Kragujevac J. Sci. 40 (2018) 113-129. UDC 581.192.2:582.893.6

THE INVESTIGATION OF BIOACTIVE SECONDARY METABOLITES OF THE METHANOL EXTRACT OF *ERYNGIUM AMETHYSTINUM*

Nenad L. Vuković[1*], Milena D. Vukić[1], Gorica T. Đelić[2], Miroslava M. Kacaniova[3], Mirjana Cvijović[4]

[1] *University of Kragujevac, Faculty of Science, Department of Chemistry, P.O. Box 60, 34000 Kragujevac, Serbia*
[2] *University of Kragujevac, Faculty of Science, Department of Biology and Ecology, P.O. Box 60, 34000 Kragujevac, Serbia*
[3] *Slovak University of Agriculture in Nitra, Department of Microbiology, Faculty of Biotechnology and Food Science, Nitra, Slovakia*
[4]*University of Belgrade, Faculty of Medicine, Department for Clinical Microbiology and Immunology, Dr Subotića 1, 11000 Belgrade, Serbia*
[*]Corresponding author; E-mail: nvukovic@kg.ac.rs

(Received June 22, 2017; Accepted September 10, 2017)

ABSTRACT. *Eryngium amethystinum* L. belonging to the *Apiaceae* family, is a perennial plant distributed in Southeast Europe. Even though this plant is used in traditional medicine, its phytochemical characterization is still incomplete. In this study composition of bioactive constituents of the methanol extract are reported for the first time. By means of the UPLC-LTQ-Orbitrap-MS^n method, altogether sixty-three constituents were characterized: eight hydroxybenzoic acid derivatives (**7**-**13**, **32**), fifteen cinnamic acid derivatives (14, 17-19, 21, 24-26, 28, 30, 39-42 and 44), four flavonoid aglycones (45, 51, 52, 54), twenty-four flavonoid derivatives (23, 27, 29, 31, 33-38, 43, 46-50, 53, 55-59, 61 and 62), three coumarin derivatives (15, 16 and 22) and nine other compounds (1-6, 20, 60 and 63).

Keywords: metabolic profiling, LC-HRMS, phenolic compounds.

INTRODUCTION

The genus *Eryngium* L. is distributed all around the world, and with more than 200 species represents the taxonomically most complex genus of the family *Apiaceae* (WORZ, 2004; CALVINO *et al.*, 2007).

Many plants from this genus have valuable ethnopharmacological and nutritional values (FACCIOLA, 1990; ZHANG *et al.*, 2008).

Eryngium amethystinum L., commonly known as amethyst sea holly, is distributed in western and central Serbia on calcareous and arid soil, up to 1600 m above the sea level. In folk medicine of Southeast Europe *E. amethystinym* is used for its diuretic and laxative

properties, in treatment of edemas, urinary ailments, and acidosis. It is also useful as an aid to digestion (FLAMINI *et al.*, 2008).

Previous chemical investigations of *Eryngium* species revealed the presence of various class of natural products: polyphenols, coumarins, saponins, acetylenes, essential oils (CROWDEN *et al.*, 1969; DRAKE and LAM, 1972; KARTAL *et al.*, 2006; FLAMINI *et al.*, 2008; ZHANG *et al.*, 2008).

Linear ion trap quadrupole-Orbitrap-mass spectrometry (LTQ-Orbitrap-MS) delivers single-stage mass analysis providing molecular mass information, two-stage mass analysis (MS^2) and multi-stage mass analysis (MS^n) delivering structural information. Exact mass measurements and elemental composition assignment are essential for the characterization of small molecules. Accurate mass measurement of the product ions formed in MS^n experiments facilitates the elucidation of the structures of unknown compounds (TCHOUMTCHOUA *et al.*, 2013).

The aim of the present study was to characterize the secondary bioactive metabolites in the methanol extract of *E. amethystinum* by using UPLC-$HRMS^n$. The chemical structures of the identified compounds and their glycoside derivatives are important to reveal information on possible bioactive effect of examined plant.

In this paper we presented for the first time chemical profiling and levels of antioxidant activitiy of the methanol extracts of wild growing *E. amethystinum*.

MATERIALS AND METHODS

Chemicals and reagents

LC-MS grade formic acid and acetonitrile were purchased from Thermo Scientific Pierce (Thermo Fisher Scientific, Pierce Biotechnology, Rockford, IL, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Brea, CA, USA).

Plant material and sample preparation

Eryngium amethystinum aerial parts were collected (Jun 2014) in the region of Raška (south Serbia, altitude 350 m, 43º30’53’’N, 20º37’07’’E) and identified at the Department of Biology and Ecology, Faculty of Science in Kragujevac, University of Kragujevac (Serbia). Voucher specimen (17088, BEOU) was deposited at the Department of Botany, Faculty of Biology, University of Belgrade, Serbia.

The collected plant material was air-dried in darkness at ambient temperature. A portion of the sample (20 g) was ground to a thin powder, and extracted three times (at room temperature, 24h) with HPLC grade methanol (200 mL). Methanol extracts were combined and evaporated under reduced pressure by means of rotary evaporator at 40 ºC. A portion of crude extract (50 mg) was dissolved in methanol, filtered through a membrane filter and used directly for LC-MS analysis.

UPLC-LTQ-Orbitrap-MS for metabolomic analysis

Chromatographic separations were performed using an ultrahigh-performance liquid chromatography (UPLC) system consisting of a quaternary Accela 600 pump and Accela Autosampler (ThermoFisher Scientific, Bremen, Germany). Analytical column used for separations was a Syncronis C_{18} column (100 x 2.1 mm, 1.7 μm particle size, ThermoFisher Scientific). The mobile phase consisted of (A) ultrapure water with 1% formic acid and (B)

acetonitrile (MS grade). The injection volume for the sample was 10 μL, elution gradient programme was 5-95 % B for 20 min, with the flow rate of 0.3 mL/min.

The UPLC system was coupled to a linear-trap quadrupole (LTQ) orbitrap hybrid mass spectrometer equipped with a heated-electrospray ionisation probe (HESI-II, ThermoFisher Scientific, Bremen, Germany). The mass spectra were obtained in negative and positive ion modes. Operation parameters were as follows: source voltage 4.5 kV (4.2 kV in positive mode), capillary voltage -10 V (42 V in positive mode), tube lens voltage -35 V (110 V in positive mode), capillary temperature 300 ºC, sheath and auxiliary gas flow (N_2) 32 and 8 (arbitrary units), respectively. The MS spectra were acquired by full range acquisition covering 100-1000 m/z, and for the fragmentation study, a data dependent scan was performed by deploying collision-induced dissociation (CID): the normalized collision energy of the CID cell was set to 35 eV.

Compounds were identified according to the corresponding spectral characteristics: mass spectra, accurate mass, characteristic fragmentation and characteristic retention time. ThermoFisher Scientific Xcalibur software (Version 2.1) was used for instrument control, data acquisition and data analysis.

RESULTS AND DISCUSSION

UPLC-LTQ-Orbitrap-MS^n identification of secondary metabolites of E. amethystinum

In the present work, a total of sixty-three compounds were tentatively identified in the sample, mainly phenolic compounds such as: simple phenols, flavonoids, coumarins and other compounds. UPLC-LTQ-Orbitrap MS^n chromatograms were given in Figure 1 and Figure 2. The tentatively identified metabolites are summarized in Table 1, in negative and positive ionization modes, including retention times, experimental and calculated $[M-H]^-$ and $[M+H]^+$, errors in ppm, MS^2 and MS^3 fragment ions (in negative and positive modes), molecular formula, together with their proposed identities. The compounds were identified by interpreting their mass spectra obtained via LTQ-Orbitrap high resolution mass spectrometer and taking into account the data reported in the literature.

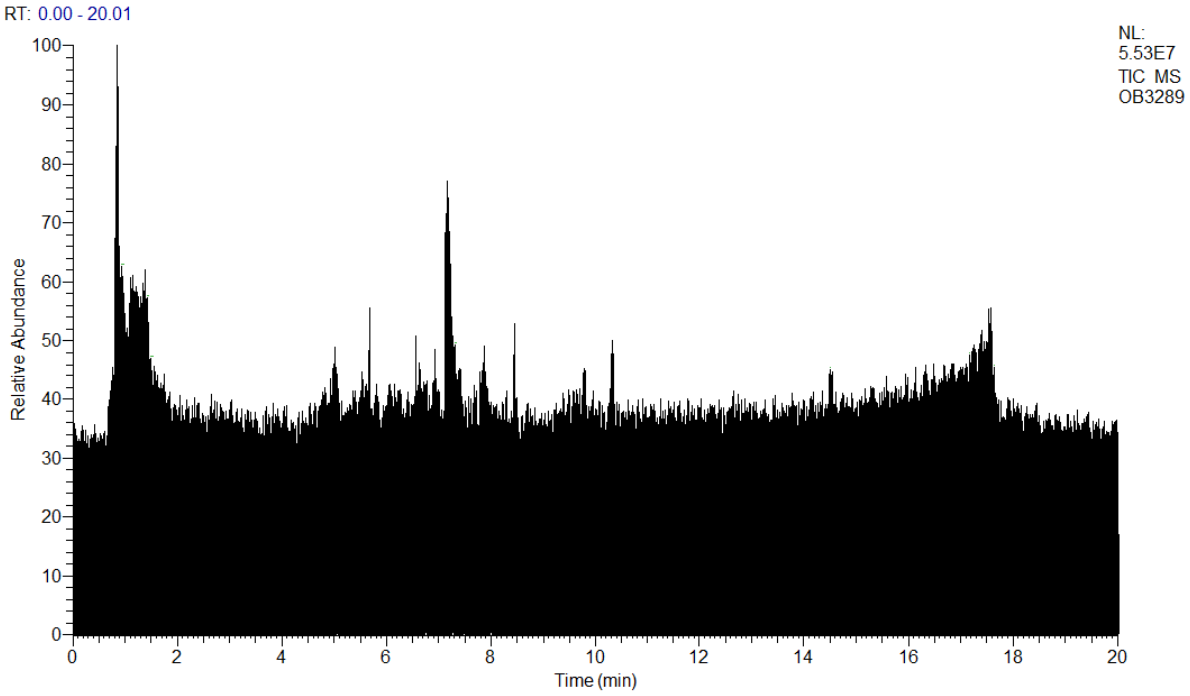

Figure 1. UPLC-LTQ-Orbitrap MS^n in negative mode of methanol extract of *E. amethystinum.*

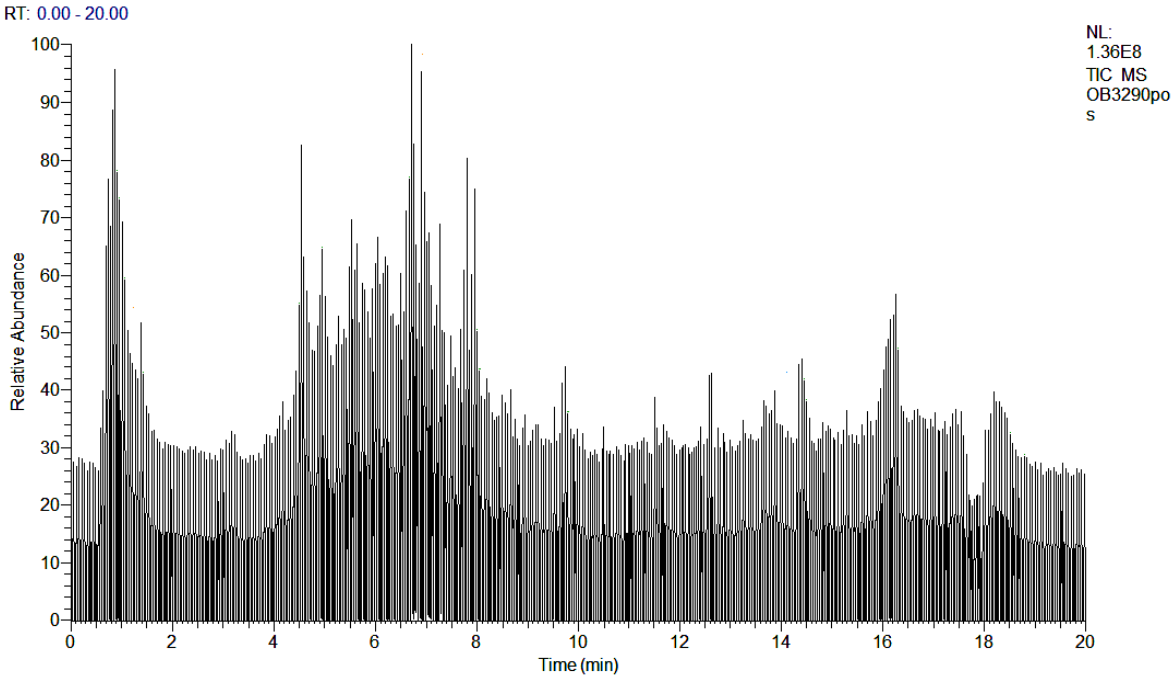

Figure 2. UPLC-LTQ-Orbitrap MS^n in positive mode of methanol extract of *E. amethystinum.*

Table 1. List of identified compounds with peak numbers, retention times (t_r), experimental and calculated masses, error (ppm), MS^2 and MS^3 fragments, and molecular formulas.

Peak	Proposed compounds	t_r (min)	Exp. *m/z* $[M-H]^-$/ $[M+H]^+$	Calc. *m/z* $[M-H]^-$/ $[M+H]^+$	Error (ppm)	MS^2 fragments (%); MS^3 fragments (%) negative mode/ positive mode	Mol. formula
1	Gluconic acid	1.09	195.0496/ n. o.	195.0505/ 197.0661	-1.482/ n. o.	177 (31), 159 (72), 129 (100); 85 (92), 57 (100)/ n. o.	$C_6H_{12}O_7$
2	Quinic acid	1.15	191.0554/ n. o.	191.0556/ 193.0712	1.965/ n. o.	127 (100)/ n. o.	$C_7H_{12}O_6$
3	Malic acid	1.43	133.0133/ n. o.	133.0137/ 135.0293	0.979/ n. o.	115 (100), 89 (11), 87 (10), 71 (6); 71 (100)/ n. o.	$C_4H_6O_5$
4	Succinic acid	1.49	117.0185/ n. o.	117.0188/ 119.0344	2.263/ n. o.	59 (100)/ n. o.	$C_4H_6O_4$
5	Hydroquinone-*O*-glucoside	1.56	271.0818/ n. o.	271.0818/ 273.0974	1.958/ n. o.	109 (100)/ n. o.	$C_{12}H_{16}O_7$
6	Hydroxymethylglutaric acid	2.44	161.0446/ 163.0594	161.0450/ 163.0606	1.243/ -4.476	117 (100)/	$C_6H_{10}O_5$
7	Glucosyringic acid	3.17	359.0975/ n. o.	359.0978/ 361.1135	0.632/ n. o.	197 (100), 179 (23)/ n. o.	$C_{15}H_{20}O_{10}$
8	1-Galloyl-*O*-glucoside	3.25	331.0644/ n. o.	331.0665/ 333.0822	-4.872/ n. o.	152 (100), 124 (7)/ n. o.	$C_{13}H_{16}O_{10}$
9	Protocatechuic acid glucoside (isomer)	3.35	315.0721/ n. o.	315.0716/ 317.0873	3.147/ n. o.	153 (100)/ n. o.	$C_{13}H_{16}O_9$
10	Syringic acid	3.49	197.0447/ n. o.	197.0450/ 199.0606	1.320/ n. o.	153 (100)/ n. o.	$C_9H_{10}O_5$
11	Protocatechuic acid	3.68	153.0185/ n. o.	153.0188/ 155.0344	1.862/ n. o.	109 (100)/ n. o.	$C_7H_6O_4$
12	4-(β-D-Glucosyloxy)benzoic acid	3.71	299.0755/ n. o.	299.0767/ 301.0923	-2.153/ n. o.	137 (100), 93 (27)/ n. o.	$C_{13}H_{16}O_8$
13	1-*O*-(4-Hydroxy-3,5-dimethoxybenzoyl)-glucoside	4.17	359.0966/	359.0978/	-1.819/	197 (100), 182 (3); 182 (100), 153	$C_{15}H_{20}O_{10}$

			361.1122	361.1135	-1.892	(23), 138 (8)/ n. o.	
14	Chlorogenic acid (isomer)	4.69	353.0856/ 355.1017	353.0873/ 355.1029	-3.055/ -1.911	191 (100), 179 (39), 135 (7); 173 (81), 127 (92), 111 (31), 93 (47), 85 (100)/ 309 (11), 165 (100), 147 (77); 147 (100)	$C_{16}H_{18}O_9$
15	Esculetin-6-*O*-glucoside	4.72	339.0710/ 341.0864	339.0716/ 341.0873	-0.231/ -0.817	177 (100)/ n. o.	$C_{15}H_{16}O_9$
16	Scopoletin-7-*O*-glucuronide	4.76	367.0659/ 369.0805	367.0665/ 369.0822	-0.145/ -3.124	191 (100)/ n. o.	$C_{16}H_{16}O_{10}$
17	Chlorogenic acid (isomer)	4.86	353.0855/ 355.1016	353.0873/ 355.1029	-3.394/ -2.080	191 (100), 179 (<3); 173 (83), 127 (96), 111 (30), 93 (49), 85 (100)/ 309 (10), 165 (100), 147 (63); 147 (100)	$C_{16}H_{18}O_9$
18	4-*O*-Glucosyl-*p*-coumaric acid	4.97	325.0910/ 327.1078	325.0923/ 327.1080	-2.350/ 1.150	163 (100)/ n. o.	$C_{15}H_{18}O_8$
19	Chlorogenic acid (isomer)	5.06	353.0851/ 355.1019	353.0873/ 355.1029	-4.612/ -1.376	191 (100), 179 (15), 161 (3); 173 (84), 127 (94), 111 (33), 93 (52), 85 (100)/ 309 (11), 165 (100), 147 (87); 147 (100)	$C_{16}H_{18}O_9$
20	Nonyl-*O*-maltoside	5.10	467.2493/ 469.2637	467.2492/ 469.2649	1.373/ -1.360	n. o. / 451 (7), 307 (100), 289 (27), 271 (29); 288 (94), 271 (100)	$C_{21}H_{40}O_{11}$
21	Caffeic acid	5.52	179.0339/ n. o.	179.0344/ 181.0501	-0.085/ n. o.	135 (100)/ n. o.	$C_9H_8O_4$
22	(4-Methylumbelliferone)-7-*O*-glucoside	5.69	337.0924/ 339.1071	337.0923/ 339.1080	1.828/ -0.867	175 (100)/ 177 (100)	$C_{16}H_{18}O_8$
23	Quercetin-3-*O*-rutinoside	5.72	609.1461/ 611.1601	609.1456/ 611.1612	1.722/ -0.918	463 (6), 301 (100), 300 (32)/ n. o.	$C_{27}H_{30}O_{16}$
24	5-*O*-*p*-Coumaroylquinic acid	5.76	337.0911/ 339.1069	337.0923/ 339.1080	-2.177/ -1.516	191 (20), 163 (100)/ n. o.	$C_{16}H_{18}O_8$
25	Feruloylquinic acid (isomer)	5.94	367.1017/ 369.1176	367.1029/ 369.1186	-1.848/ -1.080	191 (100), 173 (<2); 173 (74), 171 (31), 127 (100), 111 (41), 93 (60), 85 (92)/	$C_{17}H_{20}O_9$

26	Feruloylquinic acid (isomer)	6.14	367.1013/ 369.1177	367.1029/ 369.1186	-2.993/ -0.890	191 (100), 173 (5); 173 (72), 127 (100), 111 (54), 93 (51), 85 (87)/ n. o.	$C_{17}H_{20}O_9$
27	Quercetin-3-*O*-neohesperidoside	6.26	609.1452/ 611.1599	609.1456/ 611.1612	0.310/ -1.229	301 (100); 273 (14), 271 (71), 193 (7), 179 (100), 151 (62)/ 465 (19), 303 (100); 285 (41), 257 (100), 229 (88), 165)	$C_{27}H_{30}O_{16}$
28	3'-*O*-Glucopyranosyl rosmarinic acid	6.34	521.1284/ 523.1439	521.1295/ 523.1452	-1.165/ -1.333	359 (100), 197 (7), 161 (4); 223 (14), 197 (92), 179 (49), 161 (100)/ n. o.	$C_{24}H_{26}O_{13}$
29	Kaempferol-3-*O*-neohesperidoside	6.36	593.1500/ 595.1651	593.1506/ 595.1663	-0.129/ -1.086	447 (17), 429 (49), 285 (100); 255 (100), 227 (10)/ 449 (17), 287 (100); 258 (42), 241 (100), 213 (72), 165 (94), 153 (53)	$C_{27}H_{30}O_{15}$
30	*p*-Coumaric acid	6.38	163.0388/ n. o.	163.0395/ 165.0552	-1.292/ n. o	119 (100); 92 (40), 91 (100)/ n. o	$C_9H_8O_3$
31	Quercetin-3-*O*-glucoside	6.47	463.0859/ 465.1021	463.0877/ 465.1033	-2.510/ -1.446	301 (100); 271 (31), 255 (22), 179 (100), 150 (49)/ 303 (100); 285 (41), 257 (100), 247 (31), 229 (63), 165 (48)	$C_{21}H_{20}O_{12}$
32	Methyl 4-hydroxybenzoate	6.52	151.0390/ 153.0540	151.0395/ 153.0552	0.128/ -4.251	136 (100); 108 (23), 92 (100)/ n. o.	$C_8H_8O_3$
33	Kaempferol-*O*-rhamnoside-*O*-xyloside	6.55	563.1401/ 565.1543	563.1401/ 565.1557	0.973/ -1.560	n. o. / 433 (100), 419 (13), 287 (11); 287 (100)	$C_{26}H_{28}O_{14}$
34	Kaempferol-3-*O*-glucoside	6.57	447.0927/ 449.1072	447.0927/ 449.1084	1.235/ -1.465	285 (100)/ 287 (100)	$C_{21}H_{20}O_{11}$
35	Isorhamnetin-*O*-rutinoside	6.63	623.1614/ 625.1751	623.1612/ 625.1769	1.154/ -1.921	315 (100), 300 (12); 300 (100), 285 (27) / n. o.	$C_{28}H_{32}O_{16}$
36	Isorhamnetin-*O*-glucoside	6.75	477.1013/ 479.1175	477.1013/ 479.1190	-3.128/ -1.905	315 (100); 300 (100)/ 317 (100); 302 (100)	$C_{22}H_{22}O_{12}$
37	Syringetin-*O*-glucoside	6.86	507.1127/	507.1139/	-1.276/	n. o. /	$C_{23}H_{24}O_{13}$

			509.1282	509.1295	-1.566	347 (100); 332 (100)	
38	Luteolin-7-*O*-glucoside	6.93	447.0903/ 449.1065	447.0927/ 449.1084	-4.289/ -3.023	285 (100); 255 (100), 227 (11)/ n. o.	$C_{21}H_{20}O_{11}$
39	Cichoric acid	6.97	473.0706/ n. o.	473.0720/ 475.0877	-1.886/ n. o.	311 (52), 293 (100), 179 (10); 275 (19), 219 (100)/ n. o.	$C_{22}H_{18}O_{12}$
40	Dicaffeoylquinic acid (isomer)	7.06	515.1191/ 517.1333	515.1190/ 517.1346	1.315/ -1.455	353 (100), 235 (18), 179 (8)/ n. o.	$C_{25}H_{24}O_{12}$
41	Dicaffeoylquinic acid (isomer)	7.11	515.1194/ 517.1332	515.1190/ 517.1346	2.034/ -1.571	353 (100), 335 (3)/ n. o.	$C_{25}H_{24}O_{12}$
42	Rosmarinic acid	7.09	359.0750/ 361.0915	359.0767/ 361.0923	-3.325/ -0.759	197 (31), 179 (39), 161 (100)/ n. o.	$C_{18}H_{16}O_8$
43	Isorhamnetin-*O*-pentoside	7.26	447.0907/ 449.1068	447.0927/ 449.1084	-3.395/ -2.422	315 (100), 272 (11); 300 (100)/ 317 (100); 302 (100)	$C_{21}H_{20}O_{11}$
44	Dicaffeoylquinic acid (isomer)	7.32	515.1195/ 517.1335	515.1190/ 517.1346	2.849/ -1.107	353 (70), 235 (75), 179 (100)/ n. o.	$C_{25}H_{24}O_{12}$
45	Isorhamnetin	7.34	315.0495/ 317.0652	315.0505/ 317.0661	-1.362/ -1.322	n. o. / 302 (100); 301 (38), 274 (100), 256 (62), 228 (53), 152 (7), 124 (3)	$C_{16}H_{12}O_7$
46	Kaempferol-3-*O*-pentoside	7.47	417.0800/ 419.0967	417.0822/ 419.0978	-3.916/ -1.344	285 (100)/ 287 (100)	$C_{20}H_{18}O_{10}$
47	Kaempferol-3-*O*-(*p*-coumaroyl)-glucoside (isomer)	8.19	593.1286/ 595.1437	593.1295/ 595.1452	-0.619/ -1.575	n. o. / 449 (14), 309 (67), 291 (27), 287 (100)	$C_{30}H_{26}O_{13}$
48	Kaempferol-3-*O*-(feruloyl)-glucoside (isomer)	8.29	623.1370/ 625.1541	623.1401/ 625.1557	-4.128/ -1.715	n. o. / 339 (70), 321 (22), 287 (100), 195 (18), 177 (38)	$C_{31}H_{28}O_{14}$
49	Kaempferol-3-*O*-(feruloyl)-glucoside (isomer)	8.34	623.1423/ 625.1544	623.1401/ 625.1557	1.169/ -1.219	n. o. / 339 (67), 321 (24), 287 (100), 195 (19), 177 (45)	$C_{31}H_{28}O_{14}$
50	Kaempferol-3-*O*-(*p*-coumaroyl)-glucoside (isomer)	8.38	593.1293/ 595.1437	593.1295/ 595.1452	0.511/ -1.474	n. o. / 449 (9), 309 (5), 291 (3), 287 (100)	$C_{30}H_{26}O_{13}$
51	Luteolin	8.46	285.0396/ 287.0547	285.0399/ 287.0556	0.756/ -1.325	241 (100), 199 (78), 175 (82)/ n. o.	$C_{15}H_{10}O_6$

52	Quercetin	8.52	301.0336/ 303.0493	301.0348/ 303.0505	-2.156/ -2.109	179 (100), 151 (63), 121 (12)/ n. o.	$C_{15}H_{10}O_7$
53	Quercetin-3-*O*-(di-*p*-coumaroyl)-glucoside (isomer)	9.35	755.1666/ 757.1748	755.1612/ 757.1769	7.904/ -1.996	n. o. / 455 (100), 437 (12); 437 (56), 291 (14), 147 (100)	$C_{39}H_{32}O_{16}$
54	Kaempferol	9.50	285.0390/ 287.0547	285.0399/ 287.0556	-1.384/ -0.922	257 (11), 229 (100), 213 (67), 151 (62)/ n. o.	$C_{15}H_{10}O_6$
55	Quercetin-3-*O*-(di-*p*-coumaroyl)-glucoside (isomer)	9.57	n. o. / 757.1752	755.1612 / 757.1769	n. o. / -1.507	n. o. / 455 (100), 437 (15); 437 (62), 291 (16), 147 (100)	$C_{39}H_{32}O_{16}$
56	Kaempferol-3-*O*-(di-*p*-coumaroyl)-glucoside (isomer)	9.73	739.1678/ 741.1803	739.1663/ 741.1819	2.765/ -1.480	n. o. / 455 (100), 437 (8), 287 (<3); 437 (39), 147 (100)	$C_{39}H_{32}O_{15}$
57	Kaempferol-3-O-(*p*-coumaroyl)-(feruloyl)-glucoside (isomer)	9.83	769.1747/ 771.1909	769.1769 / 771.1925	-2.043/ -1.428	n. o. / 485 (100), 467 (9); 467 (52), 177 (100), 147 (19)	$C_{40}H_{34}O_{16}$
58	Kaempferol-3-*O*-(di-*p*-coumaroyl)-glucoside (isomer)	9.92	739.1695/ 741.1799	739.1663/ 741.1819	5.064/ -1.979	n. o. / 455 (100), 437 (9), 287 (11); 437 (32), 147 (100)	$C_{39}H_{32}O_{15}$
59	Kaempferol-3-O-(*p*-coumaroyl)-(feruloyl)-glucoside (isomer)	10.02	769.1746/ 771.1906	769.1769 / 771.1925	-2.277/ -1.830	n. o. / 485 (100), 467 (9); 467 (32), 177 (100), 147 (16)	$C_{40}H_{34}O_{16}$
60	Dihydroxyhexadecanoic acid	10.07	287.2208/ 289.2368	287.2222/ 289.2379	-2.980/ -1.680	n. o. / 271 (100), 253 (11); 253 (100), 235 (54)	$C_{16}H_{32}O_4$
61	Kaempferol-3-*O*-(di-*p*-coumaroyl)-glucoside (isomer)	10.12	739.1685/ 741.1798	739.1663/ 741.1819	3.671/ -1.587	n. o. / 455 (100), 437 (8), 287 (11); 437 (32), 147 (100)	$C_{39}H_{32}O_{15}$
62	Isorhamnetin-*O*-(*p*-coumaroyl)-(feruloyl)-glucoside	10.18	n. o. / 801.2009	799.1874. / 801.2031	n. o. / -2.067	n. o. / 485 (100), 467 (5); 467 (49), 177 (100)	$C_{41}H_{36}O_{17}$
63	Hexadecanedioic acid	10.49	n. o./ 287.2211	285.2066/ 287.2222	n. o./ -2.040	n. o./ 269 (100); 251 (100)	$C_{16}H_{30}O_4$

Benzoic acid and hydroxycinnamic derivatives

As can be seen from Table 1, twenty-three compounds were identified. Among them, eight hydroxybenzoic acid and fifteen cinnamic acid derivatives were found.

Compound **7** gave a $[M-H]^-$ ion at *m/z* 359 and its MS^2 fragmentation resulted in fragment ions at *m/z* 197 $[M\text{-glucose}]^-$ and *m/z* 179 $[M\text{-glucose-}H_2O]^-$, suggesting glucosyringic acid. Syringic acid (**10**) was identified on the basis of $[M-H]^-$ ion at *m/z* 197 and characteristic loss of CO_2 during formation of MS^2 ion at *m/z* 153. Compound **8** exhibited an $[M-H]^-$ ion at *m/z* 331, and its fragmentation showed base peak ion at *m/z* 152 $[M\text{-glucose-OH}]^-$. This compound corresponds to a 1-galloyl-*O*-glucoside. Protocatechuic acid glucoside, as compound **9**, yielded a $[M-H]^-$ ion at *m/z* 315 and its MS^2 fragmentation gave the aglycone fragment ion at *m/z* 153 (-162 Da, loss of glucose residue). Elimination of 44 Da from deprotonated molecular ion and MS^2 ion at *m/z* 109, indicated that compound **11** was protocatechuic acid. Compound **12**, 4-(β-D-glucosyloxy)benzoic acid, was characterized by loss of 162 Da from $[M-H]^-$ ion at *m/z* 299, as well as from MS^2 ion at *m/z* 93 $[M\text{-glucose-}CO_2]^-$. After examination of MS^2 (ion at *m/z* 197, loss of glucose residue) and MS^3 spectra (ion at *m/z* 182, loss of methyl group; ion at *m/z* 153, loss of CO_2), compound **13** was assigned as 1-*O*-(4-hydroxy-3,5-dimethoxybenzoyl)-glucoside. The molecular ions in negative and positive modes indicated that compound **32** had molecular formula $C_8H_8O_3$. Loss of methyl group during formation of MS^2 ion at *m/z* 136, and further elimination of CO_2 during formation of a base peak ion at *m/z* 92 in MS^3 spectra, suggested that peak **32** belonged to methyl 4-hydroxybenzoate.

Observed deprotonated molecular ion at *m/z* 179 and MS^2 ion at *m/z* 135 (loss of 44 Da) indicated that compound **21** was caffeic acid. On the basis of the obtained values of $[M-H]^-$ and $[M+H]^+$, as well as MS^2 and MS^3 fragmentation patterns (in both negative and positive modes, respectively), compounds **14**, **17** and **19** were assigned as isomers of caffeoyl quinic acid or chlorogenic acid. All these compounds showed a negative product ion at *m/z* 179 due to loss of a deprotonated molecule of caffeic acid, as well as positive product ion at *m/z* at 165 due to loss of quinic acid residue (Misic *et al.*, 2015). Comparing MS^2 fragmentation patterns obtained in negative ionization mode with previously published data (Misic *et al.*, 2015; Gardana *et al.*, 2007; Gouveia and Castilho, 2011), three isomeric dicaffeoylquinic acids (compounds **40**, **41** and **44**) and rosmarinic acid (compound **42**) were identified in our sample. The analysis in the LTQ-Orbitrap confirmed presence of **40**, **41** and **44** with $[M-H]^-$ errors of 1.315 ppm, 2.034 ppm and 2.849 ppm, respectively. The ion at *m/z* 353 or $[M-H-162]^-$ corresponded to loss of a caffeic acid unit, while ion at *m/z* 191 indicated deprotonated quinic acid. Compound **39** (t_r 6.97 min) gave an $[M-H]^-$ ion at *m/z* 473 (error -1.886 ppm). The MS^2 fragmentation gave a fragment ion at *m/z* 311 (loss of 162 Da) as deprotonated caftaric acid, base peak ion at *m/z* 293 (neutral loss of caffeic acid) and ion at *m/z* 179, which corresponds to deprotonated caffeic acid (Figure 3 and Figure 4). Thus compound **39** was characterized as cichoric acid.

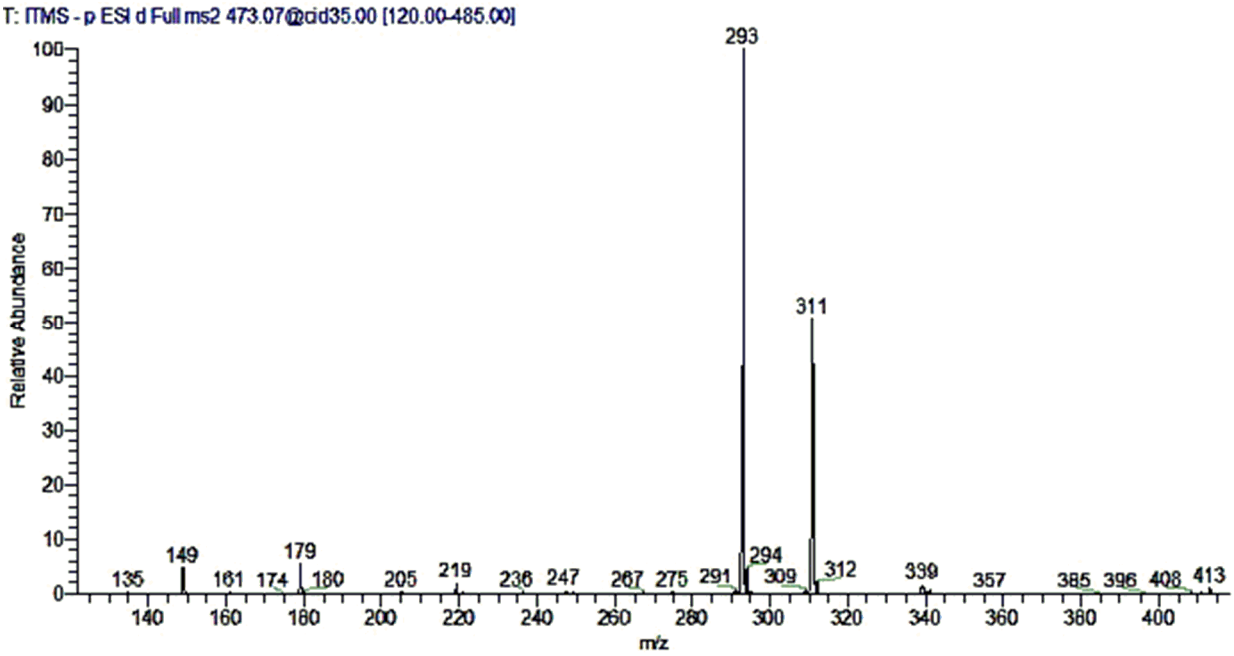

Figure 3. The MS^2 spectra in negative mode of cichoric acid.

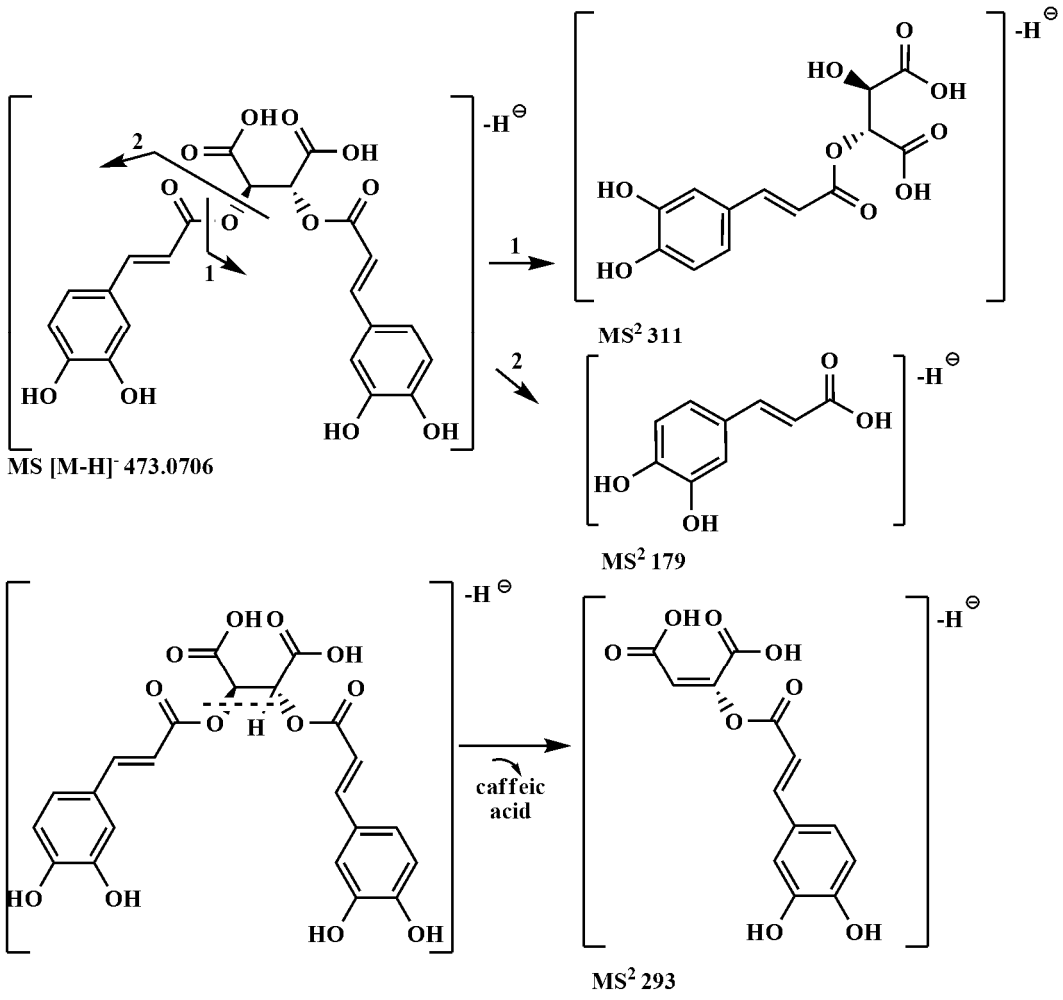

Figure 4. Possible fragmentation pathways of cichoric acid.

Peak **28** with $[M-H]^-$ at *m/z* 521 (error -1.165 ppm) and MS^2 ion at *m/z* 359 as deprotonated compound **42** $[M-H\text{-glucose}]^-$ was characterized as 3'-*O*-glucopyranosyl rosmarinic acid. By showing $[M-H]^-$ ion at *m/z* 325, as well as MS^2 ion at *m/z* 163 (-162 Da glucosyl residue), compound **18** was identified as 4-*O*-glucosyl-*p*-coumaric acid. Compounds **30** (*p*-coumaric acid) and **24** (5-O-*p*-coumaroylquinic acid) exhibited $[M-H]^-$ at *m/z* 163 and at *m/z* 337, respectively. The typical loss of CO_2 was observed for compound **30** (MS^2 base peak ion at *m/z* 119), while compound **24** in the MS^2 spectra revealed peaks at *m/z* 191 and 163 corresponding to the deprotonated quinic acid $[M-H-146]^-$ and coumaric acid $[M-H-174]^-$, respectively. Two isomeric feruoylquinic acids (compounds **25** and **26**) with $[M-H]^-$ at *m/z* 337 were detected at t_r 5.76 min and t_r 5.96 min, respectively. In both cases, MS^2 of *m/z* 337 showed a base ion peak at *m/z* 191 corresponding to the formation of deprotonated quinic acid.

Flavonoid aglycones and their derivatives

Among twenty-eight compounds, four aglycones and twenty-four flavonoid derivatives were identified in methanol extract of *E. amethystinum*.

Peak **51** (t_r 8.46 min, $[M-H]^-$ at *m/z* 301, error 0.756 ppm) was identified as luteolin. Observed fragment ions are at *m/z* 241 $[M-H-CO_2]^-$, 199 $[M-H-C_2H_2O-CO_2]^-$ and 175 $[M-H-C_3O_2-C_2H_2O]^-$. Compound **52** (quercetin) yielded an $[M-H]^-$ at *m/z* 301, while MS^2 fragmentation gave fragment ions from retro-Diels-Alder (RDA) reaction at *m/z* 179 $[^{1,2}A]^-$, 151 $[^{1,2}A\text{-}CO]^-$ and 121 $[^{1,2}B]^-$ (Fabre *et al.*, 2001). Flavonol kaempferol was identified as peak **54** at t_r 9.50 min, by comparing literature data with deprotonated ion at *m/z* 285, as well as MS^2 ions at *m/z* 257 $[M-H-CO]^-$, 229 $[M-H-2CO]^-$, 213 $[M-H-CO_2-CO]^-$ and 151 $[^{1,2}A]^-$ (Fabre *et al.*, 2001). Presence of isorhamnetin (compound **45**) was confirmed by its MS^n spectra in positive ionization mode. MS^2 of $[M+H]^+$ at *m/z* 317 (error -1.322 ppm) showed peak at *m/z* 302, which corresponds to loss of methyl group (CH_3). Further fragmentation showed ions at *m/z* 301 (loss of H), 274 $[M+H-CH_3-CO]^+$, 256 $[M+H-CH_3-CO-H_2O]^+$ and 228 $[M+H-CH_3-C_2O_2H-OH]^+$, as well as two RDA fragmentation ions at *m/z* 152 $[^{1,3}A]^+$ and 124 $[^{1,3}A]^+$.

Three quercetin glycosides-**23**, **27** and **31** were found in our sample. Quercetin-3-*O*-rutinoside (**23**) with an error of 1.722 ppm yielded $[M-H]^-$ ion at *m/z* 609. The MS^2 fragmentation gave ions at *m/z* 463 (loss of 164 Da, rhamnosyl residue) and aglycone fragment ion of deprotonated quercetin (Y_O^-) at *m/z* 301 (loss of 162 Da, glucose residue). Compound **27**, quercetin-3-*O*-neohesperidoside, was observed both in negative and positive ionization modes, respectively. The molecular ions were at *m/z* 609/611 (error 0.310 ppm/-1.229 ppm). MS^2 in negative mode showed ion at *m/z* 301 (loss of 308 Da, sugar residue), while MS^3 fragment ions confirmed presence of quercetin moiety. Further, presence of neohesperoside residue was confirmed by MS^2 fragment ions in positive mode. Observed ion at *m/z* 465 corresponds to quercetin-3-*O*-glucoside (-146 Da indicated on rhamnose part), while base peak ion at *m/z* 303 was protonated quercetin (Y_O^+) (-308 Da, neohesperidoside residue). Quercetin-3-*O*-glucoside (compound **31**) with $[M-H]^-/[M+H]^+$ at *m/z* 463/465 (error -2.510 ppm/-1.446 ppm) gave origin to quercetin aglycone, by loss of glucose moiety (*m/z* 301 in negative mode; *m/z* 303 in positive mode) (Aaby *et al.*, 2012). Two quercetin derivatives (compounds **53** and **55**) with observed $[M+H]^+$ ions at *m/z* 757 (errors -1.996 ppm and -1.507 ppm, respectively) and similar MS^2 and MS^3 spectra were identified as quercetin-3-*O*-(di-*p*-coumaroyl)-glucoside isomers. The MS^2 mass spectra showed base peak ion at *m/z* 455, which corresponds to loss of quercetin $[M+H-302]^+$ and ion at *m/z* 437 [M+H-quercetin-

$H_2O]^+$. The MS^3 mass spectra displayed ion at *m/z* 437, indicating neutral loss of H_2O, ion at *m/z* 291 which corresponds to coumaroyl glucoside residue (loss of 164 Da or *p*-coumaric acid), and ion at *m/z* 147 which confirmed presence of coumaroyl moiety (Figure 5).

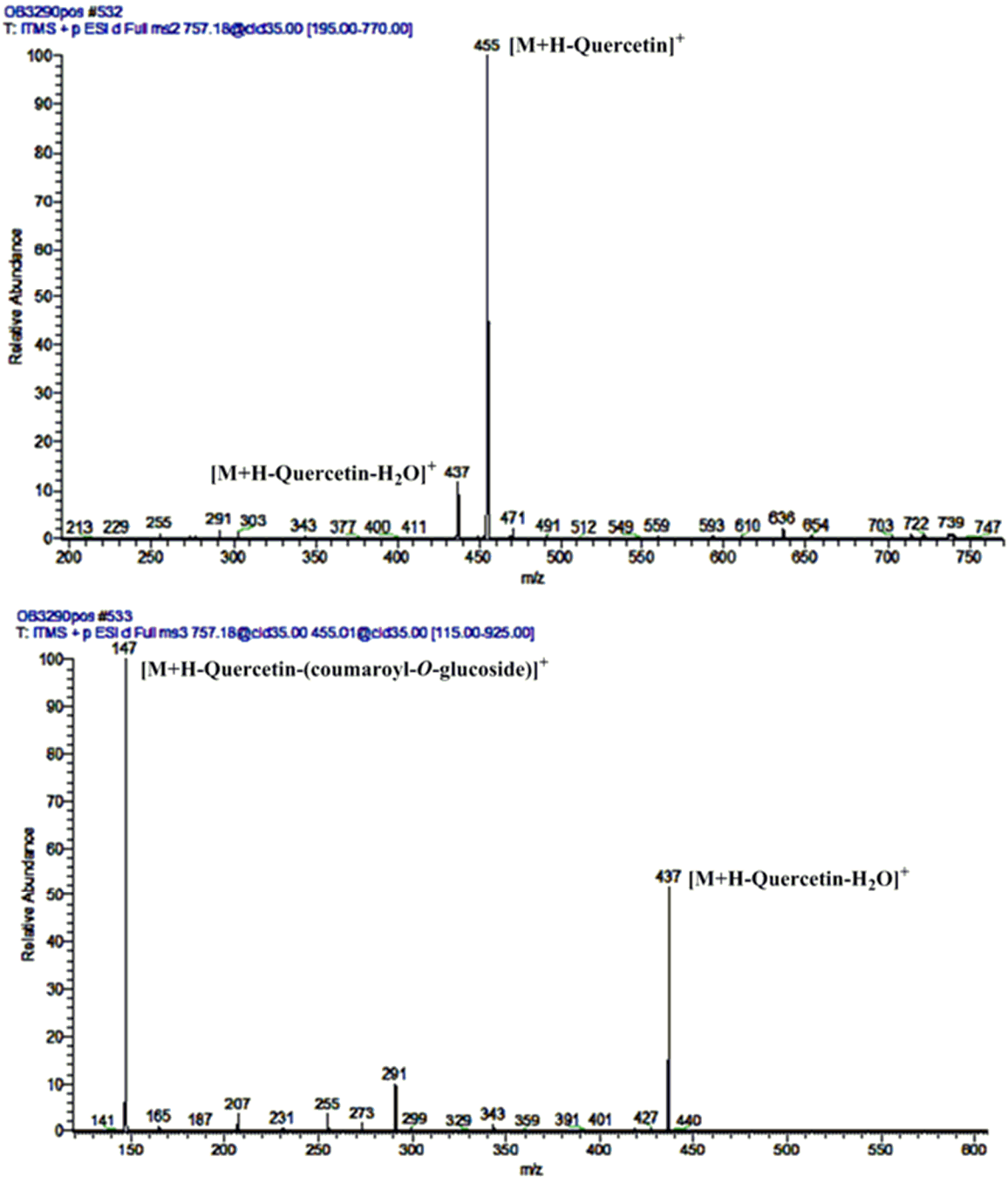

Figure 5. The MS^2 and MS^3 spectra in positive mode of quercetin-3-*O*-(di-*p*-coumaroyl)-glucoside.

Compound **29** exhibited an $[M-H]^-/[M+H]^+$ at *m/z* 593/595 (error -0.129 ppm/-1.086 ppm). The presence of neohesperoside residue was confirmed by MS^2 fragment ions. Observed ions at *m/z* 447/449 correspond to kaempferol-3-*O*-glucoside (-146 Da indicated on rhamnose part), while base peak ions at *m/z* 285/287 correspond to kaempferol residue (loss of 308 Da, neohesperidoside residue). Compound **33** gave $[M+H]^+$ ion at *m/z* 565 and in the

MS^2 fragmentation a neutral loss of 146 Da (rhamnosyl residue) was observed to produce a fragment ion at *m/z* 419. Similarly, loss of xylosyl residue (-132 Da) during formation of ion at *m/z* 433 was found. With the facts that MS^2 and MS^3 spectra showed protonated ions from kaempferol, and that under used experimental condition it was not possible to determine substitution of flavonoid hydroxyl groups, compound **33** was tentatively identified as kaempferol-*O*-rhamnoside-*O*-xyloside. Compound **34**, kaempferol-3-*O*-glycoside, displayed $[M-H]^-/[M+H]^+$ ions at *m/z* 447/449, as well as characteristic MS^2 fragmentation ions (in negative and positive modes) at *m/z* 285/287 (-162 Da, loss of glucose residue) (Ornelas-Paz *et al.*, 2013). Another kaempferol glycoside (compound **46**) was tentatively identified as kaempferol-3-*O*-pentoside. Its $[M-H]^-/[M+H]^+$ ions were observed at *m/z* 417/419 (error, -3.916 ppm/-1.344 ppm), while neutral loss of 132 Da during formation of MS^2 fragment ions at *m/z* 285/287 indicated pentose residue. Compounds **47** (t_r 8.19 min) and **50** (t_r 8.38 min) exhibited the same $[M+H]^+$ at *m/z* 595 (errors, -1.575 ppm and -1.474 ppm, respectively). The MS^2 fragmentation lead to a base peak ion at *m/z* 287 due to loss of 308 Da which was characterized as a combined loss of 162 Da + 146 Da proved by the $[M+H-146]^+$ ion at *m/z* 449. The high retention time of these compounds suggests that the neutral loss of 146 Da is due to a coumaroyl group rather than a rhamnose unit. The ion at *m/z* 287 corresponds to the protonated aglycone ion of kaempferol, while intense fragment ion at *m/z* 309 was assigned as coumaroyl-*O*-glucoside part (HANHINEVA *et al.*, 2008). Compounds **48** and **49**, kaempferol-3-*O*-(feruloyl)-glucoside isomers, showed protonated ion of ferulic acid at *m/z* 195, as well as ion of kaempferol at *m/z* 287. Again, MS^2 fragment ion at *m/z* 339 belongs to feruloyl-*O*-glucoside. Three kaempferol-3-*O*-(di-*p*-coumaroyl)-glucoside isomers (compounds **56**, **58** and **61**) were observed at t_r 9.73 min, 9.92 min and 10.12 min, respectively. MS^2 fragment ion at *m/z* at 287 indicated presence of kaempferol. The MS^2 base peak ion at *m/z* 455 $[M+H\text{-kaempferol}]^+$ corresponds to the di-*p*-coumaroyl-*O*-glucoside residue. The presence of one coumaroyl residue was confirmed by formation of MS^3 base peak ion at *m/z* 147, while neutral loss of 308 Da (coumaroyl-*O*-glucoside) $[M+H\text{-kaempferol-308}]^+$ indicated occurrence of other coumaroyl residue. Compounds **57** and **59** gave the same $[M+H]^+$ at *m/z* 771 (errors, -1.428 ppm and -1.830 ppm, respectively). The MS^2 spectra showed base peak ion at *m/z* 485 $[M+H-286]^+$, indicating the neutral loss of kaempferol. Further fragmentations of ion at *m/z* 485 lead to the formation of MS^3 ions at *m/z* 177 (-308 Da, coumaroyl-*O*-glucoside part) and *m/z* 147 (-338 Da, feruloyl-*O*-glucoside part), indicating occurrence of feruloyl and coumaroyl residues, respectively. Thus, those two isomers were identified as kaempferol-3-*O*-(*p*-coumaroyl)-(feruloyl)-glucosides. Compound **35** exhibited $[M-H]^-$ ion at *m/z* 623 (error 1.154 ppm) showing base peak at *m/z* 315 (isorhamnetin aglycone), by loss of rutinoside residue (-308 Da). Hence, this compound was tentatively identified as isorhamnetin-*O*-rutinoside. Compounds **36** and **43** showed $[M-H]^-/[M+H]+$ ions at *m/z* 477/479 and 447/449, respectively, and losses of different sugar moieties (-162 Da, glucoside residue; -132 Da, pentoside residue) resulting in isorhamnetin aglycone (*m/z* 315/317) (Rivera-Pastrana, Yahia, & Gonzalez-Aguilar, 2010). Compound **62** gave $[M+H]^+$ ion at *m/z* 801 (error -2.067 ppm). The MS^2 spectra showed base peak ion at *m/z* 485 (coumaroyl-feruloyl-*O*-glucoside), corresponding to the loss of isorhamnetin. Further fragmentations lead to the formation of ion at *m/z* 467 $[M+H\text{-isorhamnetin-}H_2O]^+$. MS^3 base peak ion at *m/z* 177 corresponds to the feruloyl residue, while neutral loss of 308 Da confirmed presence of coumaroyl-*O*-glucoside part. Thus, compound **62** was identified as isorhamnetin-*O*-(*p*-coumaroyl)-(feruloyl)-glucoside.

Compound **37** exhibited an $[M+H]^+$ ion at *m/z* 509 (error -1.566). The MS^2 fragmentation revealed a neutral cleavage of 162 Da (glucoside residue), leading to the fragment ion at *m/z* 347, which corresponds to the protonated molecule of syringetin. Thus, this compound was identified as syringetin-*O*-glucoside. Compound **38** was characterized as

luteolin-7-*O*-glucoside. This compound showed an $[M-H]^-$ ion at *m/z* 447 and the MS^2 experiment resulted in a neutral loss of 162 Da (ion at *m/z* 285) and typical fragment of luteolin.

Coumarin derivatives

Compound **15** (esculetin-6-*O*-glucoside, t_r 4.72 min) displayed a deprotonated molecular ion at *m/z* 339 and MS^2 fragmentation gave a neutral fragment of 162 Da (glucoside residue), forming the aglycone ion (Y_O^-) at *m/z* 177 (esculetin). Scopoletin-7-*O*-glucuronide was identified as compound **16**; [M-H]- at *m/z* 367 (error of -0.145 ppm). The MS^2 spectra showed deprotonated ion of scopoletin (*m/z* 191), while neutral loss of 176 Da corresponded to the glucuronide residue. (4-Methylumbelliferone)-7-*O*-glucoside (compound **22**) was identified in negative and positive ionization modes. Molecular ions were observed at *m/z* 337 (error 1.828 ppm) and at *m/z* 339 (error -0.867 ppm), respectively. The MS^2 spectra showed neutral loss of 162 Da, which indicated presence of glucoside residue, while ions at *m/z* 175/177 confirmed presence of 4-methylumbelliferone.

Other compounds

The analysis in the LTQ-Orbitrap confirmed presence of five organic acids (**1**, **2**, **3**, **4** and **6**). Gluconic acid (compound **1**) gave a $[M-H]^-$ ion at *m/z* 195 (error -1.482 ppm) and characteristic MS^2 fragment ions at *m/z* 177 $[M-H-H_2O]^-$, *m/z* 159 $[M-H-2H_2O]^-$, and *m/z* 129 $[M-H-2H_2O-CH_2O]^-$. Compound **2** with deprotonated molecular ion at *m/z* 191 and fragment ion at *m/z* 127 (loss of CO and H_2O) was identified as quinic acid. Peak **3**, characterized as malic acid, showed an $[M-H]^-$ at *m/z* 133 (error 0.979 ppm), with an MS^2 ion at *m/z* 115 corresponding to the loss of water. Neutral loss of $C_2H_2O_2$ (*m/z* at 59) from the $[M-H]^-$ at *m/z* 117 confirmed presence of succinic acid (compound **4**). Peak **6**, with a $[M-H]^-$ at *m/z* 161 (error 1.243 ppm), was identified as hydroxymethylglutaric acid, and its MS^2 spectra showed ion at *m/z* 117 (loss of 44 Da, CO_2). Compound **5** corresponded to hydroquinone-*O*-glucoside ($[M-H]^-$ at *m/z* 271) with a fragmentation pattern at *m/z* 109 $[M-H\text{-glucose}]^-$. Compound **20**, which showed protonated molecular ion at *m/z* 469 (error -1.360 ppm), was tentatively identified as nonyl-*O*-maltoside. MS^2 spectra revealed loss of H_2O (*m/z* 451) and glucoside residue (*m/z* 307). On the basis of observed $[M+H]^+$ ions, as well as MS^n spectra, compounds **60** and **63** were characterized as dihydroxyhexadecanoic acid and hexadecanedioic acid, respectively.

CONCLUSIONS

In this study we performed for the first time UPLC-LTQ-Orbitrap-MS^n non-targeted metabolic analysis of methanol extract of *E. amethystinum*. The approach used allowed identification of sixty-three secondary metabolites, most of them belonging to the phenolics class (simple phenolics, flavonoids and coumarins). These data suggest that this medicinal plant might be a valuable source of bioactive secondary metabolites from classes of phenolic acids, flavonoids and coumarins with beneficial proprieties, and a promising source of health products for functional food or nutraceutical industries.

Acknowledgments

This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (grants III41010, OI172016 and OI175039). The authors acknowledge the support of the FP7 RegPot project FCUB ERA GA No. 256716. The EC does not share responsibility for the content of the article.

References:

[1] AABY, K., MAZUR, S., NES, A., SKREDE, G. (2012): Phenolic compounds in strawberry (*Fragaria* x *ananassa* Duch.) fruits: Composition in 27 cultivars and changes during ripening. *Food Chemistry* **132** (1): 86-97. doi: 10.1016/j.foodchem.2011.10.037

[2] CALVINO, C.I., MARTÍNEZ, S.G., DOWNIE, S.R. (2007): The evolutionary history of *Eryngium* (*Apiaceae, Saniculoideae*): rapid radiations, long distance dispersals, and hybridizations. *Molecular Phylogenetics and Evolution* **46** (3): 1129-1150. doi: 10.1016/j.ympev.2007.10.021

[3] CROWDEN, R.K., HARBORNE, J.B., HEYWOOD, V.H. (1969): Chemosystematics of the Umbelliferae-a general survey. *Phytochemistry* **8** (10): 1963-1984. doi: 10.1016/S0031-9422(00)88084-X

[4] DRAKE, D., LAM, J. (1972): Seseli acetylene from *Eryngium bourgatti*. *Phytochemistry* **11** (8): 2651-2652. doi: 10.1016/S0031-9422(00)88584-2

[5] FABRE, N., RUSTAN, I., DE HOFFMANN, E., QUETIN-LECLERCQ, J. (2001): Determination of flavone, flavonol, and flavanone aglycones by negative ion liquid chromatography electrospray ion trap mass spectrometry. *Journal of the American Society for Mass Spectrometry* **12** (6): 707-715. doi: 10.1016/S1044-0305(01)00226-4

[6] FACCIOLA, S. (1990): *Cornucopia: A Source Book of Edible Plants*. Vista, California: Kampong Publications.

[7] FLAMINI, G., TEBANO, M., CIONI, P.L. (2008): Composition of the essential oils from leafy parts of the shoots, flowers and fruits of *Eryngium amethystinum* from Amiata Mount (Tuscany, Italy). *Food Chemistry* **107** (2): 671-674. doi: 10.1016/j.foodchem.2007.08.064

[8] GARDANA, C., SCAGLIANTI, M., PIETTA, P., SIMONETTI, P. (2007): Analysis of the polyphenolic fraction of propolis from different sources by liquid chromatography-tandem mass spectrometry. *Journal of Pharmaceutical and Biomedical Analysis* **45** (3): 390-399. doi: 10.1016/*j*.jpba.2007.06.022

[9] GOUVEIA, S., CASTILHO, P.C. (2011): Antioxidant potential of *Artemisia argentea* L`Her alcoholic extract and its relation with the phenolic composition. *Food Research International* **44** (6): 1620-1631. doi: 10.1016/j.foodres.2011.04.040

[10] HANHINEVA, K., ROGACHEV, I., KOKKO, H., MINTZ-ORON, S., VENGER, I., KARENLAMPI, S., AHARONI A. (2008): Non-targeted analysis of spatial metabolite composition in strawberry (*Fragaria* x *ananassa*) flowers. *Phytochemistry* **69** (13): 2463-2481. doi: 10.1016/j.phytochem.2008.07.009

[11] KARTAL, M., MITAINE-OFFER, A.C., PAULULAT, T., ABU-ASAKER, M., WAGNER, H., MIRJOLET, J.F., GUILBAUD, N., LACAILLE-DUBOIS M.A. (2006): Triterpene saponins from *Eryngium campestre*. *Journal of Natural Product* **69** (7): 1105-1108. doi: 10.1021/np060101w

[12] MIŠIĆ, D., ŠILER, B., GAŠIĆ, U., AVRAMOV, S., ŽIVKOVIĆ, S., NESTOROVIĆ ŽIVKOVIĆ, J., MILUTINOVIĆ, M., TEŠIĆ Z. (2015): Simultaneous UHPLC/DAD/(+/-)HESI-MS/MS Analysis of phenolic acids and nepetalactones in methanol extracts of *Nepeta* species: A possible application in chemotaxonomic studies. *Phytochemical Analysis* **26** (1): 72-85. doi: 10.1002/pca.2538

[13] ORNELAS-PAZ, J.D., YAHIA, E.M., RAMIREZ-BUSTAMANTE, N., PEREZ-MARTINEZ, J.D., ESCALANTE-MINAKATA, M.D., IBARRA-JUNQUERA, V., ACOSTA-MUÑIZ, C., GUERRERO-PRIETO, V., OCHOA-REYES, E. (2013): Physical attributes and chemical composition of organic strawberry fruit (*Fragaria* x *ananassa* Duch, Cv. Albion) at six stages of ripening. *Food Chemistry* **138** (1): 372-381. doi: 10.1016/j.foodchem.2012.11.006

[14] RIVERA-PASTRANA, D.M., YAHIA, E.M., GONZALEZ-AGUILAR, G.A. (2010): Phenolic and carotenoid profiles of papaya fruit (*Carica papaya* L.) and their contents under low temperature storage. *Journal of the Science of Food and Agriculture* **90** (14): 2358-2365. doi: 10.1002/jsfa.4092

[15] TCHOUMTCHOUA, J., NJAMEN, D., MBANYA, J.C., SKALTSOUNISA, A.L., HALABALAKI, M. (2013): Structure-oriented UHPLC-LTQ Orbitrap-based approach as a dereplication strategy for the identification of isoflavonoids from *Amphimas pterocarpoides* crude extract. *Journal of Mass Spectrometry* **48** (5): 561-575. doi: 10.1002/jms.3167

[16] WORZ, A. (2004): On the Distribution and Relationships of the South-West Asian Species of *Eryngium* L. (Apiaceae-Saniculoideae). *Turkish Journal of Botany* **28** (1-2): 85-92.

[17] ZHANG, Z.Z, LI, S.Y., OWNBY, S., WANG, P., YUAN, W., ZHANG, W., SCOTT BEASLEY, R. (2008): Phenolic compounds and rare polyhydroxylated triterpenoid saponins from *Eryngium yuccifolium*. *Phytochemistry* **69**: 2070-2080. doi: 10.1016/j.phytochem.2008.03.020