, Shabestar County, East-Azerbaijan province, Iran) at their flowering stage in June 2012. The plants were authenticated by botanist Dr. Yousef Ajani (Institute of Systematic Botany, Johannes Gutenberg University, Mainz, Germany).

Extraction and fractionation

The air-dried and ground aerial parts (200 g each) were individually macerated with a methanol-water mixture (8:2) (5× 1L each) at the room temperature. The obtained total hydroalcoholic extracts were concentrated using a rotary evaporator at 45 °C. A portion of each extracts (30 g) was then dissolved in a methanol-water mixture (6:4) (200 ml) and subjected to fractionation using liquid-liquid extraction method with enough volumes of chloroform to get two polar and non-polar fractions.

Essential oils extraction

Essential oils were extracted from the air-dried and comminuted plants (100 g each) using hydrodistillation method for 4 h by a Clevenger-type apparatus (Yields (v/w); *Z. tenuior*; 0.2%, *S. orientalis* subsp. *virens*; 0.15%, *E. laciniata* subsp. *iberica*; 0.2% and *P. herba-venti* subsp. *pungens*; 0.1%). The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 °C until analysis in umber glasses.

GC and GC-MS analyses

A Hewlett-Packard 6890 gas chromatograph with HP-5MS column (30m ×0.25mm id, 0.25µm film thickness) equipped with a mass detector (Hewlett-Packard model 5973 HP) was applied for essential oils analyses. The flow rate of carrier gas (Helium) was 1 ml/min. The initial oven temperature was 40 °C and was then raised at a rate of 3 °C per a minute to 250 °C. The injection temperature was 250 °C and the oil samples (1 µl) were injected with a split ratio of 1:90. The mass spectra were obtained by electron ionization at 70 eV. The retention indices (RI) of the compounds were calculated using a homologous series of n-alkanes injected in conditions equal to the samples.

Constituents of the essential oils were identified using computer matching with the Wiley7n.L library, and also by comparison of the retention indices and fragmentation pattern of the mass spectra with those published in the literature for standard compounds.[31]

The essential oils were also analyzed on an Agilent HP-6890 gas chromatograph coupled with a FID detector to quantify relative amounts of the separated compounds. The FID detector temperature was 290 °C and the operation was performed under the same conditions as described for GC-MS analyses.

DPPH free radical-scavenging assay

Free radical-scavenging potentials of the total extracts and fractions were evaluated using 2,2-diphenyl-1-picryl-hydrazyl (DPPH) method.[32] Briefly, 2 ml of freshly prepared sample solutions (10 µg ml^{-1}) were serially diluted with methanol to get concentrations ranging from 0.5 to 7.75×10^{-3} mg ml^{-1}. 2 ml of DPPH (Sigma) solution (80 µg ml^{-1} in methanol) was then added to diluted solutions and were kept 30 min at 25 °C in dark for any reaction to take place. UV absorptions were recorded at 517nm. Butylated

hydroxytoluene (BHT) was used as a positive control. The test was performed in triplicate and IC_{50} value was reported as means ± SEM.

Table 1. Chemical composition of essential oils obtained from the aerial parts of four Lamiaceae taxa from northwest Iran.

No.	Compounds[e]	RI[f]	ZT[a] %	SO[b] %	EL[c] %	PH[d] %	No.	Compounds	RI	ZT %	SO %	EL %	PH %
1	α-thujene	926	0.5	-	-	-	**34**	aromadendrene	1442	0.5	-	-	-
2	α-pinene	934	4.3	4.0	9.3	7.3	**35**	α-humulene	1455	0.3	1.2	-	0.6
3	camphene	948	0.8	0.3	-	-	**36**	geranyl acetone	1456	-	-	2.0	-
4	sabinene	971	2.4	1.1	2.1	-	**37**	(E)-β-farnesene	1457	-	-	-	1.7
5	1-octen-3-one	974	-	-	6.7	-	**38**	germacrene D	1488	4.9	16.5	1.4	11.7
6	1-octen-3-ol	976	1.0	7.3	-	5.8	**39**	(E)-β-ionone	1491	0.2	-	6.4	-
7	β-pinene	976	5.4	-	-	-	**40**	bicyclogermacrene	1505	2.0	-	-	3.1
8	2-pentylfuran	986	-	-	2.5	-	**41**	α-muurolene	1505	0.2	-	-	-
9	myrcene	990	7.6	0.4	1.5	-	**42**	(E,E)-α-farnesene	1510	-	3.5	-	-
10	3-octanol	990	-	-	-	0.6	**43**	γ-cadinene	1518	0.6	-	-	-
11	n-decane	1000	-	-	2.6	0.5	**44**	(Z)-calamenene	1532	0.4	-	-	-
12	δ-3-carene	1010	-	-	-	0.7	**45**	δ-cadinene	1541	1.9	1.7	-	1.5
13	α-terpinene	1015	0.3	-	-	-	**46**	α-calacorene	1547	0.2	-	-	-
14	p-cymene	1023	0.6	0.9	9.1	2.0	**47**	spathulenol	1580	12.2	6.6	-	7.6
15	limonene	1027	-	-	5.0	1.2	**48**	caryophyllene oxide	1586	-	1.3	-	-
16	1,8-cineol	1028	19.6	-	-	-	**49**	isospathulenol	1642	1.4	-	-	-
17	(E)-β-ocimene	1046	0.5	-	3.2	-	**50**	α-cadinol	1656	-	0.7	-	-
18	γ-terpinene	1056	0.7	1.6	4.1	-	**51**	oplopenone	1743	0.5	-	-	-
19	terpinolene	1088	-	15.6	-	9.1	**52**	HHFA[g]	1858	-	1.0	6.0	6.0
20	linalool	1097	1.0	-	10.2	-	**53**	hexadecanoic acid	1969	0.7	1.9	7.9	7.4
21	terpinen-4-ol	1176	0.7	-	1.7	-	**54**	HAME[h]	1990	-	-	-	5.2
22	myrtenal	1197	0.2	-	-	-	**55**	oleic acid	2138	0.4	-	1.1	-
23	α-terpineol	1188	-	-	1.2	1.2	**56**	tricosane	2300	2.2	-	-	-
24	pulegone	1235	2.4	-	-	-	**57**	heptacosane	2700	-	-	2.6	-
25	citronellyl formate	1273	-	4.6	-	-	**58**	nonacosane	2900	-	-	1.7	-
26	thymol	1291	-	6.4	-	-				**ZT**	**SO**	**EL**	**PH**
27	neryl acetate	1361	-	3.4	-	-	Hydrocarbone monoterpenes			23.1	23.9	34.3	20.3
28	α-copaene	1376	3.3	-	-	-	Oxygenated monoterpenes			23.9	10.2	15.1	1.2
29	geranyl acetate	1381	-	2.2	-	-	Hydrocarbone sesquiterpenes			25.8	36.3	2.1	31.7
30	(E)-β-damascenone	1386	-	-	3.5	1.4	Oxygenated sesquiterpenes			14.1	9.6	6.0	13.6
31	β-bourbonene	1390	4.0	-	-	7.3	Hydrocarbone non-terpenes			2.2	-	8.0	0.5
32	(Z)-α-bergamotene	1415	-	-	-	0.8	Oxygenated non-terpenes			2.3	15.6	27	20.4
33	β -caryophyllene	1421	7.5	13.4	0.7	5.0	Total identified			91.4	95.6	92.5	87.7

[a]*Ziziphora tenuior;* [b]*Scutellaria orientalis* subsp. *virens*; [c]*Eremostachys laciniata* subsp. *iberica*; [d]*Phlomis herba-venti* subsp. *pungens*; [e]Identified compounds listed in order of elution from HP-5MS column; [f] Retention indices to C8-C24 n-alkanes on HP-5MS column; [g]Hexahydrofarnesyl acetone; [h]Hexadecanoic acid methyl ester.

Total phenolic contents evaluation

Total phenolic content (TPC) of the total extracts and their fractions were measured by a colorimetric method using Folin-Ciocalteu reagent.[33] Briefly, 1.5 ml of tenfold water-diluted Folin-Ciocalteu reagent (Merck) was added to 200 µl of prepared extracts/fractions solution (500 µg ml^{-1}) and allowed to stand at the room temperature for 5 min. 1.5 ml of Sodium bicarbonate solution (60 g l^{-1}) was then added to the mixture and stored 90 min at 22 °C. The absorptions of the final solution were recorded on a Cecil CE7250 spectrophotometer at 725 nm. TPCs were quantified using a calibration curve obtained from absorbance measuring of the gallic acid concentrations (50-200 µg

ml^{-1}) as standard. The experiment performed in triplicate and the results were expressed as milligrams of gallic acid equivalents (GAE) per gram of dry matter (total extracts and fractions) as means ± SEM.

Results and discussion

Essential oils composition

Z. tenuior oil

Thirty-five compounds, representing 91.4% of the total oil, were identified as a result of GC and GC-MS analyses of *Z. tenuior* essential oil, among them 1,8-cineol (19.6%) and spathulenol (12.2%) were the main compounds (Table 1). The results showed that the oil was dominated by the presence of hydrocarbon monoterpenes and sesquiterpenes (48.9%), mainly myrcene (7.6%) and β-caryophyllene (7.5%). Two respective reports on essential oil of *Z. tenuior* from western Anatolia and central Iran (Kerman province) have introduced it as a potential source of pulegone (71-87%), whereas this compound was characterized at the level of 2.4% in our studied *Z. tenuior* oil.[9,10] A review of the literature revealed that pulegone has also been reported at high levels from essential oils of *Z. persica* and *Z. clinopodioides*.[34,35] However, a report on essential oil constituents of nine populations of *Z. clinopodioides* subsp. *rigida* from Hamedan province (west of Iran), has identified pulegone and 1,8-cineol at the ranges of 0.7-44.5% and 2.1-26.0%, respectively.[36] The former study described four chemotypes for this subspecies according to the high variation observed in constituents of the analysed oils.[36] Therefore, regarding to the wide distribution of *Z. tenuior*, existence of possible chemotypes caused either by genetic differences, or as a result of different climatic factors could be considered as responsible of differences in essential oil constituents of this species.

S. orientalis subsp. virens oil

GC and GC-MS analyses of *S. orientalis* subsp. *virens* oil resulted in identification of twenty-two compounds, representing 95.6% of the oil. The results showed that the essential oil was rich in hydrocarbon sesquiterpenes (36.3%) and hydrocarbon monoterpenes (23.9%) with germacrene D (16.5%), terpinolene (15.6%) and β-caryophyllene (13.4%) as the main compounds. To our knowledge this is the first report on essential oil composition of this subspecies. However, *S. orientalis* subsp. *alipine* from Khorasan Province (Northeast of Iran) has been reported to contain germacrene D and β-caryophyllene with relative percentage of 39.7% and 15.0%, respectively.[37] A review of studies on essential oils composition of *Scutellaria* species showed that β-caryophyllene has been characterized as the main compound of *S. brevibracteata* (14.4%), *S. hastifolia* (12.9%), *S. galericulata* (29.4%) and *S. albida* subsp. *velenovskyi* (20%).[38-40] Thymol, an aromatic principle of our analysed oil sample (6.0%), has been reported only from *S. barbata* (1.4%) and *S. brevibracteata* (trace).[38,41]

E. laciniata subsp. iberica oil

Twenty-three compounds were characterized as a result of GC and GC-MS analyses of *E. laciniata* subsp. *iberica* essential oil, accounting for 92.5% of the total oil. The results revealed that hydrocarbon and oxygenated monoterpenes (49.4%) were the predominant portion of the oil, of which linalool (10.2%), α-pinene (9.3%) and p-cymene (9.1%) were the main compounds. Although there is no report distinctly on chemical constituents of this subspecies, *E. laciniata* collected from Elburz province (north of Iran) has been reported to contain dodecanol (72.5%) as the main component of its essential oil.[42] Moreover, a study on essential oil composition of this species from Jordan has indicated that hydrocarbon monoterpenes (16.0-24.3%) and hydrocarbon non-terpene derivatives (80.5%) were the main groups of constituents during the flowering and post-flowering stages, respectively.[43] In agreement to the results of the mentioned study, hydrocarbon monoterpenes were identified at the level of 34.3% in our analysed oil sample. The oxygenated non-terpens, mainly hexadecanoic acid (7.9%) and hexahydrofarnesylacetone (6.0%) were also identified as the main constituents of *E. laciniata* subsp. *iberica*. Hexadecanoic acid has been previously reported as the main compound of *E. adenantha* and *E. macrophylla* essential oils with relative percentage of 9.9% and 13.5%, respectively.[44]

P. herba-venti subsp. pungens oil

Twenty-two compounds, representing 87.7% of the total oil, were identified in aerial parts oil of *P. herba-venti* subsp. *pungens* by GC and GC-MS analyses. The oil was characterized by a high concentration of hydrocarbon monoterpenes and sesquiterpenes (52.0%), among them germacrene D (11.7%), terpinolene (9.1%) and α-pinene (7.3%) were the most abundant components. Previous study on essential oil of this subspecies from Mazandaran province (north of Iran) has reported germacrene D (31.1%), T-muurolol (11%) and α-pinene (7.1%) in its leaves oil and germacrene D (39.2%), α-pinene (9.3%) and 2-pentadecanone (7.6%) in its flower oil, as the main components.[45] The results of another study on *P. herba-venti* collected from Kerman province (south of Iran) have also introduced germacrene D (24.5%), bicyclogermacrene (14.1%), α-pinene (13.5%) and (E)-α-farnesene (13.4%) as its main constituents.[46] Germacrene D and α-pinene have been reported in noticeable levels in essential oil of some other Iranian *Phlomis* species such as *P. persica*, *P. olivieri*, *P. lanceolata* and *P. brugeri*.[30]

Antioxidant activity and total phenolic content

The results of free radical-scavenging activity assay and total phenolic content measurement of the plant total extracts and their fractions were summarized in Table 2. Among the tested samples, polar fractions of

E. laciniata subsp. *iberica* and of *Z. tenuior* exhibited the highest free radical-scavenging activity in DPPH assay with the IC_{50} values of 11.0 ± 2.3 and 12.7 ± 2.7 µg ml^{-1}, respectively. The abilities of the former fractions in scavenging free radicals were comparable with BHT, a synthetic commercial antioxidant (IC_{50}; 10.8 ± 2.1 µg ml^{-1}). In total phenolic contents measurement by Folin-Ciocalteu method, polar fractions of *E. laciniata* subsp. *iberica* and of *Z. tenuior* were also found to be contained the highest amounts of total phenolic contents, 231.9 ± 9.3 and 214.1 ± 11.3 mg GAE/g (milligrams of gallic acid equivalents per gram of dry fraction), respectively. Phenolic compounds have been confirmed as potent free radical-scavenging principles of plant extracts.[47] So, flavonoid and phenylethanoid glycosides derivatives, as the main phenolic compounds identified in these plant species could be attributed to their noticeable free radical-scavenging activity.[8,13,14,25,29] Considering the role of oxidative stresses in pathogenesis of diabetes, cancers, atherosclerosis, rheumatoid arthritis and neurodegenerative diseases and also aging, natural antioxidants have recently received special attention for their potential role in the prevention of such diseases.[48] Moreover, natural antioxidants could be appropriate substitutes for synthetic antioxidants (BHT and BHA), which have been questioned for their safety in food industrial.[49]

Table 2. Total phenolic content (TPC) and free radical-scavenging activity (FRSA) of the extracts and fractions of four Lamiaceae taxa.

Sample		TPC (mg EGA/g)[a]	FRSA (IC_{50}; µg ml^{-1})[b]
ZT[c]	Total extract	125.3 ± 8.1	23.3 ± 3.2
	polar fraction	214.1 ± 11.3	12.7 ± 2.7
	Non-polar	18.8 ± 2.7	86.9 ± 7.4
SO[d]	Total extract	143.7 ± 6.2	36.5 ± 3.3
	polar fraction	164.6 ± 4.6	31.3 ± 3.0
	Non-polar	34.2 ± 2.0	156.7 ± 8.2
EL[e]	Total extract	87.7 ± 5.6	24.9 ± 2.3
	polar fraction	231.9 ± 9.3	11.0 ± 2.3
	Non-polar	21.4 ± 2.4	115.6 ± 7.1
PH[f]	Total extract	112.6 ± 6.9	46.6 ± 4.2
	polar fraction	182.7 ± 10.1	24.1 ± 3.0
	Non-polar	47.2 ± 5.3	103.2 ± 5.6
BHT[g]		-	10.8 ± 2.1

[a]Milligrams of gallic acid equivalent per gram of dry extract; [b]Concentration providing 50% inhibition; [c]*Ziziphora tenuior*; [d]*Scutellaria orientalis* subsp. *virens*; [e]*Eremostachys laciniata* subsp. *iberica*; [f]*Phlomis herba-venti* subsp. *pungens*. [g]Butylated hydroxytoluene.

Conclusions

The present study on four medicinal Lamiaceae taxa growing in northwest of Iran provides useful information about their essential oils composition which could be applied for further biological, pharmacological and taxonomical studies on these taxa. The results of our study also introduce these plants as potential source of phenolic free radical-scavenging compounds, and suggest them as appropriate candidates for the studies related to the natural antioxidants and their usage in disease prevention and health promotion.

Acknowledgments

The authors are grateful to Dr. Yousef Ajani for identification of the plants. This research was supported by Tehran University of Medical Sciences and Health Services grant (No. 13148).

References

1. Naghibi F, Mosaddegh M, Mohammadi-Motamed M, Ghorbani A. Labiatae family in folk medicine in Iran: from ethnobotany to pharmacology. *Iran J Pharm Res*. 2010;2:63-79.
2. Manafi M, Bahreiny A. *An introduction to flora of Mishu-Dagh*. Tabriz: Tabriz University Press; 1997.
3. Jamzad Z. *Flora of Iran: Lamiaceae*. Tehran: Research Institute of Forests and Rangelands; 2012.
4. Meshkibaf MH, Abdollahi A, Ramandi MF, Sadati SJA, Moravvej A, Hatami S. Antibacterial effects of hydro-alcoholic extracts of *Ziziphora tenuior*, *Teucrium polium*, *Barberis corcorde* and *Stachys inflata*. *Koomesh J Semnan UMS*. 2010;1:240-245.
5. Ozturk Y, Aydm S, Tecik B, Baser K. Effects of essential oils from certain *Ziziphora* species on swimming performance in mice. *Phytotherapy Res*. 1995;9:225-227.
6. Ghahhari J, Vaezi G, Shariatifar N, Zendehdel Kh M. The study of hydroalcoholic extract of *Ziziphora tenuior* on visceral pain with writhing test in mice. *Horizon Med Sci*. 2009;15:24-29.
7. Naeini A, Khosravi A, Tadjbakhsh H, Ghazanfari T, Yaraee R, Shokri H. Evaluation of the immunostimulatory activity of *Ziziphora tenuior* extracts. *Comp Clin Pathol*. 2010;19:459-463.
8. Mehmood R, Imran M, Malik A, Tareen RB. Ziziphorins A and B, New Flavonoids from *Ziziphora tenuior*. *Z Naturforsch B*. 2010;65:1397-1400.
9. Sezik E, Tumen G, Baser K. *Ziziphora tenuior* L., a new source of pulegone. *Flavour Frag J*. 1991;6:101-103.
10. Pirbalouti AG, Amirkhosravi A, Bordbar F, Hamedi B. Diversity in the chemical composition of essential oils of *Ziziphora tenuior* as a potential source of pulegone. *Chemija*. 2013;2:234-239.
11. Hedge I. Labiatae of South-West Asia: diversity, distribution and endemism. *Proc R Soc Edinburg*. 1986;89:23-35.
12. Ozdemir C, Altan Y. Morphological and anatomical investigations on endemic *Scutellaria*

orientalis L. subsp. *bicolor* (Hochst.) edmund and subsp. *santolinoides* (Hausskn ex Bornm). *Pak J Bot*. 2005;37:213-226.
13. Oganesyan G. Phenolic compounds from the aerial part of *Scutellaria orientalis*. *Chem Nat Compd.* 2010;46:447-466.
14. Calis I, Saracoglu I, Basaran AA, Sticher O. Two phenethyl alcohol glycosides from *Scutellaria orientalis* subsp. *pinnatifida*. *Phytochemistry.* 1993;32:1621-1623.
15. Malakov PY, Papanov GY, Spassov SL. Scutorientalin D, a neo-clerodane diterpenoid from *Scutellaria orientalis* subsp. *pinnatifida*. *Phytochemistry*. 1997;44:121-124.
16. Ezer N, Akcos Y, Rodrguez B. Neo-clerodane diterpenoids from *Scutellaria orientalis* subsp. *sintenisii*. *Phytochemistry.* 1998;49:1825-1827.
17. Karabacak C, Tılkı T, Cengız M. Two diterpenoids from *Scutellaria orientalis* L. subsp. *porphyrostegia* Edmondson. *Asian J Chem.* 2009;21:2253-2258.
18. Delazar A, Sarker SD, Nahar L, Jalali SB, Modaresi M, Hamedeyazdan S, Babaei H, Javadzadeh Y, Asnaashari S, Moghadam SB. Rhizomes of *Eremostachys laciniata*: Isolation and Structure Elucidation of Chemical Constituents and a Clinical Trial on Inflammatory Diseases. *Adv Pharm Bull*. 2013;3:385-393.
19. Said O, Khalil K, Fulder S, Azaizeh H. Ethnopharmacological survey of medicinal herbs in Israel, the Golan Heights and the West Bank region. *J Ethnopharmacol*. 2002;83:251-265.
20. Erdemoglu N, Turan NN, Cakoco I, Sener B, Aydon A. Antioxidant activities of some Lamiaceae plant extracts. *Phytotherapy Res.* 2006;20:9-13.
21. Modaressi M, Delazar A, Nazemiyeh H, Fathi-Azad F, Smith E, Rahman MM, Gibbons S, Nahar L, Sarker SD. Antibacterial iridoid glucosides from *Eremostachys laciniata*. *Phytotherapy Res*. 2009;23:99-103.
22. Nisar M, Khan S, Dar A, Rehman W, Khan R, Jan I. Antidepressant screening and flavonoids isolation from *Eremostachys laciniata* (L) Bunge. *Afr J Biotechnol*. 2013;10:1696-1699.
23. Khan S, Nisar M, Rehman W, Khan R, Nasir F. Anti-inflammatory study on crude methanol extract and different fractions of *Eremostachys laciniata. Pharm Biol*. 2010;48:110 .1105-1118
24. Delazar A, Asl BH, Mohammadi O, Afshar FH, Nahar L, Modarresi M, Nazemiyeh H, Sarker SD. Evaluation of analgesic activity of *Eremostachys laciniata* in mice. *J Nat Remedies*. 2009;9:1-7.
25. Calis I, Guvenc A, Armagan M, Koyuncu M, Gotfredsen C, Jensen S. Secondary metabolites from *Eremostachys laciniata*. *Nat Prod Commun*. 2007;3:117-124.
26. Delazar A, Modarresi M, Nazemiyeh H, Fathi-Azad F, Nahar L, Sarker SD. Furanolabdane diterpene glycosides from *Eremostachys laciniata*. *Nat Prod Commun*. 2008;3:873-876.
27. Morteza-Semnani K, Saeedi M, Shahani S. Antioxidant activity of the methanolic extracts of some species of Phlomis and Stachys on sunflower oil. *Afr J Biotechnol*. 2006;5:2428-2432.
28. Morteza-Semnani K, Saeedi M, Mahdavi MR, Rahimi F. Antimicrobial Studies on Extracts of Three Species of Phlomis. *Pharm Biol*. 2006;44:426-429.
29. Saracoglu I, Kojima K, Harput US, Ogihara Y. A new phenylethanoid glycoside from *Phlomis pungens* Willd. var. *pungens*. *Chem Pharm Bull*. 1998;46:726-727.
30. Amor IL-B, Boubaker J, Sgaier MB, Skandrani I, Bhouri W, Neffati A, Kilani S, Bouhlel I, Ghedira K, Chekir-Ghedira L. Phytochemistry and biological activities of Phlomis species. *J Ethnopharmacol*. 2009;125:183-202.
31. Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry*. Carol Stream: Allured Publishing Corporation; 2007.
32. Delazar A, Delnavazi M-R, Yassa N, Parkhideh S, Delazar N, Nahar L, Sarker SD. Essential oil composition and isolation of free radical-scavenging phenolic glycosides from the aerial parts of *Ajuga chamaepitys* growing in Iran. *Rev Bras Farmacogn*. 2012;22:399-405.
33. Khodaie L, Bamdad S, Delazar A, Nazemiyeh H. Antioxidant, total phenol and flavonoid contents of two pedicularis L. species from Eastern Azerbaijan, Iran. *BioImpacts*. 2012;2:47-53.
34. Ozturk S, Ercisli S. The chemical composition of essential oil and in vitro antibacterial activities of essential oil and methanol extract of *Ziziphora persica* Bunge. *J Ethnopharmacol*. 2006;106:372-376.
35. Behravan J, Ramezani M, Hassanzadeh M, Eskandari M, Kasaian J, Sabeti Z. Composition, antimycotic and antibacterial activity of *Ziziphora clinopodioides* Lam. essential oil from Iran. *J Essent Oil Bear Pl*. 2007;10:339-345.
36. Sonboli A, Atri M, Shafiei S. Intraspecific variability of the essential oil of *Ziziphora clinopodioides* ssp. *rigida* from Iran. *Chem Biodivers*. 2010;7:1784-1789.
37. Ghannadi A, Mehregan I. Essential oil of one of the Iranian skullcaps. *Z Naturforsch C*. 2003;58: 316-318.
38. Formisano C, Rigano D, Senatore F, Arnold NA, Simmonds MS, Rosselli S, Bruno M, Loziene K. Essential oils of three species of Scutellaria and their influence on *Spodoptera littoralis*. *Biochem Syst Ecol*. 2013;48:206-210.
39. Lawrence B, Hogg J, Terhune S, Morton J, Gill L. Terpenoid composition of some Canadian Labiatae. *Phytochemistry*. 1972;11:2636-2638.

40. Cicek M, Demirci B, Yilmaz G, Ketenoglu O, Baser KHC. Composition of the essential oils of subspecies of *Scutellaria albida* L. from Turkey. *J Essent Oil Res*. 2010;22:55-58.
41. Yu J, Lei J, Yu H, Cai X, Zou G. Chemical composition and antimicrobial activity of the essential oil of *Scutellaria barbata*. *Phytochemistry*. 2004;65:881-884.
42. Navaei MN, Mirza M. Chemical composition of the oil of *Eremostachys laciniata* (L.) Bunge from Iran. *Flavour Frag J*. 2006;2:645-646.
43. Al-Jaber HI, Al-Qudah MA, Barhoumi LM, Abaza IF, Afifi FU. Variation in the essential oil composition of *Eremostachys laciniata* from Jordan at different flowering stages. *J Essent Oil Res*. 2012;24:289-297.
44. Javidnia K, Miri R, Soltani M, Khosravi A. Essential oil composition of two species of Eremostachys from Iran (*E. adenantha* Jaub. Et Spach and *E. macrophylla* Montbr. et Auch.). *J Essent Oil Res*. 2008; 20: 226-228.
45. Khalilzadeh MA, Tajbakhsh M, Rineh A. Study of the essential oils composition of leaves and flowers of two subspecies *Phlomis herba-venti* (*Pungens* and *Lenkoranica*) from Iran. *J Essent Oil Res*. 2008;20:46-48.
46. Masoudi S, Rustaiyan A, Azar PA, Larijani K. Composition of the essential oils of Cyclotrichium straussii (Bornm.) Rech. f. and *Phlomis pungens* Willd. from Iran. *J Essent Oil Res*. 2006;18:16-18.
47. Shahidi F, Janitha P, Wanasundara P. Phenolic antioxidants. *Crit Rev Food Sci*. 1992;32:67-103.
48. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell. Biol* 2007;39:44-84.
49. Barlow SM. Food Antioxidants. In: Hudson BJF, ed. *Toxicological aspects of antioxidants used as food additives*. London: Elsevier; 1990:253-307.