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Chemotaxonomy, Morphology and Chemo Diversity of *Scutellaria* (Lamiaceae) Species in Zagros, Iran

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Abstract

This study concerns to evaluate the morphological and flavonoid variations, and chemotaxonomy among seven *Scutellaria* species. The limits of *Scutellaria* species were disturbed by different factors including hybridization and polymorphism. For this purpose, 39 *Scutellaria* accessions were collected from different natural habitats of Zagros region, Iran. A total of 15 quantitative and 20 qualitative morphological characters were studied. Leaf flavonoids were extracted using MeOH solution. The flavonoid classes were investigated using thin layer chromatography, column chromatography, *UV*-spect and *LC-MS/MS* (liquid chromatography mass spectrometry). To detect the taxonomic status of *Scutellaria* species, statistical analyses such as cluster, dissimilarity tree, and ordination methods were applied. The results of this research showed five flavonoid classes in different *Scutellaria* species including isoflavone, flavone, flavanone, flavonol and chalcone. Based on the cluster analysis of flavonoid and morphological data, the members of *Scutellaria* section *Scutellaria* were accurately separated from those of *Scutellaria* section *Lupulinaria*. Our study revealed a relationship between *Scutellaria patonii* and *Scutellaria multicaulis*. Moreover, the trichomes such as strigose, lanate, tomentose, pannous in leaf and stem, petiole, calyx, the form of leaf apex, and inflorescence length were found as diagnostic characters. Based on our results, the flavonoid and morphological markers display the taxonomic status of inter and intra-specific levels in *Scutellaria*.

Keywords: Flavonoid; Iran; Lamiaceae; Morphology; *Scutellaria*.

Introduction

The genus *Scutellaria* Linnaeus belonging to Lamiaceae family and Scutellarioideae (Dumort.) Caruel sub-family has 425 species throughout the world [1]. It is distributed in the northern hemisphere, South Africa, North of central Asia, deserts of the North Pole, and temperate mountains of southern continents [1]. The major diversity and speciation centers of this genus were reported to be mainly in Irano-Touranian region, and Eastern Mediterranean [1]. The highest number of endemic species was reported from East Asia and Irano-Touranian region. Central Asia is likely the origin of *Scutellaria* species [1]. This genus is represented by 22 species in Iran from which 10 species are endemic [2, 3]. Most of the species are observed in particular

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habitats such as altitude, damp meadows, waterside, dry rocky steppes, folded areas, and semi- desert. It grows as perennial herbaceous, erect shrubs, suffrutescent, and in cushion forming and cliff dwelling forms [1].

Scutellaria species were introduced as a medicinal herb with different properties. They are widely used in traditional medicine to treat inflammation, pyrexia, hepatitis, hypertension, pneumonia, dysentery, intestinal catarrh, and pyogenic infection [4]. Its species are also used for medicinal properties such as anticancer, antibacterial, antiviral, and antioxidant [5].

Based on the taxonomic point of view, hybridization, introgression, geographical convergence, intermediate species, morphological similarities, and polymorphism in the species lead to disturbing species limits [1, 3, 6]. In this regard, there are different classifications for infra-generic levels. *Hamilton* (1832) identified three sections (*Lupulinaria* A. Hamilton, *Stachymacris* A. Hamilton, *Galericularia* A. Hamilton) for this genus [7, 8]. Based on inflorescence morphology, *Bentham* (1834) illustrated four sections (*Lupulinaria*, *Heteranthesia* Benth., *Stachymacris*, and *Galericularia*) and three sub-sections. Later on, *Bentham* (1876) introduced three sections. *Briquet* (1896) identified two sub-genus and three sections (*Lupulinaria*, *Heteranthesia*, *Vulgares* Benth.) [7, 8]. *Rechinger* (1982) also categorized four sub-genus and three sections (*Lupulinaria, Stachymacris, Galericularia*) [3]. *Epling* (1942) divided the genus into 18 sections while *Paton* (1990a) recognized two sub-genus (*Scutellaria* and *Apeltanthus*) and seven sections (*Scutellaria*, *Anaspis* (Rech. f.) Paton, *Salazaria* (Torrey) Paton, *perilomia* (Kunth) Epling emend Paton, *Salviifoliae* (Boiss.) Edmonson, *Apeltanthus* Nevski ex Juz., *Lupulinaria*) [7]. All the classifications were based on morphological characters such as inflorescence, calyx, and corolla. Moreover, *Jamzad* (2012) identified two sub-genus and three sections (*Scutellaria*, *Anapsis*, and *Lupulinaria*) [2]. It is noted that *Paton* (1990a) considered the wide concept for *Scutellaria* including *Perilomia* Kunth, *Harlanlewisia* Epling, and *Salazaria* Torrey [1].

Based on morphological studies, there are some reports for this genus. A taxonomic revision was described for *Sc. multicaulis* Boiss. by *Safikhani* et al. (2017) [6]. These researches recognized three new taxa for Iran including *Sc. patonii* Jamzad & Safikhani, *Sc. multicaulis* subsp. *multicaulis* var. *multicaulis* and *Sc. multicaulis* subsp. *multicaulis var. gandomanensis* Jamzad & Safikhani. *Zhao* et al. (2017) reported the taxonomic position of some *Scutellaria* species in China using macro and micromorphology of pollen and trichomes [9]. *Ozdemir* and *Altan* (2005), and *Dereboylu* et al. (2012) investigated the anatomical features belonging two subspecies from *Sc. orientalis* L. and one variety of *Sc. cypria* Rech. f. and *Sc. sipthorpii* (Benth.) Hal. in Turkey and Cyprus [10, 11]. Since these features were different in subspecies, they discriminate these taxa. Comparative morphological studies were conducted in *Sc. salvifolia* Benth. and *Sc. diffusa* Benth. from Turkey [12]. Further efforts have been made on micromorphology of pollen and nutlet [7, 8, 13]. *Hasani-Nejad* et al. (2009) identified five types of nutlet among three sections of *Scutellaria* [13]. Moreover, the pollen of these sections showed two different types [8, 13].

According to phytochemical studies, there are different chemical compounds in *Scutellaria* species. However, flavonoid compounds are mainly identified in this genus. The compounds such as wogonin, wogonoside, apigenin derivatives, baicalein, baicalin [14, 15], luteolin, luteolin 7-*O*-glucoside, chrysin [5], scutellarin [15, 16], flavanone, flavonol, chalcone, lignoflavonoid derivatives, patuletin, pinobankasin [4], oroxylin A, and norwogonin [17, 18], have been reported in *Sc. pinnatifida* A. Ham., *Sc. baicalensis* Georgi, *Sc. rubicunda* Hornem., *Sc. albida* L., *Sc. alpina* L., and *Sc. altissima* L. Moreover, different essential oils such as sesquiterpenes and monoterpenes have been reported in *Sc. orientalis* [19].

To the best of authors' knowledge, there is no study conducted on morphometric and chemo-taxonomical characteristics of *Scutellaria* species in Iran. In this regards, Zagros region is one of the greatest genetic resources in Iran and includes high diversity and variations of the *Scutellaria* species. Consequently, the aim of this research is to 1) study the taxonomic status and morphological diversity among the *Scutellaria* species using morphological characters, 2) investigate the chemotaxonomic positions of *Scutellaria* species using flavonoid patterns, 3) study the flavonoid variations in *Scutellaria* accessions, and 4) identify the flavonoid classes of each species. All the obtained data are reported for the first time for Iran.

Materials and Methods

Morphologic study

In this work, 39 accessions of seven *Scutellaria* species belonging to *Sc.* sub-genus *Scutellaria*; sect. *Scutellaria* and *Sc.* sub-genus *Apeltanthus*; sect. *Lupulinaria* were collected from their natural habitats including the center, south-west, and west of Zagros region (Table 1). All specimens were deposited in the Herbarium of Shahr-e Kord University. In order to conduct morphological studies, 15 quantitative

Table 1. The locality of *Scutellaria* species in Zagros, Iran

Species/no. accession	Locality	Height (m)	Herbarium no.	Date	Latitude, longitude
	Chaharmahal va Bakhtiari				
Sc. farsistanica Rech. f.	Dorahan, 45 km Lordegan	1683	Sc1	Jun 2016	31°37′N, 51°11′E
Sc. farsistanica	Dorahan, 45 km Lordegan	2180	Sc2	Jun 2016	31°37′N, 51°11′E
Sc. farsistanica	Boroujen, Hamz-e Ali Emamzadeh	2250	Sc5	Jun 2016	31°56′N, 51°0′E
	Isfahan				
Sc. farsistanica	Bardekan, Gharghach	2130	Sc7	Jun 2016	31°28′N, 51°35′E
Sc. farsistanica	Bardekan, Gharghach	2170	Sc8	Jun 2016	31°28′N, 51°35′E
Sc. farsistanica	Bardekan	2185	Sc9	Jun 2016	31°28′N, 51°35′E
Sc. farsistanica	Gharghach village	2200	Sc10	Jun 2016	31°28′N, 51°35′E
Sc. farsistanica	Semirom- Vanak, Dalan-kouh	1897	Sc11	Jun 2016	31°29′N, 51°17′E
Sc. farsistanica	Semirom- Ghorogh-e Vanak	1800	Sc12	Jun 2016	31°24′N, 51°34′E
	Chaharmahal va Bakhtiari				
Sc. tomentosa Betrol.	Sahrekord- Farokhshahr, Tang-e Sayad	2180	Sc3	Jun 2016	32°16′N, 50°58′E
Sc. tomentosa	Sahrekord- Tang-e Sayad	2230	Sc4	Jun 2016	32°9′N, 51°7′E
	Isfahan				
Sc. tomentosa	Hajiabad, Bardekan	2130	Sc6	Jun 2016	32°39′E, 51°15′E
	Isfahan				
Sc .nepetifolia Benth.	Khansar- Damaneh	2120	Sc13	Jun 2016	33°9′N, 50°24′E
Sc. nepetifolia	Khansar- Damaneh	2130	Sc14	Jun 2016	33°9′N, 50°24′E
Sc. nepetifolia	Khansar- Damaneh	2150	Sc15	Jun 2016	33°9′N, 50°24′E
Sc. nepetifolia	Khansar- Damaneh	2420	Sc16	Jun 2016	33°9′N, 50°24′E
Sc. nepetifolia	Analoujeh village- Dalankouh	2200	Sc17	Jun 2016	31°31′N, 51°19′E
Sc. nepetifolia	Dalankouh	2900	Sc18	Jun 2016	31°31′N, 51°19′E
	Chaharmahal va Bakhtiari				
Sc. nepetifolia	Samsami- 65 km Bazoft, Safaabad	2082	Sc19	July 2019	32°8′N, 50°24′E
	Chaharmahal va Bakhtiari				
Sc. patonii	Bazoft- Siyavashabad, Chenar	2033	Sc20	Jun 2016	32°14′N, 49°59′E
Sc. patonii	Samsami- Abbarik, Marboreh	2093	Sc21	Jun 2016	32°10′N, 50°16′E
Sc. patonii	Samsami- Abbarik, Marboreh	2018	Sc22	Jun 2016	32°10′N, 50°16′E
Sc. patonii	Kouhrang	2042	Sc23	Jun 2016	32°29′N, 50°4′E
Sc. patonii	Kouhrang road	2150	Sc24	Jun 2016	32°30′N, 50°12′E
	Chaharmahal va Bakhtiari				
Sc. multicaulis Boiss. var. *multicaulis*	Talab-e Gandoman, Nasirabad	2038	Sc25	Jun 2016	31°50′N, 51°6′E
Sc. multicaulis var. *multicaulis*	Talab-e Gandoman, Nasirabad	2170	Sc26	Jun 2016	31°50′N, 51°6′E
Sc. multicaulis var. *multicaulis*	Talab-e Gandoman, Nasirabad	2200	Sc27	Jun 2016	31°50′N, 51°6′E
Sc. multicaulis var. *multicaulis*	Talab-e Gandoma, Chirou	1918	Sc28	Jun 2016	31°49′N, 51°9′E
Sc. multicaulis var. *multicaulis*	Talab-e Gandoma, Chirou	1950	Sc29	Jun 2016	31°49′N, 51°9′E
Sc. multicaulis var. *multicaulis*	Lorgegan- Glougerd	1908	Sc30	Jun 2016	31°54′N, 50°51′E
Sc. multicaulis var. *multicaulis*	Lorgegan- Glougerd	1920	Sc31	Jun 2016	31°54′N, 50°51′E
Sc. multicaulis var. *multicaulis*	Jouneghan- Tang-e Darkesh	2000	Sc32	Jun 2016	32°9′N, 50°41′E
Sc. multicaulis var. *multicaulis*	Jouneghan- Tang-e Darkesh	2030	Sc33	Jun 2016	32°9′N, 50°41′E
	Kurdestan				
Sc. pinnatifida A. Ham. subsp. *pichleri*	Marivan- Oraman	1450	Sc34	Jun 2016	35°15′N, 46°15′E
Sc. pinnatifida subsp. *pichleri*	Marivan	1464	Sc35	Jun 2016	35°30′N, 46°12′E
Sc. condensata Rech. f. subsp. *condensata*	Marivan- Darvian	1850	Sc36	Jun 2016	35°31′N, 46°10′E
Sc. condensata subsp. *condensata*	Marivan- Darvian	2400	Sc37	Jun 2016	35°31′N, 46°10′E
Sc. condensata subsp. *condensata*	Marivan	1700	Sc38	Jun 2016	35°30′N, 46°12′E
Sc. condensata subsp. *condensata*	Marivan- Darvian	1768	Sc39	Jun 2016	35°31′N, 46°10′E

characters and 20 qualitative characters were studied using Olympus SZX-ZB12 research stereo microscope (Table 2). Moreover, the taxonomical position of each species was estimated using Simple Matching coefficient and *UPGMA* (Unweighted Pair Group Method with Arithmetic Mean) method with *NTSYS pc v*.2.2 software. Afterward, the dissimilarity tree was estimated using Ward method, Dice coefficient, and *DARwin* 6 software. An Analysis of Variance (*ANOVA*) was also applied in morphological characters. The collected specimens were determined using Flora Iranica and Flore of Iran [2, 3].

Plant material

In this section, the leaf of seven *Scutellaria* species was used in chemical studies. The species were

Table 2. List of quantitative and qualitative characters in *Scutellaria* species.

Characters	Characters
Stem length (cm)	Stem width (mm)
Petiole length (mm)	Leaf length (mm)
Leaf width (mm)	Inflorescence axis length (cm)
Bract length (mm)	Bract width (mm)
Calyx length (mm)	Calyx width (mm)
Length of corolla tube (cm)	Length of corolla lip in upper surface (mm)
Length of corolla lip in lower surface (mm)	Filament length (cm)
Anther length (mm)	
Indumentum of stem in lower surface	Indumentum of stem in upper surface
Petiole indumentum	Leaf form
Leaf margin	Leaf base
Leaf apex	Indumentum of leaf in upper surface
Indumentum of leaf in lower surface	inflorescence indumentum
Bract apex	Indumentum of bract in upper surface
Indumentum of bract in lower surface	Calyx indumentum
Indumentum of calyx apex	Indumentum of corolla tube
Indumentum of corolla lip in upper surface	Indumentum of corolla lip in lower surface
Corolla color	Anther indumentum

collected at the same phenological phase such as flowering period in June and July 2016. The number of repetition in each experiment was from 3-5 ranges.

Phytochemical and Chemotaxonomic study

Flavonoid extraction was initiated using the method proposed by *Rahman* (2005) [20]. The total flavonoid of leaves (10.5 g) from seven *Scutellaria* species was extracted with crude 100% MeOH at 50°C. The flavonoid solution was condensed under a rotary evaporator *EYELA*/Japan at 70°C for removal of the total solvent. Flavonoid purification was done using n-BuOH and consecutively analyzed by silica gel 60F 254 (17 mg, 80 ml H_2O) thin layer chromatography (TLC; 5 µM, 20 ×20 cm). The chromatogram was run in a solvent system including MeOH-H_2O (70:30), $CHCl_3$-MeOH (75:25), and BuOH-CH_3COOH-H_2O (16:28:56) [16, 18, 21]. Flavonoid spots were demonstrated with natural product identifiers (H_2SO_4 5% in MeOH) and ultraviolet-366 nm [20, 21]. The flavonoid solution was purified by column chromatography (50×4 cm), followed by Sephadex LH_{20} Sigma-Aldrich (Sephadex and MeOH 20% mixture) in 100 mL MeOH solution, and extracted in fractions. Recognition of purified compounds was accomplished on the basis of their ultraviolet spectra (200-400 nm), MeOH solution, and shift reagents such as $AlCl_3$, $AlCl_3$/HCl, NaOAc, NaOAc/H_3BO_3, and MeOH. Moreover, all fractions were analyzed using *LC-MS/MS* (liquid chromatography mass spectrometry) on a triple quadrupole mass spectrometer (TQMS) to detect the m/z (mass) value of each species. Chromatography condition was prepared on an Agilent Zorbax SB-C18 column (15 cm, 3.5µm) and 25°C. LC-MS grade methanol, acetonitrile, MS grade acetic acid (98%) and ultra-pure water were used for mobile phase at a mode ESI (Electrospray Ionization) [22]. In addition, flavonoid compound standard (apigenin) from SIGMA- Aldrich Chemical Co. was used.

The flavonoid variations among 39 *Scutellaria* accessions and chemotaxonomic position were assayed by statistical methods such as cluster analysis and distance method with a Simple Matching coefficient, Dice coefficient, and *UPGMA* method using *NTSYS v*.2.2 software and Cluster *Vis* 1.8.2. Moreover, Principle Coordinate analysis (*PCoA*) was designed using Variance-Covariance (*VARCOV*) coefficient and Eigen-vector with Square Root Lambda (*SQRT*) vector scaling and *NTSYS pc v*.2.2 software. The presence and absence of color spots were surveyed during this process. In addition, the retention factor (Rf) of spots belonging to each species was considered.

Results

According to the morphological studies, the quantitative characters including the length of leaf, petiole, bract, corolla, inflorescence axis, and leaf width display high variations (Table 3). The high variations of qualitative characters were found in leaf shape, leaf margin, leaf base, leaf apex, indumentum of stem, leaf, petiole, bract, calyx, corolla tube and corolla lips, and inflorescence axis (Table 3). The morphological characters such as strigose, lanate, tomentose, pannous in leaf and stem, petiole, calyx, the form of leaf apex, and inflorescence length were found as diagnostic characters. Based on *ANOVA* analysis, the morphological characters revealed significant differences in *Sutellaria* species (**P*<0.05; *F*-value=

Table 3. The quantitative and qualitative morphological characters in *Scutellaria* species.

Characters	***Sc. farsistanica***	***Sc. tomentosa***	***Sc. pinnatifida* subsp. *pichleri***	***Sc. patonii***	***Sc. multicaulis* var. *multicaulis***	***Sc. nepetifolia***	***Sc. condensata* subsp. *condensata***
Stem length (cm)	10-27	20-24	14-28	23-48	23-48	25-33	25-30
Stem width (mm)	1-2	1-2	2	1-2	1-2	1-2	2-3
Petiole length (mm)	5-8	5-15	8-10	3-9	3-9	4-7	20-27
Leaf length (mm)	10-25	12-15	10-20	8-16	8-16	10-18	40-42
Leaf width (mm)	6-13	9-10	5-7	8-3	3-10	6-13	25-29
Inflorescence axis length (cm)	5-12.5	4.5-16	6-7	11-30	11-30	10-15	11-12
Bract length (mm)	8-11	6-10	7-8	5-8	5-8	4-5	4
Bract width (mm)	5-6	4-7	4	3-5	3-5	3	3
Calyx length (mm)	3-4	3	3-4	3	2-3	1-3	4-5
Calyx width (mm)	2	2	3	2-3	1-3	1-3	4
Length of corolla tube (cm)	2-3	1.5-3	1	1.9-2	1.3-2	1.5-2	2-3
Length of corolla lip in upper surface (mm)	5-10	5-6	2.5-2.6	6-10	4-10	3-8	1.5-1.7
Length of corolla lip in lower surface (mm)	3-6	3-5	8-10	5-8	3-8	3-6	4-5
Filament length (cm)	3-3.2	2-3.2	5-6	2	1.4-2	1.5-3	3-4
Anther length (mm)	1	1	1	1	1	1	1
Indumentum of stem in lower surface	Glandular stipitate, strigose	Pannous, glandular stipitate	Short simple	Short simple, glandular, pilose	Short simple, glandular, pilose, stigose	Lanate, pilose, tuberculate, glandular, strigose	Short simple
Indumentum of stem in upper surface	Glandular, pilose, strigose	Pannous, pilose, tomentose, short simple, glandular stipitate	Short simple, tuberculate,	Short simple, pilose, strigose	Short simple, pilose, strigose	Tuberculate, strigose, lanate, glandular	Short and long simple
Petiole indumentum	Glandular, pilose, strigose	Pilose, pannous, short simple, glandular stipitate	Short and long simple, glandular stipitate	Short simple, glandular, pilose	Short simple, pilose, glandular	Strigose, tuberculate, lanate, glandular, tomentose, pilose, short simple	Long simple, glandular
Leaf form	Ovate, oblong	Ovate, oblong	Oblong	Ovate	Ovate	Ovate	Ovate

2.870-78.826). The highest amount of *F*-value was observed in length of upper corolla lip. The morphological characters with **P*<0.05 were observed in length of stem, inflorescence, bract, the width of bract, length of upper corolla lip, indumentum of the stem, leaf, inflorescence, bract, calyx, corolla tube, upper corolla lip, and the form of leaf margin, leaf base, and bract apex.

The results of cluster analysis with morphological data showed two groups (Fig. 1). Moreover, three groups of *Sc. tomentosa* Betrol., five groups of *Sc. farsistanica* Rech. f., four groups of *Sc. nepetifolia* Benth., three groups of *Sc. patonii*, six groups of *Sc. multicaulis*, two groups of *Sc. condensata* Rech. f. and one group of *Sc. pinnatifida* were identified (Fig. 1). The highest morphological variations were observed in *Sc. multicaulis*, *Sc. farsistanica* and *Sc. nepetifolia*. It was identified that *Sc. nepetifolia* accessions are clearly separated from *Sc. multiculis*. Moreover, *Sc. farsistanica* accessions were discriminated from *Sc.*

Table 3. Ctd

Characters	***Sc. farsistanica***	***Sc. tomentosa***	***Sc. pinnatifida*** **subsp. *pichleri***	***Sc. patonii***	***Sc. multicaulis*** **var. *multicaulis***	***Sc. nepetifolia***	***Sc. condensata*** **subsp. *condensata***
Leaf margin	Serrate	Serrate	Pinnatifid	Crenate	Crenate	Serrate, dentate, crenate	Dentate, crenate
Leaf base	Obtuse	Obtuse	Acute, truncate	Cuneate, obtuse	Cuneate, obtuse	Obtuse, truncate	Truncate, cuneate, obtuse
Leaf apex	Acute, obtuse	Acute	Obtuse	Acute	Rounded, acute	Rounded, obtuse	Acute, rounded
Indumentum of leaf in upper surface	Glandular, glandular stipitate, pilose, tomentose	Pannous, glandular stipitate, pilose	Pubescent	Short simple, strigose, glandular, pilose	Short simple, glandular, pilose	Tuberculate, strigose, glandular stipitate, short simple, tomentose	Short simple
Indumentum of leaf in lower surface	Glandular stipitate, glandular, pilose, tomentose, short simple	Pannous, tomentose, short simple, glandular, pilose	Short simple, glandular	Glandular, short simple, tuberculate	Glandular, short simple, pilose, strigose, tuberculate	Tuberculate, strigose, glandular stipitate, short simple, tomentose	Long simple, glandular stipitate
Inflorescence indumentum	Glandular stipitate	Pannous, pilose, glandular stipitate, short simple	Short simple, glandular stipitate, tuberculate	Short simple, glandular stipitate, pilose, strigose	Short simple, glandular stipitate, pilose, strigose, lanate	Lanate, tubrculate, strigose, glandular, pilose, tomentose, short simple	Glandular stipitate, long simple
Bract apex	Aristate	Aristate	Acute	Attenuate	Attenuate	Attenuate	Acute
Indumentum of bract in upper surface	Glandular, short simple	Pannous, pilose, glandular stipitate	Short simple	Pilose, glandular stipitate, glandular	Glandular, pilose, short simple	Tuberculate, strigose, glandular stipitate, pilose, short simple	Long simple, glandular stipitate
Indumentum of bract in lower surface	Glandular, short simple, tuberculate, pannous	Pannous, pilose, glandular, short simple	Short simple	Short simple, glandular stipitate, glandular, pilose	Short simple, glandular stipitate, glandular, pilose	Tuberculate, strigose, glandular, tomentose, short simple, lanate	Long simple, short glandular stipitate
Calyx indumentum	Glandular, pilose, short simple	Pannous, pilose, short simple, glandular stipitate	Short simple, glandular stipitate	Glandular stipitate, glandular, short simple, pilose	Glandular, glandular stipitate, pilose, short simple	Tuberculate, strigose, short simple, pilose, glandular, lanate, tomentose	Long and short simple, glandular stipitate
Indumentum of calyx apex	Glandular, pilose, tomentose, short simple	Pannous, pilose, short simple, glandular stipitate	Short simple	Glandular, pilose, short simple	Glandular, glandular stipitate, short simple, pilose	Tuberculate, glandular, tomentose, lanate, strigose	Short simple, short glandular stipitate
Indumentum of corolla tube	Glandular, pilose, short simple	Pannous, short simple, glandular stipitate	Pubescent	Pilose, short simple, glandular,	Pilose, short simple, glandular, glandular stipitate	Tuberculate, pilose, strigose, glandular, short simple, tomentose	Short and long simple

tomentosa accessions. *Sc. patonii* with accession no. 23 was clustered separately but it was clustered with *Sc. multicaulis* with accession no. 26 and 27. Different features in *Sc. patonii* 23 were related to the presence of pilose at the lower surface of leaf and base of the stem, simple trichome in bract and lower corolla lip, and the presence of strigose at the upper surface of the stem. Different groups were identified in *Sc. tomentosa* with accession no. 3, which is associated with the presence of features such as pilose at the surface of the leaf,

Table 3. Ctd

Characters	***Sc. farsistanica***	***Sc. tomentosa***	***Sc. pinnatifida* subsp. *pichleri***	***Sc. patonii***	***Sc. multicaulis* var. *multicaulis***	***Sc. nepetifolia***	***Sc. condensata* subsp. *condensata***
Indumentum of corolla lip in upper surface	Glandular, pilose, short simple	Pannous, pilose, short simple, glandular stipitate	Short simple, glandular	Short simple, glandular stipitate	Short simple, glandular stipitate, pilose	Tuberculate, strigose, glandular, short simple, pilose	Short and long simple
Indumentum of corolla lip in lower surface	Glandular, pilose, short simple	Short simple, pannous, glandular stipitate	Short simple	Glandular stipitate, pilose, glandular	Short simple, glandular stipitate, pilose, glandular	Tuberculate, strigose, glandular, short simple, pilose	Short and long simple
Corolla color	yellow	Yellow, brown-purple	Yellow	Violet-yellow	Violet-yellow	Violet-yellow	Creamy
Anther indumentum	Glandular	Glandular	Glandular	Glandular	Glandular	Glandular	Glandular, pubescent

pannous in corolla tube, corolla lip, and petiole, and simple hairs in corolla tube. In addition, *Sc. tomentosa* with accession no. 6 was found to be different in terms of oblong leaf, the presence of tomentose and glandular trichomes at lower surface of leaf and petiole, simple hairs at upper surface of bract, upper surface of stem and petiole, and the presence of tomentose in inflorescence axis and upper surface of stem. Moreover, *Sc. pinnatifida* was definitely separated from the other members of *Sc.* sect. *Lupulinaria*. In addiotion, *Sc. condensata* (*Sc.* sect. *Scutellaria*) was separated from the members of *Sc.* sec. *Lupulinaria*.

Despite high similarity among *Sc. multicaulis*, *Sc. patonii* and *Sc. nepetifolia*, these species were definitely separated using morphological data and dissimilarity tree (Fig. 2). As shown in Fig. 2, one accession of *Sc. patonii* shows a relationship with *Sc. multicaulis*. There might be a hybridization among them or existence of intermediate species.

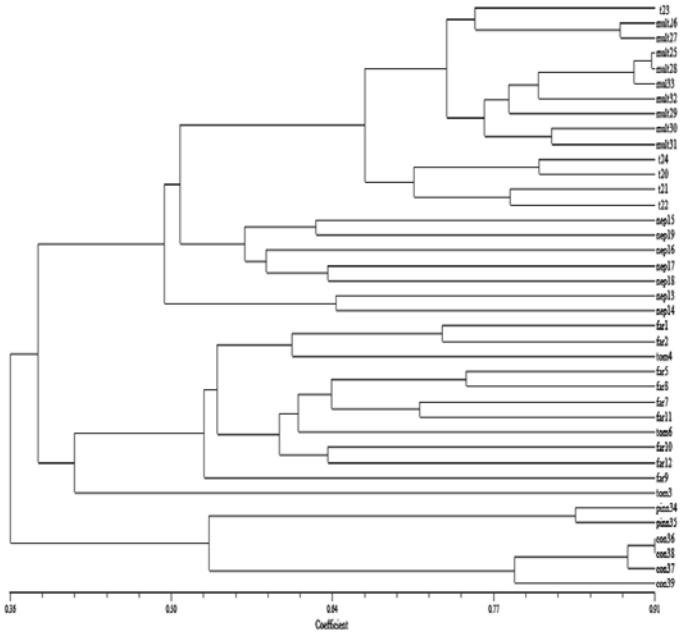

Figure 1. The cluster analysis using morphological data in *Scutellaria* species. far: *farsistanica*, tom: *tomentosa*, nep: *nepetifolia*, mult: *multicualis*, t: *patonii*, pinn: *pinnatifida*, con: *condensata*

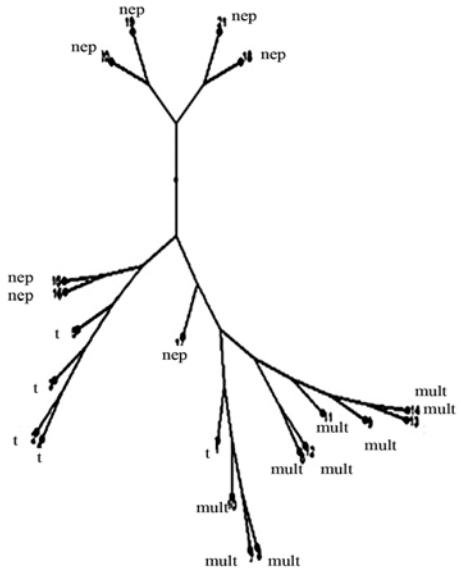

Figure 2. The dissimilarity tree of *Sc. patonii*, *Sc. multicaulis* and *Sc. nepetifolia* using morphological data. nep: *nepetifolia*, mult: *multicualis*, t: *patonii*

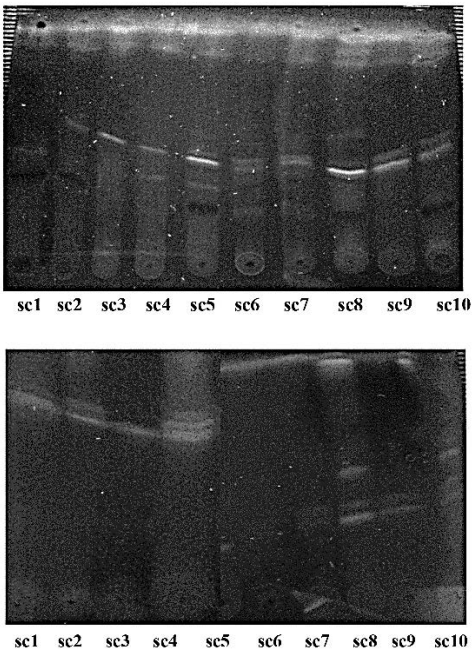

Figure 3. The chromatogram of $CHCl_3$/ MeOH system in different accessions of *Sc. tomentosa* and *Sc. farsistanica* (sc1-sc10). The number of each accession is mentioned in table 1.

Based on flavonoid data, three solvent systems were applied for *Scutellaria* species. MeOH-H_2O (70:30), $CHCl_3$-MeOH (75:25), and BuOH-CH_3COOH-H_2O (16:28:56) represented a developing solvent system. The appropriate solvent systems were $CHCl_3$-MeOH (75:25) and MeOH-H_2O (70:30). There were 166 and 152 spots in $CHCl_3$-MeOH and MeOH-H_2O solvent systems, respectively. Moreover, different color spots were observed in the chromatogram of TLC from *Scutellaria* species. These spots were mainly yellow, dark yellow, light yellow, fluorescent yellow, blue, light blue, fluorescent blue, violet, orange, and brown (Table 4). Also, extra color spots were identified after detection of natural product identifiers including blue, light blue,

Table 4. The spot colors and Rf value of *Scutellaria* species using different solvent systems including MeOH-H_2O, $CHCl_3$-MeOH and BuOH-CH_3COOH-H_2O. a: the color spots after detection of natural products

Species/spot color	Blue	Light blue	Blue florescent	Violet	Yellow	Dark yellow	Light yellow	Yellow florescent	Orange	Brown	Rf
Sc. farsistanica 1	+	+	-	+	+	+	+	-	-	+	0.14-1
Sc. farsistanica 2	+	+	-	+	+	-	+	-	-	+	
Sc. farsistanica 5	+	+	+	+	+	-	+	-	-	+	
Sc. farsistanica 7	+, +a	+	-	+a	-	+	+	-	-		
Sc. farsistanica 8	+	+	+	+	+	-	+	-	-	+	
Sc. farsistanica 9	+	+	-	-	-	-	+	-	-		
Sc. farsistanica 10	+, +a	+	-	+, +a	-	+	+	-	-	+	
Sc. farsistanica 11	+	-	-	-	+a	-	+	-	-	-	
Sc. farsistanica 12	+	-	-	-	-	-	-	-	-	-	
Sc. tomentosa 3	+a	+	+	-	+	-	+	-	-	-	0.14-1
Sc. tomentosa 4	+, +a	+	-	+	-	+	+	-	-	-	
Sc. tomentosa 6	+	+	-	-	-	+	+	-	-	-	
Sc. nepetifolia 13	+a	-	-	-	-	-	+	-	-	-	0.45-1
Sc. nepetifolia 14	-	-	-	-	-	-	+	-	-	-	
Sc. nepetifolia 15	-	-	-	-	-	+	-	+a	-	-	
Sc. nepetifolia 16	-	-	-	-	+	-	-	+a	-	-	
Sc. nepetifolia 17	-	-	-	-	+	-	-	+a	-	-	
Sc. nepetifolia 18	+	+a	-	-	-	-	-	-	-	-	
Sc. nepetifolia 19	+	-	-	-	+a	-	-	-	-	-	
Sc. patonii 20		-	-	-	-	-	+	-	-	-	0.16-0.97
Sc. patonii 21	+	-	+	-	-	-	-	+	-	-	
Sc. patonii 22	+	-	+	-	-	-	+	-	-	-	
Sc. patonii 23	+	-	-	-	-	-	-	-	-	-	
Sc. patonii 24	+	-	-	-	+	-	+	+	-	-	
Sc.multicaulis 25	-	-	-	-	+	-	+	+	-	+	0.16-1
Sc.multicaulis 26	-	-	-	-	-	-	+	-	-	-	
Sc.multicaulis 27	-	-	-	-	-	-	+	-	-	-	
Sc.multicaulis 28	+	-	-	-	-	-	-	-	-	-	
Sc.multicaulis 29	-	-	-	-	+	+	-	-	-	-	
Sc.multicaulis 30	+a	-	+	-	-	-	+	-	-	-	
Sc.multicaulis 31	-	-	-	+a	-	+	-	-	-	-	
Sc.multicaulis 32	+, +a	-	-	-	-	-	+	-	-	-	
Sc.multicaulis 33	+	+	-	-	+, +a	-	-	-	-	-	
Sc. pinnatifida 34	-	-	+	-	-	-	-	-	-	-	0.64-1
Sc. pinnatifida 35	-	-	-	-	-	-	+	-	-	-	
Sc. condensata 36	-	-	-	-	+	-	-	-	+	-	0.41-0.96
Sc. condensata 37	-	-	-	-	+	-	-	-	+	-	
Sc. condensata 38	-	-	-	-	-	-	+	-	-	-	
*Sc. condensata*39	-	-	-	-	-	-	+	-	-	-	

violet, yellow, and fluorescent yellow. The Rf values were ranged from 0.14-1 (Table 4, Fig. 3).

Based on cluster analysis using flavonoid data, two groups were comprised (Fig. 4). In these results, *Sc. patonii* accessions were definitely separated from *Sc. multicaulis*, but *Sc. patonii* with accession no. 24 was clustered with *Sc. multicaulis* with accession no. 25. Some *Sc. nepetifolia* accessions were grouped with *Sc. multicaulis*. There are some relations between these species. *Scutellaria condensata* from *Sc.* sect. *Scutellaria* seems to be definitely separated. Moreover, *Sc. tomentosa* accessions were definitely grouped. It was observed that *Sc. farsistanica* with accession no. 7 and *Sc. tomentosa* with accession no. 3 were different from other members of *Sc.* sect. *Lupulinaria*. Different groups were also identified including six groups of *Sc. farsistanica*, two groups of *Sc. tomentosa*, three groups of *Sc. nepetifolia*, three groups of *Sc. patonii*, seven groups of *Sc. multicaulis*, one group of *Sc. pinnatifida*, and two groups of *Sc. condensata*.

The *PCoA* analysis was in accord with cluster analysis. In this analysis, *Sc. multicaulis* included three groups (Fig. 5). Both sections were grouped separately.

A distance dendrogram was separately accomplished for *Sc. multicaulis*, *Sc. nepetifolia* and *Sc. patonii* using flavonoid data (Fig. 6). These species were definitely grouped but two accessions of *Sc. patoni* and one accession of *Sc. nepetifolia* were grouped with *Sc.*

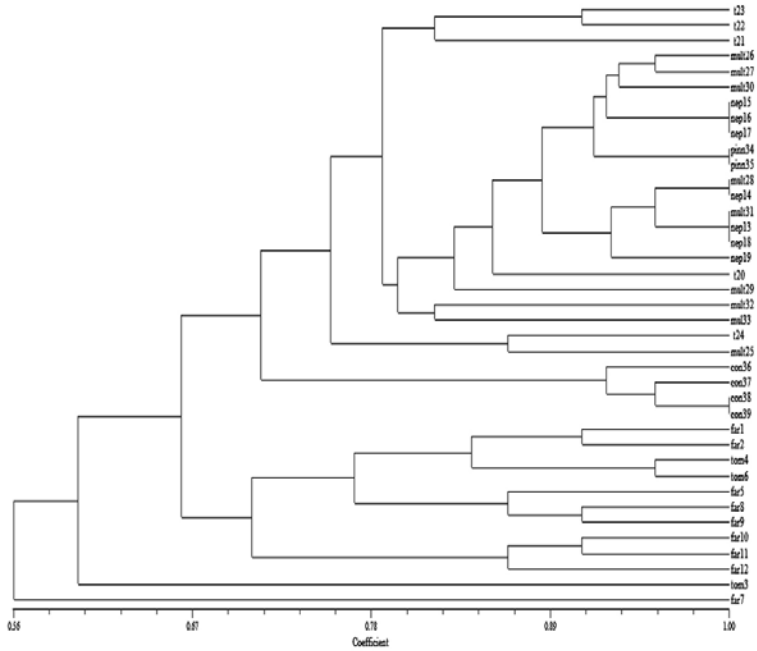

Figure 4. The cluster analysis of *Scutellaria* species using flavonoid data. far: *farsistanica*, tom: *tomentosa*, nep: *nepetifolia*, mult: *multicualis*, t: *patonii*, pinn: *pinnatifida*, con: *condensata*

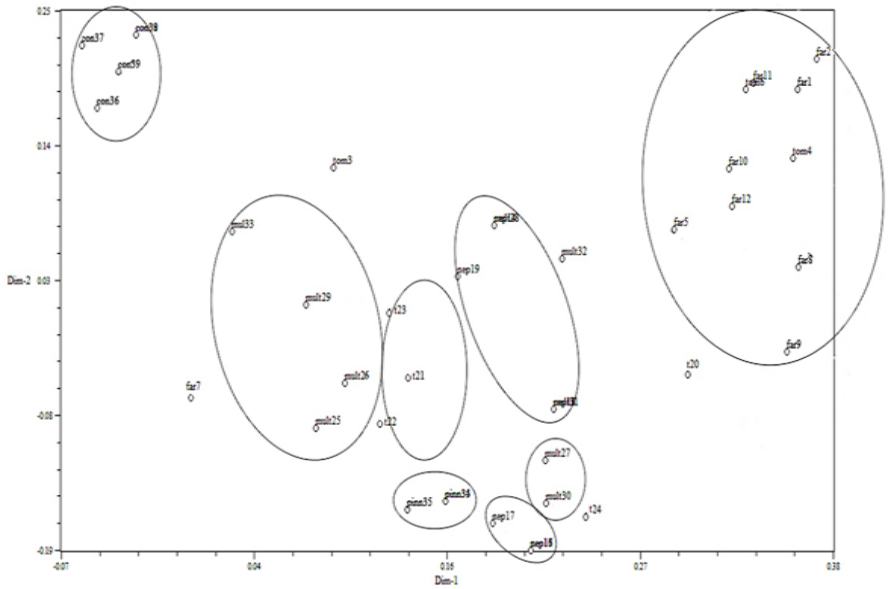

Figure 5. The PCoA analysis in *Scutellaria* species using flavonoid data. far: *farsistanica*, tom: *tomentosa*, nep: *nepetifolia*, mult: *multicualis*, t: *patonii*, pinn: *pinnatifida*, con: *condensata*

multicualis. As shown in Fig. 6, flavonoid profiles can strongly display infra-specific relations.

Based on flavonoid classes, a total of five groups were recognized including 11 isoflavones, 28 flavones, 4 flavanones, one flavonol, and one chalcone (Table 5). Isoflavone class was found to be *Sc. tomentosa*, *Sc. farsistanica*, *Sc. nepetifolia*, *Sc. patonii*, *Sc. pinnatifida*, and *Sc. condensata*. Moreover, flavone class was observed in all species. It is presented that flavanone class was recognized in *Sc. tomentosa*, *Sc. nepetifolia*, *Sc. patonii*, and *Sc. pinnatifida*. Flavonol class was observed in *Sc. tomentosa*, *Sc. patonii*, and *Sc. nepetifolia*. Chalcone class was also identified in *Sc. tomentosa* and *Sc. patonii*. Different flavonoid classes discriminated *Sutellaria* species comprising of isoflavones 4 and 5 (*Sc. tomentosa*), isoflavone 3 (*Sc.*

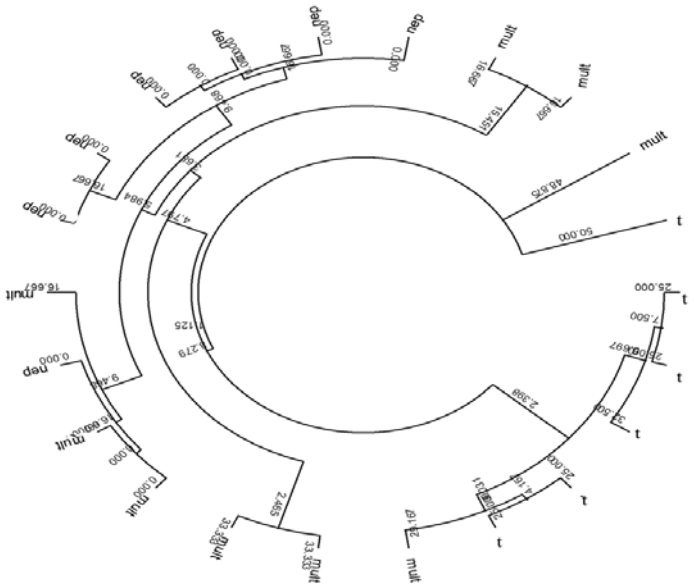

Figure 6. The dendrogram of *Sc. patonii, Sc. multicaulis* and *Sc. nepetifolia* using flavonoid data. nep: *nepetifolia*, mult: *multicualis*, t: *patonii*

farsistanica), isoflavones 6 and 11 (*Sc. condensata*), isoflavone 10 (*Sc. patonii*), isoflavone 7 (*Sc. nepetifolia*), flavone 3 (*Sc. farsistanica*), flavones 4, 6, 7, 10, 11 and 13 (*Sc. tomentosa*), flavones 14 and 15 (*Sc. nepetifolia*), flavone 17 (*Sc. patonii*), flavones 18, 22, 23 and 24 (*Sc. pinnatifida*), flavones 27 and 28 (*Sc. condensata*), flavanone 1 (*Sc. tomentosa*), flavanone 4 (*Sc. patonii*) and flavonol 1 (*Sc. patonii*). The highest diversities of flavonoid classes were found to be *Sc. tomentosa*, *Sc. nepetifolia*, *Sc. pinnatifida*, *Sc. condensata* and *Sc. patonii* (Table 5).

Based on shift reagents, the highest shifts were found to be isoflavone and flavone with 55 and 79 nm. Moreover, ortho-dihydroxylation A and B-ring were observed in isoflavone (5-12 nm, NaoAc/H_3BO_3), flavone (5-10 nm, NaoAc/H_3BO_3), flavanone (9 nm, NaoAc/H_3BO_3) and flavonol (10 nm, NaoAc/H_3BO_3) (Table 6).

The maximum absorption was observed at 398 nm (flavone) and the minimum was found to be isoflavone (262 nm) (Table 6).

Table 5. Flavonoid class identified and its m/z in each *Scutellaria* species

Species	Flavonoid class	Ms1; m/z [M-H]$^{-/+}$	Species	Flavonoid class	Ms1; m/z [M-H]$^{-/+}$
	Isoflavones			Flavanones	
Sc. farsistanica	Isoflavones 1, 2, 3	297, 577, 357	*Sc. farsistanica*	-	-
Sc. tomentosa	Isoflavones 2, 4, 5	577, 447, 285	*Sc. tomentosa*	Flavanone 1	287
Sc. nepetifolia	Isoflavones 7, 8, 9	253, 431, 285	*Sc. nepetifolia*	Flavanones 2, 3	255, 271
Sc. patonii	Isoflavones 1, 8, 10	297, 431, 445	*Sc. patonii*	Flavanones 3, 4	271, 303
Sc.multicaulis	-	-	*Sc.multicaulis*	-	-
Sc. pinnatifida	-	-	*Sc. pinnatifida*	Flavanone 2	255
Sc. condensata	Isoflavones 6, 9, 11	237, 283, 589, 253	*Sc. condensata*	-	-
	Flavones			Flavonols	
Sc. farsistanica	Flavones 1, 2, 3	267, 283, 283	*Sc. farsistanica*	-	-
Sc. tomentosa	Flavones 1, 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13	267, 283, 283, 283, 445, 415, 253, 283, 269, 445, 331, 417	*Sc. tomentosa*	-	-
Sc. nepetifolia	Flavones 5, 9, 12, 14, 15, 16	445, 269, 269, 431, 253, 329	*Sc. nepetifolia*	-	-
Sc. patonii	Flavones 5, 8, 9, 16, 17	445, 283, 269, 253, 341	*Sc. patonii*	Flavonol 1	303
Sc.multicaulis	Flavone 16	253	*Sc.multicaulis*	-	-

Table 5. Ctd

Species	Flavonoid class	Ms1; m/z $[M-H]^{-/+}$	Species	Flavonoid class	Ms1; m/z $[M-H]^{-/+}$
Sc. pinnatifida	Flavones 18, 19, 20, 21, 22, 23, 24, 25, 26	445, 445, 461, 287, 267, 417, 331, 475, 377	*Sc. pinnatifida*	-	
	Chalcones		*Sc. condensata*	-	-
Sc. farsistanica	-	-			
Sc. tomentosa	Chalcone 1	207			
Sc. nepetifolia	Chalcone 1	207			
Sc. patonii	-	-			
Sc.multicaulis	-	-			
Sc. pinnatifida	-	-			
Sc. condensata	-	-			

Table 6. The shift reagents with UV-absorption in each flavonoid class

Flavonoid class/shift reagent	$AlCl_3$	$AlCl_3$/HCl	NaOAc	NaOAc/H_3BO_3	λ (nm)
Isoflavone 1	1	2	27	5	320, 319, 318, 347, 325
Isoflavone 2	27	55	4	5	325, 382, 380, 321, 320
Isoflavone 3	40	13	20	12	323, 363, 336, 343, 335
Isoflavone 4	1	1	7	2	326, 325, 325, 366, 328
Isoflavone 5	-	1	2	4	317, 317, 316, 315, 321
Isoflavone 6	-	-	6	4	305, 305, 305, 336, 301
Isoflavone 7	4	2	12	2	315, 367, 367, 327, 317
Isoflavone 8	1	-	3	1	301, 300, 301, 304, 302
Isoflavone 9	10	10	23	8	311, 301, 301, 334, 303
Isoflavone 10	10	12	2	2	262, 272, 274, 264, 264
Isoflavone 11	3	1	7	-	303, 300, 302, 330, 303
Flavone 1	-	68	21	2	323, 323, 391, 344, 325
Flavone 2	16	8	55	-	320, 336, 328, 375, 320
Flavone 3	17	11	31	4	327, 382, 379, 358, 331
Flavone 4	7	-	9	-	323, 330, -, 332, -
Flavone 5	28	23	79	3	315, 343, 338, 394, 318
Flavone 6	3	2	8	4	328, 384, 398, 370, 332
Flavone 7	1	-	8	1	383, 328, 396, 369, 329
Flavone 8	-	-	25	-	315, -, -, 395, 315
Flavone 9	30	22	1	3	329, 359, 351, 328, 332
Flavone 10	23	-	12	5	350, 327, -, 362, 355
Flavone 11	-	-	-	-	315, -, -, 315, 315
Flavone 12	52	23	-	10	323, 375, 346, -, 333
Flavone 13	-	-	1	-	310, -, -, 311, -
Flavone 14	23	-	-	-	306, 329, -, 306, -
Flavone 15	-	29	11	4	331, 331, 331, 320, 327
Flavone 16	-	2	1	2	317, 317, 319, 318, 319
Flavone 17	-	1	1	1	325, 352, 324, 324, 324
Flavone 18	24	-	-	-	309, 333, -, -, -
Flavone 19	6	15	20	-	310, 304, 330, 330, -
Flavone 20	22	18	13	2	336, 398, 398, 380, 338
Flavone 21	-	-	40	-	336, -, -, 376, -
Flavone 22	3	1	7	-	311, -, -, -, -
Flavone 23	-	-	-	-	310, 310, 310, -, -
Flavone 24	-	-	-	-	315, -, -, 315, 315
Flavone 25	15	-	30	-	310, 325, 370, 340, 310
Flavone 26	25	22	5	6	303, 380, 380, 308, 309
Flavone 27	-	36	35	-	349, -, 385, 384, -
Flavone 28	17	13	46	2	313, 380, 381, 359, 315
Flavanone 1	14	15	1	9	324, 378, 373, 325, 333
Flavanone 2	1	1	15	-	312, 311, 311, 335, 312
Flavanone 3	14	15	1	4	326, 375, 371, 323, 322
Flavanone 4	1	1	25	4	308, 345, 307, 333, 312
Flavonol 1	48	15	-	10	327, 375, 375, 327, 337
Chalcone 1	-	-	16	2	318, 318, 318, 375, 320

Discussion

Based on the literature, there were a few morphological variation reports in *Scutellaria* species. *Ozdemir* and *Altan* (2005) [10], *Ezer* and *Renda* (2012) [12] and *Zhao* et al. (2017) [9] reported capitate glandular hair with head, stalked cell, and eglandular

hairs in petiole, leaf, calyx, and corolla of *Sc. wuana* C. L. Xiang & F. Zhao, *Sc. mairei* H. Lev., *Sc. orientalis* and *Sc. diffusa* Benth. The glandular and eglandular multicellular trichomes were observed in studied *Scutellaria* species. Moreover, hirsute, lanate and pubescence trichomes were found in some species [9, 10]. In our research, *Sc. farsistanica*, *Sc. tomentosa*, *Sc. nepetifolia*, *Sc. pinnatifida*, *Sc. patonii*, *Sc. multicaulis*, and *Sc. condensata* confirm previous results [7, 9]. It is known that the trichome variations were observed among different subspecies of *Sc. orientalis* and varieties of *Sc. cypria* Rech. f [10, 11]. The type of glandular hair in various organs such as leaf, stem, petiole, bract, pedicel, calyx, and corolla with various numbers of base cells and stalk cells was reported by *Ozdemir* and *Altan* 2005; whose results are in line with those of us. These features are valuable in taxonomical aims of this genus [10]. The presence of pilose and tomentose trichomes in petiole and leaves [12] is in accordance with our results, especially in leaves, stem, petiole, inflorescence, calyx, bract and corolla. A high variation of corolla length in different locations was approved in *Scutellaria tomentosa*, which ranged from 15-35 mm [3]. Our results were consistent with previous results. *Safikhani* et al. (2017) also reported new species and varieties in *Sc. multicaulis* using different trichomes in leaf, stem, and inflorescence axis [6]. In this research, more variations were observed in *Sc. multicaulis* including the length of stem, petiole, length and width of leaf, width of calyx, length of corolla lips and bract, width of bract, form of leaf apex, leaf base, and indumentum of calyx, corolla tube, corolla lips, bract, and petiole. Furthermore, these variations were identified in *Sc. tomentosa* accessions comprising of the length of stem, leaf, petiole, inflorescence, length and width of bract, length of corolla tube, lower lip of corolla and filament, leaf form, indumentum of leaf and stem, petiole, bract, inflorescence axis, calyx, corolla tube and corolla lips.

In our research, the highest morphological variations were observed in qualitative and quantitative characters such as indumentum of leaf, stem, petiole, inflorescence axis, bract, calyx and corolla, length of stem, petiole, leaf, and width of the leaf, length of inflorescence axis, bract and corolla lips. The different types of trichomes such as strigose (*Sc. farsistanica*, *Sc. nepetifolia* and *Sc. multicaulis*), lanate (*Sc. nepetifolia* and *Sc. multicaulis*), tuberculate (*Sc. farsistanica*, *Sc. nepetifolia*, *Sc. multicauilis*, *Sc. pinnatifida* and *Sc. patonii*), and pannous (*Sc. tomentosa* and *Sc. farsistanica*) were observed. Moreover, different features such as the presence of strigose at the stem, lower surface of leaf and leaf apex, pilose at the lower surface of the leaf, and the upper surface of corolla lip, and lanate at inflorescence axis discriminated *Sc. multicaulis* and *Sc. patonii*. Besides, there was a few relation between *Sc. patonii* and *Sc. multicaulis*, which is not consistent with the results of *Safikhani* et al. (2017) [6].

Based on palynological studies, the pollen characters such as polar axis, colpus membrane with an operculum, equatorial axis, and length of culpi were different in *Sc. tomentosa* and *Sc. farsistanica*. These two species were similar in pollen shape, ornamental, lumen shape and muri, and mesocolpium width [8, 13]. It is of note that in our research there were similar morphological characters between two species including the width of the calyx, leaf form, leaf margin, leaf base, bract apex, indumentum of leaf, stem, calyx, and corolla lips. Using morphological characters, the possible relations between *Sc. tomentosa* and *Sc. farsistanica* were also in accorded with *Jamzad* and *Hasani-Nejad* (2014) [13]. However, both species were definitely separated. Also, it is noteworthy that the taxonomic position of other *Scutellaria* species is based on previous results [13]. Polar and equatorial axis, length of culpi, and muri width were different in *Sc. nepetifolia* and *Sc. multicaulis*. In another case, both species were similar in shape of pollen and lumen, and ornamental features [8]. The previous results confirmed the different morphological features of both studied species. Lumen shape and ornamental pollen were also similar in *Sc. pinnatifida* and *Sc. multicaulis* [8]. In our research, *Sc. pinnatifida* was different from *Sc. multicaulis*, which is not based on previous researches [8].

The variations of nutlet size were observed in some of the members of *Sc.* sect. *Lupulinaria* [7]. In this research, there were different morphological characters in those members, particularly in *Sc. multicaulis*. Based on the ornamentation of nutlet, there is no difference between the members of *Sc.* sect. *Lupulinaria* [7]. This relation was also identified in our morphological results.

The pollen features such as bireticulate-perforate with primary reticulum and regular muri were reported in *Sc.* sect. *Scutellaria*; *Sc. condensata* subsp. *pycnotricha*. Micro reticulate with curved muri was observed in *Sc.* sect. *Lupulinaria*. Lumina is rounded or angular and does not show a uniform perforation. In the case of cluster analysis using morphological data, the members of *Sc.* sect. *Scutellaria* were separated from *Sc.* sect. *Lupulinaria*. These documents were in accordance with *Rechinger* (1982) [3] and *Paton* (1989) [23] classifications. *Paton* (1990a) clarified the *Scutellaria* genus with the morphology of calyx, nutlet, and adaptive mechanisms [1]. The inflorescence is the main characters for systematic treatments in this genus.

Cluster analysis using flavonoid data is in accordance

with previous classification [3, 7, 8, 23]. The members of sect. *Lupulinaria*; sub-sect. *Lupulinaria* showed high variations in flavonoid profiles, which can be discriminated in two distinct groups. Consequently, flavonoid information was an appropriate marker to display the taxonomic relations at infra-specific levels. It is recognized that *Sc. patonii* with accession no. 20 was different from other *Sc. patonii* accessions. Moreover, there is a high variation in its accessions, which is related to the type of flavonoids. *Sc. patonii* with accession no. 24 shows a correlation with *Sc. multicaulis* with accession no. 25, which is not consistent with results of *Safikhani* et al. (2017) [6].

There was no report of chemotaxonomic context in previous investigations. Therefore, the flavonoid results were discussed with pollen and nutlet information. In this connection, *Jamzad* and *Hasani-Nejad* (2014) reported the variation of pollen type, shape of pollen and lumina, and ornamental exine in *Sc.* sub-genus *Scutellara*; sect. *Scutellaria* [13]. In this research, the variation of *Sc. condensata* was observed in cluster analysis with flavonoid data. Morphological and flavonoid characters appear to have stronger relationships and discriminations. *Scutellaria condensata* is definitely separated from the members of *Sc.* sub-sect. *Lupulinaria*. Its ornamental exine, lamina shape, pollen type, and ornamental nutlet in dorsal view show dissimilarity with other species [7, 8, 13]. The pollen exine in *Sc.* sub-genus *Apeltanthus* and sub-genus *Scutellaria* is of high importance for infra-generic classification. *Jamzad* and *Hasani-Nejad* (2014) stated that the other pollen characters of *Sc.* sub-genus *Scutellaria* were similar with sub-genus *Apeltanthus* [13]. The chemotaxonomic position of *Sc. condensata* also confirmed the presence of intermediate features between both sections *Scutellaria* and *Lupulinaria*. The presence of intermediate pollen features [13] confirmed our suggestion. Smaller groups may be designated within pollen type of both sections, which needs further studies. Using morphological and flavonoid profiles, the presence of different groups in *Sc.* sect. *Lupulinaria*; sub-sect. *Lupulinaria* was in agreement with previous studies [13].

Based on the chemotaxonomic point of view, there were relations between *Sc. nepetifolia* (accessions no. 13 and 4) and *Sc. multicaulis* (accessions no. 28 and 31). Based on previous works, the ratio of polar/equatorial, pollen shape, apocolpiun index and muri width of both species display imbricate features, which are in line with our flavonoid results [8, 13].

It is recognized that *Sc. farsistanica* and *Sc. tomentosa* display relations between their accessions, which are supported by *Jamzad* and *Hasani-Nejad* (2014) [13] and *Hasani-Nejad* et al. (2009) [7] using pollen characters such as mesocolpium and muri width, and ornamental nutlet in dorsal view. They varied with pollen shape, polar/equatorial axis, apocolpium index, and colpus length; these changes were also clearly observed in our flavonoid results. *Scutellaria pinnatifida* was separated from other species from the *Sc.* sub-sect. *Lupulinaria*. Based on previous results, this species was differed using polar/equatorial axis, pollen shape, apocolpium index and mesocolpium width [8, 13].

A total of five flavonoid classes with 318 color spots were recognized for *Scutellaria* species. The highest proportion was observed in flavones and the lowest was found in chalcone. *Malikov* and *Yuldashev* (2002) approved the flavones, flavanones, flavonols, chalcones and isoflavones in *Scutellaria* genus [24]. The results of this research were highly consistent with previous results [24]. Moreover, there were different hydroxylation of A and B-ring in flavonoid classes, which are consistent with previous reports [24]. High degree of oxidation leads to absorbing longer wavelength. The spectra of band II were influenced by the degree of oxidation of A-ring [24], which is based on the flavone derivatives in this research. The shifts 3-10, 5-15, and 12-17 nm reflect the 4'-OH, 5-OH, and 3-OH, respectively [24], which are in accord with the present research. It has been determined that there is a correlation between the type of color spots in flavonoids and altitude of each habitat. This correlation was identified in the accessions of each species representing the adaptation forces at different altitudes. Moreover, the flavonoid classes are clearly different in *Sc. tomentosa*, *Sc. nepetifolia*, *Sc. multicaulis* and *Sc. patonii*. The altitudinal variation of flavonoid was also provided in previous researches [25].

The flavone compounds such as acacetin, luteolin, apigenin, baicalin, baicalein derivatives [15, 16], chrysin derivatives, wogonin derivatives, methoxyflavone derivatives, dimethoxyflavone, trimethoxyflavone, tetramethoxyflavone, hispiduloside, oroxylin A, oroxylin A 7-*O*-glucoside, tetrahydroxyflavone derivatives, dihydroxyflavone derivatives [4, 24], different flavonoid *O*-glycosides [17], ovatin [24], and salvigenin [18] were reported in previous results in different *Scutellaria* species namely *Sc. baicalensis*, *Sc. rubicunda* Hornem. , *Sc. albida*, *Sc. alpina*, *Sc. barbata* L., *Sc. altissima*, *Sc. woronowii* Juz., and *Sc. ramosissima* Papov., root of *Sc. pinnatifida* and *Sc. incana*. In the case of UV spectra/MeOH of this research, flavone 2 (320 nm), flavone 3 (327 nm), flavone 5 (315 nm), flavone 7 (328 nm), flavone 8 (315 nm), flavone 9 (329 nm), flavone 10 (350 nm),

flavone11 (315 nm), flavone 12 (323 nm), flavone 13 (310 nm), flavone 14 (306 nm), flavone 15 (331 nm), flavone 17 (325 nm), flavone 18 (309 nm), flavone 19 (310 nm), flavone 20 (336 nm), flavone 21 (336 nm), flavone 22 (311 nm), flavone 23 (310 nm), flavone 24 (315 nm), flavone 25 (310 nm), flavone 26 (303 nm), flavone 27 (349 nm), and flavone 28 (313) were definitely in accordance with previous investigations. Recently, Liquiritigenin was reported by *Jiang* (2015) in *Scutellarai baicalensis*. It is noted that flavanone 2 (312 nm) was consistent with *Jiang* (2015)' results [26].

Other flavonoid compounds such as flavonol glucoside, flavone rhamnoglucoside [27], vitexin derivatives, dihydroxyisoflavone derivatives, formononetin derivatives [27], genistein derivatives, daidzein, eriodictyol, pomiferin, naringenin [21], dihydrorobinetin, taxifolin, and chalcone derivatives [28] were reported in the other genera of Lamiaceae family; *Salvia* L. and *Phlomis* L. species. Based on UV-absorption/MeOH of the present research, we identified flavanone 1 (324 nm), flavanone 2 (274 nm), flavanone 3 (326 nm), flavanone 4 (308 nm), flavone 3 (325 nm), flavone 4 (328 nm), flavone 6 (328 nm), isoflavone 7 (315 nm), isoflavone 8 (301 nm), isoflavone 9 (311 nm), isoflavone 10 (262 nm), isoflavone 11 (303 nm), flavonol 1 (327 nm), and chalcone 1 (318 nm), which were in agreement with the results of previous works. Moreover, nine flavonoid compounds including flavones 1, 4, 16, and isoflavones 1, 2, 3, 4, 5, and 6 were first reported for *Scutellaria* species.

Conclusion

The effectiveness of flavonoids for systematic purposes has been documented by many studies. The morphological characters and flavonoid profiles were introduced as appropriate markers in the taxonomy of *Scutellaria* species. Moreover, flavonoid profiles displayed infra-specific relations in this genus. However, it deserves supplementary consideration to determine the types of flavonoid compounds of *Scutellaria* species.

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