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CHARACTERIZATION OF FLAVONOID COMPONENTS IN *SCUTELLARIA* L. SPECIES (LAMIACEAE) USING FINGERPRINTING ANALYSIS

FARZANEH JAFARI DEHKORDI[1], NAVAZ KHARAZIAN[1]*, ZAHRA LORIGOOINI[2]

[1]*Department of Botany, Faculty of Sciences, Central Laboratory, Shahrekord University, Shahrekord, Iran*

[2]*Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran*

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The genus *Scutellaria* L., Lamiaceae family, Scutellarioideae sub-family is known as the most important medicinal plant in the world. This research aimed to investigate the flavonoid content of seven *Scutellaria* species from the center, southwest, and west of Iran. Via thin layer and column chromatography, the flavonoid was extracted from leaves and then purified. To screen the flavonoid compositions, a chromatographic method was applied by liquid chromatography mass spectrometry on a triple quadrupole mass spectrometer (LC/TQMS/MS). Fingerprint analysis was implemented so as to characterize a total of 73 chemical compounds, from which 71 compounds were flavonoids belonging to different classes. Flavone with 37 derivatives possessed major values. The most abundant flavonoid compounds were observed in *S. multicaulis* (23 compounds) and *S. patonii* (22 compounds). Flavonoid composition, including apigenin, kaempferol, quercetin, and hydroxyl-flavones represented remarkable derivatives. A total of 45 flavonoids, one tannin and one anthraquinone compound, were observed to be primarily separated and identified for *Scutellaria* species. Moreover, six categorized chemical groups were identified in this genus and proposed as chemical barcodes. The specific chemical groups strongly provided the boundaries of *Scutellaria* species, the pharmacological value enhancement, breeding programs, and comprehensive documents of the species. According to the results, LC/TQMS/MS was proven a dominant method regarding genus *Scutellaria*.

Keywords: chemical groups, flavone, liquid chromatography, *Scutellaria multicaulis*, Scutellarioideae

INTRODUCTION

Lamiaceae is a flowering plant, including 236 genera and 6900–7200 species with extensive distribution. Its members are mainly annual, perennial, herbaceous, or shrub plants distributed in temperate regions (Taamalli et al., 2015). This family is classified as medicinal plants containing basic sources of chemical compounds (Kozlowska et al., 2015).

Belonging to Lamiaceae family and Scutellarioideae (Dumort.) Caruel sub-family, the genus *Scutellaria* L. comprises around 425 species throughout the world (Paton, 1990a). Its species are distributed in the northern hemisphere, South Africa, north of central Asia, deserts of the North Pole, and temperate mountains of southern continents (Paton, 1990a; Minareci and Pekonur, 2017). *Scutellaria* is further known to have 22 species in Iran, out of which 10 species are endemic (Rechinger, 1982; Jamzad, 2012). It grows as a perennial herbaceous, erect shrub, suffrutescent. and forms cushion and cliff dwellings (Paton, 1990a). The importance of this genus is attributed to its high medicinal properties and different chemical compounds (Lin et al., 2013). *Scutellaria* species are globally utilized to treat HIV-infection, inflammation, fever, hepatitis, hypertension, pneu-

* Corresponding author, e-mail: nkharazian@gmail.com

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monia, dysentery, intestinal barrier function, pyogenic infection, and high cholesterol levels. They also possess medicinal properties, including anti-allergic, anti-cancer, anti-bacterial, anti-viral, anti-thrombotic, anti-spasmodic, anti-SARS, anti-corona-virus, anti-fibrotic activity in the liver, antitumor, antioxidant, anti-anxiety, and anticonvulsant effects (Tang et al., 2014; Madani Mousavi et al., 2015; Zhao et al., 2016).

According to the previously published works, there are different reports on chemical compounds of *Scutellaria* species. Via HPLC, UPLC, GC and GC-MS methods, essential oils (such as monoterpenes, diterpenes and sesquiterpenes) and phenylethanoids (such as verbascosides) were extracted from *S. orientalis* subsp. *virens* (Boiss. Kotschy) J.R.Edm, *S. barbata* D. Don. (Delnavazi et al., 2014; Wang et al., 2018a,b), *S. pinnatifida* A. Ham (Ghanadi and Mehregan, 2003), *S. alpina* L. (Grzegorczyk-Karolak et al., 2016), *S. altissima* L. (Georgieva et al., 2019), and *S. laterifolia* L. (Kawka et al., 2017). Furthermore, flavonoid compounds were identified in different *Scutellaria* species, e.g., baicalin, laterifolin, dihydrobaicalin, and baicalein (Gafner et al., 2003) in *S. lateriflora*, wogonin in *S. baicalensis* Georgi (Gao et al., 2008), isorhamnetin derivatives, galangin, norwogonin, and skulcapflavone II in *S. baicalensis*, *S. immaculata* Nevski ex Juz., and *S. ramosissima* Popov (Mamadalieva et al., 2011; Luo et al., 2012), tectoridin, scutellarin, liquiritigenin and isoliquiritigenin, and oroxylin A in *S. baicalensis* (Jiang, 2015), patuletin, pinobanksin, chrysin derivatives, methoxyflavone and hydroxyflavone derivatives in *S. baicalensis* and *S. barbata* (Olennikov et al., 2010; Wang et al., 2011; Lin et al., 2013; Tang et al., 2014), flavonoid-glycosides, and flavonoid aglycones in *S. incana* L. (Nurul Islam et al., 2013) using HPLC, HPLC-ESI-MS/MS, LC-MS/MS, LC-UV-ESI-Q/TOF/MS, 1H and 13C NMR, MS, and UV techniques. Different flavonoid classes were mainly reported including flavones, flavanones, flavanonols, flavonols, chalcones, isoflavones, biflavonoids, lignoflavonoids, and lignan glycosides in various *Scutellaria* species using UV-spect, LC-MS/MS, PMR, FAB-MS, and EI/MS (Malikov and Yuldashev, 2002; Jafari Dehkordi and Kharazian, 2019).

Over the recent years, different chromatography techniques have been employed, among which liquid chromatography mass spectrometry (LC-MS/MS) has been one of the most efficient, accurate and applicable methods in phytochemical analyses (Hossain et al., 2010). It applies to a number of organic and inorganic, large, polar, ionic, non-volatile compounds, measures low molecular weight, and identifies metabolites in pharmacokinetic, clinical, and biological research (Anderson and Markham, 2006; Hossain et al., 2010; Haneef et al., 2013).

The abovementioned technique is also a potent and precise analytical method for identifying different compounds such as phenol and flavonoids. To the best of our knowledge, there are no reports regarding the chemical compounds of Iranian *Scutellaria* species via liquid chromatography mass spectrometry (LC-MS/MS). In particular, it would be of interest to detect chemical profiles. Consequently, the current research was conducted to fully characterize chemical compounds such as flavonoids in seven *Scutellaria* species using LC-MS/MS, to reveal specific flavonoid compounds and different chemical groups in this genus, and to apply the compounds in a chemotaxonomic approach.

MATERIAL AND METHODS

STUDY OF SPECIES

Seven *Scutellaria* species including 22 accessions were collected from the west, northwest, center and south-west of Iran during 2016–2017 (Table 1). The collected specimens were identified using Flora Iranica (Rechinger, 1982) and Flora of Iran (Jamzad, 2012). The authenticity of species was verified by Dr. N. Kharazian, Department of Botany, Shahrekord University. All specimens were deposited in the Herbarium of Shahrekord University (HSU).

CHROMATOGRAPHY SECTION

The crude extract of air-dried leaves (10.5 g) from seven *Scutellaria* species was separated using 85% MeOH at 60°C. The extract was concentrated via a rotary evaporator at 70°C to remove the total solvent. Total flavonoids were separated through the use of n-Butanol and sequentially analyzed by silica gel 60F 254 (15 mg) and thin layer chromatography (TLC; 5 μm, 20 × 20 cm). The chromatogram was transferred to MeOH-H_2O (70:30), $CHCl_3$-MeOH (75:25), and BuOH-CH_3COOH-H_2O (16:28:56) as a solvent system (Olennikov et al., 2010; Hawryl et al., 2016). Spots were detected

TABLE 1. The locality of *Scutellaria* species, voucher numbers, altitude, and geographical position in different natural habitats of Iran

Species/no. accession	Locality	Altitude (m)	Geographical position
	Chaharmahal va Bakhtiari		
S. farsistanica Rech. f. 1	Dorahan, 45 km Lordegan	1683	51°11'E,31°37'N
S. farsistanica 5	Boroujen, Hamz-e Ali Emamzadeh	2250	50°59'E, 32° 5'N
	Isfahan		
S. farsistanica 7	Bardekan, Gharghach	2130	51°37'E, 31°27'N
S. farsistanica 10	Gharghach village	2200	51°37'E, 31°27'N
S. farsistanica 11	Semirom- Vanak, Dalan-kouh	1897	51°19'E, 31°31'N
	Chaharmahal va Bakhtiari		
S. tomentosa Betrol. 3	Shahrekord- Farokhshahr, Tang-e Sayad	2180	50°59'E, 32°11'N
S. tomentosa 4	Sahrekord- Tang-e Sayad	2230	50°59'E, 32°11'N
	Isfahan		
S. tomentosa 6	Hajiabad, Bardekan	2130	51°36'E, 31°32'N
S. nepetifolia Benth. 13	Khansar- Damaneh	2120	50°29'E, 33°1'N
S. nepetifolia 17	Analoujeh village- Dalankouh	2200	51°19'E, 31°31'N
S. nepetifolia 18	Dalankouh	2900	51°19'E, 31°31'N
	Chaharmahal va Bakhtiari		
S. nepetifolia 19	Samsami- 65 km Bazoft, Safaabad	2082	50°24'E, 32°8'N
	Chaharmahal va Bakhtiari		
S. patonii 20	Bazoft- Siyavashabad, Chendar	2033	50°18'E, 32°10'N
S. patonii 21	Samsami- Abbarik, Marboreh	2093	50°16'E, 32°9'N
S.. patonii 23	Kouhrang	2042	50°7'E, 32°27'N
	Chaharmahal va Bakhtiari		
S. multicaulis Boiss. 25	Talab-e Gandoman, Nasirabad	2038	51°5'E, 31°48'N
S. multicaulis 28	Talab-e Gandoma, Chirou	1918	51°5'E, 31°48'N
S. multicaulis 30	Lordegan- Glougerd	1908	50°51'E, 31°55'N
S. multicaulis 33	Jouneghan- Tang-e Darkesh	2030	50°40'E, 32°5'N
	Kurdestan		
S. pinnatifida A. Ham. 34	Marivan- Oraman	1450	46°15'E, 35°15'N
S. condensata Rech. f. 36	Marivan- Darvian	1850	46°27'E, 35°56'N
S. condensata 38	Marivan	1700	46°10'E, 35°31'N

using natural product reagents (H_2SO_4 5% in MeOH; and diphenyl boric acid 2-aminoethyl ester; Sigma Chemical; in methanol, followed by 5% solution of polyethylene glycol 400 in ethanol) under ultraviolet-366 nm (Rahman, 2005). To accomplish the purification process of flavonoids, column chromatography (65 × 3 cm) followed by Sephadex LH20 Sigma-Aldrich eluting with MeOH in H_2O (20%–100% MeOH) was done and flavonoids were extracted in different fractions. In this process, a total of 12 aqueous methanol fractions were obtained (50 ml each).

LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY (LC-MS/MS) SECTION

The fractions were evaluated via liquid chromatography-tandem mass spectrometry (LC-MS/MS) on a triple quadrupole mass spectrometer (TQMS). LC-MS/MS was accomplished on an Agilent Zorbax SB-C18 column (15 cm, 3.5 μm) and its temperature was maintained at 25°C. LC-MS grade methanol, acetonitrile, MS grade acetic acid (98%), and ultra-pure water were utilized for the mobile phase. This phase was elected at A = 0.1% (v/v) acetic acid in water and B = 0.1% acetic acid in acetonitrile (0.1% acetic acid in water/acetonitrile 0.1% acetic acid). The gradient elution was verified in a timeframe of 0–75 min, B% of 10–100, and flow rate of 0.3 ml/min. The injection volume was 5 μL (Falcao et al., 2012; Taamalli et al., 2015).

MASS SPECTROMETRY (MS) CONDITION

The MS1 was detected at a negative mode ESI (Electrospray Ionization) basis by using the Agilent G6410 triple quadrupole mass spectrometer through the acquisition method and full-scanning over an m/z range of 254–952 AMU. MS data were performed in the total ion chromatogram (TIC) and the extracted ion chromatogram (EIC). Zero grade air was used as the nebulizer gas (15 psi) and turbo gas for solvent drying (300°C, gas flow: 6 ml/min). The ion spray voltage was arranged as capillary voltage 4000 V, dwell time 500 msec and fragmentor voltage 135 V. The extractions were filtered over a 0.2 μm filter for LC-MS/MS analysis.

In the MS/MS spectrum, the most influential ions obtained from MS1 were designated for dissociation. The MS/MS detection was accomplished at a negative mode ESI. The nebulizer gas was applied at 15 psi, 300°C drying gas with a flow rate of 6 ml/min, ion spray capillary voltage of 4000 V, and product ion method (collision energies of 10 and 25 eV using nitrogen as the collision gas). The deprotonated molecular ions were detected using collision induced dissociation (CID) in the MS/MS.

Commercial standards were not available for all the flavonoid compounds. We used flavonoid compound standards from SIGMA-Aldrich Chemical Co., including apigenin, quercetin, and kaempferol with 98% purity. Stock solution (1mg/mL) was prepared in methanol.

STATISTICAL ANALYSIS

In order to determine the chemical groups in each *Scutellaria* species, we applied multivariate analysis, comprised of clustering with Neighbor-Joining method, Dice similarity index, and PAST 3.18. The significance of the measured values was estimated via T-test and SPSS V. 20.

RESULTS

The results of analytical profile spectra showed that there were different flavonoid compounds in each *Scutellaria* species. A total of 73 chemical compounds were identified, from which 71 compounds were flavonoids. We further specified different flavonoid classes such as flavone (37), flavonol (11), isoflavone (9), flavanone (6), flavan (4), anthocyanin (2), biflavonoid (1), and chalcone (1). The most abundant flavonoid belonged to *S. multicaulis* (23 compounds) and *S. patonii* (22 compounds). A low number of flavonoids were present in *S. tomentosa* (11 compounds), *S. condensata* (11 compounds), *S. farsistanica* (4 compounds), *S. pinnatifida* (3 compounds) and *S. nepetifolia* (2 compounds). The molecular mass of each compound was determined with EIC (Extracted Ion Chromatogram) (Fig. 1a). The ESI negative mode was considered with multilevel collision energy (10–25 eV). The proposed chemical compounds were accurately investigated or tentatively assigned with the number of product ions vs. mass to charge (m/z) in MS/MS spectra (Fig. 1b) and 12 fractions (Table 2). The mass measurement of each fraction was approved by the reference standards (massbank.eu; mona.fiehnlab.ucdavis.edu; metlin.scripps.edu). The m/z value was recorded in the range of 254–624 AMU. Table 2 shows all the information related to MS/MS fragmentation, retention times, high-intensity peaks, and proposed

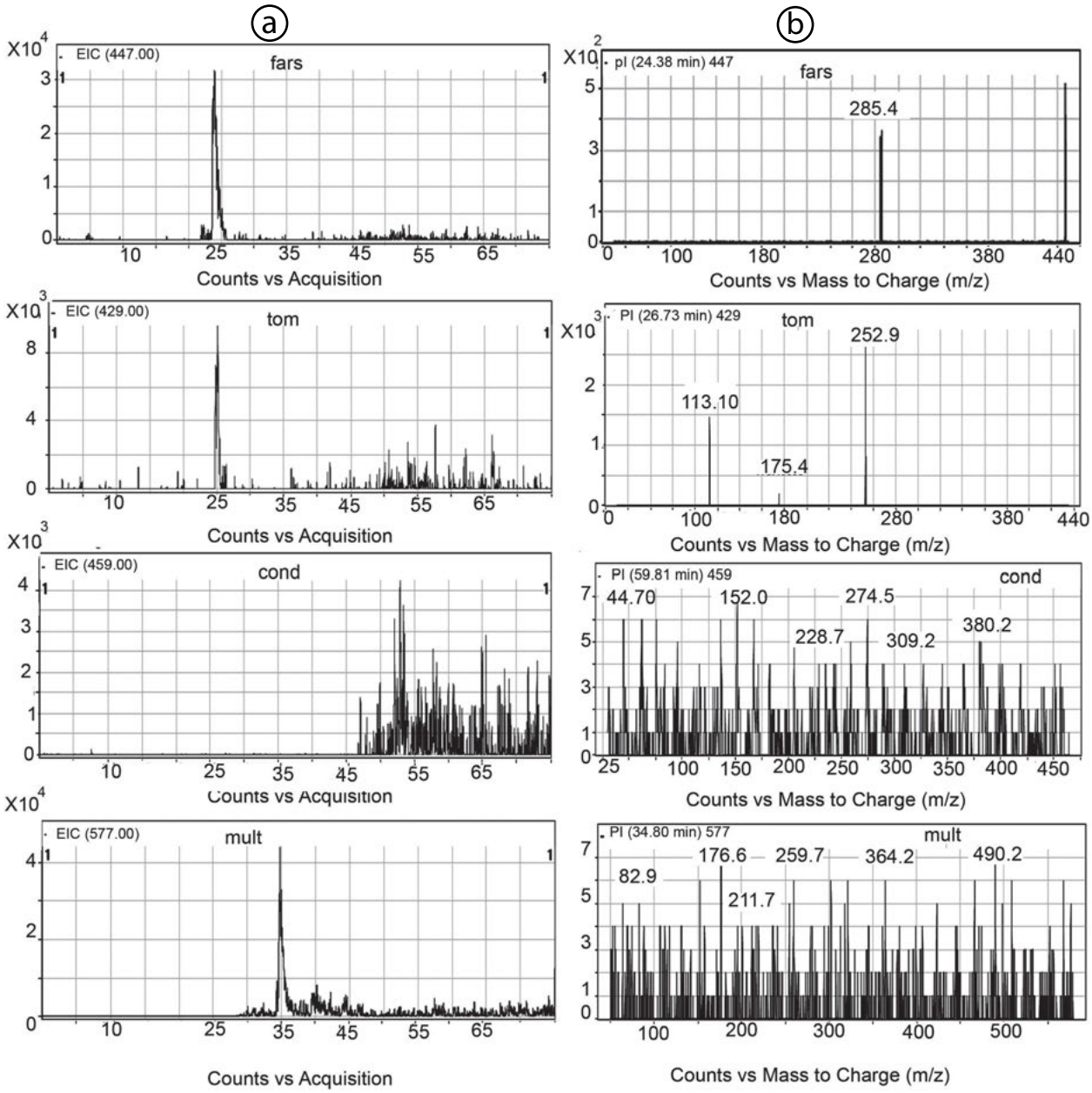

Fig. 1. Representative of (**a**) Extracted Ion Chromatogram (EIC) and (**b**) MS/MS spectra of some flavonoid compounds; m/z 447, 429, 459, and 577. fars – *S. farsistanica*, tom – *S. tomentosa*, cond – *S. condensata*, mult – *S. multicaulis*.

identities. A total of 45 flavonoids, one tannin and one anthraquinone compound were first detected to be separated and identified for *Scutellaria* species.

Statistical significance was estimated between mass to charge and abundance of ions for each species. The results showed that the statistical significance ranged between of 0.001 and 0.043 ($p < 0.05$).

In this research, there were specific identified compounds in *Scutellaria* species. The flavonoid compounds, including hydroxy-dimethoxyflavone, taxifolin, luteolin-dimethyl ether, and vicenin-2 were similar in certain species, namely *S. nepetifolia, S. tomentosa, S. pinnatifida, S. multicaulis, S. patonii,* and *S. condensata*. A total of 68 chemical compounds were recognized as special

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TABLE 2. The identities of chemical compounds in *Scutellaria* species followed by peak number, retention time, molecular weight, [M-H]-, MS/MS, identified compound and classification.

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
S. nepetifolia	1	11.69, 11.70, 11.80	304	303	303.4, 285, 275, 259, 245, 141.3, 139.8, 112.3, 111.9	Dihydroquercetin	Flavonols
	2	42.84, 43.13	298	297	297.4, 296.8, 282, 269, 267, 183.2	3-hydroxy-3',4'-dimethoxyflavone	Flavone
S. tomentosa	3	5.31, 5.32	240	239	118, 165, 151, 151.2, 194.1, 104, 240, 239.9, 211, 156.2, 133, 195, 120, 211, 157	2',4-dihydroxychalcone	Chalcone
	4	12.16, 12.17, 12.30, 12.31	346	345	345, 330, 326.9, 315, 300, 285.4, 270, 268.9, 241.1, 169.1, 163.8, 156, 138.5, 125.3, 117	Catechin tetramethyl ether	Flavan
	5	26.71, 26.72	300	299	299.2, 283.7, 260.4, 259.9, 251.8, 230.5, 200, 188.6, 179, 151, 150.6, 135.1, 104.9	Diosmetin	Flavone
	6	26.73, 26.74	430	429	253.5, 175.4, 113.1	Chrysin-7-*O*-*β*-D-glucuronide	Flavone
	7	33.68, 33.69	306	305	306, 276, 256, 238.4, 216.4, 205.4, 175.2, 151, 134.6, 110.7, 115.8, 112.2, 106.7	Gallocatechin	Flavan
	8	39.01, 42.52, 42.53	592	591	592, 591, 471, 367, 325, 305, 298.9, 297, 296, 283.8, 283, 269.1, 268, 267.9, 240, 211	Acacetin-7-*O*-neohesperidoside	Flavone
	9	42.54, 42.55	298	297	327.5, 327.4, 297.2, 282, 269, 267,185.2, 183	3-hydroxy-3',4'-dimethoxy flavone	Flavone
	10	68.90	460	459	444, 429.2, 307.8, 297, 186.6, 156, 140.3	Wistin	Isoflavone
	11	68.87, 68.88	594	593	576, 447, 430, 308, 285, 286.1, 255	Scutellarein-7-*O*-neohesperidoside	Flavone
	12	36.77, 36.78	658	657	546, 515, 495, 481, 479, 401, 370, 332, 317, 305, 269	Myricetin–*O*-hexoside	Flavone
	13	36.79, 36.80	314	313	313.5, 312.9, 285, 283.2, 298.4, 297.5	Luteolin-7,3'-dimethyl ether	Flavone
S. pinnatifida	14	42.901, 42.909	298	297	297.6, 282, 269, 267, 183	3-hydroxy-3',4'-dimethoxy	Flavone

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
						flavone	
	15	42.91, 42.92	328	327	314, 312, 297, 285, 284.4, 282, 270, 112	Kaempferol-3,7,4'-trimethyl ether	Flavonol
	16	48.66	298	297	285, 282, 269, 267.6, 258, 211.4, 111.3	7', 4'-dimethoxy-3-hydroxyflavone	Flavone
S. condensata	17	15.94, 15.95	578	577	491.4, 470.8, 450, 431, 299, 285, 274.4, 199.9	Kaempferol-3,7-di-*O*-rhamnoside	
	18	15.96, 15.97	300	299	297, 284.5, 269, 257, 255.8, 185.7, 156.1, 139.6, 130, 111.1, 108	Scutellarein-7-methyl ether	Flavone
	19	18.03, 18.04	578	577	576, 432, 413, 371, 293, 269, 268, 312, 151, 75	Apigenin-7-*O*-neohesperidoside	Flavone
	20	18.05, 18.06	300	299	302.5, 300, 285.6, 284, 271, 261.2, 255, 242, 241.6, 238, 215.6, 186.3, 144.9, 139.8, 119.1, 116	Hispidulin	Flavone
	21	20.11	594	593	593, 473, 414.9, 370.9, 365, 325, 294.3, 284, 269	Saponarin	Flavone
	22	22.62, 22.63	594	593	596, 593, 545.2, 472.5, 447, 366.2, 326.3, 285, 260.1, 183.3, 131.3, 116	Kaempferol-3-*O*-neohesperidoside	Flavonol
	23	22.64, 22.65	578	577	578, 577.1, 269.2	Apigenin-7-*O*-rutinoside	Flavone
	24	22.66, 22.67	300	299	283.4, 271, 269, 225.5	5-hydroxy-3',4'-dimethoxyflavanone	Flavanone
	25	30.14, 30.15	298	297	297, 281.8, 276.6, 269, 267, 253, 234.9, 226.3, 214.4, 211, 198.8, 195, 184, 169.5, 168, 155, 147.3, 103.1	3',7-dimethoxy-3-hydroxyflavone	Flavone
	26	36.36, 36.37	314	313	313.5, 298.4, 297.5, 283.2	Luteolin-7,3'-dimethyl ether	Flavone
	27	59.81	460	459	459, 283, 297, 269, 267, 240, 198, 167.3, 165, 156.5, 152, 136.4, 119, 116.7	Wogonin-7-*O*-glucuronide	Flavone
S. farsistanica	28	24.35, 24.36	300	299	300, 284, 271, 255, 242,	Tectorigenin	Isoflavone

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
					230.9, 170.5, 167, 118, 104.9		
	29	24.36, 24.37	448	447	447.3, 285.3, 285. 4, 284.9, 284.5	Kaempferol-3-*O*-glucoside	Flavonol
	30	42.69, 42.70	306	305	307.6, 288.8, 277, 261, 249, 219.1, 216.6, 165.6, 124	Epi-gallocatechin	Flavan
	31	42.72, 42.73	432	431	341, 323, 312, 311, 282.4, 270.1, 269	Vitexin	Flavone
S. patonii	32	28.75	610	609	608, 591, 301, 270.7, 255, 177	Quercetin-3-*O*-rutinoside	Flavonol
	33	35.35	594	593	502.4, 473, 424.5, 365.1, 353, 322	Vicenin-2	Flavone
	34	42.08, 42.09	418	417	417, 285, 283.4, 270, 255.3, 188.8, 158.8, 144.3, 117	Kaempferol-3-*O*-arabinoside	Flavonol
	35	42.10, 42.11	298	297	297.1, 269, 197.1, 183.2	Apigenin-7, 4'-dimethyl ether	Flavone
	36	62.69, 62.70, 63.65	432	431	285, 283, 259, 254.8, 226.7, 211, 195, 186,160.4, 151.4, 132	Kaempferol-3-*O*-rhamnoside	Flavonol
	37	62.72	490	489	474, 461, 459, 313, 297.9	5-hydroxy-8, 2'-dimethoxy-7-*O*-β-glucuronylflavone	Flavone
	38	62.72, 62.73, 63.68, 63,69	418	417	418, 389, 349.8, 334, 298, 254.6, 241, 226, 147.8, 136, 130, 199, 119	Liquiritin	Flavanone
	39	18.140, 18.149	594	593	431, 419, 308, 285, 265, 241, 229, 210, 200, 185, 151, 146.4	Kaempferol-3-*O*-glucoside-2"-*P*-coumaroyl	Flavonol
	40	22.93, 22.95	449	448	447, 286, 283, 182, 137.5, 125, 121, 119	Cyanidin-3-*O*-galactoside	Anthocyanin
	41	22.96, 22.97	610	609	608, 490, 403, 367, 357, 315, 310, 301, 287, 258, 217, 163, 125	Hesperetin-7-*O*-neohesperidoside	Flavanone
S. patonii	42	22.98	430	429	403, 340, 283, 274.6, 260.3,	Calycosin-7-*O*-β-D-rhamnoside	Isoflavone

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
					180, 154, 137.7		
	43	25.52, 25.53, 25.92, 25.93, 26.38, 26.39	432	431	343, 311, 295, 282.4, 270, 269.3, 268, 252.4, 240, 238, 159.6	Isovitexin	Flavone
	44	25.54, 26.37, 26.40	449	448	448.5, 447, 286, 283, 272.3, 271.4, 182, 175, 125.4, 137.5	Cyanidin-3-*O*-glucoside	Anthocyanin
	45	25.55, 25.94, 25.95, 26.41, 26.42	610	609	608, 343, 325.3, 311.9, 301, 290, 287.5, 284.2, 268, 257.8, 216.4, 178.9, 165.2, 138, 126.8, 112	Hesperetin-7-*O*-rutinoside	Flavanone
	46	25.56, 25.57, 25.96, 25.97, 26.42, 26.43	430	429	428, 355, 340.2, 334, 292, 270, 267, 253, 233, 222, 211, 195, 179.4, 163, 134, 112	Formononetin-7-*O*-glucoside	Isoflavone
	47	27.25	432	431	338, 272.1, 269, 240.1, 211, 181.4, 168, 150, 148, 130, 120, 117, 115.9, 105	Apigenin-7-*O*-glucoside	Flavone
	48	27.27, 27.28	610	609	608, 580, 462, 445, 388, 340, 301, 288.3, 284, 272, 216, 187, 158, 107	Quercetin-3-*O*-neohesperidoside	Flavonol
	49	32.70	330	329	327.4, 313.5, 301, 299.6, 285, 272, 243, 228, 216, 204.6, 199, 170, 161	Tricin	Flavone
	50	32.71, 32.72	332	331	316.5, 315, 300, 179, 151	Myricetin-3'-methyl ether	Flavone
	51	39.50, 39.51	256	255	255.3, 220.5, 213, 196, 183.9, 177, 169, 158.5, 150.5, 141.4, 132.2, 123	Pinocembrin	Flavanone
	52	39.52, 39.53	332	331	270, 241.2, 170.8, 211	Galloyle-*O*-glucose	Tannin
	53	39.54, 39.55	432	431	430.9, 361.8, 311.7, 283, 267.3, 253.1, 246, 239, 222, 172, 143, 105	Sophoricoside	Isoflavone
	54	39.55, 39.56	254	253	253, 225, 209.2, 154.9, 143.5	7, 3'-dihydroxyflavone	Flavone
S. multicaulis	55	12.47, 12.48	304	303	303.3, 141.2, 141.1, 140.3, 101.3	Dihydroquercetin	Flavonol

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
	56	30.18	270	269	253.6, 252, 241, 239, 226.3, 225, 214, 212, 195.6, 186.9, 159.6, 148.3, 140, 120	3, 7, 4'-trihydroxyflavone	Flavone
	57	32.41, 32.42, 33.36, 33.37	578	577	577.6, 432, 431, 412, 377, 325, 310, 307, 292, 282.6, 268, 249, 100	Vitexin-2-*O*-rhamnoside	Flavone
	58	32.43, 32.44, 33.38, 33.39	564	563	545.3, 533, 527, 515.6, 505.2, 455, 426, 381, 325.4, 192	Isoschaftoside	Flavone
	59	32.44, 32.45	450	449	450, 318.7, 317, 270.4, 260, 227, 219, 214, 210, 175.9, 164, 143.7	Myricetin-3-arabinoside	Flavone
	60	32.46, 32.47	270	269	269.2, 154.9, 151.1, 121.2, 117.1, 106.9	Apigenin	Flavone
	61	34.84, 34.85, 35.63, 35.64	446	445	430, 429.4, 417, 416, 400, 380, 370, 349, 339.6, 323.1, 320.9, 310.7, 283, 271.1, 240.07, 150, 117.9	7-*O*-β-glucopyranosyl-4'-hydroxy-5-methoxyisoflavone	Isoflavone
	62	34.80, 34.81	578	577	490.2, 446, 431, 400, 326, 302.4, 298.7, 285, 259.7, 254, 229, 215, 200, 151, 108	Procyanidin B1	Biflavan
	63	36.09, 36.10	594	593	577, 536, 502.4, 473, 467, 455, 438, 424.5, 395, 382, 365.1, 353, 322, 311	Vicenin-2	Flavone
	64	36.11, 36.12	446	445	448, 444, 283, 271, 270.4, 269, 268.1, 250.5, 210, 200, 175, 150, 136, 117	Baicalein-7-*O*-glucuronide	Flavone
	65	38.65	446	445	445, 430, 385, 353, 340, 338.1, 325, 310, 298, 273.9, 269, 117	6-*C*-glucopyranosyl-7-*O*-methylapigenin	Flavone
	66	41.08, 41.87, 42.06, 42.07, 42.70, 42.71	564	563	563.6, 425.4, 414.8, 347.4, 283.5, 273.8, 181.3, 176, 171.5, 141, 86.9	Theaflavin	Flavan
S. multicaulis	67	41.88, 41.89, 42.08, 42.72, 42.73	756	755	755, 728, 611, 609, 490, 447, 416, 401, 387, 354, 344.5, 313, 302, 300, 283.7, 226,	Quercetin-3-neohesperidoside-7-rhamnoside	Flavonol

Species	Peak no.	Retention time	MW	[M-H]-	MS/MS	Identified compound	Classification
					242, 199.3, 180, 151, 107		
	68	41.90, 41.91, 42.09, 42.10	626	625	623, 463, 301, 299, 271, 255, 243, 228, 214.3, 202, 192.7, 180, 165, 151, 121	Quercetin-3, 4'-diglucoside	Flavonol
	69	41.91, 41.92, 42.11	622	621	621, 574.3, 500, 563, 557, 538, 458.9, 423, 338, 313, 283, 187, 146.8, 137, 119	Pectolinarin	Flavone
	70	45.54	432	431	255, 175.2	Liquiritigenin -4'-*O*-glucuronide	Flavanone
	71	46.75, 46.76	432	431	431.7, 270, 268, 227, 224, 210, 195.9, 160, 133.5, 131	Genistin	Isoflavone
	72	46.77	672	671	656, 643, 627, 614, 599, 583, 551, 508, 431, 389, 229	3, 5, 7, 8, 3', 4'-hexahydroxy-6-methoxy flavone diglucopyranoside	Flavone
	73	46.80, 46.81	328	327	324, 312, 296.1, 240.4, 195.3, 185, 171.7, 128, 119.1, 102	Isotectorigenin-7-methyl ether	Isoflavone
	74	48.29, 48.30	432	431	431, 314.1, 311, 296.2, 280, 267, 239, 221.7, 196, 173.4, 159.4, 133.9, 123	Genistein-*O*-glucoside	Isoflavone
	75	50.23, 50.24	432	431	270, 269, 227, 224, 197, 150, 124	Apigenin-4'-*O*-hexoside	Flavone
	76	54.58, 54.59	432	431	431, 311, 294, 283.4, 281, 269, 265, 240, 224, 180	Emodin-8-*O*-β-D-glucoside	Anthraquinone
	77	54.61, 54.62	314	313	298, 285, 283, 280, 279.7, 269, 214, 256.4, 182, 171, 155, 145, 132, 121.7, 108.7	3, 7-dihydroxy-3', 4'-dimethoxyflavone	Flavone
	78	54.63, 54.64	328	327	327.6, 312, 299, 297, 282, 193, 161, 149, 108, 106.3	3-hydroxy-3', 4', 5'-trimethoxyflavone	Flavone

characters for each species comprising 20 compounds in *S. multicaulis*, 22 compounds in *S. patonii*, 10 compounds in *S. condensata*, 10 compounds in *S. tomentosa*, 4 compounds in *S. farsistanica*, and 2 compounds in *S. pinnatifida*. These characteristics were assigned to the presence of six chemical groups proposed as chemical barcodes for *Scutellaria* species according to mass range, MS/MS spectra, and cluster analysis (Fig. 2). Compared with other *Scutellaria* species, *S. nepetifolia* showed only two flavonoid compounds which were not properly independent. However, a specific group was detected. Further investigation is required to corroborate the existence of more flavonoid compounds.

DISCUSSION

The identification of all chemical compounds, their detected molecular ions, chemical groups of *Scutellaria* species, and botanical viewpoints are listed and discussed as follows:

FLAVONE DERIVATIVES

A total of 37 flavone compounds were described for *Scutellaria* species.

In full MS spectrum, the molecular ions at m/z 253, 269, 297, 313, 327, 329, 489, and 671 were found in *S. nepetifolia*, *S. pinnatifida*, *S. condensata, S. patonii, S. multicaulis*, and *S. tomentosa* (Table 2). The obtained fragmentation patterns entailed the successive loss of hydroxyl, glucuronyl, and di-glucopyranosyl moieties. Consequently, the flavone compounds were proposed as methoxyl-hydroxylated flavone derivatives and di-glucopyranosides. Compared with the reference standards, these compounds were accurately identified as formerly separated in *S. baicalensis*, Lamiaceae (Malikov and Yuldashev, 2002; Rahman, 2005; Lin et al., 2013; massbank.eu; mona.fiehnlab.ucdavis.edu). According to published data, hydroxyl-methoxylated flavone derivatives were first attributed to *S. baicalensis, S. incana,* and *S. moniliorhiza* Kom. (Nurul Islam et al., 2013; Han et al., 2017; Wang et al., 2018).

In line with the study of Hussain et al. (2010) and Brito et al. (2014), MS/MS spectra of *S. tomentosa* and *S. condensata* at m/z 299 and 313 with the neutral loss of methyl ether were attributed to diosmetin and luteolin-dimethyl ether (Table 2). As shown in previously published works, luteolin-methyl ether was characterized in *Phlomis cashmeriana* Royle ex Benth. (Hussain et al., 2010; mona.fiehnlab.ucdavis.edu). It has further been evidenced that luteolin is a common flavone in different medicinal plants.

One of the best-known flavone compounds and its derivatives were first found in *S. multicaulis, S. condensata, S. patonii,* and *S. farsistanica*. The fragmentation patterns of molecular ions at m/z 269, 297, 431, 455, 563, 577, and 593 showed the different neutral losses of neohesperidoside, *O*-glucosyl or *O*-hexosyl, 6-*C*-glucopyranosyl, methyl ether, rhamnoside, and xylose or arabinose units (Table 2). Subsequently, the identified compounds were attributed to apigenin and its derivatives, namely apigenin-*O*-neohesperidoside, apigenin-*O*-rutinoside, apigenin-*O*-glucoside, apigenin-dimethyl ether, glucopyranosyl-methylapigenin, vitexin and vitexin-*O*-rhamnoside, isovitexin, vicenin-2, and isoschaftoside. These findings are consistent with the approved reports on *S. salvifolia* Benth., *S. hastifolia* L., *S. baicalensis*, and *S. incana* (Nurul Islam et al., 2013; Lin et al., 2013; Xu et al., 2018; Dogan et al., 2019; Bardakci et al., 2019; massbank.eu; mona.fiehnlab.ucdavis.edu). In the ESI-MS spectra of *S. condensata,* a molecular ion at m/z 593 was first identified as saponarin, previously reported in *Salvia officinalis* L., Lamiaceae (Zimmermann et al., 2011; Uritu et al., 2018; mona.fiehnlab.ucdavis.edu). Regarding the obtained results, apigenin derivatives are mostly present as *O*-hexoside in structure.

The MS/MS spectra of negative ionization mode showed the fragment ions at m/z 299 and 593 in *S. tomentosa* and *S. condensata* (Table 2). The presence of neohesperidoside and methyl ether units was primarily attributed to scutellarein-*O*-neohesperidoside and scutellarein-methyl ether in structure. Scutellarein-methyl ether and its *O*-neohesperidoside derivative were previously reported in *S. repens* Buch. Ham ex D. Don., *S. baicalensis,* and *S. przewalskii* Juz. (Atif et al., 2015; metlin.scripps.edu; mona.fiehnlab.ucdavis.edu). Scutellarein compound was commonly attributed to the genus *Scutellaria* (Olennikov et al., 2010).

In the MS/MS spectra of *S. tomentosa, S. patonii, S. condensata,* and *S. multicaulis*, the [M-H]- ions at m/z 429, 445, 449, 459, 591, and 657 revealed different flavone xylosides or hexosides as the neutral loss of the glucuronide residue, neohesperidoside unit, hexose, and xylose or arabinose

moieties (Table 2). According to the previous reports and accurate MS/MS, these compounds were considered to be chrysin-*O*-glucuronide, acacetin-*O*-neohesperidoside, wogonin-*O*-glucuronide, myricetin-*O*-hexoside, myricetin-arabinoside, and baicalein-*O*-glucuronide. Chrysin, wogonin, acacetin, and myricetin compounds were specified in *S. incana*, *S. baicalensis,* and *S. supina* L. (Malikov and Yuladachev, 2002; Olennikov et al., 2010; Luo et al., 2012; Nurul Islam et al., 2013; mona.fiehnlab.ucdavis.edu; massbank.eu). Noteworthy, certain derivatives of myricetin such as myricetin-3-*O*-galactoside were found in *S. baicalensis* (Xu et al., 2018). Consequently, *O*-xyloside and *O*-hexoside flavones were reported for most medicinal plants.

In ESI-MS spectra, a molecular ion at m/z 621 was primarily observed in *S. multicaulis* (Table 2). Compared with the reference standards, this compound was identified as pectolinarin in structure (Zhang et al., 2018; mona.fiehnlab.ucdavis.edu; massbank.eu).

FLAVONOL DERIVATIVES

In this work, the identities of different flavonol derivatives were distinguished as follows:

The seven kaempferol derivatives were assessed in *S. pinnatifida, S. condensata, S. farsistanica,* and *S. patonii*. MS/MS spectra of negative ionization mode showed fragment ions at m/z 327, 417, 431, 447, 577, and 593 (Table 2). The fragmentation patterns revealed successive loss of methyl ether, rhamnosyl and di-rhamnosyl units, neohesperidoside, hexosyl or glucosyl, xylosyl or arabinosyl units, and coumaroyl residue. In agreement with authentic standards (massbank.eu; mona.fiehnlab.ucdavis.edu), among these MS/MS spectra and reference standards, the flavonol compounds were identified as kaempferol-methyl ether, kaempferol-di-*O*-rhamnoside, kaempferol-*O*-rhamnoside, kaempferol-*O*-neohesperidoside, kaempferol-*O*-glucoside, kaempferol-*O*-arabinoside, and kaempferol-*O*-glucoside-2"-*P*-coumaroyl in structure. Some derivatives of kaempferol were previously reported in *Micromeria fruticosa* (L.) Druce and *Salvia officinalis,* Lamiaceae (Azevedo et al., 2010; Xu et al., 2018). According to the published data, kaempferol-3-*O*-glucoside and kaempferol-3-glucuronide were identified in *S. baicalensis* (Xu et al., 2018). Regarding the obtained results, kaempferol derivatives, *O*-hexoside in structure, frequently revealed variability.

Based on MS/MS spectra dissociation, five quercetin derivatives were assessed in *S. patonii, S. nepetifolia,* and *S. multicaulis.* The most influential ions obtained from fragmentation patterns at m/z 303, 609, 625, and 755 corresponded to different neutral losses, including rutinosyl, neohesperidoside, and rhamnosyl moieties and the successive loss of hexoside or glucoside unit (Table 2). The product ions were assumed to be taxifolin or dihydroquercetin, quercetin-*O*-rutinoside, quercetin-*O*-neohesperidoside, quercetin-neohesperidoside-rhamnoside, and quercetin-diglucoside (Chen et al., 2016; massbank.eu; mona.fiehnlab.ucdavis.edu). More derivatives were previously reported as quercetin-di-glucopyranoside and taxifolin-glucoside in *S. baicalensis,* and *Marrubium parviflorum* Fisch. & C.A.Mey., Lamiaceae (Delnavazi et al., 2017; Xu et al., 2018). It is of note that quercetin derivatives in the studied species were mostly present as hexosides.

FLAVAN DERIVATIVES

Four flavan derivatives were identified for *S. tomentosa, S. farsistanica,* and *S. multicaulis*. The MS/MS spectra at m/z 305, 345, and 563 represented the successive loss of methyl ether. This compound was attributed to catechin tetramethyl ether. Compared with the accurate MS/MS data standard and the published reports about *Mentha pulegium* L., Lamiaceae (Hossain et al., 2010), gallocatechin, epi-gallocatechin, and theaflavin were further identified (Table 2). Catechin and gallocatechin were commonly characterized in *Phlomis* L. species, Lamiaceae (Hossain et al., 2010; Taamalli et al., 2015; Aghakahni et al., 2018; mona.fiehnlab.ucdavis.edu).

ISOFLAVONOID DERIVATIVES

The fragment ions at m/z 299, 327, and 459 were observed in *S. tomentosa, S. farsistanica,* and *S. multicaulis* (Table 2). The precursor ions were attributed to the residue of methoxyl and hexosyl or glucosyl units, and the sequential loss of hydroxyl. The recognized isoflavones were considered as wistin, tectorigenin, and isotectorigenin-7-methyl ether in structure as corroborated by the reference standards (mona.fiehnlab.ucdavis.edu; massbank.eu)

Other isoflavonoid derivatives were first identified in *S. patonii* and *S. multicaulis*. Fragment ions at m/z 429, 431, and 445 (Table 2) represented the residue of rhamnosyl, hexoside or glucoside, methoxyl, hydroxyl, and *O*-glucopyranosyl moieties. ESI-MS spectra and authentic stan-

dards supported the isoflavone compounds as calycosin-*O*-rhamnoside, formononetin-*O*-glucoside, sophoricoside, *O*-glucopyranosyl-hydroxymethoxyisoflavone, genistin, and genistein-*O*-glucoside. These findings are consistent with reference standards and literature data on *S. scordiifolia* Fisch. ex Schrank (Polya, 2003; Olennikov and Chirikova, 2013; Uritu et al., 2018; mona.fiehnlab.ucdavis.edu). As mentioned above, isoflavones are generally present in *Scutellaria* species.

FLAVANONE DERIVATIVES

In *S. condensata* and *S. patonii*, the fragmentation patterns of ions at m/z 255 and 299 (Table 2) revealed the neutral loss of 28 AMU or hydroxyl unit and the consecutive loss of methoxyl moiety. These compounds were identified as hydroxydimethoxyflavanone and pinocembrin in structure as previously reported for *Phlomis burguieri* Desf. and *S. baicalensis*, Lamiaceae (Aghakhani et al., 2018; Xu et al., 2018; massbank.eu; mona.fiehnlab.ucdavis.edu).

In the ESI-MS spectra of *S. patonii* and *S. multicaulis*, first the fragment ions at m/z 417, 431, and 609 were recognized (Table 2). The fragmentation patterns in the following ions were attributed to the residue of hydroxyl, glucuronide, neohesperidoside, rutinosyl, and glucosyl or hexosyl. These compounds were considered to be liquiritin, liquiritigenin-*O*-glucuronide, hesperetin-*O*-neohesperidoside, and hesperetin-*O*-rutinoside in structure as confirmed by published literature (Li et al., 2016; Han et al., 2017; mona.fiehnlab.ucdavis.edu). Liquiritigenin and hesperetin were previously separated from *S. baicalensis* (Olennikov et al., 2010; Jiang et al., 2015). According to our results, flavanone-*O*-hexosides were mostly found in *Scutellaria* species.

BIFLAVONOID, CHALCONE, AND ANTHOCYANIN DERIVATIVES

In the ESI-MS spectra of *S. multicaulis*, a deprotonated molecule at m/z 577 was known as procyanidin B1, a biflavan derivative (Table 2). Based on the published spectral data, this compound was separated from *Scutellaria* species (Malikov and Yuldashev, 2002; massbank.eu).

The fragmentation pattern of ion at m/z 239 (Table 2) corresponded to the consecutive loss of hydroxyl group. Based on accurate MS/MS and literature reports about *Scutellaria* species (Polya, 2003; Olennikov et al., 2010; massbank.eu; mona.fiehnlab.ucdavis.edu), this compound was considered to be dihydroxychalcone for *S. tomentosa.*

An anthocyanin derivative was characterized in *S. patonii*. The MS/MS spectra of molecular ion at m/z 448 (Table 2) were attributed to the neutral loss of galactoside and glucoside (hexoside) moieties. These compounds were tentatively identified as cyanidin-galactoside and cyanidin-glucoside in structure, which is in line with the proposed fragmentation (mona.fiehnlab.ucdavis.edu). According to published works, cyanidin-glycosides were found in *Ajuga* species, Lamiaceae (Inomata et al., 2013). Therefore, chalcone, biflavan and anthocyanin derivatives were in low proportions in *Scutellaria* species.

ADDITIONAL COMPOUNDS

One of the additional compounds, classified as tannin in structure, was observed in *S. patonii*. The fragmentation pattern of molecular ion at m/z 331 (Table 2) was considered to be galloyle-*O*-glucose or mono-galloyl glucose as confirmed by the recent published works (Wang et al., 2016).

One of the anthraquinone derivatives was observed in *S. multicaulis* with a deprotonated molecule at m/z 431 (Table 2). The MS/MS spectrum revealed 162 mass units (hexosyl or glucosyl). Based on previous reports combined with MS/MS information, this additional compound was likely to correspond with emodin-8-*O*-β-D-glucoside in structure (Chang et al., 2016; massbank.eu).

BOTANICAL VIEWPOINTS

From a botanical perspective, high morphological similarities were reported in *S. patonii, S. multicaulis, S. nepetifolia,* and *S. farsistanica* and *S. tomentosa* belonging to the section *Lupulinaria* (Rechinger, 1982; Jamzad, 2012). The presence of different pilose, tomentose, and glandular trichomes at the surface of the leaf, pannouse in petiole, corolla tube and lip, simple hairs in corolla tube, petiole and bract, tomentose in inflorescence axis and stem, the length of petiole, corolla lip and inflorescence, leaf size, and oblong leaf form, discriminated both *S. tomentosa* and *S. farsistanica* species (Jafari Dehkordi and Kharazian, 2019). Noticeably, these two species were chemically discriminated. Moreover, different features such as pilose at leaf and stem, simple trichome in bract and corolla lip, lanate at inflorescence axis, the presence of strigose at the

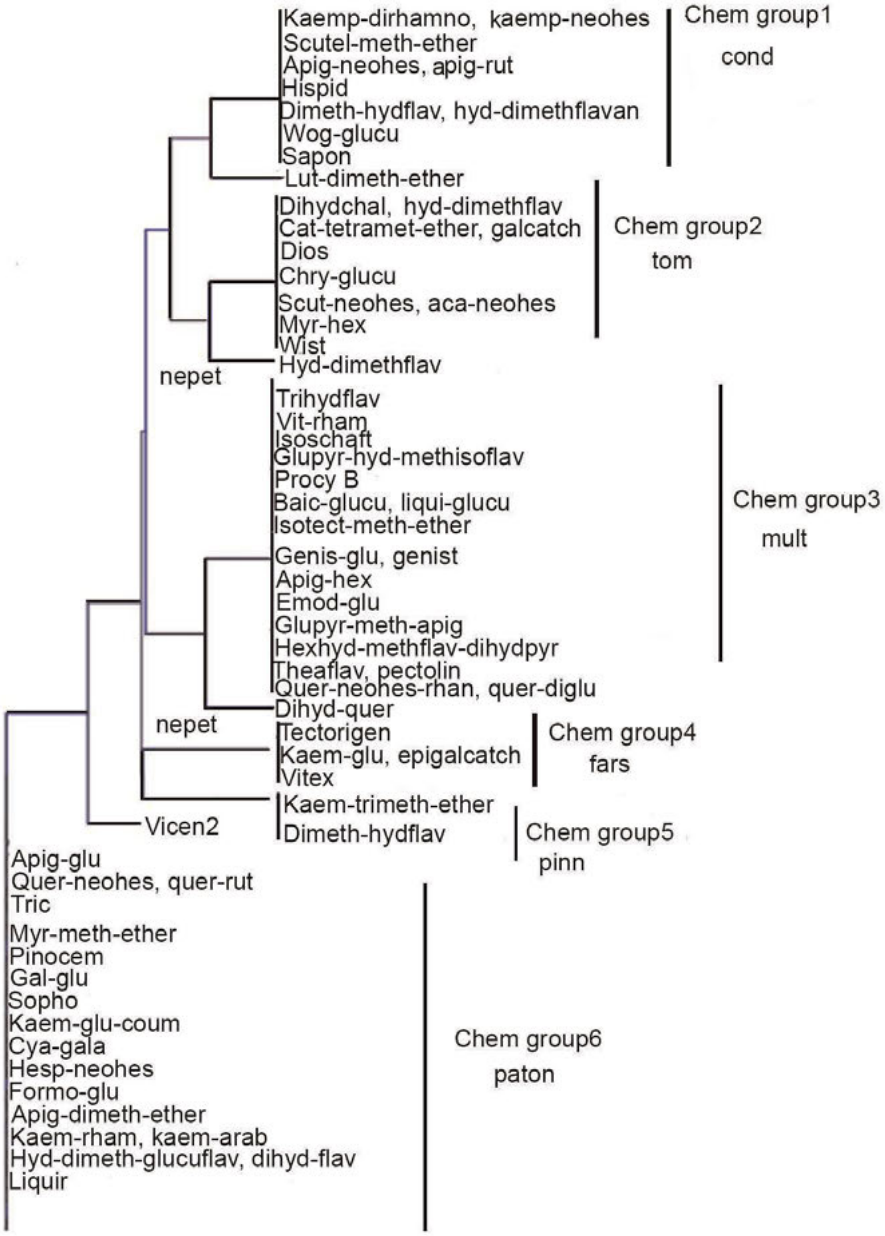

Fig. 2. The Representative of chemical groups based on flavonoid compounds in *Scutellaria* species. nepet – *S. nepetifolia*, tom – *S. tomentosa*, pinn – *S. pinnatifida*, cond – *S. condensata*, fars – *S. farsistenica*, paton – *S. patonii*, mult – *S. multicaulis*. The abbreviated chemical compounds are cited in Table 2.

upper surface of the stem, leaf margin (serrate, dentate), leaf apex (acute, obtuse), leaf size, the length of inflorescence, bract, and corolla lip separated three species, namely *S. multicaulis, S. nepetifolia,* and *S. patonii* (Jafari Dehkordi and Kharazian, 2019), which supports our fingerprinting analysis. *S. pinnatifida* was reported to be morphologically different from the other members of this section with pinnatifid margin leaf, the length of petiole (8–10 mm), inflorescence (6–7 cm), corolla lip in upper and lower surface (2.5–2.6 and 8–10 mm, respectively), and filament (5–6 mm) (Jafari Dehkordi and Kharazian, 2019). This evidence was corroborated by the presence of two different flavonol and flavone derivatives. In addition, the members of S. sect. *Scutellaria* (*S. condensata*) were

separated from *S.* sect. *Lupulinaria* using the presence of pubescent trichomes in anther, the length of petiole (20–27 mm), corolla lip in upper surface (1.5–1.7 mm) and filament (3–4 mm), leaf size (40 × 25 mm), and calyx size (4 × 5 mm). It is concluded that fingerprinting analysis is in line with morphological reports (Jafari Dehkordi and Kharazian, 2019).

CHEMICAL GROUPS OF *SCUTELLARIA* SPECIES

The molecular weight and mass to charge of flavonoid compounds were applied for the limitation of the members of each *Scutellaria* section. Regarding morphological features, in section *Lupulinaria*, *S. tomentosa* and *S. farsistanica* had considerable similarities. In some cases, infra-specific relations such as hybridization were provided (Rechinger, 1982; Jafari Dehkordi and Kharazian, 2019). In our results, both species showed separated taxa and were definitively identified by 14 flavonoid compounds. In the same way, *S. multicaulis* and *S. patonii* with high morphological similarity were largely dominated by 42 specific flavonoid compounds (Fig. 2). Indeed, *S. patonii* was previously considered as *S. multicaulis* (Rechinger, 1982). Using flavonoid class and thin layer chromatography, some relationships were found between both species in terms of morphology and chemotaxonomic status (Jafari Dehkordi and Kharazian, 2019). Compared with the previous reports, both species possess only one flavonoid compound (vicenin-2) as the related case. Similarly, the presence of dihydroquercetin compound in *S. multicaulis* and *S. nepetifolia* could be attributed to their relationships, but both species were definitely discriminated.

CONCLUSIONS

As demonstrated in this research, LC-MS/MS technique proved to be a highly powerful analytical and chemical fingerprint method for infra genus and infra specific boundaries in the genus *Scutellaria*. This property also makes it suitable for taxa identity. In the present work, the specified compounds were precisely conducted as chemical barcodes of the species. It is known that the presence of chemical barcodes in fingerprinting extracts is basically important for industrial production, pharmacological value enhancement, mass production of flavonoid compounds for medicinal uses, and comprehensive documentation of species for botanical purposes. The ecological conditions and environmental influences in different habitats could reflect the adaptation in each species, as confirmed by LC-M/MS technique. The current study introduced the most detailed features of the detected flavonoid compounds, which are appropriate for chemotaxonomic values.

AUTHORS' CONTRIBUTIONS

Farzaneh Jafari Dehkordi: accomplished this research, collected the species and analyzed data. Dr. N. Kharazian: accomplished this research, collected the species and analyzed data. Zahra Lorrigooini: collaboration on analytical tools.

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