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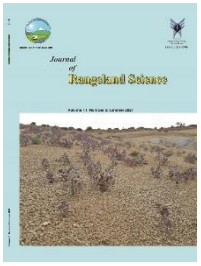

Research and Full Length Article:

Chemotaxonomy of Wild Lamiaceae Taxa Based on Their Flavonoids Profiles

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Abstract. The study presents flavonoids compounds patterns of thirty two Lamiaceae (Mint) taxa from Oshtorankoh located on Zagros Mountains, Iran for understanding flavonoids role in mint chemotaxonomy and their usages as food additive, edible, spices and medicine. This is a novel report of some Iranian Mint taxa flavonoids using two-dimensional paper chromatography and thin layer chromatography methods. Results showed all of the studied taxa contained flavone *C*-&*C*-/*O*-glucosides and flavonoid sulphates. Eight taxa had aglycones while the rest lacked. Quercetin was found in all of taxa except *Lamium album* ssp. *crinitum* and *Nepeta persica*. *Stachys setifera* had not myricetin while others had. Rhamnetin, tricin and morin were not detected in all taxa exceptional *Salvia brachycalyx* and *Salvia staminea* those had. Kaempferol was found in all taxa except *Ajuga chamaecistus*, *Lamium amplexicaule* var. *amplexicaule*, *Nepeta persica* and *Stachys pilifera*. All of taxa except six species had luteolin. These results showed aerial parts flavonoids compounds variation in studied taxa can be useful for studying relationships within relatively narrow taxonomic limits, e. g. at the species and genus levels and their importance in chemotaxonomic surveys of mint genera. Also flavonoids compounds presence in studied taxa increase their quality and antioxidant activity as edible, spices and medicinal plants.

Key words: Chemosystematics, Mint, Polyphenolic, Compounds, Zagros

Introduction

Plant chemosystematics is the application of chemical data to systematic problems. It is a rapidly expanding interdisciplinary field concerned with using chemical constituents for explaining relationships between plants and inferring phylogeny (Jones and Luchsinger, 1987). Flavonoids are popular compounds for chemotaxonomic surveys of plant genera and families because of their almost ubiquitous presence in vascular plants, structural variety, ease of detection and relative ease of identification (Harborne and Turner, 1984). They are the most numerous of the phenolic and are found throughout the plant kingdom (Harborne, 1993). Flavonoids chemical stability in herbarium samples can help to study the compounds in many of herbarium plant species and support revisions of existing classifications at the lower genus and species levels. Flavonoids presence/absence and their kind can be more useful for studying relationships within relatively narrow taxonomic limits, e. g. at the infraspecific level (Noori, 2014). Lamiaceae flavonoids can be used as key and marker compounds in ecological adaptations, plant defending, plant resistance and plant chemo-diversity studies (Noori, 2012). Mint (Lamiaceae or Labiatae) is a family with about 236genera (Raymond *et al*., 2004) and 6900 species (Heywood *et al*., 2007) to 7200 species in the world(Raymond *et al*., 2004) that about 124 species and subspecies (30%) are endemic to Iran. They are rich in secondary metabolites such as essential oils and flavonoids. Wide studies have been done on mint flavonoids profiles that almost are valuable in cosmetic, flavouring, fragrance, perfumery, pesticide and pharmaceutical industries but the study want to show flavonoid composition as separator factor in mint taxa as (Kharazian and Mohammadi, 2014; Coisin *et al*., 2012; Nickavar and Abolhasani, 2013; Asghari *et al*., 2015) showed in their works. The study was done for identification of flavonoids content of 32 collected Lamiaceae taxa from different parts of "Oshtorankoh" protected area in Lorestan Province located on Zagros Mountains, Iran for understanding flavonoids role in the family chemotaxonomy.

Materials and Methods

Plant Collection, Preparation and Extraction

Mature fresh aerial part of 32 Lamiaceae taxa were collected from different parts of "Oshtorankoh" protectedarea, Lorestan Province, Iran during 2014-2015 (Table 1). Lorestan with 28,294Km^2 area is located on western Iran in the Zagros Mountains (33°58´N, 48°39´E) (Mostafavi *et al*., 2017). Samples were identified using available references (Ghahreman, 1978-2008, Rechinger, 1963-2005, Jamzad *et al*., 2012). Voucher specimens of each sample were prepared for reference as herbarium vouchers and deposited at the Arak University Herbarium (not listed in herbarium index). Samples were air dried and extracted using 70% EtOH for detection and identification of their flavonoids by Two-Dimensional Paper Chromatography (2-DPC) (Markham, 1982).

Two-Dimensional Paper Chromatography (2-DPC)

For the detection of flavonoids, ca 20 µl of each of the small extracts was applied to the Whatman No 1 chromatography paper as a concentrated spot. Then chromatograms were developed in BAW (n-BuOH-AcOH-H2O=4:1:5; V/V and AcOH15% with rutin (quercetin 3-*O*-rutinoside) as a standard. Chromatograms were viewed in UV light (366 nm) and any dark absorbing and fluorescent spots were marked. R_f values in BAW and 15% AcOH were calculated.

Flavonoids Identification

After obtaining sufficient amounts of purified flavonoids, as in the case of the flavonoids from aerial part of the taxa, they were

identified by means of UV spectroscopy using shift reagents to investigate the substitution patterns of the flavonoids (Mabry *et al*., 1970, Markham, 1982) and by acid hydrolysis to identify the aglycone and sugar moieties. Chromatography and thin layer chromatography with standards were also performed where possible. Flavonoid standards available for comparison during the study were apigenin, chrysin, genistein, isorhamnetin, kaempferol, luteolin, morin, myricetin, naringenin, quercetin, rhamnetin, rutin, tricin and vitexin (all obtained commercially from Merck, apigenin and luteolin from Sigma and the rest from Fluka). Developed TLC chromatograms in CAW solvent studied in UV 254 nm and any dark absorbing and fluorescent spots were marked and calculated (color and R_f).

Data Analysis, Principal Component Analysis (PCA) and Hierarchical Cluster Analysis

Nineteen qualitative and quantitative phytochemical characters were studied. Qualitative characters were coded as multistate characters and the quantitative characters were used that tricin and rhamnetin characters were deleted in analysis (Table 3). Data were analyzed using the SPSS for windows release 16.0 statistical package for social scientists by principal component analysis (PCA) test (Tables 4 and 5). Then cluster analysis using Ward, Average Linkage (between gtableroups) and Median methods were performed on standardized photochemical data. Ward method was the best. Fit of the clusters to the original data was checked using cophenetic correlation. Scored characters for cluster analysis were based on existence, variation and concentration of flavonoids (Tables 1-3 & Fig.1).

Results

All of obtained results were recorded in Tables 1 and 2. Studies of aerial part flavonoids of 32 Lamiaceae taxa from Iran using two-dimesional paper chromatography showed all of the studies samples contained flavone *C*-&*C*-/*O*-glucosides and flavonoid sulphates.

Table 1. Collection information and aerial part two-dimensional paper chromatographical data of 32 studied Lamiaceae taxa from Iran

Voucher samples	Taxon	Altitude (m)	Total flavonoids number	Flavone *C*-&*C*-/*O*-glucosides number	Flavonoid sulphates number	Aglycones number
*CEF41	*Ajuga chamaecistus* Ging.et Benth. ssp. *chamaecistus*	2243	3	1	2	0
CEF66	*Eremostachys laevigata* Bunge, Mem.	2293	8	5	3	0
CEF63	*Lamium album* L. ssp. *crinitum*	2796	3	1	2	0
CEF33	*Lamium amplexicaule* L. var. *amplexicaule*	1803	3	2	1	0
CEF29	*Marrubium anisodon* C. Koch.	1762	5	3	1	1
CEF60	*Marrubium astracanicum* Jacq	2281	6	1	5	0
CEF80	*Mentha longifolia* L.var. *Asiatica* (Boriss.) Rech.f	2200	6	3	2	0
CEF57	*Nepeta cataria* L.	1937	4	2	2	0
CEF51	*Nepeta heliopifolia* Lam	2190	8	3	5	0
CEF58	*Nepeta persica* Boiss	2293	7	2	4	1
CEF30	*Phlomis olivieri* Benth	1762	10	6	3	1
CEF68	*Phlomis pungens* Wild	2012	4	3	1	0
CEF17	*Salvia acetabulosa* L.var. *Szovitsiana* (Bunge) Bornm	2237	9	5	4	0
CEF38	*Salvia brachycalyx* Boiss.	1785	7	2	5	0
CEF78	*Salvia* nemorosa L.	2600	4	2	1	1
CEF20	*Salvia* reuterana Boiss	1917	9	3	5	1
CEF42	*Salvia* staminea Montbr	2219	7	3	2	2
CEF46	*Salvia virgata* Jacq.	2188	6	2	4	0
CEF21	*Stachys benthamiana* Boiss	1922	4	3	1	0
CEF67	*Stachys inflata* Bth	2770	6	5	1	0
CEF34	*Stachys kurdica* Boiss et Honen	1792	6	5	1	0
CEF02	*Stachys lavandulifolia* vahl	2400	6	3	3	0
CEF37	*Stachys pilifera* Benth.in Dc.	1790	11	4	7	0
CEF48	*Stachys setifera* C.A.Mey	2180	4	3	1	0
CEF79	*Stachys spectabilis* Choisy ex DC.	2219	4	3	1	0
CEF72	*Teucrium orientale* L.	2237	6	2	3	1
CEF71	*Teucrium orientale* L. ssp. *Taylori* (Boiss.) Rech.f.	2237	6	4	1	1
CEF55	*Teucrium polium* L.	1951	6	2	4	0
CEF70	*Thymus daenensis* Celak	2235	9	5	4	0
CEF10	*Thymus serpyllum* L. var. *Squarrosus* (Fisch et Mey) Boiss.	1922	7	4	3	0
CEF73	*Ziziphora clinopodioides* Lam.	2223	3	2	1	0
CEF32	*Ziziphora tenuior* L.	1810	7	4	3	0

*C=Collection number Elham Faryabi

As Table 1 shows *Marrubium anisodon*, *Nepeta persica*, *Phlomis olivieri*, *Salvia nemorosa*, *Salvia reuterana*, *Salvia staminea*, *Teucrium orientale* L. ssp. *Taylori* and *Teucrium orientale* taxa had aglycones while the rest taxa lacked. The most flavonoids number were observed in *Stachys pilifera* species aerial part and *Ajuga chamaecistus*, *Lamium album* L. ssp. *crinitum*, *Lamium amplexicaule* L. var. *amplexicaule* and *Ziziphora clinopodioides* taxa showed the lowest (Table 1). Flavone *C*-&*C*-/*O*-glucosides, flavonoid sulphates and aglycones are identified flavonoids series in 2-DPC and kind of flavonoids are in TLC. Results of TLC chromatograms using UV light (254 and 336nm), comparing spot color and R*f* values of each plant samples to flavonoids standards were recorded in Table 2.

Table 2. Thin Layer Chromatographical data of 32 studied Lamiaceae taxa aerial parts flavonoids from Iran

Voucher samples	Flavonoids Identification														
	Apigenin	Chrysin	Genistein	Isorhamnetin	Kaempferol	Luteolin	Morin	Myricetin	Naringenin	Orientin	Quercetin	Rhamnetin	Rutin	Tricin	Vitexin
*CEF41	+	-	-	-	-	-	-	+	-	++	±	-	-	-	-
CEF66	-	±	-	+	+	+	-	+	±	-	++	-	-	-	+
CEF63	-	±	-	+	+	+	-	+	±	+++	-	-	+	-	-
CEF33	-	-	-	-	-	-	-	±	-	++	+	-	-	-	-
CEF29	-	+	-	-	+	+	-	±	++	+	+	-	+	-	-
CEF 60	++	+++	++	-	++	+++	-	++	-	++	++	-	++	-	+++
CEF80	+	-	-	+	+	-	-	++	+	-	++	-	+	-	+
CEF57	+	+	+	+	+	+	-	++	-	+	++	-	+	-	++
CEF51	+	-	-	++	++	+	-	++	-	-	+	-	++	-	+
CEF58	-	-	-	±	-	-	-	+	-	-	-	-	-	-	+
CEF30	-	+	-	-	+	+	-	++	++	++	++	-	++	-	-
CEF68	++	++	+	-	++	++	-	++	-	+	±	-	±	-	++
CEF17	++	++	++	-	++	+++	-	+++	-	+++	+++	-	+++	-	+++
CEF38	-	+	-	-	++	++	++	+++	++	++	+++	-	++	-	-
CEF78	-	+	-	+	+	+	-	++	-	-	+	-	+	-	-
CEF20	++	++	++	-	++	++	-	++	-	++	+++	-	+++	-	+++
CEF42	+++	-	-	-	+++	++	++	+++	+++	+++	-	-	++	-	-
CEF46	++	+++	++	+++	++	++	-	+++	-	++	++	-	++	-	++
CEF21	+++	++	+++	-	+	++	-	++	-	++	+++	-	+++	-	+++
CEF67	-	-	-	-	+	+	-	++	±	+	+	-	-	-	-
CEF34	-	-	-	-	++	+	-	+	+++	++	+++	-	+++	-	-
CEF02	+	-	-	+	+	±	-	++	-	++	+	-	++	-	++
CEF37	-	-	-	-	-	+	-	+++	-	+	+	-	+	-	-
CEF48	-	-	-	+++	++	++	-	-	-	+	++	-	++	-	++
CEF79	-	-	-	+	++	++	-	+	+	+	++	-	+	-	++
CEF72	-	±	-	±	+	-	-	++	±	-	++	-	+	-	+
CEF71	-	-	-	+	+	+	-	++	+	+	+++	-	+	-	+
CEF55	++	+	++	++	++	+	-	-	-	+	++	-	++	-	++
CEF70	-	-	-	+	+	+	-	+++	+	++	+	-	+	-	-
CEF10	+	++	±	+	+	+	-	+++	-	+++	++	-	++	-	++
CEF73	++	+++	++	+	+	+	-	+	-	+	+	-	-	-	+
CEF32	-	+	-	-	+	-	-	++	+	++	+++	-	-	-	-

*CEF=Elham Faryabi collection number; for species name refer to Table 1; Concentration of flavonoids:-(non flavonoid), ± (non or a few flavonoid), + (few flavonoid), ++ (middle concentration of flavonoid), +++ (high concentration of flavonoid).

Table 3. Nineteen scored qualitative and quantitative phytochemical characters in 32 studied Lamiaceae taxa aerial parts from Iran

No.	Characters	Abbreviations
1	Apigenin: absence (1), presence (2)	Ap
2	Chrysin: absence (1), presence (2)	Ch
3	Genistein: absence (1), presence (2)	G
4	Isorhamnetin: absence (1), presence (2)	Iso
5	Kaempferol: absence (1), presence (2)	Ka
6	Luteolin: absence (1), presence (2)	Lu
7	Morin: absence (1), presence (2)	Mo
8	Myricetin: absence (1), presence (2)	My
9	Naringenin: absence (1), presence (2)	Na
10	Orientin: absence (1), presence (2)	O
11	Quercetin: absence (1), presence (2)	Qu
12	Rhamnetin: absence (1), presence (2)	Rh
13	Rutin: absence (1), presence (2)	Ru
14	Tricin: absence (1), presence (2)	Tr
15	Vitexin: absence (1), presence (2)	Vi
16	Aglycones number	AN
17	Flavon *C*-&*C /O*-glucosides number	FCN
18	Flavonoid sulphates number	FSN
19	Total flavonoids number	TFN

As the table shows quercetin was found in all of studied taxa aerial part with the exception of *Lamium album* L. ssp. *crinitum* and *Nepeta persica*. *Stachys setifera* had not myricetin while others had. Rhamnetin, tricin and morin were not detected in all taxa exceptional *Salvia brachycalyx* and *Salvia staminea* those had. Kaempferol was found in all of studied taxa with the exception of *Ajuga chamaecistus*, *Lamium amplexicaule* var. *amplexicaule*, *Nepeta persica* and *Stachys pilifera*. All of studied taxa with the exception of 6 species (*Ajuga chamaecistus* ssp. *chamaecistus*, *Lamium amplexicaule* var. *amplexicaule*, *Menta longifolia* L. var. *asiatica*, *Nepeta persica*, *Teucrium orientale* and *Ziziphora tenuior*) had luteolin. For other flavonoids is referred to Table 2. Factor analysis results of phytochemical characters are shown in Tables 4 and 5. Figure 1 shows cluster analysis of phytochemical data using cophenetic correlation in studied Lamiaceae taxa.

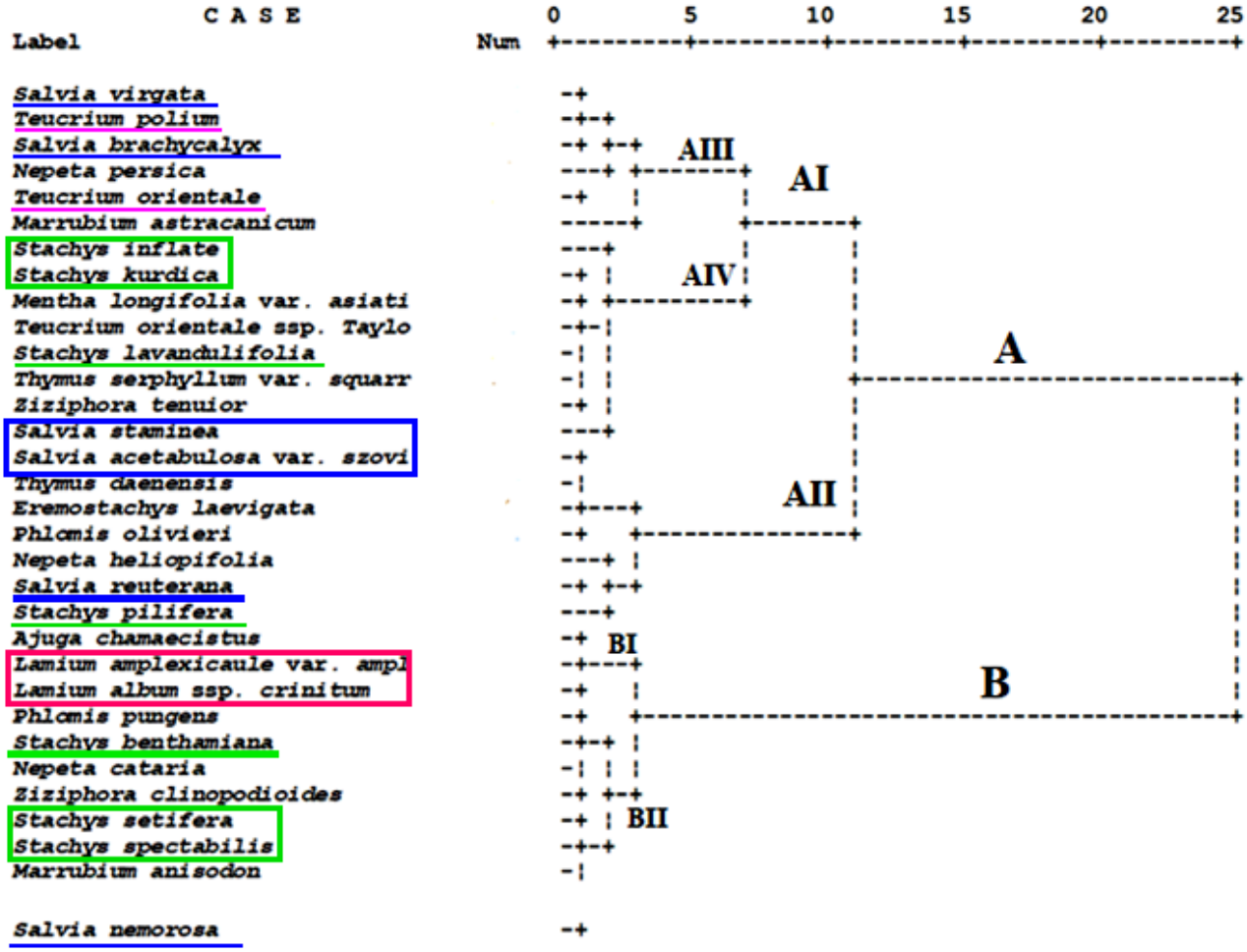

Fig. 1. Cluster analysis (Ward Method) of 19 phytochemical characters of studied Lamiaceae taxa in Iran. Scored characters for cluster analysis have been shown in Tables 1-3

Table 4. Total variance explained for principal component analysis for studied Lamiaceae taxa phytochemical characters

Total Variance Explained

Component	Initial Eigen values			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %	Total	% of Variance	Cumulative %
1	3.336	19.624	19.624	3.336	**19.624**	19.624	2.524	14.849	14.849
2	2.618	15.397	35.021	2.618	**15.397**	35.021	2.156	12.685	27.534
3	1.985	11.677	46.698	1.985	**11.677**	46.698	2.139	12.584	40.118
4	1.877	11.039	57.737	1.877	**11.039**	57.737	2.088	12.281	52.399
5	1.516	8.919	66.655	1.516	**8.919**	66.655	1.948	11.459	63.858
6	1.252	7.363	74.019	1.252	**7.363**	74.019	1.535	9.030	72.889
7	1.090	6.411	80.430	1.090	**6.411**	80.430	1.282	7.541	**80.430**
8	.729	4.288	84.718						
9	.659	3.875	88.593						
10	.483	2.840	91.433						
11	.373	2.192	93.625						
12	.314	1.848	95.473						
13	.279	1.644	97.117						
14	.247	1.453	98.570						
15	.138	.810	99.380						
16	.104	.612	99.992						
17	.001	.008	100.000						

Extraction Method: Principal Component Analysis

Table 5. Seven components of PCA test and correlating flavonoid characters of 32 studied Lamiaceae taxa aerial parts in Iran. Bold values are positive significant *P<0.01*

Rotated Component Matrix[a]

	Component						
Characters	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Genistein	**.866**						
Chrysin	**.859**						
Apigenin	.628						
Vitexin	.507						
Aglycones number		**.788**					
Morin		**.764**					
Naringenin		.543		.510			
Rutin			**.762**				
Isorhamnetin			.690				
Kaempferol			.681				
Flavone *C*-&*C*-/*O*-glucosides number				**.898**			
Quercetin				.530			
Flavonoid sulphates number					**.954**		
Total flavonoids number					**.810**		
Orientin						**.861**	
Luteolin						.530	
Myricetin							**.845**

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
a. Rotation converged in 21 iterations.

Discussion

Two flavone glycosides, chrysoeriol 7-*O*-glucopyranoside (3'-methoxy-luteolin 7-*O*-glucopyranoside) and apigenin 7-*O*-rhamno pyranoside, were isolated from aerial parts of *Ajuga chamaepitys* a medicinal plant in Iran (Delazar *et al*., 2012). Three flavonol glycosides, myricetin 3-*O*-rutinoside-4'-*O*-rutinoside, myricetin 3-*O*-rutinoside-3'-*O*-rutinoside and isorhamnetin 3-*O*-rutinoside-7-*O*-rutinoside-4'-*O*-beta-glucoside have been isolated from the aerial parts of *Ajuga* (Lawrence *et al.*, 2006*)*. Our data showed apigenin, myricetin and orientin existence in aerial part of *A. chamaecistus* ssp. *chamaecistus* (Table 2). Luteolin and chrysoeriol glycosides were recorded in *Phlomis* and *Eremostachys* genera (Azizian and Cutler, 1982). Bajalan *et al*. (2017) showed a good antioxidant activity of *Eremostachys laciniata* phenolic and flavonoid contents collected from Zagros. Isorhamnetin, kaempfrol, luteolin, myricetin, quercetin and vitexin were found in *Eremostachys laevigata* (Table 2). Two new flavonol glycosides of kaempfrol and quercetin were isolated from *Lamium amplexicaule* aerial part (Nugroho *et al*., 2009) while a small amounts of aglycones was found in *Lamium album* (Paduch *et al*., 2008). Prescence of Kaempferol in *L. amplexicaule* and *L. album* species and its absence in *L. amplexicaule* var. *amplexicaule* is a phytochemical factor for their separation. Aglycones were not found in two studied *Lamium* taxa and both had orientin (Table2). Quercetin and kaempferol 3-*O*-glucosides were isolated from *Lamium album* flowers (Budzianowski and Skrzypczak, 1995). Verbascoside and isoscutellarein derivatives having health benefits were recorded main components of *L. album* ethanolic (Pereira *et al*., 2012). Kharazian and Hashemi (2017) found the highest flavonoid diversity in *Marrubium anisodon* and *M. vulgare*. Hussain *et al*., (2009) identified apigenin 4′-0-β-D-glucopyranoside, kaempferol 3-0-β-D-glucopyranoside and β-sitosterol 3-0-β-D-glucopyranoside in *Marrubium anisodon*. Our results showed high concentrations of apigenin, chrysin, genistein, kaempfrol, loteoline, myricetin, orientin, quercetin, rutin and vitexin in *Marrubium astracanicum* in comparison with *Marrubium anisodon*. Apigenin, isorhamnetin, kaempfrol, myricetin, naringenin, quercetin, rutin and vitexin were found in *Mentha longifolia* var. *asiatica* (Table 2). In addition apigenin 7-*O*-glucoside having genotoxic potency was isolated from *Mentha longifolia* ssp. *longifolia* (Gulluce *et al*., 2015). Also apigenin-7-*O*-rutinoside and apigenin-7-*O*-glucuronide were isolated from these taxa (Baris *et al*., 2011).Two flavone glycosides, apigenin 7-*O*-glucuronide and apigenin 7-*O*-glucopyranoside were isolated from *Nepeta heliotropifolia* aerial parts in addition of other chemical compounds (Güvenalp *et al*., 2009). Our studies confirmed apigenin presence in *N. cataria* and *N. heliopifolia*. Apigenin (4′, 5, 7-trihydroxyflavone), as a flavone has some potential health benefits (Viola *et al*., 2009).

Antinociceptive effect of three *Phlomis* species extracts were examined (Sarkhail *et al*., 2003). Chrysoeriol-7-*O*-β-D-glucoside and verbascoside were obtained from *Phlomis olivieri* aerial part methanolic extract using column chromatography (Sarkhail *et al*., 2006). All of studied *Salvia* species had luteoline (Table 2) as (Asghari *et al*., 2015) reported luteolin 7-*O*-glucoside, luteolin 7-*O*-glucuronide, diosmetin 7-*O*-glucuronide and salvigenin in *Salvia chloroleuca* (Asghari *et al*., 2015). Also luteolin and luteolin glycosides isolated of *Salvia palaestina* and *S. sclarea* (Miski *et al*., 1983, Ulubelen *et al*., 1994). Our results in Table 2 show chrysin (chrysoeriol) existence in all of studied *Salvia* species exceptional *Salvia staminea* as (Nickavar and Abolhasani, 2013) isolated and identified chrysin from *Salvia virgata.*

A strong positive correlation was observed between total phenolic content and

antioxidant activity in *Stachys inflata*. So the species can be used potentially as a readily accessible source of natural antioxidant (Eghdami *et al*., 2011). Nine *Stachys* species were examined for their antioxidant activity and total phenolic content (Khanavi *et al*., 2009). Rahimi-Khoigani *et al*. (2017) identified 32 flavonoids in *Stachys lavandulifolia* methanolic extract. Luteolin was reported from *Teucrium* species (Kadifkova Panovska *et al*., 2005). Luteolin-7-*O*-rutinoside, luteolin-7-*O*-glucoside, hesperetin-7-*O*-rutinoside, 8-*O*-acetyl harpagide and 8-*O*-methyl harpagide having antioxidant activities were identified in *Teucrium orientale* var. *orientale* (Cakir *et al*., 2006). Salvigenin, cirsiliol, and luteolin were known in methanolic extract of *Teucrium polium* (Rizk *et al*., 1986). As Table 2 shows both *T. orientale* ssp. *Taylori* and *T. polium* had luteolin. Luteolin, 3',4',5,7-tetrahydroxyflavone, is a common flavonoid that have been used in Chinese traditional medicine for treating various diseases such as hypertension, inflammatory disorders, and cancer. It has antioxidant activity (Lin *et al*., 2008). Amiri (2010) studies on *Teucrium orientale* ssp. *Taylori* showed a positive correlation between antioxidant activity and total phenolicscontent (Amiri, 2010).

Identification and quantification of eighteen *Thymus serpyllum* phenolic compounds were done that luteolin, luteolin 7-*O*-glucoside and rosmarinic acid were the most evident of them (Sonmezdag *et al*., 2016). Luteolin glucuronide isolated from *Thymus broussonettii*, *T. vulgaris* and *T. willdenowii* (Ismaili *et al*., 2001, Dapkevicius *et al*., 2002). Also luteolin and luteolin-7-*O*-glucoside were found in *Thymus broussonettii* and *T. piperella* (Barberan *et al*., 1985, Ismaili *et al*., 2001). As Table 2 shows in addition luteolin, isorhamnetin, kempfrol, myricetin, orientin, quercetin and rutin were found in *Thymus daenensis* and *T. serphyllum* var. *squarrosus*.

The highest total phenolic content were found in aerial parts of *Ziziphora tenuior*, *Scutellaria orientalis* ssp. *virens*, *Eremostachys laciniata* ssp. *iberica* and *Phlomis herba-venti* ssp. *pungens* collected from Northwest of Iran (Delnavazi *et al*., 2014). Apigenins and two new flavonoids, ziziphorins A and B were isolated from *Ziziphora tenuior* (Mehmood *et al*., 2010). Acacetin, natural flavones that selectively inhibits human atrial repolarization potassium currents and prevents atrial fibrillation in dogs were identified in *Ziziphora clinopodioides* (Li *et al*., 2008, Tian *et al*., 2011, Yang *et al*., 2014). Chrysin, kaempferol, myricetin, orientin and quercetin were found in *Ziziphora clinopodioides* and *Z. tenuior*. Apigenin also was found in the second species (Table 2).

Factor analysis results of phytochemical characters in Tables 4 and 5 showed that the first seven factors describe about 80% of total variance. First component with 20% total variation was found positively correlated with genistein and chrysin presence in plant aerial parts. Secondary component with 15% total variation was positive and significantly correlated with aglycones number and morin presence. Third component with 12% total variation was correlated positively and significantly with rutin existence. Fourth component with 11% total variations was correlated positively and significantly with flavone *C*-&*C*-/*O*-glucosides, number. Component five with 9% total Variance was correlated positively and significantly with total flavonoids and flavonoid sulphates numbers in studied mint aerial parts. Sixth component with 7% of variance was correlated positively and significantly with orientin presence in studied plant aerial part and component seven with 6% total variation showed positive correlation with Myricetin existence in aerial part of mint taxa ($P \leq 0.01$). Fig. 1 cluster analysis of phytochemical data using cophenetic correlation showed two main clades A and B. Clade A consists of two

AI and AII subclades that first one contained AIII and AIV and AII contain seven mint taxa. Secondary main clade B consists of BI and BII two subclades that two *Lamium* taxa are in BI and three *Stachys* species are in BII. As the figure 1 shows flavonoids composition are good separator factors for the studied *Lamium*, *Salvia*, *Stachys* and *Teucrium* taxa. Apigenin, chrycin, genistein and vitexin presence in *Stachys bentamina* and also *Teucrium polium* are good factors for separation them from the other taxa in their genera. *Lamium album* ssp. *crinitum* because having quercetin is separated of *Lamium amplexicaule* var. *amplexicaule* (Tables 4 and 5, Fig. 1). These studies show that plant phenolic patterns appear to be more useful for studying relationships within relatively narrow taxonomic limits, e. g. at the lower than species level (sub species, variety, cultivar or chemotype) as found in the previous works (Harborne, 1993, Moore and Giannasi, 1994, Noori *et al*., 2009, Noori, 2014). Based on the obtained results it is concluded that the quantities and presence of important metabolites such as flavonoids depend on plant species and their ecological conditions. Therefore depth and further study of mint morphological characters is needed additional their chemical composition.

Conclusion and Suggestion

Lamiaceae (Mint) member having different classes of secondary metabolites are valuable in cosmetic, flavoring, fragrance, perfumery, pesticide and pharmaceutical industries. As the study showed they are good sources of different flavonoids. Flavonoids can be used as key and marker compounds in ecological adaptations, plant defending, plant resistance and plant chemo-diversity studies. Although flavonoid compounds are taxonomically important for their stability in herbarium samples and often show correlations with existing classifications at the family, genus, and species but rarely provide key characters since the flavonoid may be absent in one or more members of the taxon and the same flavonoid may occurs in an unrelated taxon. Also plant flavonoid pattern depends on genetics factors and ecological conditions. It is believed that one organ flavonoid patterns cannot always reveal the taxa differences. More work on flavonoids profiles of other species organs that collected from different regions is needed. It is suggested that for more subtle results, studying other biosystematics characters would be required. In addition, molecular marker application along with the current research strategies could be useful and is recommended. Finally depth study of mint medicinal and food additive potentials can provide the basis for further development and utilization.

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کموتاکسونومی تاکسون‌های وحشی خانواده نعناع بر اساس پروفایل فلاونوئیدی آنها

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چکیده. در این مطالعه الگوهای فلاونوئیدها از ۳۲ تاکسون Lamiaceae از اشترانکوه واقع در کوه‌های زاگرس، ایران برای درک نقش فلاونوئیدها در شیمیوتاکسونومی نعناع و کاربردهای آنها به عنوان افزودنی غذایی، خوراکی، ادویه و دارویی استفاده شده است. این گزارش جدیدی از فلاونوئیدهای برخی تاکسون‌های ایرانی خانواده نعناع با استفاده از کروماتوگرافی های کاغذی دو بعدی و لایه نازک است. نتایج نشان داد که همه تاکسون های مورد مطالعه حاوی فلاون C- &O / -C- گلوکوزیدها و فلاونوئید سولفات ها هستند. هشت گونه دارای آگلیکون و بقیه فاقد آن بودند. کوئرستین در تمام تاکسون‌ها به جز *Lamium album* ssp. *crinitum* و *Nepeta persica* یافت شد. *Stachys setifera* فاقد میرستین بود در حالی که بقیه دارا بودند. رامنتین، تریسین و مورین در همهٔ تاکسون ها به جز *Salvia brachycalyx* و *Salvia staminea* وجود داشت. کامفرول در همه گونه ها به جز *Ajuga chamaecistus*، *Lamium amplexicaule* var. *amplexicaule*، *Nepeta persica* و *Stachys pilifera* مشاهده شد. همه گونه‌ها به جز شش گونه دارای لوتئولین بودند. این نتایج نشان داد که تنوع فلاونوئیدهای بخش هوایی در تاکسون‌های مورد مطالعه می تواند برای مطالعه روابط در محدوده تاکسونومیکی نسبتاً باریک در سطح جنس و گونه مفید بوده و در بررسی‌های کموتاکسونومیکی جنس‌های خانواده نعناع اهمیت دارند. همچنین وجود فلاونوئید در تاکسون‌های مورد مطالعه باعث افزایش کیفیت و فعالیت آنتی اکسیدانی آنها به عنوان خوراک، ادویه‌ها و گیاهان دارویی می‌شود.

کلمات کلیدی: کموسیستماتیک، نعناع، ترکیبات پُلی فنلیک، زاگرس