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Untargeted metabolite profiling and phytochemical analysis of *Micromeria fruticosa* L. (Lamiaceae) leaves

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ABSTRACT

Micromeria fruticosa is an important crop, is widely used in the Mediterranean basin as food and in folk medicine, owing to its health-promoting properties, partially due to the secondary metabolite composition. However, complete information on the phyto-metabolites in *M. fruticosa* is still lacking. Plant leaves were extracted in methanol (80%), then the phyto-metabolites were separated on C18 column and an extensive characterization using UHPLC–DAD-ESI–QTOF–MS^2 method in two ionization modes was established. A total of 215 phenolics and other compounds were tentatively identified, offering the first comprehensive study available on the phytochemicals from *M. fruticosa*. Over 180 phytochemicals (87 flavonoids, 41 phenolic acids, 16 terpenoids, 8 sulfate derivatives, 7 iridoids, and others) are reported in Micromeria for the first time. *M. fruticosa* can be a promising source of functional ingredients and has use in the food, pharma and nutraceutical industries.

1. Introduction

White micromeria [*Micromeria fruticosa* L.: family Lamiaceae] is an aromatic herb widely distributed in the Mediterranean bordering countries, including the Levant. *M. fruticosa* usually grows in clefts of fissured rocks in the dry open habitats. Fresh or dried herbal parts of this plant are frequently used as a seasoning in soups and foods (Güllüce et al., 2004). It is traditionally used to replace mint. Owing to its minty odor, this herb used for tea consumption gives a feeling of coolness during the hot summer seasons (Yaniv & Dudai, 2014). *M. fruticosa* is endemic in Palestine and is known by local citizens with some common names such as “Zaa’tar balat”, “Zie’ttman”, “Ghurniah” and “Ishbat Al-Shay”. Toxicity studies on its aqueous extract were previously reported *in vivo* and the LD_{50} was reported to be 5 g per kg (Abu-Gharbieh & Ahmed, 2016). Extracts, herbal teas, spice and infusions of *M. fruticosa* are widely used in the traditional folk medicine to treat a variety of diseases, including gastrointestinal ailments like stomach ache and diarrhea, to treat respiratory problems (i.e. cough and colds), open

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wounds, eye infections, heart disorders, fever, headaches, exhaustion, asthma, weariness, inflammation, urinary diseases, diabetes and hypertension (Abu-Gharbieh, Shehab, & Khan, 2013; Azab, 2016; Shehab & Abu-Gharbieh, 2012;Yaniv & Dudai, 2014; Abu-Gharbieh & Ahmed, 2016). Furthermore, the aerial parts of the plants were reported to possess antirheumatic, hepatoprotective antiviral, antiseptic, antibacterial, anaesthetic, anti-inflammatory, anti-cancer, sedative, CNS-stimulant and abortifacient properties (Abu-Gharbieh & Ahmed, 2016; Dudai, Larkov, Ravid, Putievsky, & Lewinsohn, 2001; Koc, Ozdemir, Kizilkaya, Sengul, & Turkez, 2017; Shehab & Abu-Gharbieh, 2012). These biological activities are thought to result from the phytochemical composition of the plants (i.e. phenolic compounds). Polyphenols or phenolic compounds form a large group of secondary compounds including flavonoids and phenolic acids (Balasundram, Sundram, & Samman, 2006).. Phenolics are considered one of the most widely distributed groups of compounds in the plant kingdom with about 10,000 structures identified to date (Harnly, Bhagwat, & Lin, 2007).

Despite the importance of this plant in the field of food and medicine, a review of the literature revealed no reports on the chemical composition except for some reports about the occurrence of some flavonoid glycosides and essential oils (Formisano et al., 2007; Güllüce et al., 2004; Telci & Ceylan, 2007).

Liquid chromatography with diode array detector coupled with quadruple-time-of flight tandem mass spectrometry (UHPLC-DAD-QTOF-MS^2) is an important analytical tool and is thought to be the method of choice for profiling of phytochemicals in the plant extracts (Wolfender, Marti, Thomas, & Bertrand, 2015). This method is also considered to be a precise, effective, convenient and comprehensive technique in the analysis of complex samples. It combines the highly efficient separation of the UHPLC and high-resolution, sensitivity and selectivity of ESI-QTOF-MS. So, the tandem MS can determine the exact mass of main and product ions besides providing the possible elemental compositions, which is very useful for structural elucidation and confirmation in case of unavailability of commercial standards (Kachlicki, Piasecka, Stobiecki, & Marczak, 2016).

Thus, the aim of this work was to carry out an extensive profiling of the hydro-methanolic extract of *M. fruticosa* for their phytochemical composition by using UHPLC-ESI-QTOF-MS in two ionization modes as a powerful analytical technique. To our knowledge, no such comprehensive study is available concerning the phytochemical constituents of *M. fruticosa* plant by means of UHPLC-MS^2.

2. Materials and methods

2.1. Chemicals and reagents

All the chemicals used in the present study have been of analytical grade and they were used as received. Acetic acid was acquired from Fluka (Buchs, Switzerland). HPLC-grade acetonitrile and methanol were purchased from LabScan (Dublin, Ireland). External standards: apigenin, luteolin, rosmarinic acid and quercetin, chlorogenic acid, syringic acid, rutin and kaempferol were obtained from Sigma and Sigma-Aldrich (St. Louis, MO, USA). Double-deionized water (conductivity of < 18.0 MΩ) was obtained using a Milli-Q system (Millipore, Bedford, MA).

2.2. Sample and standard solution preparation

The areal parts of 15 plants of *M. fruticosa* were collected in the summer of 2012 from their wild habitat in the mountains of Nablus district (three villages: Qusra, Jalood, and Talfit) situated at 800–880 m above sea level and authenticated at the Department of Biology & Biotechnology, Faculty of Science, An-Najah National University. The leaves of the plants were separated from the areal parts, air-dried at room temperature (RT) and ground to a coarse powder using a household mill and stored at RT until they were used for extraction. Standard solutions of concentrations 1 mg/mL were prepared in methanol and filtered through a 0.45 μm (Millipore) filter prior to analysis by UHPLC-MS analysis.

2.3. Extraction of phytochemicals

Extraction of the leaves was performed as reported by Simirgiotis, Caligari, and Schmeda-Hirschmann (2009), with some variations. The air-dried and ground *M. fruticosa* leaves (0.5 g) was mixed with hexane to remove the non-polar fraction, then the plant residue was extracted (turraxed) using aqueous methanol (80%) and sonicated for 60 min at RT. The mixture was centrifuged for 15 min at 3800*g*. The extraction process was repeated twice, and the supernatant liquid was decanted into a round-bottom flask. The solvent was then evaporated using a rotary evaporator R-210 from Büchi Labortechnik AG (Flawil, Switzerland) under vacuüm at 38 °C and the dry deposit was re-dissolved in aqueous methanol. Finally, the extract was centrifuged again and the supernatant liquid was filtered through a 0.2-μm syringe filter and kept in the freezer at −20 °C until injection into the UHPLC-MS^2 system.

2.4. Ultra-high-performance liquid chromatography coupled to diode array detection and electrospray time-of-flight tandem mass spectrometry (UHPLC–DAD-ESI-QTOF-MS^2) analysis

Separation of phytochemicals from *M. fruticosa* extracts was carried out on an Agilent 1200 series Rapid Resolution LC (Agilent Technologies, Santa Clara, CA) consisting of an auto-sampler, a vacuum degasser, a binary pump and diode-array detector (DAD). This instrument was equipped with an Agilent Zorbax C_{18} column (4.6 × 150 mm, 5 μm particle size) (Agilent Technologies, Palo Alto, CA, USA). Acidified water (0.5% acetic acid, v/v) (A) and acetonitrile (B) were used as Mobile phases. The gradient was performed as follows: 0 min, 0% B; 20 min, 20% B; 30 min, 30% B; 40 min, 50% B; 50 min, 75% B; 60 min, 100% B; 63 min 0% B, and finally, the initial conditions were used for 7 min as a re-equilibration step. The flow rate was set at 0.2 mL/min throughout all the gradient. The injection volume was 10 μL, and the column temperature was kept at 25 °C.

The UHPLC system was attached to a quadrupole-time-of-flight (micrOTOF-QII™, Bruker Daltonik GmbH, Bremen, Germany), a Q-TOF (quadrupole-time-of-flight) mass spectrometer, equipped with a model G1607A ESI interface (Agilent Technologies) operating in the positive and negative ion modes, with spectra acquired over a mass range from *m/z* 50 to 1100. The optimum values of the ESI-MS parameters were as follows: capillary voltage, 3.5 and +4000 V; drying gas flow, 9.0 L/min; drying gas temperature, 190 °C; nebulizing gas pressure, 29 psi; transfer time 70 μs; collision RF, 150 Vpp and pre-pulse storage, 5 μs. In addition, the automatic MS^2 experiments were performed by adjusting the collision energy values as follows: *m/z* 100, 20 eV; *m/z* 500, 30 eV; *m/z* 1000, 35 eV, using Nitrogen as the collision gas. The MS data were handled through Data Analysis 4.1 software (Bruker Daltonics, Bremen, Germany) which provides a list of possible elemental formulas by using the Generate-Molecular-Formula™ editor. The qualitative analysis was performed using isotope distribution, exact mass, UV absorption, and tandem MS fragmentation pattern, also as described from previously reported spectra of the same species or/and the botanical family (Kachlicki, Piasecka, Stobiecki, & Marczak, 2016).

3. Results and discussion

3.1. Characterization of the (poly)phenols and other phytoconstituents

3.1.1. General

Table 1 lists the 215 compounds tentatively identified via UHPLC–DAD–ESI–QTOF–MS^2 analysis including their retention times (*Rt*), molecular formula, detected accurate mass ([M ± H] $^{\pm}$ ionization modes), mass error (ppm) of each chemical, mSigma value, in

Table 1

Peak	R_t (min.)	$[M + H]^+$ (m/z)	$[M-H]^-$ (m/z)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	Tentative identification	Family
Organic acids and derivatives									
1	2.19	193.0705	191.0565	C 7H 12O 6	−2.1	2	127.0393(8), 171.0309(4)[a]	Quinic acid	Organic acid
3	2.79	_	133.0144	C 4H 6O 5	−1	4	115.0043(1 0 0)	Malic acid	Organic acid
10	4.60	_	191.0202	C 6H 8O 7	−2.7	1	111.0090(1 0 0)	Citric acid	Organic acid
Sacchrides and derivatives									
2	2.49	_	341.1086	C12H22O11	1	4.8	149.0451(67), 191,0562(1 0 0)	(+)-Sucrose	Saccharide
4	3.26	_	665.2154	C 24H 42O 21	−1.2	4	503.1605(2), 341.1076(9), 179.0565(5)	D-Stachyose	Saccharide
5	3.66	_	503.1620	C 18H 32O 16	−0.4	6.6	341.1030(11), 179.0558(1 0 0)	Raffinose	Saccharide
6	3.80	280.1396	_	C 11*H* 21 N O 7	−1.7	6.5	216.1229(1 0 0), 198.1139(40), 114.0880(32)	Fructosyl-valine	Saccharide derivative
7	3.88	295.1012	_	C 11*H* 18O 9	3.8	9	173.9605(15), 142.9543(1 0 0), 132.9578(43), 111.0442(19)	Tuliposide B	Saccharide derivative
Nitrogen containing derivatives									
8	4.11	136.0614	_	C 5H 5 N 5	2.5	4.3	119.0350(1 0 0), 92.0229(13)	Adenine	Nucleotide
11	7.18	182.0812	180.0670	C 9H 11 N O 3	−2.4	2.4	163.0403(80), 119.0505(1 0 0)[a]	Tyrosine	Amino acid
12	7.82	170.0813	_	C 8H 11 N O 3	−0.6	52.2	152.0696(1 0 0)	Pyridoxine hexoside	Saccharide derivative
13	8.04	294.1547	_	C 12H 23 N O 7	0.3	7.1	230.1380(1 0 0), 212.1277(28), 142.9541(79), 132.9568(33)	Fructose-L-leucine I	Peptide
14	8.24	_	243.0629	C 9H 12 N 2O 6	−2.7	5.7	152.0359(45), 110.0244(1 0 0)	Uridine	Nucleotide
15	8.27	_	292.1394	C 12H 23 N O 7	−2	13.4	130.0874(1 0 0)	Fructose-L-leucine II	Peptide
16	9.89	268.1044	_	C 10*H* 13 N 5O 4	−1.3	7.9	136.0614(1 0 0)	Adenine riboside	Nucleotide
18	10.24	_	282.0852	C10H13N5O5	2.9	12.6	150.0416 (1 0 0)	Guanine ribonucleoside	Nucleotide
19	10.77	_	266.0898	C 10*H* 13 N 5O 4	−1.4	3.8	134.0470 (11)	Adenosine	Nucleotide
20	10.90	286.0910	_	C 12H 15 N O 7	4	28.6	124.0386(1 0 0)	p-Nitrophenyl fucoside	Nitrogen derivative
21	11.10	166.0851	_	C 9H 11 N O 2	6.9	2.6	120.0793(53),103.0546(1 0 0)	Phenylalanine	Amino acid
31	15.24	205.0973	_	C 11*H* 12 N 2O 2	−0.8	9.2	143.0719(53), 132.0799(23), 118.0644(1 0 0)	Tryptophan	Amino acid
91	26.64	399.1562	_	C 21H 22 N 2O 6	−2.9	25.6	253.0893(1 0 0), 207.0837(68), 153.0716(40), 181.0682(39)	Criofolinine	Alkaloid
Phenolic acids and derivatives									
23	11.26	_	301.0908	C 13H 18O 8	3.9	23	139.0409(1 0 0)	(Iso)tachioside	Phenolic acid derivative
24	11.33	_	331.0669	C13H16O10	0.5	11.3	169.0085(73), 125.0255(35)	Galloylhexose	Phenolic acid derivative
26	12.38	_	315.0729	C 13H 16O 9	−2.4	4.2	153.0180(44), 152.0129(1 0 0)	Dihydroxybenzoic acid hexoside	Phenolic acid derivative
27	12.67	_	197.0461	C9H10O5	2.8	4.1	179.0357(15), 151.0398(7), 135.0442(1 0 0), 123.0450(58)	Syringic acid[c]	Phenolic acid
29	14.20	517.1351	_	C25 H24 O12	−1.9	10.7	163.0393(1 0 0)	Caffeic acid derivative	Phenolic acid derivative
30	14.91	355.1012	353.0874	C 16H 18O 9	3.3	4.9	163.0388(1 0 0), 135.0438(20)	Caffeoylquinic acid I	Phenolic acid derivative
33	16.50	_	359.0985	C15H20O10	0.3	15.3	197.0479(1 0 0)	Syringic acid hexoside	Phenolic acid derivative
34	16.52	277.1661	_	C14 H20 N4 O2	−0.6	1.7	147.0433(1 0 0), 119.0485(33)	Coumaroylagmatine I	Phenolic acid derivative
36	17.06	_	299.0791	C13H16O8	6.1	29.1	137.0249(1 0 0)	Hydroxybenzoic acid hexoside	Phenolic acid derivative

(*continued on next page*)

Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (*m/z*)	$[M\text{-}H]^-$ (*m/z*)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
37	17.17	_	341.0884	C15H18O9	1.7	6.8	179.0352(1 0 0)	Caffeoylhexose	Phenolic acid derivative
38	17.46	375.0911	_	C 15H 18O 11	3	13.1	127.0379(1 0 0)	Trihydroxybenzene malonate	Phenolic acid derivative
39	17.64	_	337.0931	C16H18O8	−0.7	2.3	191.0564(10), 173.0442(19), 163.0403(1 0 0), 119.0502(22)	Coumarylquinic acid I	Phenolic acid derivative
40	18.06	355.1022	353.0882	C 16H 18O 9	0.4	5.1	163.0387(1 0 0)	Caffeoylquinic acid II	Phenolic acid derivative
41	18.08	_	515.1394	C22H28O14	−2.3	30.9	353.0900(12), 341.0873(1 0 0), 179.0353(31)	Caffeoylquinic acid hexoside	Phenolic acid derivative
43	18.89	355.1020	353.0879	C 16H 18O 9	1	31.6	163.0385(1 0 0)	Chlorogenic acid[c,d]	Phenolic acid derivative
46	19.05	277.1660	_	C14 H20 N4 O2	−0.5	3.5	147.0435(1 0 0), 119.0486(10)	Coumaroylagmatine II	Phenolic acid derivative
50	20.67	_	337.0911	C16H18O8	5.4	21.2	191.0591(22), 173.0469(1 0 0), 163.0397(22)	Coumaroylquinic acid II	Phenolic acid derivative
53	20.82	_	297.0635	C13H14O8	−6.5	15.6	135.0315(1 0 0)	O-Caffeoyl-threonic acid	Phenolic acid derivative
54	21.34	_	337.0933	C16H18O8	1.1	5.9	191.0559(1 0 0), 173.0460(15), 163.0414(11)	Coumaroylquinic acid III	Phenolic acid derivative
56	21.47	_	355.1016	C16H20O9	5.2	10.7	193.0514(30)	Ferulic acid hexoside	Phenolic acid derivative
58	21.69	_	337.0933	C16H18O8	1.3	3.5	191.0601(2), 173.0456(1 0 0), 163.0400(27),119.0495(6)	Coumaroylquinic acid V	Phenolic acid derivative
60	22.34	_	335.0778	C16H16O8	−1.7	62	179.0354(1 0 0), 135.0455(35)	Caffeoylshikimic acid	Phenolic acid derivative
69	24.02	_	537.1059	C27 H22 O12	−3.8	9	493.1187(10), 313.0740(13), 295.0610(1 0 0), 197.0444(15), 179.0354(36), 161.0247(11)	Lithospermic acid[d]	Phenolic acid
70	24.09	369.1168	_	C17 H20 O9	3.2	1.1	193.0855(18)	Feruloylquinic acid	Phenolic acid derivative
71	24.23	_	385.1141	C17 H22 O10	−0.2	4	267.0727(1 0 0), 249.0613(62), 223.0843(2)	Sinapoylhexose	Phenolic acid derivative
83	25.84	_	539.1188	C27 H24 O12	1.2	7.4	495.1311(22), 359.0776(49), 315.0877(1 0 0), 297.0786(40), 253.0852(27), 197.0459(62), 179.0349(63), 161.0241(37)	Yunnaneic acid D	Phenolic acid derivative
87	26.14	599.1395	597.1254	C29 H26 O14	0.1	20.8	287.0553(76), 265.0870(84), 223.0761(1 0 0)	Yunnaneic acid F	Phenolic acid derivative
96	27.70	_	493.1021	C 22H 22O 13	−6.7	24.6	331.0692(55), 323.0779(82), 179.0349(6)	1-O-(E)-Caffeoyl − 3-O-galloyl-β-D-glucopyranose	phenolic acid derivative
112	29.11	_	515.1193	C25 H24 O12	0.3	16.9	353.0884(1 0 0), 191.0562(53)	Dicaffeoylquinic acid I	Phenolic acid derivative
114	29.45	_	719.1618	C 36H 32O 16	−0.1	40.8	359.0789(27)	Sagerinic acid	Phenolic compound
120	30.10	_	503.1207	C24 H24 O12	−2.4	139	341.0915(19), 179.0342(1 0 0)	Di-*O*-caffeoyl-glucoside	Phenolic acid derivative
121	30.17	517.1339	515.1187	C25 H24 O12	1.5	4.2	353.0877(1 0 0), 299.0566(1), 191.0560(7), 179.0358(19), 173.0455(29)[a]	Dicaffeoylquinic acid II	Phenolic acid derivative
125	30.57	_	775.2456	C37 H44 O18	−0.2	14.5	359.0771(1 0 0), 197.0455(3), 179.0359(1), 161.0243(9)	Rosmaric acid derivative	Phenolic acid derivative
126	30.64	_	359.0770	C18H16O8	−0.7	1.9	197.0459(51), 179.0348(20), 161.0242(1 0 0)	Rosmaric acid[c,d]	Phenolic acid derivative
129	30.81	_	461.1074	C 22H 22O 11	3.2	15.1	315.0712(38), 163.0388(7), 152.0107(35)	Cinnamoyl-galloylglucose	Phenolic compound
137	31.85	_	665.2450	C 32H 42O 15	0.1	7.9	503.1936(8), 371.1497(15)	Phillygenin pentosyl-hexoside	Phenolic compound

(*continued on next page*)

Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (m/z)	$[M\text{-}H]^-$ (m/z)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
139	32.02	521.1059	_	C 27H 20O 11	3.8	1.4	295.0621(4), 289.2443(2), 163.0381(1 0 0)	BCECF acid	Phenolic compound
143	32.99	_	499.1256	C25 H24 O11	−2	46.5	337.0946(1 0 0), 179.0310(6), 163.0408(26)	Coumaroyl-caffeoylquinic acid	Phenolic acid derivative
164	38.18	_	582.2600	C34 H37 N3 O6	1.7	9	582.2605(1 0 0), 462.2024(87), 436.2225(5), 342.1456(10), 145.0289(7), 119.0493(4)	Tri-p-coumaroylspermidine	Phenolic acid derivative
170	39.69	361.0902	_	C 18H 16O 8	4.5	1.5	346.0666(34), 331.0436(1 0 0), 256.0727(19)	Trihydroxy-trimethoxyflavone I	Phenolic compound
180	41.77	361.0902	_	C 18H 16O 8	4.5	6.2	343.0772(1 0 0), 315.0863(79), 283.0608(1 0 0)	Trihydroxy-trimethoxyflavone II	Phenolic compound
Flavonoid derivatives									
44	19.00	627.1578	_	C 27H 30O 17	−3.6	19.8	465.1135(1), 303.0509(1 0 0)	Quercetin dihexoside	Flavonoid derivative
45	19.02	773.2144	771.1994	C 33H 40O 21	−1.1	19.5	465.1031(19), 303.0501(1 0 0)	G-rutin I	Flavonoid derivative
49	20.62	771.1978	_	C 33H 38O 21	0	29	465.0993(12), 303.0469(1 0 0)	Tricetin *O*-hexoside-*O*-(HMG)-hexoside I	Flavonoid derivative
51	20.67	757.2218	_	C 33H 40O 20	−4.3	15.8	449.1090(23), 287.1090(1 0 0)	Kaempferol glucosyl-rutinoside	Flavonoid derivative
57	21.61	595.1636	593.1510	C27H30O15	−0.3	5.8	503.1185(1), 383.0757(1), 353.0645(1)	Diglucosylapigenin	Flavonoid derivative
59	21.99	773.2145	_	C 33H 40O 21	−1.4	18.4	465.0973(1), 303.0502(1 0 0)	G-rutin II	Flavonoid derivative
62	22.45	743.2039	_	C 32H 38O 20	−1.3	15.5	303.0496(1 0 0)	Apiorutin	Flavonoid derivative
65	23.15	757.2186	755.2053	C 33H 40O 20	−0.1	6.4	303.0492(1 0 0)	Quercetin-*O*-di-rhamnosyl-glucoside	Flavonoid derivative
66	23.17	611.1575	_	C 27H 30O 16	5	6	303.0492(1 0 0)	Rutin[c]	Flavonoid derivative
67	23.44	_	741.1905	C 32H 38O 20	−2.8	1.1	300.0265(1), 301.0373(1)	Quercetin-*O*-pentosyl-rhamnosyl-glucoside	Flavonoid derivative
72	24.26	_	771.2327	C 34H 44O 20	3.4	19.5	609.1453(4)	Hesperidin hexoside	Flavonoid derivative
73	24.56	465.1000	_	C 21H 20O 12	6	7.1	303.0478(1 0 0)	Quercetin hexoside I	Flavonoid derivative
74	24.60	611.1579	609.1463	C 27H 30O 16	4.4	2.1	303.0499(1 0 0)	Quercetin rhamnosyl glucoside[d] I	Flavonoid derivative
75	24.63	_	595.1315	C 26H 28O 16	−1.8	12.5	301.0321(6), 300.0276(19)	Arabinoglucosyl-quercetin	Flavonoid derivative
76	24.70	741.2233	_	C 33H 40O 19	0.5	13.6	449.1107(1), 287.0550(1 0 0)	Kaempferol 3-(2G-rhamnosylrutinoside)	Flavonoid derivative
77	24.83	757.2173	_	C 33H 40O 20	1.7	8.1	465.0959(1), 303.0500(1 0 0)	Quercetin 3-(2G-rhamnosylrutinoside)	Flavonoid derivative
78	24.96	771.1980	_	C 33H 38O 21	−0.3	50	303.0485(1 0 0)	Tricetin *O*-hexoside-*O*-(HMG)-hexoside II	Flavonoid derivative
79	25.25	727.2048	_	C 32H 38O 19	4.3	18.8	287.0604(1 0 0)	Kaempferol 3-(2G-xylosylrutinoside)	Flavonoid derivative
81	25.77	611.1586	_	C 27H 30O 16	3.4	5.1	303.0499(1 0 0)	Quercetin rhamnosyl glucoside[d] II	Flavonoid derivative
82	25.79	465.1006	_	C 21H 20O 12	4.6	6.9	303.0490(1 0 0)	Quercetin hexoside II	Flavonoid derivative
84	26.04	595.1648	593.1507	C 27H 30O 15	1.7	20.1	287.0552(1 0 0)	Kaempferol- or luteolin- rutinoside I	Flavonoid derivative

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Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (m/z)	$[M-H]^-$ (m/z)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
86	26.11	741.1872	739.1721	C 32H 36O 20	0.1	9.2	303.0497(1 0 0)	Quercetin-*O*-xylopyranosyl-*O*-(HMG)-hexoside	Flavonoid derivative
88	26.37	_	695.1440	C 30H 32O 19	3.5	18.2	651.1570(1 0 0), 609.1471(5), 301.0341(2)	Quercetin malonyl-rutinoside	Flavonoid derivative
89	26.61	755.2016	753.1893	C 33H 38O 20	−1.2	8.3	609.1468(1 0 0), 301.0310(1)[a]	Quercetin-*O*-rhamnosyl-*O*-(HMG)-hexoside	Flavonoid derivative
90	26.46	609.1428	_	C 27H 28O 16	3.6	8.9	463.0869 (81), 301.0477(85)	Quercetin-*O*-(HMG)-hexoside I	Flavonoid derivative
92	26.88	465.1003	463.0879	C 21H 20O 12	5.3	6.5	303.0487(1 0 0)	Quercetin hexoside III	Flavonoid derivative
93	26.91	449.1062	447.0933	C 21H 20O 11	3.6	8.3	287.0549(1 0 0)	Kaempferol- or Luteolin- hexoside	Flavonoid derivative
94	27.32	493.1323	491.1193	C 23H 24O 12	3.6	6.7	331.0811(1 0 0)	Tricin hexoside	Flavonoid derivative
97	27.89	_	477.1036	C 22H 22O 12	0.5	18.5	315.0715(36)	Isorhamnetin hexoside	Flavonoid derivative
99	27.92	595.1636	593.1506	C 27H 30O 15	3.6	2.5	287.0550(1 0 0)	Kaempferol- or luteolin- rutinoside II	Flavonoid derivative
101	28.04	_	357.0642	C 18H 14O 8	−7.3	45.1	339.0518(26), 295.0619(52), 179.0358(24), 161.0303(31), 135.0461(1 0 0)	Santaflavone[d]	Flavonoid derivative
102	28.17	609.1424	607.1296	C 27H 28O 16	1.4	53.4	463.0873(92), 301.0357(14)[a]	Quercetin-*O*-(HMG)-hexoside II	Flavonoid derivative
103	28.07	551.1016	549.0896	C 24H 22O 15	2.8	13.3	303.0491(1 0 0)	Quercetin malonyl-hexoside	Flavonoid derivative
104	28.19	_	651.1577	C 29H 32O 17	−1.5	50.6	593.1373(3), 301.0318(5)	Rutin monoacetate	Flavonoid derivative
105	28.22	681.1631	–	C 37H 28O 13	−4.2	8.3	287.0549(1 0 0)	Kaempferol 3-O-(6″-O-malonylglucoside) − 7- O-rhamnoside	Flavonoid derivative
106	28.26	579.1682	577.1560	C 27H 30O 14	4.5	17.5	271.0597(1 0 0)	Apigenin 7-O-rutinoside	Flavonoid derivative
107	28.36	_	607.1306	C 27H 28O 16	−0.2	5.5	463.0877(73), 301.0355(12)	Quercetin-O-(HMG)-hexoside III	Flavonoid derivative
108	28.39	_	737.1936	C 33H 38O 19	1.6	23	593.1502(35), 285.0390(1)	Kaempferol-(HMG)-rutinoside	Flavonoid derivative
110	28.94	449.1048	447.0928	C 21H 20O 11	6.7	3.5	287.0529(1 0 0)	Kaempferol Hexoside or Luteolin hexoside	Flavonoid derivative
111	29.09	493.1291	_	C 30H 20O 7	−2	19.5	331.0800(1 0 0)	Tricin hexoside	Flavonoid derivative
113	29.50	609.1787	607.1661	C 28H 32O 15	1.1	8	299.0561(1 0 0)[a]	Diosmetin 7-*O*-rutinoside	Flavonoid derivative
115	29.49	433.1104	431.0978	C 21H 20O 10	5.7	24.6	271.0610(1 0 0)	Apigenin *O*-hexoside	Flavonoid derivative
116	29.78	447.0899	445.0781	C 21H 18O 11	−1	185	269.0476(1 0 0)[a]	Apigenin 7-*O*-glucuronide[d]	Flavonoid derivative
117	29.73	_	579.1137	C 29H 24O 13	−2.1	42.5	337.0728(28), 285.0390(1), 197.0458(52), 179.0361(1 0 0), 135.0448(81)[a]	Kaempferol -*O*-caffeoyl -hexoside	Flavonoid derivative
118	29.68	507.1118	505.0988	C 23H 22O 13	3	17.8	303.0509(1 0 0)	Quercetin-*O*-acetylhexoside	Flavonoid derivative
119	29.80	433.1112	_	C 21H 22O 10	4	14.4	271.0602(1 0 0)	Apigenin *O*-hexoside	Flavonoid derivative
124	30.72	593.1468	591.1359	C 27H 28O 15	−0.6	7	447.0933(56), 285.0408(31)[a]	Kaempferol-O-(HMG)-hexoside	Flavonoid derivative
127	30.67	535.1063	_	C 24H 22O 14	3.6	5.9	287.0554(1 0 0)	Kaempferol malonyl-hexoside I	Flavonoid derivative

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Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (*m/z*)	$[M\text{-}H]^-$ (*m/z*)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
130	30.82	523.1429	_	C 24H 26O 13	3.4	7.9	361.0906(1 0 0)	Irigenin hexoside	Isoflavonoid
131	30.84	_	489.1035	C 23H 22O 12	0.7	4.4	285.0410(1 0 0)	Kaempferol -O-acetylhexoside	Flavonoid derivative
132	30.87	_	533.0930	C 24H 22O 14	1.3	12.8	489.1029(1 0 0), 285.0401(90)	Kaempferol malonyl-glucoside II	Flavonoid derivative
133	30.91	579.1331	577.1201	C 26H 26O 15	2.4	56.1	331.0802(1 0 0)	Tricin-O-malonyl-hexoside	Flavonoid derivative
134	31.24	551.2097	549.1974	C 27H 34O 12	0.6	7.9	387.1653(81)[a]	Hydroxyarctigenin-O-hexoside	Flavonoid derivative
135	31.26	_	445.1140	C 22H 22O 10	0	33.8	281.0673(1 0 0)	Glycitein-*O*-hexoside	Flavonoid derivative
140	32.30	_	623.1956	C 36H 30O 10	−7.1	41	477.1250(3), 315.0846(1 0 0)[a]	Isorhamnetin-*O*-rutinoside	flavonoid derivative
141	32.96	317.0649	315.0502	C 16H 12O 7	2	7.6	302.0423(1 0 0), 168.0040(27)	Isorhamntin	Flavonoid
150	35.91	565.1201	_	C 25H 24O 15	−2.3	26.3	317.0692(1 0 0)	Isorhamnetin-*O*-malonyl-hexoside	Flavonoid derivative
151	34.59	593.1851	_	C 28H 32O 14	2.3	15.9	285.0757(1 0 0)	Acacetin − 7-*O*-rutinoside (Linarin)	Flavonoid derivative
152	35.39	347.0748	345.0617	C 17H 14O 8	3.8	6.4	332.0541(11), 317.0284(1 0 0)	Syringetin	Flavonoid derivative
155	36.16	287.0545	285.0418	C 15H 10O 6	1.8	3.9	269.0485(4), 241.0551(3), 153.0159(26), 135.0447(14)	Luteolin[c]	Flavonoid
156	36.58	303.0481	301.0340	C 15H 8O 7	4.6	27	273.0358(10), 178.9989(54), 151.0030(1 0 0)[a]	Quercetin[c]	Flavonoid
157	36.60	377.0865	375.0724	C 18H 16O 9	0.7	12.3	362.0618(55), 347.0385(1 0 0)	Tetrahydroxy-trimethoxyflavone	Flavonoid derivative
159	36.78	301.0693	299.0565	C 16H 12O 6	4.6	5.2	286.0468(1 0 0), 168.0043(36)	Chrysoriol[d]	Flavonoid
160	37.59	331.0803	329.0667	C 17H 14O 7	2.9	3.9	316.0569(1 0 0), 301.0357(6)	Tricin or Jaseocidin I	Flavonoid
161	37.66	_	461.2013	C 21H 34O 11	3.4	29.3	417.2107(1 0 0), 375.2033(43), 357.1894(26), 327.2164(13), 195.1360(4)	Patrinoside or Valerosidate	Flavonoid
162	37.72	347.0751	_	C 17H 14O 8	3	5.6	329.0610(26),301.0707(57), 269.0428(1 0 0),153.0183(85)	Dimethyl myricetin	Flavonoid
165	39.01	331.0799	329.0671	C 17H 14O 7	4.1	4.7	316.0560(10), 301.0330(1 0 0)	Tricin or Jaseocidin II	Flavonoid
167	39.42	271.0606	_	C 15H 10O 5	−1.8	6.4	153.0177(97)	Apigenin[c]	Flavonoid
168	39.51	_	329.0673	C 17H 14O 7	−1.8	3.6	299.0198(1 0 0), 314.0436(99), 229.1426(13), 211.1345(19)	Hydroxy-di-O-methylluteolin[d]	Flavonoid derivative
169	39.55	_	271.0620	C 15H 12O 5	−2.8	2.5	177.0180(10),151.0033(78), 119.0503(36)	Naringenin[d]	Flavonoid
171	40.18	_	359.0777	C 18H 16O 8	−1.4	3.8	344.0539(1 0 0), 329.0308(59)	Thymonin[d]	Flavonoid
172	40.24	287.0541	285.0407	C 15H 10O 6	−1	23.4	257.0509(1), 239.0347(1),229.0552(4), 151.0075(1)[a]	Kaempferol[c]	Flavonoid
174	40.42	331.0801	_	C 17H 14O 7	3.3	4.6	316.0555(22), 298.0469(65), 270.0512(1 0 0)	Tricin or Jaseocidin III	Flavonoid derivative
177	41.68	345.0948	_	C 18H 16O 7	6.1	1.4	329.0644(1 0 0), 315.0489(12), 301.0656(24), 284.0703(5)	Cirsilineol	Flavonoid
179	41.75	_	343.0826	C 18H 16O 7	−0.7	4.8	328.0596(1 0 0), 313.0358(79)	Dihydroxy-trimethoxyflavone[d] I	Flavonoid derivative
181	42.13	_	343.0832	C 18H 16O 7	−2.4	9.9	328.0595(1 0 0), 313.0366(72)	Dihydroxy-trimethoxyflavone[d] II	Flavonoid derivative
183	43.09	301.0697	299.0565	C 16H 12O 6	3.3	11.5	286.0477(28), 258.0527(1 0 0), 167.0334(13)	Diosmetin[d]	Flavonoid derivative
184	43.50	315.0851	_	C 17H 14O 6	4	17.8	300.0598(14), 282.0517(55), 254.0573(1 0 0)	Scrophulein	Flavonoid derivative
185	43.63	375.1050	373.0932	C 19H 18O 8	5	13.8	360.0805(19), 345.0588(1 0 0), 270.0876(16)	Casticin or Chrysosplenetin	Flavonoid derivative
187	44.06	_	373.0933	C 19H 18O 8	−1.2	3.5	358.0695(1 0 0), 359.0729(18)	Methylsudachitin[d]	Flavonoid derivative
188	44.28	315.0851	_	C 17H 14O 6	4	7.6	300.0602(1 0 0),272.0695(28)	Pectolarigenin	Flavonoid derivative

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Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (*m/z*)	$[M\text{-}H]^-$ (*m/z*)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
189	45.26	497.1764	_	C 27H 28O 9	8.5	20.6	331.0803(1 0 0)	Methoxygalloyl-tricin	Flavonoid derivative
191	46.13	345.0947	_	C 18H 16O 7	6.4	13.3	330.0730(9), 315.0462(1 0 0), 284.0679(6), 240.0766(22)	Dihydroxy-trimethoxyflavone	Flavonoid derivative
192	46.30	285.0746	283.0614	C 16H 12O 5	−0.9	3.7	268.0379(1 0 0),165.0188(1), 117.0323(1)[a]	Eupatorin or Eupatilin	Flavonoid derivative
193	46.67	527.1874	_	C 28H 30O 10	7.2	8.9	361.0894(1 0 0)	Methoxygalloyl-rosemarinic acid	Flavonoid derivative
196	47.74	359.1110	_	C 19H 18O 7	4.3	18.9	343.0802(35), 326.0805(69), 315.0906(10), 298.0834(1 0 0), 270.0843(8)	Hydroxy-tetramethoxyflavone	Flavonoid derivative
Sulfate derivatives									
25	12.05	_	409.0444	C14H18O12S	0.5	4.6	329.0877(1), 241.0027(1 0 0),167.0350(5)	Vanillic acid-O-glucopyranoside-6′-sulfate	Sulfate derivative
28	13.43	_	261.0072	C 9H 10O 7 S	0.8	7.7	181.0519(1 0 0), 96.9605(56)	Dihydrocaffeic acid-O-sulfate	Sulfate derivative
48	20.33	_	305.0708	C12H18O7S	−2	6.8	225.0839(1 0 0), 96,9592(49)	Methyl-hydroxyjasmonate sulphate	Sulfate derivative
128	30.79	_	249.0806	C 10*H* 18O 5 S	−1.4	7.1	96.9586(2)	Ethyl 7-[(methanesulfonyl) oxy]hept−2-enoate	Sulfate derivative
154	35.98	_	383.1738	C 16H 32O 8 S	1.8	12.1	303.2147(18), 217.0095(8), 96.9597(91)	Unknown sulfate derivative	Sulfate derivative
163	37.86	_	233.0853	C 10*H* 18O 4 S	−0.1	8.5	96.9591(6)	Unknown sulfate derivative	Sulfate derivative
173	40.11	_	233.0868	C 11*H* 14 N 4 S	−0.9	14.9	96.9595(12)	Unknown sulfate derivative	Sulfate derivative
198	48.02	_	365.1653	C 17H 26 N 4O 3 S	0	25	285.2092(15), 96.9588(1 0 0)	Unknown sulfate derivative	Sulfate derivative
Fatty acid and derivatives									
175	40.64	_	329.2339	C 18H 34O 5	−1.6	3.5	229.1453(47), 211.1345(72)	Trihydroxyoctadeca−10(E)-dienoic acid	Fatty acid
176	41.35	_	327.2183	C 18H 32O 5	−1.8	63.4	309.2074(19), 239.1675(1 0 0), 197.1181(43)	Trihydroxy-octadecadienoic acid I	Fatty acid
178	41.69	_	287.2231	C 16H 32O 4	−1.1	1.2	269.2135(3)	Dihydroxypalmitic acid	Fatty acid
182	42.44	_	327.2179	C 18H 32O 5	−0.6	31.9	171.1030(1 0 0),137.0974(42)	Trihydroxy-octadecadienoic acid II	Fatty acid
194	46.98	313.2373	311.2230	C 18H 32O 4	−0.7	3.6	223.1705(1 0 0)[a]	Linoleic acid hydroperoxide	Fatty acid
197	48.00	_	675.3606	C 33H 56O 14	−1.2	2.5	415.1391(32), 397.1348(1 0 0), 277.2164(34)	Gingerglycolipid A	Fatty acid
202	51.15	280.2630	_	C 18H 33 N O	1.7	3.7	119.0845(66),107.0855(69), 95.0863(1 0 0)	Linoleamide	Fatty acid
204	51.55	_	293.2129	C 18H 30O 3	−2.3	6	275.2023(1 0 0), 235.1705(84)	Oxooctadeca-dienoic acid	Fatty acid
207	52.40	_	271.2273	C 16H 32O 3	2.2	13.3	225.2189(3)	Lanopalmitic acid	Fatty acid
208	53.03	296.2569	_	C 18H 33 N O 2	5.2	7.3	196.1218(1 0 0), 95.0840(74), 109.0982(64)	Linoleylhydroxamate I	Fatty acid
210	53.43	296.2570	_	C 18H 33 N O 2	4.6	2.7	183.1357(1 0 0), 95.0839(63)	Linoleylhydroxamate II	Fatty acid
Lignan and derivatives									
85	26.08	_	581.2237	C 28H 38O 13	0.4	9	389.1582(14), 359.1524(6), 341.1391(78), 329.1396100)	(+)−5,5′-Dimethoxy−9-O-beta-D-glucopyranosyl lariciresinol (alangilignoside C)	Lignan
109	28.56	_	579.2069	C 28H 36O 13	2.5	13.8	417.1552(1 0 0)	Syringaresinol-O-glucoside	Lignan
123	30.44	_	621.2181	C 30H 38O 14	1.2	23.5	417.1535(1 0 0)	Syringaresinol acetylglucoside	Lignan
158	36.78	_	701.2455	C 35H 42O 15	−0.6	12.6	539.1921(7), 505.2052(1), 359.0968(29), 341.1392(19), 197.0457(1 0 0)	Tarennanoside G (s (þ)-isolariciresinol O-(4-hydroxy−3,5-dimethoxy)benzoyl]-hexoside)	Lignan
190	46.06	387.1769	_	C 22H 26O 6	8.6	5.8	121.0654(8), 105.0697(1 0 0)	Eudesmin	Lignan
Iridoids and derivatives									
17	10.10	_	371.0984	C 16H 20O 10	−0.2	8.7	371.0980(8), 191.0564(15)	Deacetylasperuloside I	Iridoid
22	11.20	_	371.0980	C16H20O10	1	5.9	191.0559(35), 173.0457(1 0 0), 135.0445(30)	Deacetylasperuloside II	Iridoid
32	15.90	631.2618	_	C 29H 42O 15	−3.4	6	457.2026(7), 277.1707(12), 260.1399(27), 163.0384(16), 147.0432(29)	Sylvestroside IV dimethyl acetal	Iridoid
42	18.85	_	489.1607	C21H30O13	−1.4	9.1	161.0245(17)	Acetylbarlerin	Iridoid
55	21.43	377.1445	_	C 16H 24O 10	−0.9	11.2	243.0882(1 0 0), 198.0664(14), 172.0873(18)	Loganic acid	Iridoid
63	22.81	_	371.0985	C16H20O10	−0.5	2	353.0880(2), 311.0760(3), 249.0623(1 0 0), 231.0510(11), 173.0451(19), 121.0297(78)	Deacetylasperuloside	Iridoid
145	33.30	_	751.2445	C 35H 44O 18	1.3	50	707.2533(1 0 0), 507.1579(9), 423.1791(8)	Scropolioside A	Iridoid
Terpenoids and derivatives									

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Table 1 (*continued*)

Peak	R_t (min.)	$[M + H]^+$ (*m/z*)	$[M\text{-}H]^-$ (*m/z*)	Molecular formula	Δm (ppm)	mSigma	MS/MS fragmentation[b]	**Tentative identification**	**Family**
68	23.81	429.2109		C 21H 32O 9	2.3	9.1	231.1403(17),185.1313(34),175.1137(48),157.1001(1 0 0)	Lactuside B	Sesquiterpene lactone
95	27.52	197.1171	_	C 11*H* 16O 3	0.5	5.8	179.1058(49), 161.0944(9), 135.1156(16), 107.0834(5)	Loliolide	Monoterpene lactone
122	30.29	423.2001	_	C 22H 30O 8	3	13.2	175.1479(1 0 0)	Valtratum	Terpenoid
136	31.81	475.1943	_	C 25H 30O 9	4.2	15.2	285.1132(1 0 0), 253.1111(62), 189.0903(65), 151.0733(90)	Salvinorin C I[d]	Terpenoid
138	31.98	_	717.1454	C 36H 30O 16	1	8.8	555.1159(7), 519.0919(67), 493.1124(6), 357.0606(1 0 0)	Salvianolic acid B/E/L	Terpenoid
142	32.97	609.1421	_	C 27H 28O 16	4.7	11.1	361.0904(1 0 0)	3-(6-malonyl-glucopyranosyl)-rosmarinic acid	Terpenoid derivative
144	33.19	_	537.1017	C 27H 22O 12	4	37.9	519.0931(5), 357.0614(1 0 0)	Salvianolic acid H/I	Terpenoid
146	33.63	475.1928	_	C 25H 30O 9	7.2	7.5	285.1125(1 0 0), 253.1145(71), 189.0905(75), 151.0739(94)	Salvinorin C II[d]	Terpenoid
147	33.73	_	665.2454	C 32H 42O 15	−0.5	11.2	441.1909(4), 423.1806(67), 411.1804(1 0 0)	Celangulatin E/F	Sesquiterpene
149	34.53	_	563.2133	C 28H 36O 12	0.2	21.2	387.1657(1 0 0),175.0390(15)	Bruceine C	Terpenoid
201	49.91	_	471.3467	C 30H 48O 4	2.8	38	427.3524(1)	Masilinic acid or Corosolic acid I	Triterpenoid
203	51.16	_	471.3471	C 30H 48O 4	1.8	17.2	nd	Masilinic acid or Corosolic acid II	Triterpenoid
205	51.64	553.4200	_	C 32H 52 N 6O 2	3.00	5.40	535.4097(17), 369.2750(16), 171.1137(16)	Myriconal disemicarbazone	Triterpenoid
206	52.04	541.2001	_	C 29H 32O 10	6.60	3.70	343.0798(1 0 0), 358.1019(63), 373.0893(40)	Yuanhuafine	Terpenoid derivative
211	53.76	_	295.2284	C 18H 32O 3	−1.7	3.6	277.2176(1 0 0), 195.1396(15), 171.1024(34)	Coriolic acid	Sesquiterpene
213	55.18	215.1421	_	C 15H 18O	4.2	12.2	157.1005(23), 142.0771(1 0 0)	7-Hydroxycadalin	Sesquiterpenoid
Other detected compounds									
9	4.34	_	337.0777	C 12H 18O 11	−0.1	3.6	175.0247(22)	L-Ascorbic acid hexoside	Vitamin glycoside
35	16.94	325.0915	_	C 15H 16O 8	1	23.4	163.0385(1 0 0),135.0437(25)	Umbelliferone Hexoside	Coumarin derivative
47	19.73	_	459.1501	C20H28O12	1.6	7.8	281.0665(4),161,0260(19)	Deacetylnimbin	Limonoid
52	20.80	_	387.1662	C18H28O9	0.4	10.5	225.1140(1), 207.1036(20),163.1139(3)	Tuberonic acid hexoside I	Jasmonate glycoside
61	22.36	_	471.1530	C21H28O12	−4.8	37	309.0973(1 0 0),163,0403(35)	Sibirioside A	Phenylpropanoid
64	23.12	_	399.1297	C 18H 24O 10	0	28	173.0470(21), 161.0242(77)	Regaloside A	Acylated glycerol glucosides
80	25.57	419.0955	_	C 20H 18O 10	4.3	14.2	149.0963(1 0 0)	Di(+)-(p-anisoyl)-D-tartrate	Other
100	28.04	_	387.1651	C 18H 28O 9	2.5	19.2	225.1146(3), 207.1040(1 0 0),163.1132(35)	Tuberonic acid hexoside II	Jasmonate
148	34.00	595.2354	_	C 29H 38O 13	5.3	20.9	351.1057(6), 207.0640(1 0 0)	Icariside E7	Phenylethanoid
153	35.48	407.0961	_	C 19H 18O 10	2.8	3.5	245.0452(38)	4-C-Glucosyl−1,3,7-trihydroxyxanthone (Lancerin)	Xanthone glycoside
166	39.10	_	327.2181	C 18H 32O 5	−1.3	4.2	329.0678(19), 314.0435(93), 299.0198(89), 229.1451(53), 211.1340(70)	Oxiranedioctanoic acid	Other
200	48.08	385.1982	_	C 23H 28O 5	7.2	14.5	185.1345(16), 175.1096(12), 157.1001(1 0 0)	(Acetyloxy)−17-hydroxypregna−1,4,9(11)-triene−3,20-dione	Steroid derivative
215	56.15	503.2397	_	C 31H 34O 6	6.3	8	185.1308(35), 157.0993(1 0 0)	Scilliglaucosidin 3-benzoate	Steroid derivative

*Δm: mass measurement error. HMG: 3-hydroxy-3-methylglutaryl. Nd: not detected.

[a] Fragment ions taken from the negative ion mode (in case detected in both modes).

[b] In parenthesis (relative intensity %).

[c] Confirmed by using Standard.

[d] Already identified compounds in the Micromeria family.

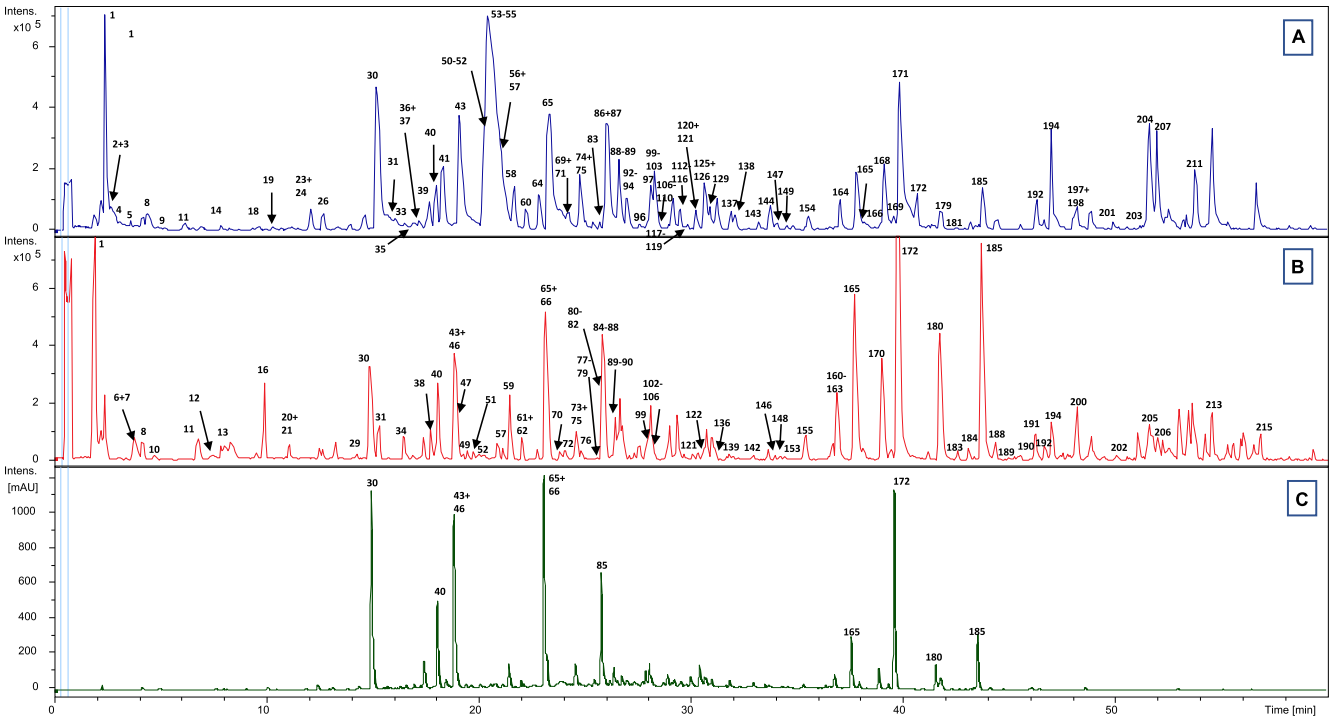

Fig. 1. Base peak chromatogram (BPC) of *M. fruticosa* leaves aqueous-methanol extract: (**A**) negative ion mode (**B**) positive ion mode and (**C**) UV spectrum (180 nm) by using HPLC-DAD-QTOF-MS^2.

addition to the MS^2 fragmentation, all of which have been used in the course of the characterization process. In the present report, a qualitative analysis of the phenolics and other phytochemicals from the hydro-methanol extract of *M. fruticosa* has been established by using UHPLC–MS^2 in two ionization modes. By using this technique, it was possible to characterize 113 & 137 compounds in the positive and negative ion modes, respectively, of which 42 compounds were detected in both ionization modes. Fig. 1 A–C corresponds to the base peak chromatograms of: A) *M. fruticosa* aqueous-methanolic extract (-ve mode), B) *M. fruticosa* aqueous-methanolic extract (+ve mode), and C) HPLC-UV profile (180 nm). A table with a list of the retention time and λ max absorption of the authentic standards used in this work is shown in Table S1.

In the chromatogram of *Micromeria fruticosa*, the elution of several compounds (**1–7**, and **10**) identified as organic acids and saccharide derivatives can be noted in the first five minutes of the analysis (Fig. 1).

3.1.2. Nitrogen containing compounds

Ten nitrogen-containing compounds identified as 5 nucleotide derivatives (**8, 14, 16, 18, and 19**), were assigned as adenine, uridine, adenosine, and adenine riboside, respectively.

Fructose-L-leucine was tentatively identified as compounds **13** and **15**, based on the MS data and the MS^2 fragmentation pattern obtained by QTOF-MS. It showed the product ions of 130.0874 and 132.9568 *m/z* (corresponding to leucine's structure). Both products were detected after the neutral loss of a fructose moiety, using the QTOF-MS analysis in the negative and positive ionization modes.

The peak **18** (***R$_t$*** 10.24 min) included the pseudomolecular ion at *m/z* 282.0852 and the fragment ion at *m/z* 150.0416 (represents guanine) with 100% relative intensity and therefore, **18** was labeled as guanine ribonucleoside.

The MS analysis of compound **20** (***Rt*** 10.90 min) displayed the molecular ion $[M + H]^+$ at *m/z* 286.0910 and the fragment ion at *m/z* 124.0386 which corresponds to nitrophenyl residue. Thus, peak **20** was tentatively assigned as *p*-nitrophenyl fucoside.

Peaks **21** and **31** were detected in the positive mode for the molecular ions at *m/z* 166.0851 and 205.0973 and characterized as phenylalanine and tryptophan, respectively.

3.1.3. Simple phenolic acid derivatives

Phenolic acids and their derivatives are widely distributed in the plants. These compounds are known to act as antioxidants owing to their ability to donate protons or electrons as well as their stable radical intermediates, which can prevent the oxidation of food components (Choe & Min, 2009).

The chromatogram of the *Micromeria fruticosa* revealed the presence of 41 phenolic acid derivatives. These derivatives were identified using accurate mass measurements in addition to the MS^2 fragmentation pattern. Otherwise, compound **23** (***Rt*** 11.26 min) was tentatively assigned to (iso)tachioside.

Peak **24** (***Rt*** 11.33 min) with a precursor ion at *m/z* 331.0669 and fragment ions at *m/z* 169.0085 (galloyl) [M-H-162]- and 125.0255 [M-H-162-44]- indicating the loss of a glucose moiety and CO_2, respectively, therefore, peak **24** was identified as glucogallin (Fig. 3a)

Peaks **30** and **40** (*m/z* 355.1012) were characterized as caffeoylquinic acid isomers, based on the mass as detected in the QTOF-MS mode. The fragmentation pattern showed the product ion at *m/z* 163.0388 (100% relative intensity) indicating the presence of caffeic acid in the structure.

Two isomers with the molecular ion at *m/z* 277.1660 were detected in the positive ion mode. In the MS^2 spectra gave two product ions at *m/z* 147.0433 and 119.0485 which correspond to a coumaroyl moiety after losing of an agmatine residue $[M + H-130]^+$. Thus, compounds **34** and **46** have been tentatively identified as coumaroylagmatine isomers. These isomers have already been found in barley and were reported to have antifungal activity (Stoessl, 1965).

Compound **38** was tentatively assigned as trihydroxybenzene malonate. This characterization was based on its MS data and the fragment ion at *m/z* 127.0379 $[M + H-86]^+$ indicating trihydroxybenzene after the loss of a malonyl moiety.

When the negative ion mode was used, four isomers (***Rt*** 17.64, 20.67, 21.34, and 21.69 min) with the molecular ion at *m/z* 337.0931

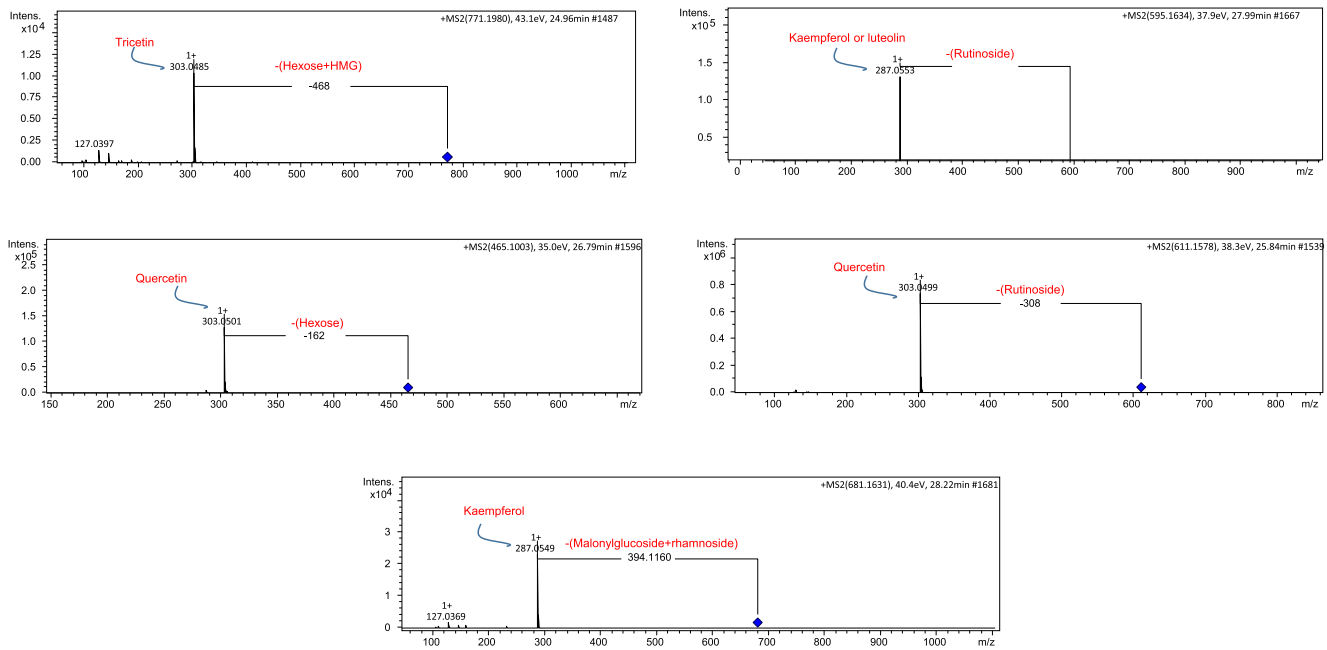

Fig. 2. MS^2 spectra and supposed fragmentation pattern of newly detected phyto-components in *M. fruticosa* by means of ESI-QTOF in the positive and negative ionization modes. **a.** Tricetin *O*-hexoside-*O*-(HMG)-hexoside, **b.** Kaempferol- or luteolin rutinoside, **c.** Quercetin hexoside, **d.** Rutin, **e.** Kaempferol 3-*O*-(6″-*O*-malonylglucoside)-7-*O*-rhamnoside.

have been detected and identified to have the same formula of $C_{16}H_{18}O_8$. All these isomers had similar fragment ion at *m/z* 191.0564 $[M\text{-}H\text{-}146]^-$ which corresponds to quinic acid after the loss of a coumaroyl moiety. Therefore, compounds **39**, **40**, **50**, and **58** have been suggested to be isomers of coumaroylquinic acid.

At the retention time, 18.89 min the precursor ion at *m/z* 355.1020 has been detected. The data from MS spectrum and the MS^2 fragmentation pattern in the positive ionization mode, which showed the product ion at *m/z* 163.0385 (indicating caffeoyl residue in structure) after a neutral loss of quinic acid. On the other hand, in the negative ionization mode, it was shown that the product ion at *m/z* 191.0565, corresponds to quinic acid. Therefore, peak **43** was identified as chlorogenic acid (Fig. 3b). Moreover, the identification was confirmed by using an external standard.

The compound **53** displayed the molecular ion at *m/z* 297.0635 using the negative ion mode. In tandem MS spectrum, it has shown the fragment ion at *m/z* 135.0315 [M-H-162]-, which denotes a threonic acid in the structure after breakage and loss of a caffeoyl moiety ([M-H-162]-). Thus, the peak **53** was assigned as *O*-caffeoyl-threonic acid (Fig. 3c).

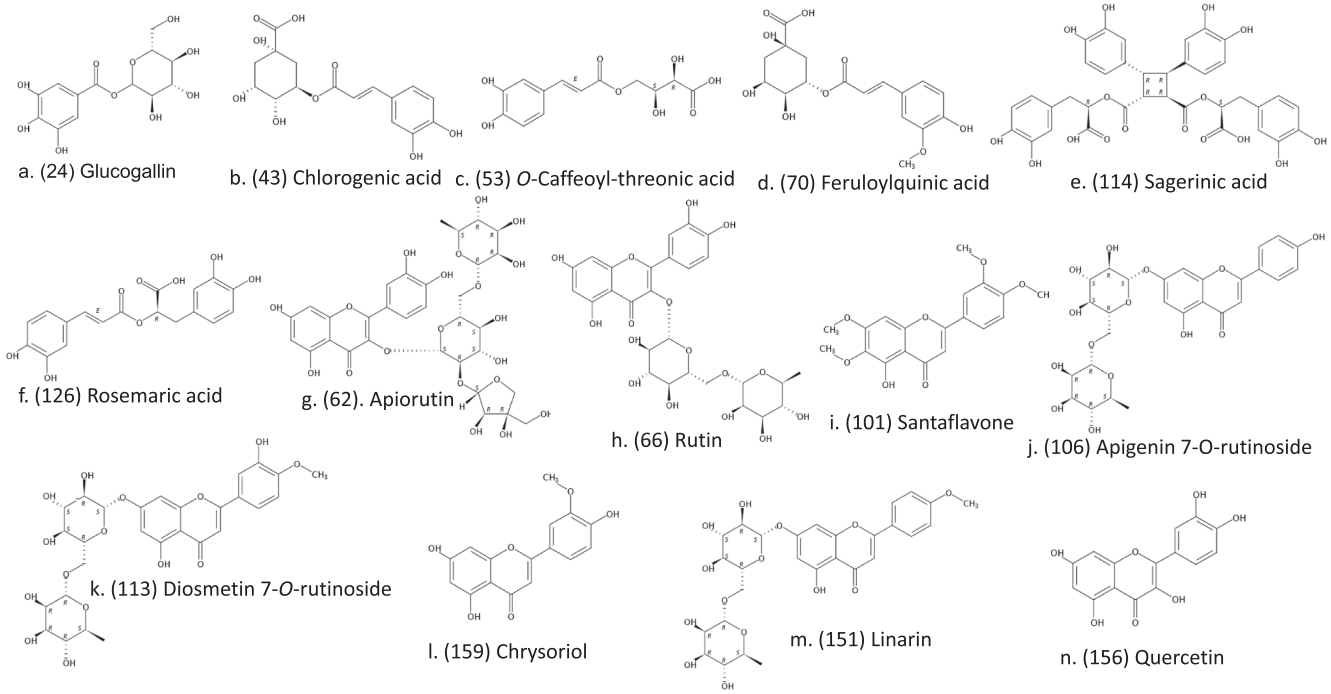

Fig. 3. Chemical structures of novel proposed compounds in *Micromeria fruticosa* leaves.

The Peaks **56** and **70** with the precursor ions at *m/z* [M-H]$^-$ 355.1016 and [M + H]$^+$ 369.1168 were identified as ferulic acid hexoside and feruloylquinic acid, respectively. In the MS^2 both compounds showed the fragment ions at *m/z* 193, which corresponds to ferulic acid [M-H-193]$^-$ after losing a hexose moiety from the compound **56**, while representing a protonated fragment ion of quinic acid [M + H-193]$^+$ for the compound **70** (Fig. 3d).

The peak **83** (*Rt* 25.84 min) exhibited the molecular ion at *m/z* 539.1188 and identified to have the molecular formula $C_{27}H_{24}O_{12}$. This compound was characterized as yunnaneic acid D. Otherwise, yunnaneic acid F has been assigned to compound **87** that was detected using both ion modes. Interestingly, these two compounds already reported in Melissa and Salvia (Lamiaceae family). (Chen, Zhang, Wang, Yang, & Wang, 2011; Barros, Dueñas, Dias, & s., Sousa, M. J. o., Santos-Buelga, C., & Ferreira, I. C. F. R. , 2013).

Compound **114** (*Rt* 29.45 min) has shown pseudomolecular ion [M-H] − at *m/z* 719.1618 and a MS^2 major fragment at *m/z* 359.0789 corresponding to [M-2H]$^{2-}$; mass characteristics coincided with those of sagerinic acid; a rosmarinic acid dimer (Fig. 3e), which was already described in other Lamiaceae plants (Rita, Pereira, Barros, Santos-Buelga, & Ferreira, 2016).

The isomers **112** and **121** at different retention times (29.11 and 30.17 min, respectively) had the characteristic molecular ion at *m/z* 517.1351/515.1193 with the molecular formula $C_{25}H_{24}O_{12}$. These compounds were identified as dicaffeoylquinic acid isomers, based on their data obtained from MS and the fragment ion at *m/z* 353.0884 [M-H-162]- and at *m/z* 191.0562 [M-H-162-162]-, which correspond to the caffeoylquinic acid and quinic acid, respectively, after the successive neutral loss of two caffeoyl residues.

Peak **126** (*Rt* 30.64 min) was identified as rosmaric acid (Fig. 3f). based on the MS data, MS^2 fragmentation pattern, it was further confirmed by using a commercial standard. This compound has been already found in other species of micromeria (Vladimir-Knezevic, Blazekovic, Stefan, & M., Alegro, A., Koszegi, T., & Petrik, J. , 2011), but is being reported here in *M. fruticosa* for the first time. Rosmarinic acid is an ester of caffeic acid. It is commonly found in species of the Lamiaceae family. It has a number of interesting biological activities, e.g. antibacterial, antiviral, antioxidant and anti-inflammatory (Petersen & Simmonds, 2003).

Compound **137** was assigned to phillygenin pentosyl-hexoside. This compound exhibited the molecular ion at *m/z* 665.2450 and the formula $C_{32}H_{42}O_{15}$. In the MS^2 spectra it showed the product ions at *m/z* 503.1936 and 371.1497 (indicating phillygenin in the structure). These ions arose after the neutral losses of hexose [M-H-162]- and pentose [M-H-132]- moieties.

3-(6-malonyl-glucopyranosyl)-rosmarinic acid has been assigned to compound **142**, where the TOF-MS analysis has shown the [M + H] + protonated ion at *m/z* 609.1421 and the fragment ion at *m/z* 361.1421 (rosmarinic acid) appeared after the neutral loss of (248 Da). This latter loss corresponds to the loss of a malonyl (−86 Da) and a glucose moiety (Fig. 4A).

Compound **143** showed a [M-H]- ion at *m/z* 499.1239 and its MS^2 spectrum yielded the fragment ion at *m/z* 337.0899 [M-H-caffeic acid-H]-. Thus, this compound was tentatively labeled as coumaroyl-caffeoylquinic acid.

3.1.4. Flavonoid derivatives

A total of 87 flavonoid derivatives have been characterized in *M. fruticosa* by using UHPLC-MS method.

For the first time, in the Micromeria and in the Lamiaceae family, this methodology allowed for the identification of 7 unusual flavonoids conjugated with 3-hydroxy-3-methylglutaryl (HMG). This fragmentation pattern was previously described by Kraut, Mues, and Sim-Sim (1993). The data obtained from MS spectra and MS^2 fragmentation pattern, a dominant neutral loss of 144 Da has been observed, which may be attributed to the loss of 3-hydroxy-3-methylglutaric acid (HMG) moiety in all compounds detected in both negative and positive ionization modes.

Thus, tricetin *O*-hexoside-*O*-(HMG)-hexoside was suggested for two isomers (**49** and **78**) which showed the [M + H]$^+$ ion at *m/z* 771.1978 and having the formula $C_{33}H_{38}O_{21}$. The MS^2 spectrum showed the fragment ion at *m/z* 465.0993 [M + H-162-144]$^+$ arose after loss of a hexose and a HMG moieties (Fig. 2a). Also, the fragment ion at *m/z* 303.0469 (representing tricetin in the structure) appeared after the loss of another hexose moiety (Kraut et al., 1993).

Similarly, peak **86** had the [M + H]$^+$/[M-H]$^-$ ions at *m/z* 741.1872/739.1721 with the molecular formula $C_{32}H_{36}O_{20}$, and it was characterized as quercetin-*O*-xylopyranosyl-*O*-(HMG)-hexoside.

Three isomers (**90, 102** and **107**) had same molecular formula $C_{27}H_{28}O_{16}$. All isomers has shown the fragment ions at *m/z* 463.0873 [M + H-144]$^+$ and at *m/z* 301.00357 [M + H-144-162]$^+$, which denoted the loss of a HMG moiety and the appearance of the quercetin after losing a hexose moiety. Thus, the three compounds were assigned as quercetin-*O*-(HMG)-hexoside isomers.

Peak **108** was proposed as kaempferol-(HMG)-rutinoside, based on the data obtained from MS and MS^2 spectra which demonstrated the fragment ions at *m/z* 593.1502 [M + H-144]$^+$ (loss of HMG) and at *m/z* 285.0390 [M + H-308]$^+$ (the loss of rutinoside and appearance of kaempferol). In the same manner, compound **124** was characterized as kaempferol-*O*-(HMG)-hexoside (Fig. 2b).

Two isomers (***Rt*** 19.02 and 21.99 min) showed the molecular ions at *m/z* 773.2144/771.1994 in the MS spectrum. Their MS^2 fragmentation pattern in the positive ion mode exhibited the product ions at *m/z* 465.0993 and at *m/z* 303.0509 (quercetin), appeared as a result of the neutral successive loss of a pentose-hexose and a hexose moieties [M + H-308-162]$^+$. Thus, compounds **45** and **59** were labeled as G-rutin isomers.

Compounds **62**, **65**, and **66** (***Rt*** 22.45, 23.15, and 23.17 min) demonstrated the same fragment ion at *m/z* 303.0492 (corresponds to quercetin in the structure) in the positive ion mode, and they were identified as apiorutin (Fig. 3g), quercetin-*O*-di-rhamnosyl-glucoside and rutin (Fig. 3h), respectively.

Quercetin hexoside was suggested for the isomers **73**, **78** and **92** at the retention times 24.56, 24.96 and 25.79 min, which displayed the same molecular formula $C_{21}H_{20}O_{12}$. MS spectrum exhibited the [M + H]$^+$ at *m/z* 465.1006 and the product ion at *m/z* 303.0490 (indicating quercetin) in the MS^2 fragmentation pattern (Fig. 2c).

Peaks **74** and **81** (***Rt*** 24.60 and 25.77 min) have been identified as isomers of quercetin rhamnosyl glucoside (Fig. 2d). Interestingly, this compound has been already described in *Micromeria cilicica* (Öztürk, Kolak, Topçu, Öksüz, & Choudhary, 2011), but reported herein in *M. fruticosa* for the first time.

The compounds **84** and **99** were tentatively identified as isomers of kaempferol-*O*-rutinoside or luteolin-*O*-rutinoside, based on the MS data and the fragmentation pattern which showed the product ion at *m/z* 278.0550 (indicates kaempferol or luteolin in the structure). It was uneasy to differentiate between the two isomers by the use of MS^2 data.

The fragment ions at *m/z* 331.0811 and 315.0715 were detected in the MS^2 spectra of peaks **94** and **97**, after each has lost a hexose moiety. Therefore, they were identified as tricin hexoside and isorhamnetin hexoside, respectively.

Peak **101** was assigned to santaflavone (Fig. 3i) based on MS and MS^2 data and the bibliography cited on the Micromeria (Tomas-Barberan, Gil, Marin, & Tomas-Lorente, 1992).

In the UHPLC-MS analysis, five novel flavonoid-malonyl-hexose conjugated compounds have been detected and characterized for the first time in *M. fruticosa*.

Compound **103** (*Rt* 28.07 min) contained the precursor ion at *m/z* 551.1016/549.0896. The MS^2 spectrum it displayed the product ion at *m/z* 303.0491 (indicating quercetin) which arose after the loss of hexose and malonyl moieties [M-H-162-86]-, respectively. Therefore, **103** has been assigned as quercetin malonyl-hexoside.

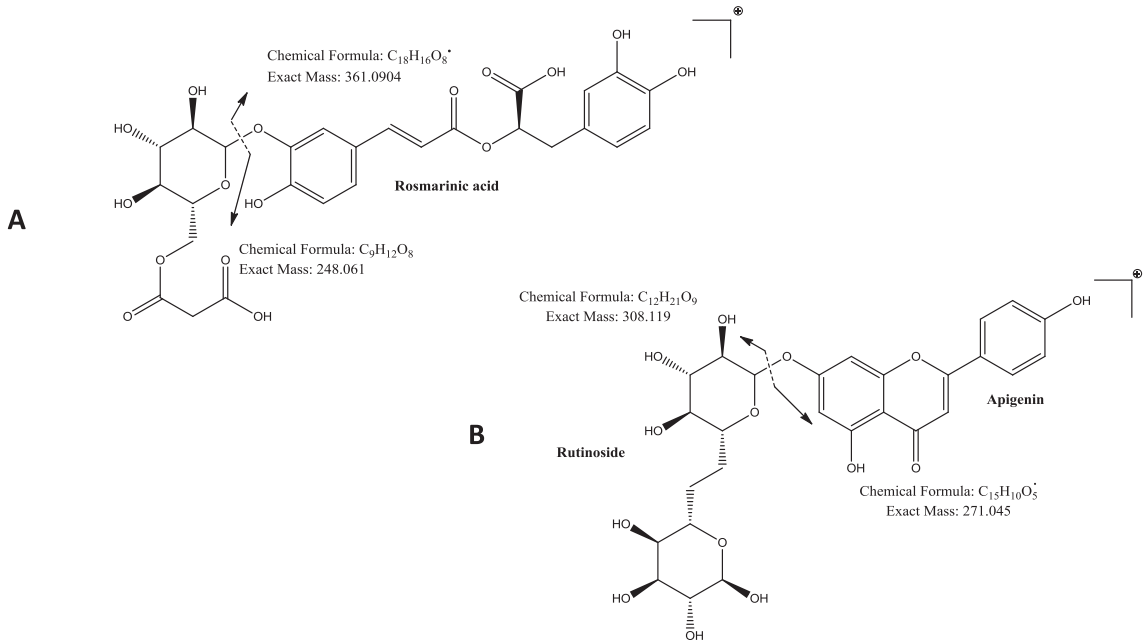

Fig. 4. Hypothesized fragmentation pattern of protonated (**A**) 3-(6-malonyl-glucopyranosyl)-rosmarinic acid and (**B**) Apigenin 7-O-rutinoside generated by ESI-QTOF analysis.

The molecular formula $C_{37}H_{28}O_{13}$ was generated by the Smart Molecular Formula™, for the protonated molecular ion at *m/z* 681.1631. The fragment ion at *m/z* 287.0549 (100% relative intensity) has been detected in the MS^2 spectrum, which may correspond to kaempferol. Thus, compound **105** was tentatively identified as kaempferol 3-*O*-(6″-*O*-malonylglucoside)-7-*O*-rhamnoside (Fig. 2e) (Veit, Beckert, Höhne, Bauer, & Geiger, 1995).

Compound **106** (*m/z* 579.1682/577.1560) has been assigned to apigenin 7-*O*-rutinoside (Fig. 3j). The tandem MS obtained from QTOF-MS^2 analysis showed the product ion at *m/z* 271.0597 (with 100% relative intensity) after the loss of rutinoside moiety $[M-H-308]^-$ (Fig. 4B).

Peak **113** (***Rt*** 29.50 min) was detected in both ionization modes with pseudomolecular ions at *m/z* 609.1787/607.1661 and had the formula $C_{28}H_{32}O_{15}$. The QTOF-MS^2 showed the fragment ion at *m/z* 299.0561 (denotes diosmetin). Thereafter, **113** was characterized to be diosmetin 7-*O*-rutinoside (Fig. 3k).

Peaks **127** and **132** were suggested as kaempferol malonyl-hexoside based on the data obtained from the MS and the MS^2 fragmentation pattern which showed the product ion after neutral loss of a hexose (−162 Da) and a malonyl moiety (−86 Da).

Similarly, compounds **133** and **150** were labeled as tricin-*O*-malonyl-hexoside and isorhamnetin-*O*-malonyl-hexoside, respectively. Tricin was represented by the fragment ion $[M + H]^+$ at *m/z* 331.0802, whereas isorhamnetin was denoted by the fragment ion $[M + H]^+$ at *m/z* 317.0692.

At the retention time 29.49 min, the pseudo-molecular ion at *m/z* 433.1104/431.0978 was detected. In the MS^2 spectrum the fragment ion at *m/z* 271 (corresponding to apigenin) has appeared after having lost a hexose moiety $[M + H-162]^+$. Thus, compound **115** has been identified as apigenin -*O*-hexoside.

On the other hand, compound **116** has been identified as apigenin 7-*O*-glucuronide depending on the MS and MS^2 data. The compound has been previously reported in *Micromeria pulegium* (Tosic et al., 2015), and is being mentioned herein for the first time in *M. fruticosa*.

The following aglycones at the retention times (36.78, 39.51, 39.55, 40.18, 41.75, and 44.06 min) have been assigned as chrysoriol (Fig. 3l), hydroxy-di-*O*-methylluteolin, naringenin, thymonin, dihydroxy-trimethoxyflavone, and methylsudachitin, respectively. These flavonoids were already noted in other Micromeria species and are being reported here in *M. fruticosa* for the first time.

In this work, the use of the UHPLC-MS method has facilitated the detection of flavonoid acylated hexose compounds. The fragmentation pattern in the MS^2 showed the loss of hexose and acetyl moieties $[M-H-162-42]^-$. Based on these data and on the appearance of the product ions at *m/z* 303.0509 (quercetin in positive ion mode) and at *m/z* 285.0410 (kaempferol in negative ion mode), compounds **118** and **131** have been characterized as quercetin-*O*-acetylhexoside and kaempferol-*O*-acetylhexoside, respectively.

Hydroxyarctigenin-*O*-hexoside has been suggested for compound **134**, based on the MS data and the MS^2 spectrum which showed the product ion at *m/z* 387.1653 (corresponds to hydroxyarctigenin) arose after having lost a moiety of hexose.

Compound **151** (*Rt* 34.59 min) displayed the precursor ion $[M + H]^+$ at *m/z* 593.1851 with the molecular formula $C_{28}H_{33}O_{14}$. In the MS^2 spectrum, the product ion at *m/z* 285.0757 (corresponds to acacetin in the structure) appeared after a loss of a rutinoside moiety. Therefore, **151** was characterized as acacetin-7-*O*-rutinoside (linarin) (Fig. 3m). This compound was already detected in other Micromeria species (Marin, Grayer, Veitch, Kite, & Harborne, 2001). This compound has been reported with anti-Alzheimer effect (Lou, Fan, Perez, & Lou, 2011).

The aglycones luteolin, quercetin (Fig. 3n), apigenin, kaempferol (**155**, **156**, **167**, and **172**) have been identified for the first time in *M. fruticosa* based on the data obtained from the MS and MS^2 data, and confirmation by using commercial standards.

In the QTOF-MS^2 analysis, compounds **189** and **193** (*Rt* 45.26 and 46.67 min) have shown the fragments signals at *m/z*: 331.0803 and at *m/z* 361.0894, $[M-H-166]^-$ (acyl-18) indicating same neutral loss of an *O*-acyl moiety (Benayad, Gomez-Cordova, & Es-Safi, 2014). Therefore,

compounds **189** and **193** have been tentatively assigned as methoxygalloyl-tricin and methoxygalloyl-rosemarinic acid, respectively.

Three isomers (**160, 165** and **174**) detected at *m/z* 331.0803/329.0667 in both ionization modes and had the same formula $C_{17}H_{14}O_7$. In the MS^2 spectra they possessed the same product ions at *m/z* 316.0569 and 301.0357, and they were tentatively labeled as tricin or jaseocidin isomers.

Based on the data obtained from MS and MS^2 spectra and on the bibliography cited (Tomas-Barberan, Husain, & Gil, 1988), peaks **183** and **191** have been identified as diosmetin and dihydroxy-trimethoxyflavone, respectively.

3.1.5. Sulfoconjugate derivatives

Mass spectrometry has proved useful to detect several novel sulfated metabolites. In the MS^2 spectra the product ion at m/z 96.9590 (HSO_4^-) which indicates the presence of the aliphatic sulfate has been observed. Otherwise, the aromatic sulfate conjugation is implied by the presence of product ion at *m/z* 80.0130 (Weidolf, Lee, & Henion, 1988). However, in our present work we only observed the product ion at *m/z* 96.9588 which corresponds to the presence of aliphatic sulfate. In addition, neutral loss (80 Da) for the elements of S—O is characteristic of the presence of aryl sulfate esters (Rudewicz & Straub, 1986).

In this work, eight naturally occurring sulfate derivatives have been detected by using the UHPLC-MS^2 method exclusively in the negative ionization mode.

The compound **25** (***Rt*** 12.05 min) showed the precursor ion at *m/z* 409.0444 and had the molecular formula $C_{14}H_{18}O_{12}S$. In the MS^2 spectrum, it had the product ions at *m/z* 329.0877 (indicating vanillic acid glucopyranoside) after the neutral loss of 80 Da (aromatic sulfate). Thus, **25** was proposed as vanillic acid-*O*-β-D-glucopyranoside-6′-sulfate. This compound is also termed "Periodic Leaf Movement Factor 5" (PLMF 5).

In the QTOF-MS^2 the fragment ions at *m/z* 181.0519 $[M-H-80]^-$ (dihydrocaffeic) and 96.9605 (HSO_4^-) have been detected for the molecular ion at *m/z* 261.0072 which corresponds to peak **28**. This compound had the molecular formula ($C_9H_{10}O_7S$) and was tentatively identified as dihydrocaffeic acid-3-*O*-sulfate (Mullen, Borges, Lean, Roberts, & Crozier, 2010).

Compounds **48** and **128** had the precursor $[M-H]^-$ ions at *m/z* 305.0708 and at *m/z* 249.0806 and were tentatively characterized as methyl-hydroxyjasmonate sulphate and ethyl 7-[(methanesulfonyl)oxy] hepta-2-enoate, respectively.

Additional 4 unknown sulfate derivatives have been detected in the *M. fruticosa* extract by using UHPLC-MS method.

3.1.6. Lignan derivatives

The lignans are bioactive, non-nutrient, non-caloric phenolic plant compounds that are found in seeds, herbs, fruits and vegetables (Peterson et al., 2010). Four lignan derivatives have been detected in the *M. fruticosa*.

Peak **85** (26.08 min) had the pseudomolecular ion at *m/z* 581.2237 and the fragment ion at *m/z* 329.1524 (characteristic of lariciresinol in structure), and thus it was identified as (+)-5,5′-dimethoxy-9-*O*-β-D-glucopyranosyl lariciresinol (alangilignoside C).

Two compounds **109** and **123** (***Rt*** 28.56 and 30.44 min) had shown the same fragment ion at *m/z* 417.1552 (100% relative intensity) which corresponds to syringaresinol in the structure. Peak **109** displayed the fragment ion after losing a hexose moiety $[M-H-162]^-$, while a neutral loss of (−42 Da) which represent loss of a hexose and an acetyl residue noted in the MS^2 spectrum of **123**. Therefore, **109** and **123** have been tentatively identified as syringaresinol-glucoside and syringaresinol-acetylglucoside, respectively.

Peak **190** has been assigned to eudesmin, based on the correct and acceptable date obtained from the QTOF-MS analysis.

3.1.7. Iridoid derivatives

Seven iridoid derivatives have been detected and identified in *M. fruticosa* for the first time by QTOF-MS mode using accurate mass measurements and MS^2 fragmentation pattern. Among them, three isomers of deacetylasperuloside (*m/z* 371.0985 in the negative ion mode) have been assigned for compounds **17**, **22**, and **63** which have shown identical molecular formula $C_{16}H_{20}O_{10}$.

Peak **32** with the pseudomolecular $[M + H]^+$ ion at *m/z* 631.2618 had the formula $C_{29}H_{42}O_{15}$ and was proposed to be sylvestroside IV dimethyl acetal. The fragmentation pattern of this compound has already been discussed elsewhere (Tomassini, Foddai, & Nicoletti, 2004).

Compounds **42** and **55** (***Rt*** 18.85 and 21.43 min) have been tentatively characterized as acetylbarlerin and loganic acid, respectively.

3.1.8. Terpenoid derivatives

A total of 16 terpenoid derivatives has been characterized in the hydro-methanolic extract of *Micromeria fruticosa*.

Two isomers (**136** and **146**) were detected in the MS-analysis (positive ion mode) at the molecular ion *m/z* 475.1943 and showed same molecular formula $C_{25}H_{30}O_9$. These isomers were assigned as salvinorin C. This compound was already reported in *Micromeria pulegium* (Tosic et al., 2015), but for the first time in *M. fruticosa*.

Compound **142** (***Rt*** 32.97 min) has been characterized as 3-(6-malonyl-glucopyranosyl)-rosmarinic acid. In the MS^2 spectrum, it was detected the product ion at *m/z* 361.0904 (corresponds to rosmarinic acid) appeared after the neutral loss of a glucopyranose and a malonyl residues $[M + H-162-86]^+$ (Selenge, Murata, Tanaka, Sasaki, Batkhuu, & Yoshizaki, 2014). This compound is reported here in *M. fruticosa* for the first time.

Peaks **138** and **144** have been tentatively identified as salvianolic acid B/E/L and salvianolic acid H/I, respectively.

Two isomers were detected at the retention time 49.91 and 51.16 min and showed the same $[M-H]^-$ molecular ion at *m/z* 471.3467. Both isomers were tentatively characterized as masilinic acid or corosolic acid isomers. These terpenoids have been previously noted in the Lamiaceae family (Hasan, Al-Jaber, Al-Qudah, & Abu Zarga, 2016).

Four sesquiterpene derivatives were detected and characterized in the *M. fruticosa* extract using QTOF-MS in the positive and negative ionization modes, and the literature cited when applicable.

Thus, compounds **68**, **147**, **211**, and **213** have been tentatively identified as lactuside B, celangulatin E/F, coriolic acid (Babovic et al., 2010), and 7-hydroxycadalin, respectively.

3.1.9. Fatty acids and derivatives

Eleven compounds were detected as belonging to fatty acids and derivatives in *Micromeria* analyzed sample. Compound **197** was suggested to be gingerglycolipid A based on the acceptable data obtained from the MS and MS^2 spectra.

Two isomers (**208** and **210**) had the same molecular ion $[M + H]^+$ at *m/z* 296.2569 and the formula $C_{18}H_{33}NO_2$. Both compounds have been tentatively identified as linoleylhydroxamate isomers. The fragmentation pattern of this fatty compound has already been identified in Sumac (Abu-Reidah, Ali-Shtayeh, Jamous, Arráez-Román, & Segura-Carretero, 2015).

Other fatty compounds also were characterized based on their correct data by QTOF-MS and the previous bibliography cited.

3.1.10. Other compounds detected

A total of 16 other compounds has been characterized in *M. fruticosa* for the first time. A glycoside compound (**9**) had the product ion at *m/z* 175.0247 (ascorbic acid), after having lost a neutral loss of a hexose residue. Therefore, **9** was suggested as L-ascorbic acid hexoside.

Peak **47** (***Rt*** 19.73 min) had the molecular $[M-H]^-$ ion at *m/z* 459.1501 and the molecular formula $C_{20}H_{28}O_{12}$. This compound has been assigned as deacetylnimbin. A limonoid which has previously detected in neem (Pandreka et al., 2015).

Two isomers (**52** and **100**) had an identical precursor ion at *m/z* 387.1651 [M-H]$^-$ and a fragment ion at *m/z* 225.1140 (corresponds to tuberonic acid in the structure). Thus, both isomers were identified as tuberonic acid hexoside.

Compound **80** has been tentatively identified as di(+)-(p-anisoyl)-D-tartrate. Its characterization was based on MS and MS^2 spectrum which displayed the product ion at *m/z* 149.0963 (relative intensity 100%) after having lost a di-anisoyl residue (−270 Da).

Finally, peak **153** was detected at the retention time (***Rt*** 35.48 min) with the molecular ion at *m/z* 407.0961 ($C_{19}H_{18}O_{10}$). Peak **153** has been assigned as trihydroxyxanthone glucoside. The MS^2 spectrum showed the product ion at *m/z* 245.0452 (indicating trihydroxyxanthone) which arose after the neutral loss of a hexose residue [M + H-162]$^+$.

4. Conclusion

In this work an extensive metabolite profiling and fingerprinting of the bioactive components in the hydro-methanolic extract obtained from the *Micromeria fruticosa* leaves has been carried out by using a UHPLC–DAD–ESI–QTOF–MS^2 method, supported with high resolution and mass accuracy, which facilitated the phyto-metabolites' untargeted characterization, based on MS and MS/MS spectra in negative and positive ion modes, together with the related data from the literature cited. The use of the proposed method has been supportive to detect and to characterize 215 metabolites, thereof, over 180 phytochemicals including mainly: flavonoids, phenolic acids, terpenoids, organic acids, iridoids, lignans, being reported herein in *M. fruticosa* leaves for the first time. By using the ESI-QTOF-MS^2 method totals of 137, 118, and 42 compounds have been identified in the negative, positive, and in (both) modes, respectively, suggesting that the use of the two modes is an indispensable tool for the complete characterization process of plant metabolites. The results highlight the importance of *M. fruticosa* as a source of functional phyto-constituents. Also, the data may encourage other uses of this plant, as potential inclusion in the food, pharma and nutraceutical industries. Further research on these identified metabolites bioactivity and quantitation is necessary.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.11.144.

References

Mullen, W., Borges, G., Lean, M. E. J., Roberts, S. A., & Crozier, A. (2010). Identification of metabolites in human plasma and urine after consumption of a polyphenol-rich juice drink. *Journal of Agricultural and Food Chemistry, 58*(4), 2586–2595.

Abu-Gharbieh, E., & Ahmed, N. G. (2016). Bioactive content, hepatoprotective and antioxidant activities of whole plant extract of *Micromeria fruticosa* (L) Druce ssp Serpyllifolia F Lamiaceae against Carbon tetrachloride-induced hepatotoxicity in mice. *Tropical Journal of Pharmaceutical Research, 15*(10), 2099–2106.

Abu-Gharbieh, E., Shehab, N. G., & Khan, S. A. (2013). Anti-inflammatory and gastroprotective activities of the aqueous extract of *Micromeria fruticosa* (L.) Druce ssp Serpyllifolia in mice. *Pakistan Journal Pharmaceutical Sciences, 26*, 799–803.

Abu-Reidah, I.M., Ali-Shtayeh, M.S., Jamous, R.M., Arráez-Román, D., & Segura-Carretero, A. (2015). HPLC–DAD–ESI-MS/MS screening of bioactive components from Rhus coriaria L. (Sumac) fruits. Food Chemistry, 166(Supplement C), 179–191.

Azab, A. (2016). Micromeria: Chemistry and Medicinal Activities, European Chemical Bulletin, 5(7), 300–307.

Babovic, N., Djilas, S., Jadranin, M., Vajs, V., Ivanovic, J., Petrovic, S., et al. (2010). Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity. *Innovative Food Science & Emerging Technologies, 11*(1), 98–107.

Balasundram, N., Sundram, K., & Samman, S. (2006). Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. *Food Chemistry, 99*(1), 191–203.

Barros, L., Dueñas, M., Dias, M. I.s., Sousa, M. J. O., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Phenolic profiles of cultivated, in vitro cultured and commercial samples of *Melissa officinalis* L. infusions. *Food Chemistry, 136*(1), 1–8.

Benayad, Z., Gomez-Cordova, C., & Es-Safi, N. E. (2014). characterization of flavonoid glycosides from Fenugreek (*Trigonella foenum-graecum*) crude seeds by HPLC-DAD-ESI/MS analysis. *International Journal of Molecular Sciences, 15*(11), 20668–20685.

Chen, H., Zhang, Q., Wang, X., Yang, J., & Wang, Q. (2011). Qualitative analysis and simultaneous quantification of phenolic compounds in the aerial parts of *Salvia miltiorrhiza* by HPLC-DAD and ESI/MS^n. *Phytochemical Analysis, 22*, 247–257.

Choe, E., & Min, D. B. (2009). Mechanisms of antioxidants in the oxidation of foods. *Comprehensive Reviews in Food Science and Food Safety, 8*(4), 345–358.

Dudai, N., Larkov, O., Ravid, U., Putievsky, E., & Lewinsohn, E. (2001). Ddevelopmental control of monoterpene content and composition in *Micromeria fruticosa* (L.) Druce. *Annals of Botany, 88*, 349–354.

Formisano, C., Mignola, E., Rigano, D., Senatore, F., Bellone, G., Bruno, M., et al. (2007). Chemical composition and antimicrobial activity of the essential oil from aerial parts of *Micromeria fruticulosa* (Bertol.) Grande (Lamiaceae) growing wild in Southern Italy. *Flavour and Fragrance Journal, 22*(4), 289–292.

Güllüce, M., Sökmen, M., Şahin, F., Sökmen, A., Adigüzel, A., & Özer, H. (2004). Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp serpyllifolia (Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *Journal of the Science of Food and Agriculture, 84*(7), 735–741.

Harnly, J. M., Bhagwat, S., & Lin, L. Z. (2007). Profiling methods for the determination of phenolic compounds in foods and dietary supplements. *Analytical and Bioanalytical Chemistry, 389*, 47–61.

Hasan, M. R., Al-Jaber, H. I., Al-Qudah, M. A., & Abu Zarga, M. H. (2016). New sesterterpenoids and other constituents from *Salvia dominica* growing wild in Jordan. *Phytochemistry Letters, 16(Supplement C)*, 12–17.

Kachlicki, P., Piasecka, A., Stobiecki, M., Marczak, Å., & u (2016). Structural Characterization of Flavonoid Glycoconjugates and Their Derivatives with Mass Spectrometric Techniques. *Molecules, 21*(11), 1494.

Koc, K., Ozdemir, O., Kizilkaya, F. O., Sengul, M., & Turkez, H. (2017). Cytotoxic activity of the aqueous extract of Micromeria fruticosa (L.) Druce subsp. serpyllifolia on human U-87 mg cell lines. Archives of Biological Sciences, 69, 449–453.

Kraut, L., Mues, R., & Sim-Sim, M. (1993). Acylated flavone and glycerol glucosides from two Frullania species. *Phytochemistry, 34*, 211–218.

Lou, H., Fan, P., Perez, R. G., & Lou, H. (2011). Neuroprotective effects of linarin through activation of the PI3K/Akt pathway in amyloid-β-induced neuronal cell death. *Bioorganic & Medicinal Chemistry, 19*(13), 4021–4027.

Marin, P. D., Grayer, R.e. J., Veitch, N. C., Kite, G. C., & Harborne, J. B. (2001). Acacetin glycosides as taxonomic markers in Calamintha and Micromeria. *Phytochemistry, 58*(6), 943–947.

Öztürk, M., Kolak, U., Topçu, G., Öksüz, S., & Choudhary, M. I. (2011). Antioxidant and anticholinesterase active constituents from *Micromeria cilicica* by radical-scavenging activity-guided fractionation. *Food Chemistry, 126*(1), 31–38.

Pandreka, A., Dandekar, D. S., Haldar, S., Uttara, V., Vijayshree, S. G., Mulani, F. A., et al. (2015). Triterpenoid profiling and functional characterization of the initial genes involved in isoprenoid biosynthesis in neem (*Azadirachta indica*). *BMC Plant Biology, 15*, 214.

Petersen, M., & Simmonds, M. S. J. (2003). Rosmarinic acid. *Phytochemistry, 62*, 121–125.

Peterson, J., Dwyer, J., Adlercreutz, H., Scalbert, A., Jacques, P., & McCullough, M. L. (2010). Dietary lignans: Physiology and potential for cardiovascular disease risk reduction. *Nutrition Reviews, 68*(10), 571–603.

Rita, I., Pereira, C., Barros, L., Santos-Buelga, C., & Ferreira, I. C. F. R. (2016). *Mentha spicata* L. infusions as sources of antioxidant phenolic compounds: Emerging reserve lots with special harvest requirements. *Food & Function, 7*(10), 4188–4192.

Rudewicz, P., & Straub, K. M. (1986). Rapid structure elucidation of catecholamine conjugates with tandem mass spectrometry. *Analytical Chemistry, 58*(14), 2928–2934.

Selenge, E., Murata, T., Tanaka, S., Sasaki, K., Batkhuu, J., & Yoshizaki, F. (2014). Monoterpene glycosides, phenylpropanoids, and acacetin glycosides from Dracocephalum foetidum. Phytochemistry, 101(Supplement C), 91–100.

Shehab, N. G., & Abu-Gharbieh, E. (2012). Constituents and biological activity of the essential oil and the aqueous extract of *Micromeria fruticosa* (L.) Druce subsp. Serpyllifolia. *Pakistan Journal Pharmaceutical Sciences, 25*, 687–692.

Simirgiotis, M. J., Caligari, P. D. S., & Schmeda-Hirschmann, G. (2009). Identification of phenolic compounds from the fruits of the mountain papaya Vasconcellea pubescens A. DC. grown in Chile by liquid chromatography-UV detection-mass spectrometry. *Food Chemistry, 115*(2), 775–784.

Stoessl, A. (1965). The antifungal factors in barley-III.: Isolation of p-coumaroylagmatine. *Phytochemistry, 4*, 973–976.

Telci, I., & Ceylan, M. (2007). Essential oil composition of *Micromeria fruticosa* Druce from Turkey. *Chemistry of Natural Compounds, 43*(5), 629–631.

Tomas-Barberan, F. A., Gil, M. I., Marin, P. D., & Tomas-Lorente, F. (1992). Flavonoids from some Yugoslavian Micromeria Species: Chemotaxonomical Aspects. *Biochemical Systematics and Ecology, 19*(8), 697–698.

Tomas-Barberan, F. A., Husain, S. Z., & Gil, M. I. (1988). The distribution of methylated flavones in the Lamiaceae. *Biochemical Systematics and Ecology, 16*(1), 43–46.

Tomassini, L., Foddai, S., & Nicoletti, M. (2004). Iridoids from *Dipsacus ferox*

(Dipsacaceae). *Biochemical Systematics and Ecology, 32*(11), 1083–1085.
Tosic, S., Stojicic, D., Stankov-Jovanovic, V., Mitic, V., Mihajilov-Krstev, T., & Zlatkovic, B. (2015). Chemical composition, antioxidant and antimicrobial activities of micropropagated and native *Micromeria pulegium* (Lamiaceae) extracts. *Oxidation Communications, 38*(1), 55–66.
Veit, M., Beckert, C., Höhne, C., Bauer, K., & Geiger, H. (1995). Interspecific and intraspecific variation of phenolics in the genus Equisetum subgenus Equisetum. *Phytochemistry, 38*(4), 881–891.
Vladimir-Knezevic, S., Blazekovic, B., Stefan, M. B., Alegro, A., Koszegi, T., & Petrik, J. (2011). Antioxidant Activities and Polyphenolic Contents of Three Selected Micromeria Species from Croatia. *Molecules, 16*(2), 1454–1470.
Weidolf, L. O. G., Lee, E. D., & Henion, J. D. (1988). Determination of boldenone sulfoconjugate and related steroid sulfates in equine urine by high-performance liquid chromatography/tandem mass spectrometry. *Biological Mass Spectrometry, 15*(5), 283–289.
Wolfender, J.-L., Marti, G., Thomas, A.l., & Bertrand, S. Current approaches and challenges for the metabolite profiling of complex natural extracts. Journal of Chromatography A, 1382, 136–164.
Yaniv, Z., & Dudai, N. (2014). *Medicinal and Aromatic Plants of the Middle-East.* Netherlands: Springer.