Article

Phytochemical Diversity in Rhizomes of Three Reynoutria Species and their Antioxidant Activity Correlations Elucidated by LC-ESI-MS/MS Analysis.

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Abstract: One of the richest natural sources of resveratrol - the rhizome of *Reynoutria japonica* in East Asia is a well-known traditional herb (Hu zhang) used in various inflammatory diseases, infections, skin diseases, scald, hyperlipidemia. Although, it has been recently included in the European Pharmacopoeia, still in Europe is an untapped resource. Some of the therapeutic effects are likely to be influenced by its antioxidant properties and this in turn is frequently associated with a high stilbene content. However, some literatures suggested that other compounds than stilbenes may add to the total antioxidant capacity. Hence, the aim of this research was to examine rhizomes of *R. japonica* and less studied, morphologically similar species, *R. sachalinensis* and *R. x bohemica* for their phytochemical composition and antioxidant activity and to clarify the relationship between the antioxidant activity and compounds by statistical methods. HPLC/UV/ESI-MS studies of three *Reynoutria* species revealed 171 compounds comprising stilbenes, carbohydrates, procyanidins, flavan-3-ols, anthraquinones, phenylpropanoids, lignin oligomers, hydroxycinnamic acids, naphthalenes and their derivatives. Our studies confirmed the presence of procyanidins with high degree of polymerization, up to decamers in the rhizomes of *R. japonica* and brings new data on the presence of these compounds in other *Reynoutria* species. A procyanidin trimer digallate was described for the first time in the studied plants. Moreover, we suggested a presence of new for these species, dianthrone glycosides and previously unrecorded phenylpropanoid disaccharide esters and hydroxycinnamic acid derivatives, mainly in *R. sachalinensis*. Furthemore, compounds tentatively annotated as lignin oligomers were observed for the first time in studied species. The rhizomes of all *Reynoutria* species exhibited strong antioxidant activity. Statistical analysis demonstrated that proanthocyanidins should be considered as important contributors to the total antioxidant capacity.

Keywords: proanthocyanidins; Polygoni cuspidati rhizoma; mass spectrometry; stilbenoids

1. Introduction

In East Asia, the rhizome of *Reynoutria japonica* Houtt. (syn. *Fallopia japonica* [Houtt.] Ronse Decr., obsolete syn. *Polygonum cuspidatum* Sieb. & Zucc.) is a well-known traditional herb (*Hu zhang*, *Polygoni cuspidatae rhizoma*) used in various inflammatory diseases, infections, skin diseases, scald, hyperlipidemia etc. [1]. It is also one of the richest natural source of resveratrol (free and glycosylated) with a proven antioxidant activity [2]. In Europe, *R. japonica* has until recently been considered primarily as a troublesome invasive species that threatens native vegetation. However, rhizome of *R. japonica* (*Polygoni cuspidati rhizoma*) has been recently included in the European Pharmacopoeia [3] among many other Traditional Chinese herbs. A morphologically similar species from this genus - *R. sachalinensis* (F.Schmidt) Nakai, (syn. *F. sachalinensis* (F.Schmidt) Ronse Decr., *P. sachalinense* F.Schmidt) and a hybrid between them, *R. x bohemica* Chrtek & Chrtková (syn. *F.* x *bohemica* (Chrtek & Chrtková) J.P.Bailey) are not considered as equivalent medicinal plants. Both are also noxious invasive weeds outside their native distribution areas, However, *R. sachalinensis* has been to some extent used traditionally as a herbal medicine in Japan and China for treatment of arthralgia, jaundice, amenorrhea, coughs, scalds and burns, traumatic injuries, carbuncles and sores [4]. Earlier studies revealed striking differences in metabolic profile between these three species. *R. sachalinensis* contains significantly less anthraquinones and no stilbenes but the highest amount of phenylpropanoid-derived disaccharide esters than *R. japonica*. The phytochemical profile of *R. x bohemica* was intermediate between the two parent species [5]. Some of the therapeutic effects of studied species are likely to be influenced by their antioxidant properties and this in turn is frequently associated with a high stilbene content [6]. However, some researchers showed no correlation between the content of resveratrol or emodin and antioxidant activity in the obtained extracts and fractions from *R. japonica* [7]. In another study of *R. japonica*, where correlation analysis for chromatographic peak areas and radical scavenging rates of compounds were performed, it revealed a high positive correlation value for flavanol gallate, resveratrol, catechin but low for piceid, questin/physcion, and no correlation to emodin-8-O-glucoside, questin/physcion and emodin [8]. Moreover, in a study by Pan et al. [9] ethanol extract of *R. japonica* had a stronger antioxidant activity than resveratrol. These data suggested that other compounds than stilbenes may add to the total antioxidant capacity. It is worth to look more closely at the phytochemical profile of rhizomes from all three *Reynoutria* species.

The aim of the present study was to examine rhizomes of the invasive *Reynoutria* species from the wild population in Poland for their phytochemical composition and antioxidant activity. To clarify the relationship between the antioxidant activity and compounds present in the extracts and fractions, the statistical analysis was performed involved the LC-MS data and results from antioxidant assays.

2. Results and Discussion

2.1. Mass spectra analysis, annotation and identification of major constituents in extracts and fractions

The LC-MS analysis studies of three *Reynoutria* species (Rj, Rs, Rb) revealed a total of 171 detectable compounds comprising stilbenes, carbohydrates, procyanidins, flavan-3-ols, anthraquinones, phenylpropanoids, lignin oligomers, hydroxycinnamic acids, naphthalenes and their derivatives. Among the detected chromatographic peaks, 37 remained unassigned and without clear indication of their (phyto)chemical nature and 4 were tentatively defined as carbohydrates. However, most of the unidentified peaks had UV spectra typical for either hydroxycinnamic (the early eluting) or anthraquinone (late eluting) derivatives. Tentative assignments were carried out based on the MS, MS^2 and MS^3 spectra obtained for major *m*/*z* signals recorded in negative ion mode. Further, analysis of UV-vis spectra of compounds and comparison with literature data were used for identification [Figure **1**,**2**,**3** and Table **1**].

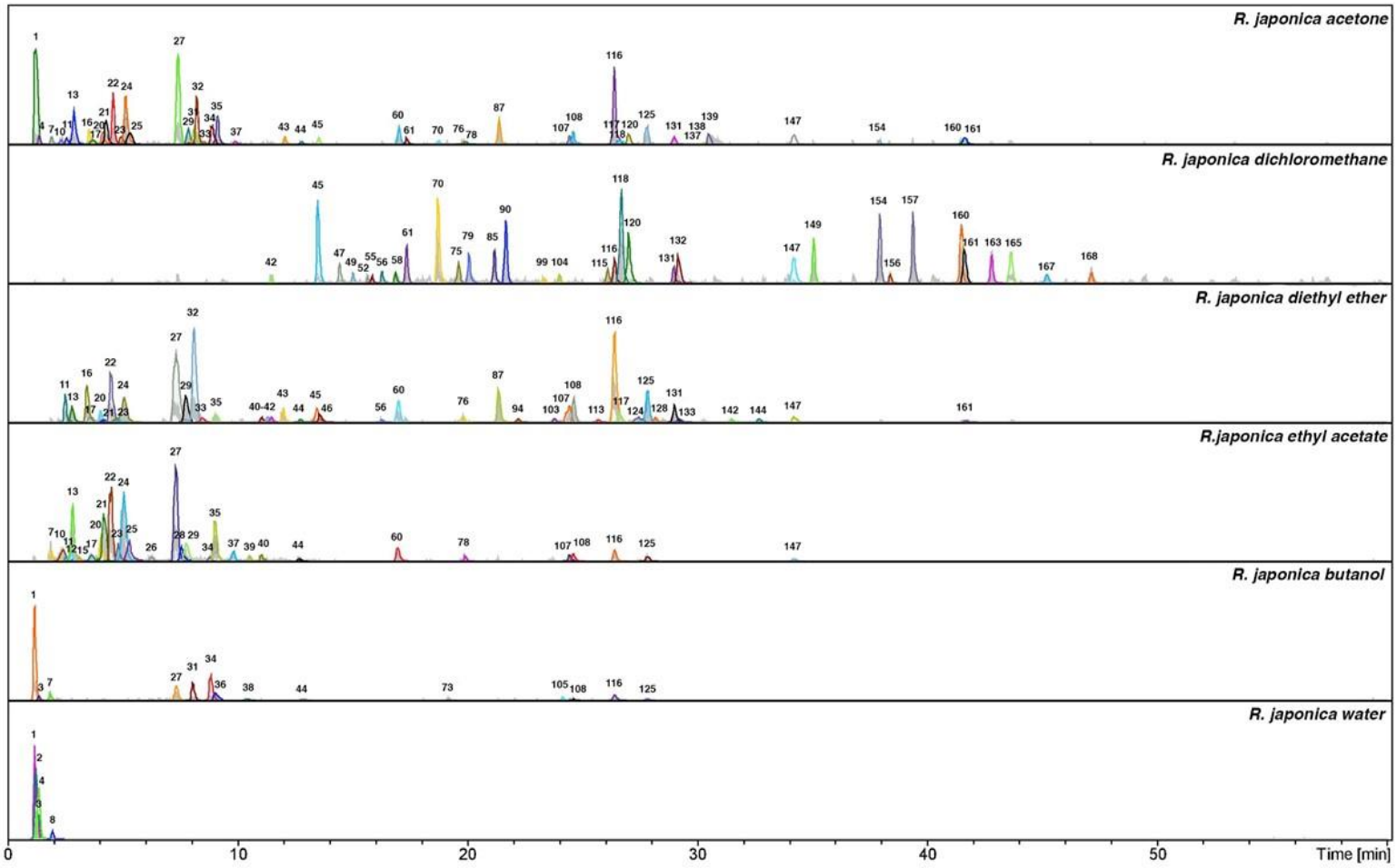

Figure 1. Total ion chromatograms in negative mode and dissect chromatograms of *Reynoutria japonica* extract and fractions. Deconvolution of an LC/MS mass chromatogram was carried out by using the Bruker's Dissect algorithm. Peak numbers are explained in Table 1.

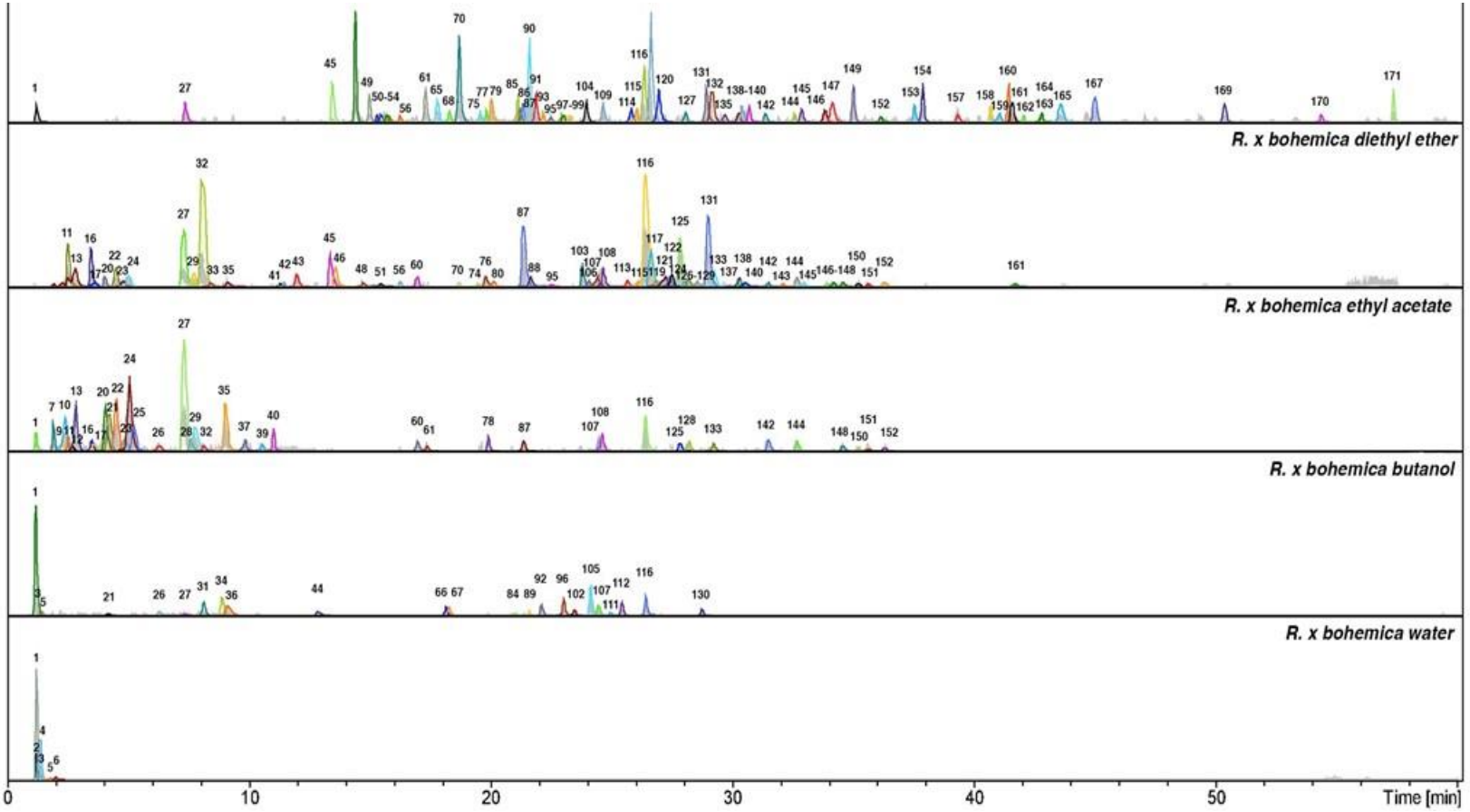

Figure 2. Total ion chromatograms in negative mode and dissect chromatograms of *Reynoutria x bohemica* extract and fractions. Deconvolution of an LC/MS mass chromatogram was carried out by using the Bruker's Dissect algorithm. Peak numbers are explained in Table 1.

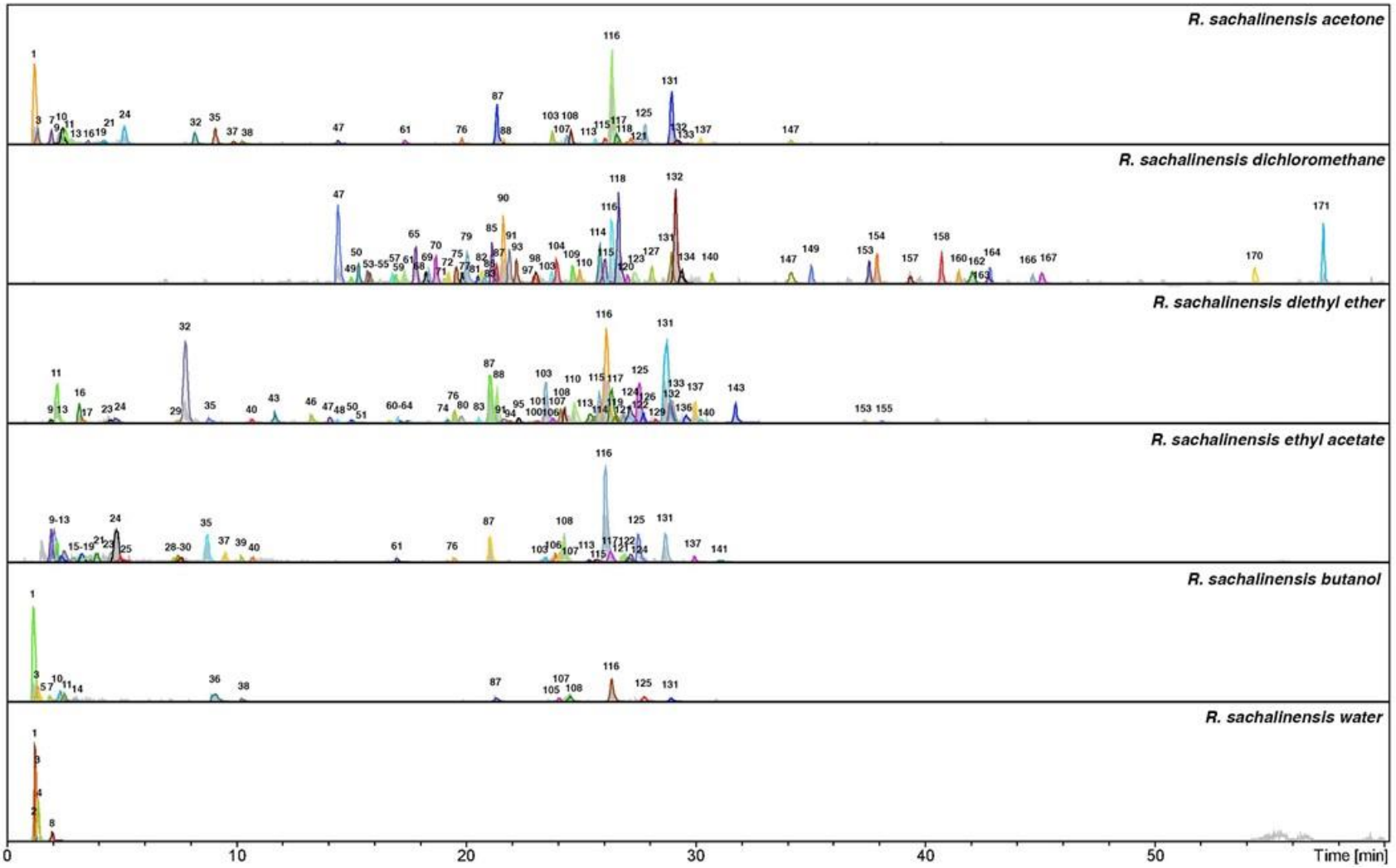

Figure 3. Total ion chromatograms in negative mode and dissect chromatograms of *Reynoutria sachalinensis* extract and fractions. Deconvolution of an LC/MS mass chromatogram was carried out by using the Bruker's Dissect algorithm. Peak numbers are explained in Table 1.

Table 1. Retention times, MS data, and UV λ_{max} values of the constituents detected in the extracts and fractions of the three *Reynoutria* species

Nr.	Identification	Rt	λ max (nm)	*m/z* [M-H]-	MS^2ions	MS^3ions	NL amu	References
1	Unknown carbohydrate/e.g. Disaccharide-sucrose	1.2	ND	341.15	**178.82b**	160.81b, 142.78	162	[10]
2	Unknown carbohydrate	1.21	ND	683.18	341.04b			
3	Unknown carbohydrate	1.3	ND	781.12	**439.02b**	420.95, 341.09[M-2H]2−, 277.01b, 178.80	162	
4	Unknown carbohydrate	1.4	ND	781.12	**439.04b**	421.04, 340.98[M-2H]2−b, 276.87, 178.83	162	
5	Galloyl-glucose	1.5	210, 276	331.13	270.72, 168.58b			[11]
6	Unknown	1.8	235, 275, 325	477.1	459.05b, 357.04, 234.83, 150.80			
7	Procyanidin dimer, Type B	1.9	225, 280	577.11	559.04, 450.99, **424.96b**, 407.15, 288.93, 286.97	406.90b, 381.02, 272.85	152	[11-13]
8	Unknown	2.0	235, 275, 325	439.00b, **425.05**	344.98, 240.80b			
9	Procyanidin dimer, Type B	2.3	225, 280	577.13	559.04, 450.97, **424.95b**, 407.09, 288.93, 286.97	406.91b, 381.12, 339.07, 272.90	152	[11-13]
10	Procyanidin trimer, Type B	2.4	225, 280	865.19	739.14, 695.12b, 577.07, 406.98, 286.87			[12, 13]
11	Catechin*	2.6	225, 280	288.99	270.90, 244.91b, 204.85, 178.83			
12	Procyanidin trimer monogallate	2.7	225, 280	1017.2	891.18, 865.18, 847.12, 729.12b, 577.11, 407.07, 287.81			[13]
13	Procyanidin dimer, Type B	2.8	225, 280	577.08	559.05, 451.00, **424.96b**, 407.00, 288.90, 286.97	406.90b, 381.11, 272.87	152	[11-13]
14	Procyanidin pentamer,	3.1	225, 280	720.55 [M-2H]2−	1315.33, 1151.29b, 1027.23, 863.22, 635.05, 577.05, 288.85			[12]
15	Procyanidin trimer, Type B	3.2	225, 280	865.21	739.13, 695.14b, 577.08, 407.00, 286.90			[12, 13]
16	Epicatechin*	3.5	225, 280	288.82	270.76, 244.75b, 230.68, 204.70, 178.65			
17	Procyanidin dimer monogallate	3.6	225, 280	729.17	**577.06b**, 425.06, 407.07, 286.92	559.05, 450.98, 424.98, 407.00b, 288.90	152	[11, 13]
18	Procyanidin trimer monogallate	3.7	225, 280	1017.2	**865.16b**, 847.15, 729.11, 577.06, 406.97	847.14, 695.12b, 577.05, 394.95, 286.81	152	[13]
19	Procyanidin trimer, Type B	4.0	225, 280	865.2	739.15, 695.14b, 577.07, 406.99, 286.89			[12, 13]
20	Piceatannol glucoside*	4.1	220, 305, 318	405.06	**242.73b**	224.70b, 214.68, 200.69, 184.64, 174.73	162	
21	Procyanidin trimer, Type B	4.2	225, 280	865.19	739.15, 695.12b, 577.08, 406.98, 286.87			[12, 13]
22	Resveratroloside*	4.6	219, 304, 315	389.07, **435.13** [M+HCOO]−	389.07b, 226.91			
23	Procyanidin trimer monogallate	4.8	225, 280	1017.19	**865.14**, 847.15, 729.16b, 603.09, 559.08, 407.06, 288.89	847.07b, 695.02, 575.94, 451.02, 286.80	152	[13]
24	Procyanidin dimer monogallate	5.1	225, 280	729.12	**577.05**, 559.05, 451.00, 441.01, 407.02b, 288.90	559.01b, 450.99, 406.95, 288.86	152	[11, 13]
25	Procyanidin tetramer, Type B	5.3	225, 280	1153.26	**1001.20**, 983.20, 865.16b, 739.12, 575.09, 449.02	983.18b, 804.93, 533.18, 382.95	152	[12, 13]
26	Procyanidin pentamer	6.3	225, 280	720.55 [M-2H]2−	1315.33, 1151.29b, 1027.23, 863.22, 635.05, 577.05, 288.85			[12]
27	Piceid*	7.4	218, 308, 318	**389.12**, 435.07 [M+HCOO]−	226.71b			
28	Procyanidin trimer digallate	7.6	225, 280	1169.24	1151.24, 999.21, 881.22b, 729.18, 603.11, 406.98			[13]

29	Procyanidin dimer digallate, Type B	7.7	225, 280	881.16	**729.12b**, 559.08, 407.01, 288.86	603.07, 577.08, 559.07, 451.01b, 407.10, 288.98	152	[13]
30	Procyanidin trimer monogallate	7.9	225, 280	1017.22	**865.17**, 847.15, 729.13b, 603.09, 575.09, 406.98, 286.88	847.07b, 739.12, 714.02, 577.04, 448.84, 288.69	152	[13]
31	Procyanidin heptamer	8.1	225, 280	1008.63[M-2H]−, 1152.74, 1021.26, 999.18, 631.20, 567.10, **499.09b**	484.00b, 452.98, 419.04, 345.92, 314.85, 288.79			[12, 14]
32	Epicatechin-3-O-gallate*	8.2	220, 280	440.95	330.82, 302.80, 288.82b, 270.81, 244.82			
33	Procyanidin dimer, Type B	8.4	225, 280	577.09	559.08, 450.96, **424.94b**, 407.06, 288.92	406.91b, 381.04, 339.00, 272.85	152	[11-13]
34	Procyanidin octamer	8.8	225, 280	1152.70[M-2H]−, 901.21, 879.11, 507.02, **439.04b**	423.93b, 392.86, 358.98, 315.84			[15]
35	Procyanidin trimer monogallate	9.0	225, 280	1017.21	**865.12**, 847.15, 729.15b, 603.08, 406.99, 288.90	847.15b, 684.05, 518.85, 451.83, 395.07, 301.69	152	[13]
36	Procyanidin octamer	9.1	225, 280	1152.69[M-2H]2-, 901.16, 864.10, 845.10, 439.02, **382.90b**	302.80b, 284.83, 176.67			[15]
37	Procyanidin tetramer monogallate	9.8	225, 280	**652.11**[M-2H]2-b, 1305.32	1179.22, 1017.23, 863.18, 729.11, 576.00, 567.07b, 440.99, 288.88, 286.86			[15]
38	Unknown	10.3	225, 280	1532.47, **382.90b**	302.77b, 284.88, 178.67			
39	Procyanidin gallate	10.5	225, 280	**796.27**[M-3H]3b,948.11[M-2H]2	1467.40, 1305.34, 1179.31, 1017.21, 863.17, 729.15b, 440.96, 288.86			[15]
40	Procyanidin trimer monogallate	11.0	225, 280	1017.2	**891.16**, 847.16, 729.14b, 603.07, 559.05, 407.03, 288.87			[13]
41	Procyanidin gallate	11.3	225, 280	**660.32**, 505.17b	1151.19, 999.17, 881.14, 584.04b, 440.96, 302.86			[12, 13, 15]
42	Resveratrol-hexoside	11.4	219, 304, 315	389.06	226.71			
43	Procyanidin dimer monogallate	12.0	225, 280	729.13	711.11, 603.05, **577.04**, 559.05, 407.01b, 288.91	559.03, 450.97, 406.93b, 288.85	152	[11, 13]
44	Emodin glucoside*	12.8	220, 247, 269, 281, 423	431.3	**268.75b**	239.63, 226.68, 224.72b	162	
45	Resveratrol*	13.5	218, 306, 318	226.78	184.60, 158.67b, 142.68			
46	Procyanidin dimer digallate, Type B	13.6	225, 280	881.13	**729.11b**, 559.12, 407.05, 288.90	603.07, 577.08, 559.04, 451.01b, 407.04, 288.98	152	[13]
47	N-trans-feruloyltyramine*	14.4	220, 281, 323	312.08	296.97b, 177.83, 134.87			
48	Acetyl lapathoside d	14.7	220, 290, 315	675.24	633.17, 615.12, **529.07b**, 511.12, 487.12, 453.11, 306.97	487.04b, 469.19, 306.96	146	[16]
49	N-Feruloyl-methoxytyramine	15.0	220, 281, 323	342.14	327.04b, 308.97, 297.01, 177.84, 134.87			[17]
50	Phenylpropanoid-derived disaccharide ester	15.3	220, 284, 315	655.21	613.18b, 595.18, 571.16, 553.10, 425.12, 306.99			

51	Cyanidin	15.4	210, 286, 332	286.9	268.79, 150.59b, 134.71, 124.75, 106.72			[18]
52	Unknown	15.6	225, 287, 315	585.28	537.17b, 371.13, 359.13			
53	Unknown	15.7	225, 287, 315	583.27	535.23b, 369.10, 357.25, 194.91			
54	Unknown	15.8	220, 280	685.23	643.20b, 625.20, 601.19, 337.12, 192.90			
55	Unknown	15.9	220, 287, 315	585.29	537.20b, 359.12, 345.14			
56	Torachrysone glucoside*	16.2	225, 267, 325	407.16	**244.87b**	229.97	162	
57	Unknown	16.8	225, 280	371.12	327.08b, 297.08			
58	Unknown	16.85	225, 280	597.26	553.13, 549.23, 383.11b, 371.12, 194.86			
59	Unknown	16.9	225, 280	583.31	553.20, 369.11b, 357.15, 194.80			
60	Emodin glucoside*	17.0	221, 247, 269, 281, 423	431.04	310.84, 292.76, **268.75b**	264.71, 240.73, 224.70b	162	
61	Dihydroksyferuloyl-O-acetoxy-p-coumaroyl-O-caffeoylquinic acid	17.4	214, 282, 325	735.27	693.19, **559.12b**, 541.18	517.11b, 499.10, 337.04, 264.90, 192.83	176	[18]
62	Tatariside e	17.5	220, 290, 315	717.39	675.19, **571.11b**, 529.20, 453.12, 288.94	529.06b, 511.05, 469.03, 306.85	146	[19]
63	Tatariside e	17.7	220, 290, 315	717.4	675.19, **571.13b**, 529.24, 453.10, 288.93	529.05b, 511.05, 469.00, 306.85	146	[19]
64	Unknown	17.8	225, 280	314.95	299.78b, 270.98, 246.72, 204.68, 178.78			
65	(diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid	17.9	214, 282, 325	777.25	735.22b, 717.25, 693.00, **601.16**, 559.13, 337.09	559.13b, 541.11, 499.05	176	[18]
66	Emodin bianthrone-hexose-(malonic acid)-hexose	18.1	220, 278	919.21	875.23, **757.10**, 713.20b, 671.25, 509.08, 458.00	713.15b, 509.04, 502.00, 457.99, 253.79	162	[20, 21]
67	Derivative of Emodin bianthrone-hexose-malonic acid	18.2	220, 278	1005.23	961.13, 917.29, 757.10, 713.23b, 458.10			[21]
68	Unknown	18.3	225, 280, 325	811.36	793.32b, 763.38, 745.34, 669.23, 567.21, 389.09, 342.99, 311.93			
69	Unknown	18.4	225, 280, 325	597.27	549.18b, 401.11, 357.12, 342.12, 194.87			
70	(diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid	18.7	214, 282, 325	777.26	735.24b, 717.25, 693.00, **601.16**, 559.20, 337.04	559.13b, 541.17, 499.13	176	[18]
71	Trimer lignin β-O-4-linked S unit with syringaresinol [S-(β-O-4')-S-(β-β')-S]	19.0	220, 280	643.29	613.22, 417.13b, 387.15, 224.93, 194.87			[22]
72	Tetramer lignin, S-(8-O- 4')-S-(8-O-4')-S- (8-8')-S	19.1	220, 280	869.39	851.34b, 821.34, 697.27, 643.22, 595.21, 417.15, 387.15			[22]
73	Emodin-O-(sulfonyl)-glucoside	19.2	214, 280,	511	430.99, 268.73b, 240.74, 224.96			[11, 20]
74	Lapathoside c	19.5	220, 290, 315	809.28	**663.13b**, 485.07, 322.98	517.04, 485.10b, 322.88, 280.89	146	[23, 16]
75	(diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid	19.6	214, 282, 325	777.3	735.24b, 717.13, 693.13, **601.17**, 559.00, 337.10	559.13b, 541.13, 499.00	176	[18]
76	Lapathoside c isomer	19.7	220, 290, 315	809.28	**663.13b**, 485.07, 322.98	517.04, 485.10b, 322.88, 280.89	146	[23, 16]
77	Unknown	19.8	220, 280, 315	**327.26**	309.12, 291.10, 228.95b, 210.95, 170.91			
78	Emodin-8-O-(6′-O-malonyl)-glucoside*	19.81	220, 282, 423	517.05	472.99b, 431.10			
79	Oligolignol-hedyotisol	20.1	220, 280	809.36	791.33, 773.34, 761.25, 743.33b, 565.21, 417.11			[24]
80	Tatariside e	20.2	220, 290, 315	717.22	675.17, **571.09b**, 529.10, 511.17, 487.09	529.05b, 511.04, 487.03	146	[19]
81	Derivative of lignin- S(8–8)S	20.5	220, 280	641.32	623.22, 611.20b, 417.13, 387.08, 347.09, 222.87			[25]
82	Unknown	20.7	220, 280, 315	1035.48	1017.45b, 999.38, 969.41, 821.41, 791.35, 595.14			

83	Tatariside a	20.8	220, 290, 315	759.22	717.21b, **613.13**, 571.13, 453.04, 288.94	571.09b, 553.10, 529.07, 511.06, 306.71	146	[19]
84	Methyl derivative of Emodin bianthrone-hexose-(malonic acid)-hexose	21.0	220, 278	933.21	889.37b, 727.21, 458.06			[21]
85	Oligolignol-e.g.hedyotisol(isomer)	21.1	220, 280	809.37	791.34b, 773.25, 761.31, 743.34, 565.21, 417.15			[24]
86	Acetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	21.2	214, 282, 325	819.29	777.29b, 759.25, **643.19**, 601.14, 513.13	601.10b, 583.16, 559.07, 337.02	176	[18]
87	Hydropiperoside*	21.3	220, 290, 315	779.26	**633.16b**, 615.19, 487.13, 469.16, 453.09	487.12b, 469.16, 453.11, 307.10, 289.03	146	
88	(3,6-*O*-di-*p*-coumaroyl)-β-fructofuranosyl-(2→1)-(2′-*O*-acetyl-6′-*O*-feruloyl)-β-glucopyranoside*	21.5	220, 290, 315	851.25	809.23, **705.20b**, 675.20, 527.07	663.22b, 645.38, 559.16, 527.16, 485.12	146	
89	Derivative of Emodin bianthrone-di-hexose	21.6	220, 278	1019.22	975.25, 931.42b, 889.25, 727.18, 458.06			[21]
90	Acetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	21.7	214, 282, 325	819.28	777.25b, 759.38, **643.18**, 601.14, 513.13	601.18b, 583.18, 559.15, 541.11, 337.02	176	[18]
91	Unknown	21.9	220, 280, 315	329.27	311.18, 293.12, 228.95b, 210.96, 170.91			
92	Emodin bianthrone-hexose-(malonic acid)-hexose	22.0	220, 278	919.2	875.24, **757.09**, 713.20b, 671.13, 509.06, 458.00	713.18b, 508.96, 501.88, 458.03	162	[20, 21]
93	Oligolignol-e.g.hedyotisol (isomer)	22.1	220, 280	809.32	791.30, 773.25, 761.28, 743.29, 611.20b, 565.18, 417.19			[24]
94	Phenylpropanoid-derived disaccharide ester	22.2	220, 290, 315	987.31	969.39b, 957.50, 851.27, 823.32, 633.18, 453.09			
95	Tatariside a	22.5	220, 290, 315	759.4	717.22,675.16, **613.14b**, 571.21, 529.18	571.09b, 553.05, 529.06, 511.06	146	[21]
96	Emodin bianthrone-hexose-(malonic acid)-hexose	23.0	220, 278	919.21	875.23, **757.10**, 713.22b, 671.25, 509.09, 458.13	713.16b, 509.00, 501.75, 458.20	162	[20, 21]
97	Unknown	23.01	220, 280, 315	837.37	819.31, 695.25, 640.23b, 579.18, 347.02			
98	Acetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	23.1	220, 288, 325	819.26	777.28b, 759.38, **643.17**, 601.25, 513.13, 361.01	601.13b, 583.13, 559.11, 336.97	176	[18]
99	Acetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	23.3	220, 288, 325	819.28	777.27b, 759.25, **643.17**, 601.25, 513.13, 361.04	601.15b, 583.10, 559.11, 336.97	176	[18]
100	Isomer of (3,6-O-di-p-coumaroyl)-β-fructofuranosyl-(2→1)-(2′-O-acetyl-6′-O-feruloyl)-β-glucopyranoside or tatariside d	23.4	220, 290, 315	851.39	809.24, **705.19b**, 663.27, 527.12	663.20b, 645.25, 559.13, 527.11, 485.10	146	[19]
101	Isomer hydropiperoside	23.45	220, 290, 315	779.36	**633.11b**, 615.25, 487.06, 469.13, 453.38, 288.86	487.06b, 469.18, 453.08, 306.90, 288.88	146	
102	Methyl derivative of Emodin bianthrone-hexose-(malonic acid)-hexose	23.5	220, 278	933.21	889.47b, 727.24, 458.09			[21]
103	Vanicoside C*	23.8	220, 290, 315	821.23	761.18, **675.16b**, 633.19, 529.10, 487.09, 288.87	633.15, 529.10b, 453.18, 288.98	146	
104	Acetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	24.0	220, 290, 315	819.31	777.29b, 759.25, **643.17**, 601.25, 583.20, 361.04	601.15b, 583.10, 559.11, 337.13	176	[18]
105	Derivative of Emodin bianthrone-hexose-malonic acid	24.1	220, 278	1005.22	961.13, 917.29, 757.12, 713.23b, 458.07			[21]
106	Phenylpropanoid-derived disaccharide esters	24.2	220, 290, 315	1181.4	1133.38, 1009.38, 955.50b, 809.41, 663.14			
107	Phenylpropanoid-derived disaccharide esters	24.5	220, 290, 315	1151.38	1133.42, 1103.35, 1009.32, 955.40b, 809.29			[23]
108	Phenylpropanoid-derived disaccharide esters	24.6	220, 290, 315	1151.4	1133.38, 1103.38, 1009.33, 955.39b, 809.29			[23]
109	Unknown	24.7	220, 280, 315	623.28	591.21, 551.26, 486.13, 460.17b, 352.16, 297.07			
110	Tatariside b*	25.0	220, 290, 315	893.27	851.24, **747.22b**, 705.27, 687.33, 569.19	705.24b, 687.25, 663.22, 569.16, 527.18, 322.96	146	

111	Methyl derivative of Emodin bianthrone-hexose-(malonic acid)-hexose	25.1	220, 278	933.2	889.42b, 727.19, 685.20, 416.06			[21]
112	Derivative of Emodin bianthrone-di-hexose	25.4	220, 278	1019.24	975.25, 931.43b, 889.25, 727.20, 458.07			[21]
113	Vanicoside B (isomer)	25.6	220, 290, 315	955.37	**809.26b**, 663.19	663.26b, 485.20, 453.09	146	
114	Unknown	25.8	220, 280, 315	801.29	759.25b, 741.50, **655.19**, 613.25, 571.13, 331.05	613.18b, 595.13, 571.15, 553.12, 330.95	146	
115	Tatariside b (isomer)	26.0	220, 290, 315	893.28	851.27, **747.21b**, 705.29, 687.31, 569.18	705.26b, 687.37, 663.34, 569.23, 527.31, 322.98	146	
116	Vanicoside B*	26.4	220, 290, 315	955.29	**809.22b**, 663.20, 453.05	663.21b, 485.20, 323.05	146	
117	Lapathoside a	26.6	220, 290, 315	985.3	839.24b, **809.24**, 663.22, 483.12	663.20b, 485.08, 322.85	176	[23, 16]
118	Diacetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	26.7	220, 288, 325	861.3	819.29b, 801.25, 777.25, 759.25, **685.20**, 643.17, 601.20, 583.18, 559.25, 513.17, 361.01	643.19b, 625.18, 601.15, 583.15	176	[18]
119	Lapathoside b	26.8	220, 290, 315	1015.31	869.23, **839.23b**, 693.19, 663.22, 483.15	693.23, 663.20b, 645.28, 499.09, 322.89	176	[26]
120	Questin*	27.0	222, 286, 430	282.94	267.89, 239.85b			
121	Phenylpropanoid-derived disaccharide esters	27.1	220, 290, 315	1193.48	1175.45, 1145.50, 1051.38, 997.44b, 851.31, 821.30			
122	Phenylpropanoid-derived disaccharide esters	27.2	220, 290, 315	1163.41	1145.45b, 1133.51, 999.37, 955.30, 851.15, 809.28			
123	Diacetyl derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	27.3	220, 288, 325	861.32	819.29b, 801.25, 777.25, 759.25, **685.20**, 643.17, 601.20, 583.18, 559.25, 513.17, 361.01	643.17b, 625.18, 601.15, 583.15	176	[18]
124	Vanicoside B (isomer)	27.4	220, 290, 315	955.28	**809.20b**, 663.19, 453.04	663.23b, 485.20, 323.06	146	
125	Dihydroferuloyl vanicoside B	27.8	220, 290, 315	1133.4	1115.49b, 1103.65, 997.32, 969.37			[23, 16]
126	Unknown	28.0	220, 290, 315	1071.38	1053.46b, 1041.64, 935.32, 907.40 ,866.38, 717.11			
127	Diacetyl derivative of (diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid	28.1	220, 288, 325	861.32	819.29b, 801.25, 777.25, 759.25, **685.20**, 643.17, 601.20, 583.18, 559.25, 513.17, 361.01	643.17b, 625.18, 601.15, 583.15	176	[18]
128	Emodin bianthrone-hexose-malonic acid	28.2	220, 278, 350	757.14	713.25b, 509.10, 458.12			[21]
129	Dihydroferuloyl vanicoside B	28.5	220, 290, 315	1133.38	1115.49b, 1103.50, 997.33, 969.38			[23, 16]
130	Derivative of Emodin bianthrone-di-hexose	28.7	220, 278	1019.24	975.38, 931.43b, 889.25, 727.20, 458.07			[21]
131	Vanicoside A*	29.0	220, 290, 315	997.31	955.29, **851.24b**, 821.28, 705.21, 453.05	809.24, 705.29b, 663.48, 527.22	146	
132	Tatariside C	29.1	220, 290, 315	935.27	893.27, **789.22b**, 747.32, 705.29, 611.17, 569.18	747.26b, 705.23, 611.26, 569.22	146	[19, 27]
133	Hydropiperoside b	29.2	220, 290, 315	1027.3	985.38, 967.30, 881.25b, **851.23**, 705.20, 453.09	809.19, 705.20b, 663.20, 527.08, 453.06, 322.96	176	[28]
134	Derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	29.4	220, 285, 325	965.36	923.31, **819.26**, 789.29b, 747.22, 643.21	777.31b, 643.08, 611.15, 569.05, 361.06	146	[18]
135	Derivative of (diacetoxy-methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid	29.7	220, 285, 325	995.37	953.33, **819.23b**, 777.25, 759.13, 611.24	777.23b, 735.18, 643.29, 611.16, 569.18	176	[18]
136	Isomer vanicoside A/ vanicoside F	29.9	220, 290, 315	997.32	955.29, **851.24b**, 821.28, 705.21, 453.06	809.22, 705.27b, 663.31, 527.20, 323.01	146	
137	Phenylpropanoid-derived disaccharide esters	30.3	220, 290, 315	1175.43	1157.52b, 1145.61, 1039.33, 1011.37			
138	Emodin bianthrone-hexose	30.35	220, 278, 350	671.17	653.18, **509.09**, 416.08b, 253.95	491.01, 253.88b	162	[21]
139	Unknown	30.4	220, 265, 325	**324.99b**, 244.93	244.88			
140	Unknown	30.7	220, 265, 325	1113.43	1095.45b, 1083.45, 977.29, 949.33			
141	Phenylpropanoid-derived disaccharide esters	31.4	220, 290, 315	954.33 [M-3H]3	881.20[M-2H]2, 809.20, **779.22b**	633.09b, 486.99	176	[23]

142	Emodin bianthrone-hexose-malonic acid	31.5	220, 278, 350	757.16	713.25b, 671.25, 509.10, 502.00, 458.12			[21]
143	Vanicoside E	32.1	220, 290, 315	1039.31	997.24, **893.25b**, 747.30, 453.05	851.27, 747.28b, 705.40, 569.24, 304.91	146	[28, 27]
144	Emodin bianthrone-hexose-malonic acid	32.7	220, 278, 350	757.16	713.21b, 671.19, 509.11, 502.00, 458.12			[21]
145	Methyl derivative of Emodin bianthrone-hexose	33.0	220, 278, 350	685.18	416.07b, 253.92			[21]
146	Methyl derivative of Emodin bianthrone-hexose	34.0	220, 278, 350	685.17	416.07b, 253.92			[21]
147	Emodin*	34.2	220, 248, 265, 288, 430	268.89	240.81, 224.93b, 181.68			
148	Methyl derivative of Emodin bianthrone-hexose-malonic acid	34.6	220, 278, 350	771.14	727.22b, 502.05, 458.07			[21]
149	Unknown	35.0	220, 278, 350	721.41	675.39b, 397.10			
150	Methyl derivative of Emodin bianthrone-hexose-malonic acid	35.2	220, 278, 350	771.15	727.24b, 502.05, 458.07			[21]
151	Methyl derivative of Emodin bianthrone-hexose-malonic acid	35.6	220, 278, 350	771.14	727.23b, 502.04, 458.08			[21]
152	Methyl derivative of Emodin bianthrone-hexose-malonic acid	36.3	220, 278, 350	771.15	727.23b, 502.04, 458.08			[21]
153	Unknown	37.5	225, 280, 325	**647.37b**, 1203.74	601.34b, 341.1			
154	Unknown	37.9	225, 280, 325	723.42	677.40, 397.09			
155	Unknown	38.3	220, 278, 350	369.18	351.12, 311.02, 292.99b, 210.79, 170.76			
156	Unknown	38.4	225, 280, 325	559.35	513.28b, 277.15, 252.98			
157	Unknown	39.4	225, 280, 325	559.36	513.29b, 277.16, 253.01			
158	Unknown	40.7	225, 275	649.39	603.37			
159	Isovitexin/vitexin diglucoside	41.0	269, 333	755.39	**593.25**, 575.29b, 477.06, 431.21	533.25, 503.21, 431.19b, 413.28	162	[29, 30]
160	Unknown	41.5	220, 278, 360	725.45	679.43b, 397.09			
161	Emodin bianthrone	41.6	220, 278, 360	509.14	491.08, 253.88b			[21]
162	Unknown	42.1	225, 280, 325	295.19	277.08b, 194.94, 170.90			
163	Unknown	42.7	225, 280, 325	561.59	515.32b, 279.20, 253.00			
164	Unknown	42.8	225, 280, 325	625.39	579.36			
165	Emodin bianthrone isomer	43.6	220, 278, 360	509.14	491.06, 253.88b			[21]
166	Unknown	44.7	225, 280, 325	651.41	605.4			
167	Unknown	45.2	220, 278, 350	757.4	**595.30**, 577.30, 477.05b, 433.22, 279.16	535.27, 505.24, 475.23, 433.22b, 279.13	162	
168	Unknown	47.2	225, 280, 325	563.39	517.34b, 281.21, 253.00			
169	Methyl derivative of emodin bianthrone	50.4	220, 278, 360	523.18	253.89			[21]
170	Alpha-carboxyethylhydroxychroman	54.4	292	277.19	259.13, 233.06b			[31]
171	Unknown	57.4	220, 278, 350	279.2	261.11b, 233.17			

b-base peak (the most abundant ion in the recorded spectrum), **in bold**-ions subjected to MS/MS fragmentation (if it's not obvious), *- isolated and/or characterised in our previous paper [5], ND-not determined

2.1.1. Stilbenoids

Almost all identified stilbenes with characteristic UV spectra with maxima about λ_{max} 220, 305, 320 nm have been previously observed in studied materials [5]. No were detected in *R. sachalinensis*. Compounds 20 (piceatannol glucoside) 22 (resveratrolside), 27 (piceid) and 45 (resveratrol), were characterized by HPLC-DAD-ESI-HR-TOF-MS and described in previous article [5]. Only compound 42 with quasi-molecular ion at *m/z* 389 $[M - H]^-$ and fragmentation ion at *m/z* 227 characteristic for resveratrol hexoside was noticed for the first time. Compound 42 was observed in small amount in *R. japonica* and *R x. bohemica* dichloromethane or diethyl ether fractions.

2.1.2. Carbohydrates

Deconvolution of an LC/MS mass chromatogram by using the Bruker's Dissect algorithm made it possible to observe several carbohydrates in very similar retention times. Furthermore, the hydrophilic character of compounds and the lack of chromophores confirmed the presence of carbohydrates. Based on quasi-molecular and fragmentation ions, compounds 1,2,3 and 4 were described as unknown carbohydrates [Table 1] [10]. Compound 1 exhibited quasi-molecular ion at *m/z* 341 $[M - H]^-$, characteristic for dissacharids e.g. sucrose, which was confirmed by the fragmentation ions at *m/z* 179 $[M - H\text{-}162]^-$, 161 and 143 characteristic for fructose. Compounds 2, 3 and 4 were more complex but contained the same fragmentation ions at *m/z* 341 and 179. More accurate analyses with using different method are needed to identify carbohydrates fully [32]. All apparent carbohydrates were observed in studied *Reynoutria* water fractions.

2.1.3. Flavan-3-ols and procyanidins

B-type procyanidins have different fragmentation patterns than A-type procyanidins and it was used to differentiate procyanidins by the type of linkages between monomeric units [12]. In studied material, there were observed only B-type procyanidins. Identified compounds possessed the same UV spectra characteristic for flavan-3-ols with maxima about λ_{max}225, 280 and characteristic fragmentation patterns presented in the Figure 4.

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Peer-reviewed version available at Molecules 2019, 24, 1136; doi:10.3390/molecules24061136

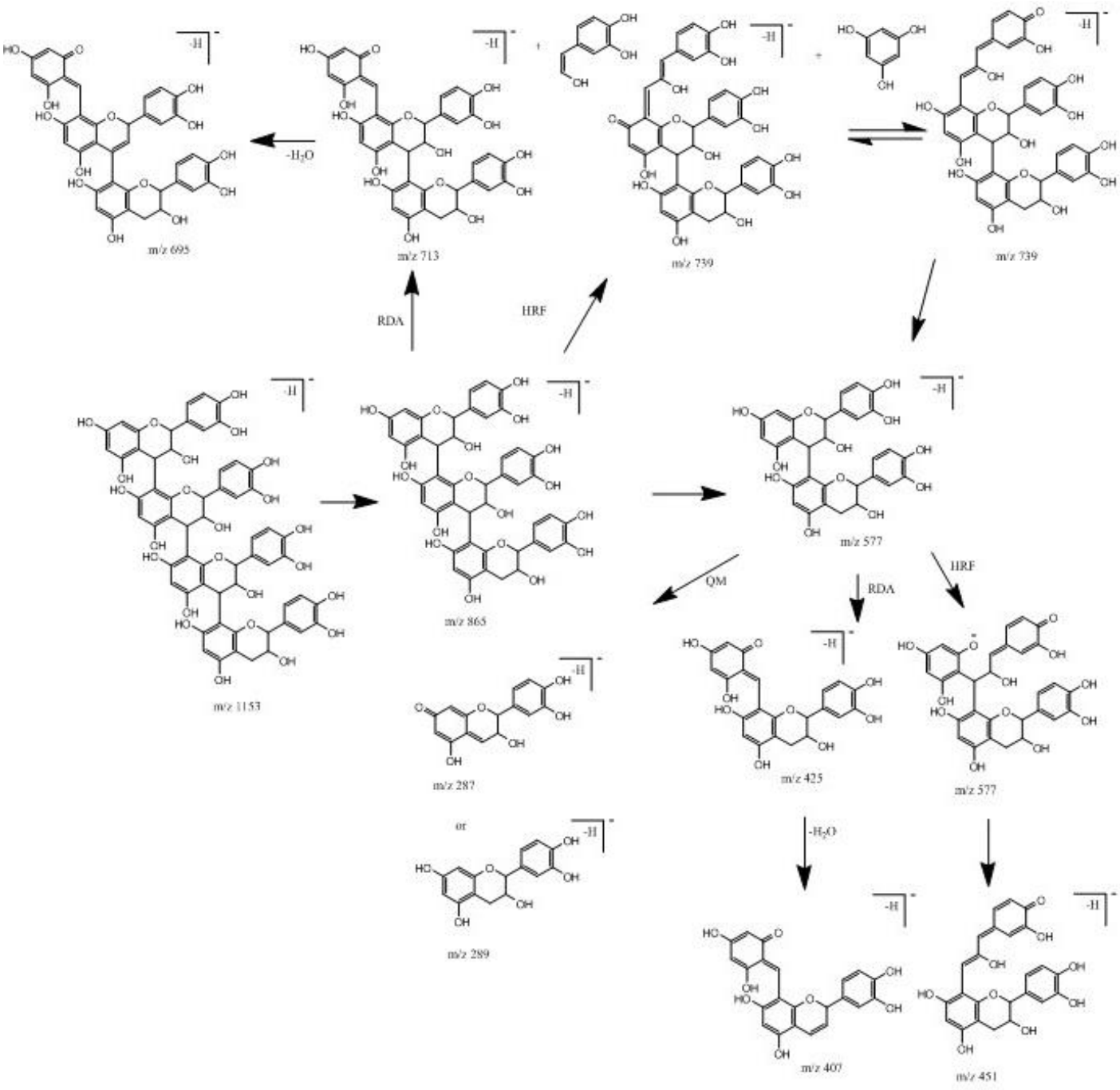

Figure 4. Fragmentation pathways of procyanidins in negative ion mode. RDA, retroDiels-Adler fragmentation; HRF, heterocyclic ring fission; QM - quinone methide cleavage.

Compound 11 was identified as catechin (quasi-molecular ion at *m/z* 289 $[M - H]^-$). Compound 16, an isomer of 11 with the same molecular mass was identified as epicatechin, both reported earlier in studied species [5] and confirmed with standards. Compounds 7, 9, 13, 33 with deprotonated ion at *m/z* 577 $[M - H]^-$ were identified as procyanidin dimers type B and compounds 10, 15, 19, 21 with deprotonated ion at *m/z* 865 $[M - H]^-$ as procyanidin trimers type B [11-13]. Compound 25 with quasi-

molecular ion at *m/z* 1153 $[M - H]^-$ and with the main product ion at *m/z* 865$[M - H\text{-}288]^-$ corresponding to procyanidin trimer type B was assigned to procyanidin tetramer type B. Procyanidins with high degree of polymerization, due to the mass range limitations of MS detector were identified by multiple charged ions. Compounds 14 and 26 possessed double- charged ion with signals at *m/z* 720 $[M-2H]^{2-}$ and compound 31, double-charged ion with signals at *m/z* 1008 $[M-2H]^{2-}$. Taking into account derivative ions [Table 1], characteristic for fragmentation patterns of pentamer and heptamer [12], [14, 15] they were tentatively assignment to procyanidin pentamers and procyanidin heptamer respectively. According literature, compounds 34 and 36 with signals at *m/z* 1152 $[M-2H]^{2-}$ were tentatively assignment to procyanidin octamers [15] .

Procyanidin gallates were distinguished by their characteristic fragment ion spectra yielding losses of galloyl moiety (-152 amu). Based on the literature [11, 13], [15] compounds 17, 24, 43 were identified as procyanidin dimer monogallates with quasi-molecular ion at *m/z* 729 $[M - H]^-$, and peaks 18, 23, 30, 35, 40 as procyanidin trimer monogallates with quasi-molecular ion at *m/z* 1017 $[M - H]^-$. Compound 37 with quasi-molecular ion at *m/z* 1305 $[M - H]^-$ and double- charged ion at *m/z* 652 $[M-2H]^{2-}$ as well as with fragmentation patterns characteristic for procyanidins, was tentatively assigned to procyanidin tetramer monogallate [15]. Compounds 29 and 46 revealed deprotonated ion at *m/z* 881 $[M - H]^-$ and had characteristic fragmentation pattern for procyanidin dimer digallate type B. Compound 28 with deprotonated ion at *m/z* 1169 $[M - H]^-$ and characteristic fragmentation pattern was tentatively assigned to procyanidin trimer digallate [13]. Compound 39 possessed triple-charged ions with signals at *m/z* 796 and fragmentation ions characteristic for procyanidin gallate like *m/z* 1305 [tetramer gallate] and others [Table 1]. It was assigned as procyanidin gallate, probably it is built with more than five monomers and one or more galloyl groups. Similar compound 41 assigned as procyanidin gallate, in their fragmentation possessed ions characteristic for procyanidin gallate, like *m/z* 881 for dimer digallate, *m/z* 1151 [15] for tetramer procyanidin type A [32], *m/z* 441 for catechin monogallate [13] and others [Table1]. Compound 32 with deprotonated ion at *m/z* 441$[M - H]^-$ and confirmed with standards was identified as epicatechin-3-O-gallate.

Procyanidins with degree of polymerization higher than dimers were described for the first time in *R. x bohemica.* Whereas most of them were earlier observed in extract of rhizome of *R. japonica* by analyzed it on HPTLC-MS [15]. Using different analytical methods-HPLC-DAD-MS, we confirmed the presence of high order procyanidins, up to decamers in the rhizomes of *R. japonica* and brought new data on the presence of these compounds in other *Reynoutria* species

The presence of a procyanidin trimer digallate has not been reported from any of the studied species

before.

2.1.4. *Anthraquinones*

Compounds 44 and 60 has been previously reported in studied species by using HR-MS analysis [5] and identified as emodin glucoside. Compounds 44 and 60 showed the most abundant product ion at *m/z* 269 $[M - H\text{-}162]^-$ (due to loss of glucosyl moiety) which was characteristic for emodin. It is supposed that peak 44 correspond to emodin-1-O-glucoside and peak 60 to emodin- 8-O-glucoside. Also compounds 78, 120 and 147 were earlier characterized using a high-resolution time-of-flight MS [5]. Here, the deprotonated ion peak at *m/z* 517 $[M - H]^-$ for compound 78 showed the most abundant product ion at *m/z* 473 $[M - H\text{-}44]^-$ and product ion at *m/z* 431 $[M - H\text{-}44\text{-}42]^-$what correspond to fragmentation pattern of emodin-8-O-(6′-O-malonyl)-glucoside, earlier identified in rhizome of *R. japonica* [11]. In our study, compound 78 was observed also in *R. bohemica*. Compound 120 with deprotonated ion peak at m/z 283 $[M - H]^-$ showed the most abundant product ion at *m/z* 240 and product ion at *m/z* 268 what correspond to fragmentation pattern of questin [11]. Questin was observed in all extract, but only in small amount in *R. sachalinensis*. The next antraquinone identified in all extracts was emodine (compound 147), due to characteristic UV spectrum and fragmentation [the most abundant product ion at *m/z* 225 and smaller product ion at *m/z* 241 and 182].

Compound 73 with deprotonated ion at m/z 511 $[M - H]^-$ a consecutive loss of SO_3 (the fragmentation ion at *m/z* 431 $[M - H\text{-}80]^-$) and glucoside *m/z* 269 $[M - H\text{-}80\text{-}162]-$ led to the formation of aglycone ion identified as emodin, proved by the diagnostic ions *m/z* 241 and 225. Based on the literature [11, 20] compound 73 was tentatively identified as emodin-O-(sulfonyl)-glucoside observed only in *R. japonica* butanol fraction.

Compounds 66, 92 and 96 were observed in studied species for the first time, all of them exhibited the same deprotonated ion at *m/z* 919 $[M - H]^-$ and similar fragment ions despite little difference in intensity. The proposed fragmentation map was shown in Figure 5.

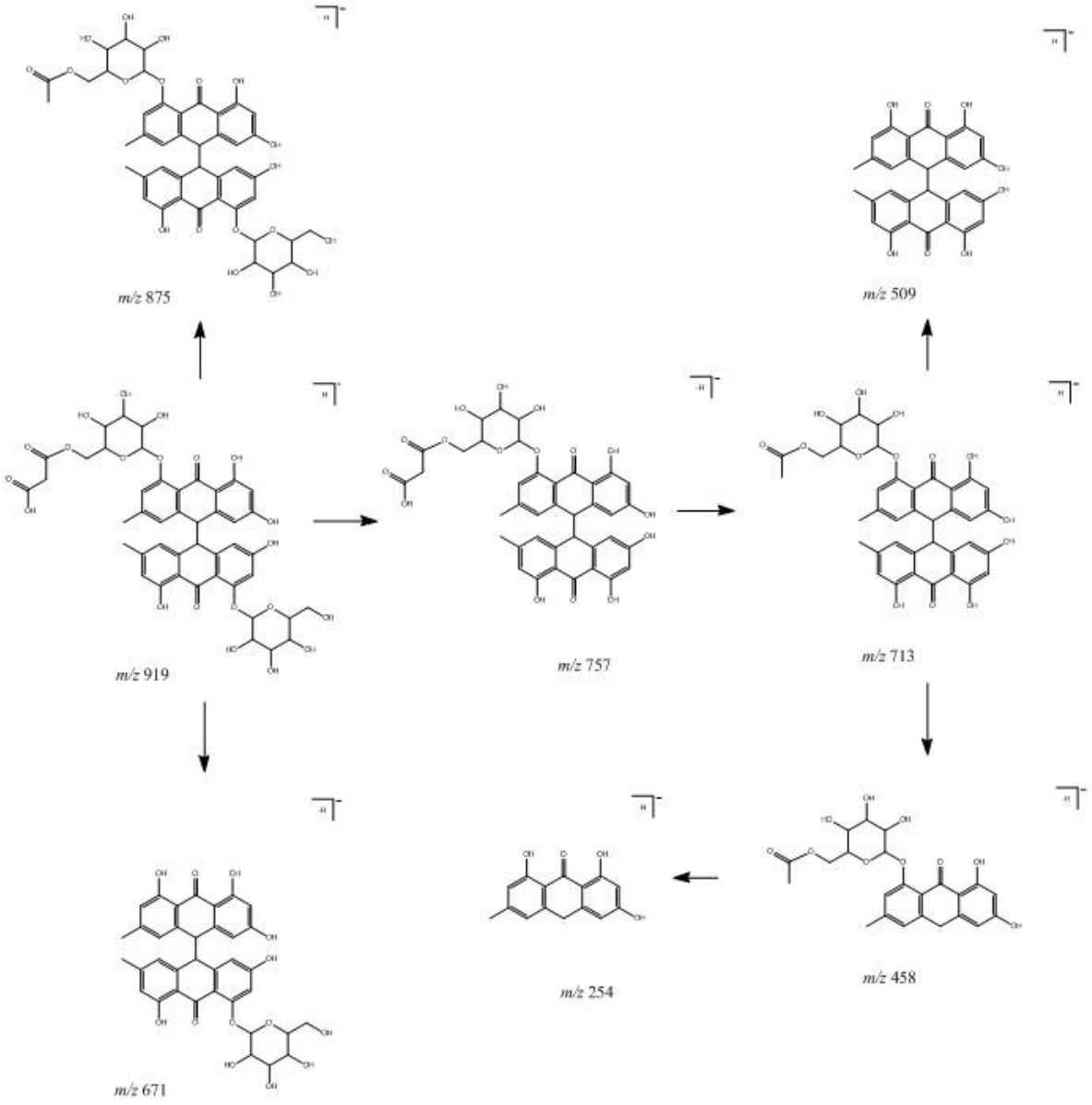

Figure 5. Proposed fragmentation pathway for peaks 66, 92, and 96.

Malonyl-substituted type glucosides were widely discovered from the *Fallopia multiflora* and *Rheum* plants. As a lack of standard compounds and no dianthrones have been found earlier in *R. japonica*, *R. bohemica* and *R. sachalinensis*, the structural characterization of the new dianthrone glycosides were referred to the literature of *Rheum* genus plants and *F. multiflora* [21, 33, 34] in which the MS fragmentation behavior of dianthrone glycosides was well described. Malonyl-substitution of dianthrone glycosides was earlier described in *F. multiflora* [21]. In our study, compounds 66, 92 and 96 tentatively assigned as emodin bianthrone-hexose-(malonic acid)-hexose were observed only in *R. bohemica* extract and *R. bohemica* butanol fraction.

Compounds 84, 102, 111 exhibited the same deprotonated ion at *m/z* 933 $[M - H]^-$ and the same most abundant fragment ion at *m/z* 889 $[M - H\text{-}44]^-$ due to loss of CO_2 and fragment ion at *m/z* 727

$[M - H\text{-}44\text{-}162]^-$ by cleavage of glucosidic bond. Deprotonated ion at *m/z* 933 $[M - H]^-$ differed from compounds 66, 92, 96 by 14 Da what corresponds to methyl moiety. Based on fragmentation pattern (similar to presented in Figure 5, but with addition of methyl moiety) and literature, these compounds were tentatively identified as methyl derivatives of emodin bianthrone-hexose-(malonic acid)-hexose [21] which were observed as small peaks only in *R. bohemica* butanol fraction. Compounds 89, 112, 130 with the same deprotonated ion at *m/z* 1019 $[M - H]^-$ and fragmentation ions like described above - *m/z* 889, 727, 458 suggested presence of methyl derivative of emodin bianthrone-hexose-(acetyl)-hexose. Mentioned compounds are fragmented to ion at *m/z* 975 $[M - H\text{-}44]^-$ due to loss of CO_2 , the most abundant product ion at *m/z* 931 $[M - H\text{-}44x2]^-$ due to lost the second CO_2. However, because of the many possible structures of compounds 89, 112, 130 , they were described as derivatives of emodin bianthrone-di-hexose. The exact structure of these compounds requires detailed research. Compounds 89, 112, 130 were observed only in *R. x bohemica* extract and butanol fraction.

Compounds 128, 142, 144 showed the same deprotonated ion at *m/z* 757 $[M - H]^-$. Due to the characteristic fragmentation ions at *m/z* 713, 509, 458 (Figure 5), they were tentatively identified as emodin bianthrone-hexose-malonic acid. Compounds were mainly observed in *R. x bohemica* extract and fractions and as a small peak in *R. japonica* diethyl ether. None of them were observed in *R. sachalinensis*.

Compounds 148, 150, 151, 152 exhibited the same quasi-molecular ion at *m/z* 771 $[M - H]^-$, differed from peaks described above-128, 142, 144 by 14 Da what could corresponds to methyl moiety loss. The most abundant product ion at *m/z* 727 $[M - H\text{-}44]^-$ was observed due to the lost of CO_2. Product ion at *m/z* 502 $[M - H\text{-}269]^-$ was caused by the 10–10′ homolytic cleavage of anthrone and product ion at *m/z* 458 $[M - H\text{-}44\text{-}269]^-$ by cleavage of anthrone and loss of CO_2. Peaks were tentatively identified as a methyl derivative of emodin bianthrone-hexose-malonic acid. Peaks were observed only in *R. x bohemica* extract and fractions.

Compounds 67 and 105 showed the same deprotonated ion at *m/z* 1005 $[M - H]^-$. Fragmentation ion at *m/z* 757 $[M - H\text{-}248]^-$ could represent emodin bianthrone-hexose-malonic acid as confirmed by subsequent fragmentation ions: the most abundant ion at *m/z* 713 $[M - H\text{-}248\text{-}44]^-$ due to the loss of CO_2 , product ion at at *m/z* 458 $[M - H\text{-}248\text{-}44\text{-}255]^-$ by cleavage of anthrone [Figure 5]. Moreover, quasi-molecular ion at 1005 $[M - H]^-$ m/z after lost CO_2 created ion at *m/z* 961 $[M - H\text{-}44]^-$ and after

more losss of CO_2 created ion at *m/z* 917 $[M - H\text{-}44x2]^-$. Due to the many possible structures of compounds 67 and 105, they were described as derivative of emodin bianthrone-hexose-malonic acid. The exact structure of the compounds requires detailed research. Compounds were observed in all studied *Reynoutria* specie of butanol fraction.

Compound 138 observed in extract of *R. japonica* and *R. x bohemica* exhibited deprotonated ion at *m/z* 671 $[M - H]^-$ and product ion at *m/z* 653 $[M - H\text{-}18]^-$, due to the loss of H_2O moiety , product ion at *m/z* 509 $[M - H\text{-}162]^-$ by loss of hexosyl moiety, the most abundant product ion at *m/z* 416 $[M - H\text{-}255]^-$ caused by the 10–10′ homolytic cleavage of anthrone and product ion at *m/z* 254 $[M - H\text{-}255\text{-}162]^-$ by cleavage of anthrone and hexosyl moiety [Figure 5]. Based on fragmentation pattern and literature, compound 138 was tentatively identified as emodin bianthrone-hexose [21]. Compounds 145 and 146 with the same quasi-molecular ion at *m/z* 685 $[M - H]^-$ differed from peak 138 by 14 Da what corresponds to loss methyl moiety. What is more, compounds 145 and 146 exhibited product ions at *m/z* 416 and 254, described above. Compounds were tentatively identified as methyl derivative of emodin bianthrone-hexose. Compounds were observed only in *R.x bohemica* dichloromethane and diethyl ether fractions.

Compounds 161 and 165 with the same deprotonated ion at *m/z* 509 $[M - H]^-$, fragmentation ion at *m/z* 491$[M - H\text{-}18]^-$, due to the loss of H_2O and fragmentation ion at *m/z* 254 $[M - H\text{-}255]^-$ caused by the 10–10′ homolytic cleavage of anthrone were tentatively identified as emodin bianthrone [Figure 5]. Compounds were observed in *R. japonica* and in *R. x bohemica* fractions.

Compound 169 exhibited the same UV spectra with maximum about λ_{max}220, 278, 360 nm, alike compounds 161 and 165. Deprotonated ion at *m/z* 523 $[M - H]^-$ fragmented to ion at *m/z* 254 $[M - H\text{-}269]^-$ caused by the 10–10′ homolytic cleavage of anthrone. Compound 169 differed from peak 161 and 165 by 14 Da what corresponds to methyl moiety. Compound was tentatively identified as methyl derivative of emodin bianthrone.

2.1.5. Phenylpropanoid Disaccharide Esters

Phenylpropanoid-derived disaccharide esters possess a core of sucrose carrying a varying number of O-substituents, including phenylpropanoid, acetyl, benzoyl, p-methoxybenzoyl, and p-hydroxybenzoyl groups. Peaks 48, 74, 76 , 87, 101, 88, 100, 103, 110, 115, 113, 116, 124, 117, 125, 129, 131, 136 corresponding to acethyl lapathoside d, lapathoside c and isomer e.g. hydropiperoside a [28], hydropiperoside and isomer, (3,6-O-di-p-coumaroyl)-β-fructofuranosyl-(2→1)-(2′-O-acetyl-6′-O-feruloyl)-β-glucopyranoside and an isomer, vanicoside C, tatariside b and an isomer, vanicoside B and isomers, lapathoside a, dihydroferuloyl vanicoside B and an isomer, vanicoside a and an isomer [Table 1] were observed in studied species previously [5, 16, 23]. The remaining phenylpropanoid disaccharide esters were detected in the present study for the first time. Identified phenylpropanoid-derived disaccharide esters possessed the same UV-Vis spectra characteristic for flavan-3-ols with maxima about λ_{max} = 220, 290, 315 nm. Compounds 62, 63 and 80 possessed the same quasi-molecular ion at *m/z* 717 $[M - H]^-$ and similar fragmentation patterns with the most abundant ion at *m/z* 571[M – H-146]$^-$ by loss of deoxyhexosyl, which gives in MS^3 analysis similar ions with the most abundant at *m/z* 529 $[M - H\text{-}42]^-$ by loss of acetyl. According to the literature [19] these compounds were tentatively identified as tatariside e and isomers. Compounds 83 and 95 with quasi-molecular ion at *m/z* 759 $[M - H]^-$ and with characteristic fragmentation pattern [Table 1], were tentatively assigned as tatariside a and isomer [19]. Both tatariside e and tatariside a were previously isolated from *Fagopyrum tataricum* [19]. Compound 119 observed in diethyl ether fraction of *R. sachalinensis* and *R. x bohemica* with quasi-molecular ion at *m/z* 1015 $[M - H]^-$ and characteristic fragments was assigned as lapathoside b, earlier isolated and described from *Polygonum lapathifolium* [26]. Compound 132 with quasi-molecular ion at *m/z* 935 $[M - H]^-$ was identified as tatariside c, earlier isolated from *Fagopyrum tataricum* [19, 27]. Compound 132 had an additional acetyl group relative to the tatariside B. Fragmentation ions of compound 132 were characteristic for tatariside b e.g. *m/z* 893 and others [Table1]. Compound 133, observed in all studied species, with quasi-molecular ion at *m/z* 1027 $[M - H]^-$ was tentatively identified as hydropiperoside b, isolated for the first time from *Polygonum hydropiper* [28]. Quasi-molecular ion at *m/z* 1027 give fragment ions at *m/z* 985, which corresponds to the loss of the acetyl group from hydropiperoside b and is the same as quasi-molecular ion of lapathoside a. Similar compound 143 with quasi-molecular ion at *m/z* 1039 $[M - H]^-$ identified as vanicoside e, after losing the acetyl group, product fragmentation ion at *m/z* 997 $[M - H\text{-}42]^-$, characteristic for quasi-molecular ion of vanicoside a [28]. Vanicoside e was observed in diethyl ether fraction of *R. sachalinensis* and in small amount in *R. x bohemica.* Compounds 107 and 108 with quasi-molecular ion at *m/z* 1151 $[M - H]^-$ were earlier observed in rhizomes of *R.sachalinensis* [23] and were

described as undefined phenylpropanoid glucoside. Compounds 107, 108 gave fragmentation ions characteristic for dihydroferuloyl vanicoside B *m/z* 1133 and for vanicoside b *m/z* 955, *m/z* 809 and were observed in all studied species. Compound 106 with quasi-molecular ion at *m/z* 1181 $[M - H]^-$, observed in small amount only in diethyl ether fraction of *R. sachalinensis* and *R. x bohemica,* was noticed there for the first time. It has been described as disaccharide ester derivatives of phenylpropanoid due to its UV-Vis spectrum and fragmentation ions, characteristic for this group of compounds [Table 1]. Compounds 121, 122 and 137 with quasi-molecular ions at *m/z* 1193, 1163 and 1175 respectively, were observed for the first time in studied species and were desribed as disaccharide ester derivatives of phenylpropanoid due to fragmentation ions such as *m/z* 997 (vanicoside a), *m/z* 955 (vanicoside b). Compound 141 which was observed only in ethyl acetate fraction of *R. sachalinensis*, possesed triple-charged ion with signal at *m/z* 954, but also fragmentation ions at *m/z* 809 characteristic for lapathoside c, *m/z* 779 characteristic for hydropiperoside, as well UV-Vis spectrum with maximum at λ_{max} 220, 290, 315 nm and this compound was described as disaccharide ester derivatives of phenylpropanoid. Compound 50 observed in *R.sachalinensis* and *R. x bohemica* fractions was tentatively assigned as disaccharide ester derivatives of phenylpropanoid because of their fragmentation ions at *m/z* 613, 571, similar to fragmentation ions of tatariside a (compound 83). Compound 94 was tentatively assigned as disaccharide ester derivatives of phenylpropanoid because its UV-Vis spectrum similarity and fragmentation ions at *m/z* 851 ((3,6-O-di-p-coumaroyl)-β-fructofuranosyl-(2→1)-(2′-O-acetyl-6′-O-feruloyl)-β-glucopyranoside) and ions at *m/z* 633, 453 similar to fragmentation ions of hydropiperoside.

2.1.6. Lignin oligomers

Compounds tentatively identified as lignin oligomers (LOs) were observed in dichloromethane fractions of studied *Reynoutria* species. All LOs were seen in studied raw materials for the first time. Identification was made based on fragmentation pattern of LOs and the UV/VIS spectrum and compared with literature.

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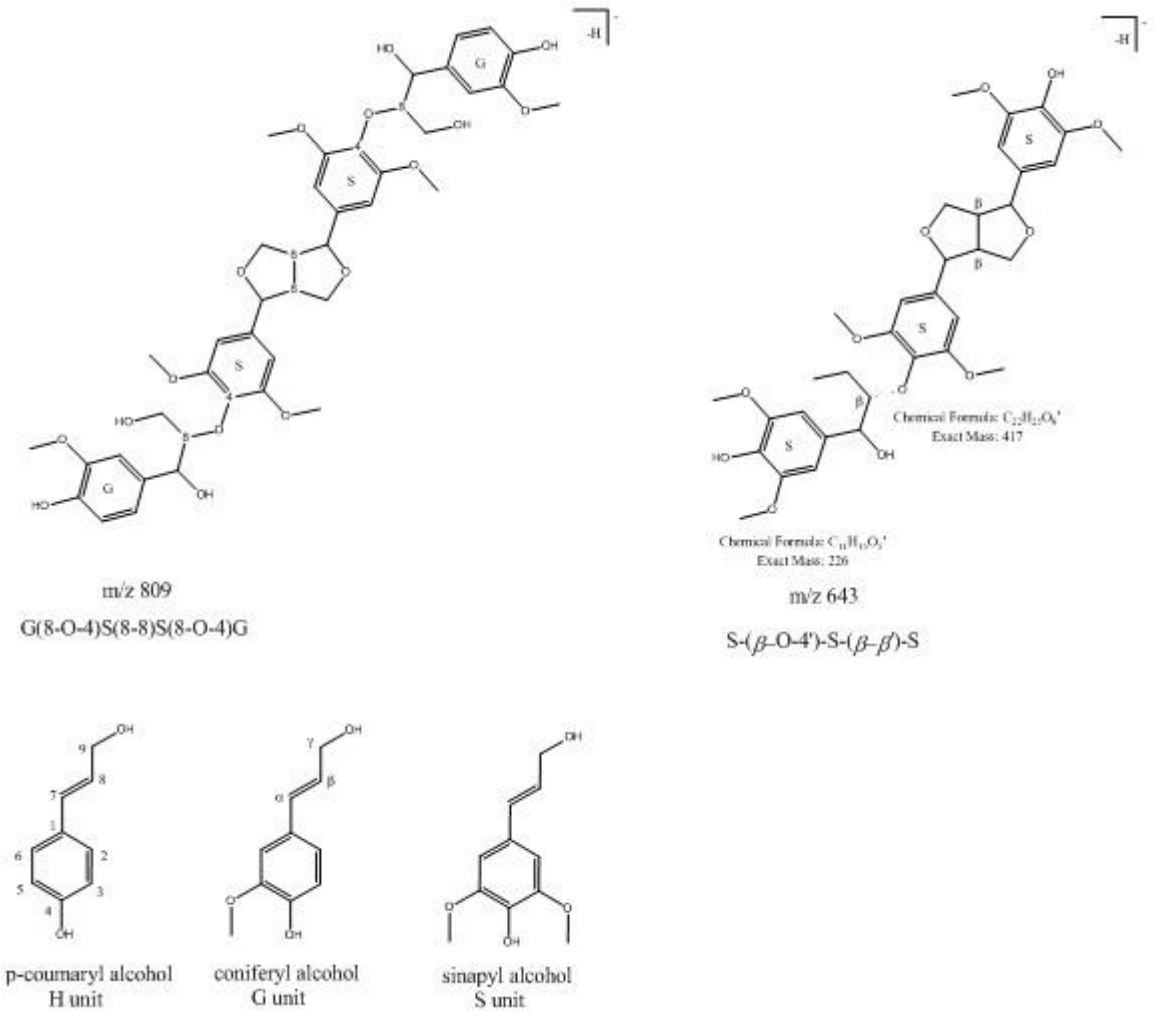

Figure 6. Lignin oligomers

Coniferyl alcohol (G unit), sinapyl alcohol (S unit) and p-coumaryl alcohol (H unit) are linked covalently, forming ether, ester and carbon–carbon bonds, which repeat to provide great complexity of lignin [25]. Degree of polymerization in natural lignin is difficult to measure because it is supposed that it fragments during extraction [35]. Therefore, the lignin fragments - oligomers of lignin are the most often identified in plant extracts. Compounds 71 and 72 were observed only in dichloromethane fraction of *R. sachalinensis* and were identified based on fragmentation pattern described in literature [25]. Quasi-molecular ion of compound 71 at *m/z* 643 $[M - H]^-$ was tentatively identified as trimer lignin β-O-4-linked S unit with syringaresinol [S-(β-O-4')-S-(β-β')-S] due to its fragmentation pattern corresponds to described by Evtuguin et al. [22]. Characteristic and the most abundant fragmentation ions at *m/z* 417 correspond to deprotonated syringaresinol [Figure 6]. Compound 72 with quasi-molecular ion at m/z 869 differ by 226 Da from compound 71, what correspond to the syringyl phenyl propane unit. Based on fragmentation pattern, which was similar to peak 71 and based on literature [22], peak 72 was assigned as tetramer lignin, S-(8-O- 4')-S-(8-O-4')-S-(8-8')-S. Compound 81, observed as very small peak in the dichloromethane fraction of *R. sachalinensis* was described as derivative of lignin- S(8–8)S . UV/VIS spectrum (λ_{max} at 220 and 280 nm) and fragmnetation ions at 417 *m/z* and 387 (-CH_2O), suggested that the compound 81 is composed of syringaresinol. Compounds

79, 85 and 93 with the same quasi-molecular ion at *m/z* 809 $[M - H]^-$ and fragmentation ions were observed in all dichloromethane fractions of *Reynoutria* species. Quasi-molecular ion at *m/z* 809 $[M - H]^-$ suggested a tetrameric structure of compound, composed of two **G** and two **S** units [Figure 6]. MS/MS spectral peaks at *m/z* 791 ($-H_2O$), 773 ($-2H_2O$), 761 ($-CH_2O$ and H_2O), 743 ($-CH_2O$ and $2H_2O$) indicated the presence of two β-aryl ether units and fragmentation ion at *m/z* 417 correspond to deprotonated syringaresinol [24]. This MS and MS/MS spectrum was similar to the spectrum of oligolignol: **G**(8–O–4)**S**(8–8)**S**(8–O–4)**G** [24] called hedyotisol [36].

2.1.7. Other Hydroxycinnamic Acid Derivatives

Quasi-molecular ion at *m/z* 735 $[M - H]^-$ for compound 61 was observed in all extracts. Peak showed a product ion at *m/z* 693 $[M - H\text{-}42]^-$, due to the loss of acetyl moiety. The most abundant product ion at *m/z* 559 was due to the loss of feruloyl or isoferuloyl group. The fragmentation pattern showed ions at *m/z* 499 and 337, which were characterized to *p*-coumarylquinic acid moiety. Based on fragmentation pattern and compare with literature, compound 61 were tentatively assigned as dihydroksyferuloyl-O-acetoxy-p-coumaroyl-O-caffeoylquinic acid [18] [Figure 7].

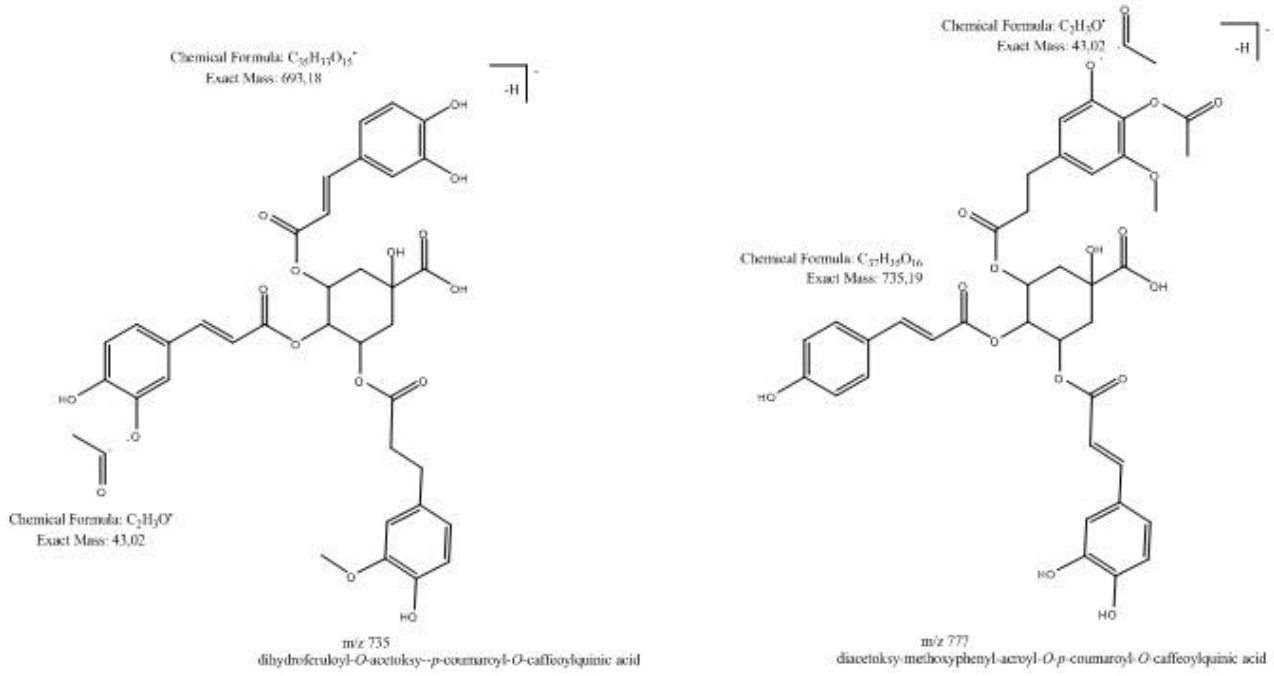

Figure 7. Examples of hydroxycinnamic acids esters.

Quasi-molecular ion at *m/z* 777 $[M - H]^-$ [Figure 7] for compounds 65, 70 and 75 showed the most abundant product ion at *m/z* 735 $[M - H\text{-}42]^-$, due to the loss of acetyl moiety, product ion at *m/z* 693 $[M - H\text{-}42x2]^-$ due to the loss next acetyl moiety , product ion at *m/z* 717 $[M - H\text{-}42\text{-}18]^-$ due to the loss acetyl moiety and H_2O. Fragmentation ions at *m/z* 499 and 337 are characterized to *p*-

coumarylquinic acid moiety [37-39]. Based on fragmentation pattern and compare with literature, compounds 65, 70, 75 were tentatively assigned as (diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid and its isomers [18].

Compounds 86, 90, 98, 99, 104 showed quasi-molecular ion at *m/z* 819 $[M - H]^-$ and similar fragmentation ions like compounds 65, 70, 75 e.g the most abundant product ion at *m/z* 777 $[M - H-42]^-$ due to the loss of acetyl group. Compounds 86, 90, 98, 99, 104 were tentatively assigned as acetyl derivative of (diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acids [18]. The most abundant peak 118, observed in all dichloromethane fractions and peaks 123, 127 with quasi-molecular ion at *m/z* 861$[M - H]^-$ were described as diacetyl derivative of (diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid. The product ion at *m/z* 819 $[M - H-42]^-$ due to the loss of acetyl moiety and the rest fragmentation ions were similar to earlier described hydroxycinnamic acid derivatives. Compound 134 observed in the dichloromethane fraction of *R. sahalinensis* with quasi-molecular ion at *m/z* 965 $[M - H]^-$ and compound 135 observed in the dichloromethane fraction of *R. bohemica* with quasi-molecular ion at *m/z* 995 $[M - H]^-$ due to the more complex structure was described as derivatives of (diacetoxy-methoxyphenyl)acroyl-O-p-coumaroyl-O-caffeoylquinic acid. However, it can be assumed that compound 134 is coumaroyl or deohexosyl derivative of compound 86 or its isomers, due to the loss of moiety at *m/z* 146 and product ion at *m/z* 819 $[M - H-146]^-$. The fragmentation of product ion at *m/z* 819 gave fragmentation ions, which were similar to compound 86. Whereas fragmentation of compound 135 gave the most abundant product ion at *m/z* 819 $[M - H-176]^-$ due to the loss of feruloyl or oxyhexosyl moiety.

2.1.8. Naphthalene derivatives

Compound 56 was characterized by HPLC-DAD-HR-MS analysis in previous article as torachrysone glucoside [5]. Peak 56 showed deprotonated ion peak at *m/z* 407 $[M - H]^-$ and product ion at *m/z* 245 $[M - H-162]^-$ by cleavage glucosidic bond and characteristic for torachrysone fragmentation ion at *m/z* 230 $[M - H-162-15]^-$. Torachrysone glucoside was noticed in acetone extract and dichloromethane fractions of *R. japonica* and *R. x bohemica.*

2.1.9. Other compounds

Compound 47 with quasi-molecular ion at *m/z* 312 $[M - H]^-$ was earlier identified as N-trans-feruloyltyramine by HPLC-DAD-HR-MS analysis and described in our previous article [5]. Using

different analytical instrument, based on compound MS, MS^2 and MS^3 spectra, its identity was confirmed. Moreover, compound 49 exhibited similar UV/VIS spectrum (λ_{max} at 220, 280, 323 nm) and fragmentation pattern to compound 47 (*m/z* 297, 178, 135) and differed from compound 47 by 30 Da what could result from methoxylation. Based on fragmentation ions and literature [17] compound 49 was tentatively assigned as N-Feruloyl-methoxytyramine, observed in the studied plants for the first time.

Compound 51, because of deprotonated ion at *m/z* 287 and product ion at *m/z* 269, the most abundant product ion at *m/z* 151 and product ion at *m/z* 135, 125, 107 was tentatively , identified as cyanidin [18]. Unfortunately due to the UV–vis spectra was recorded in the range of 200–450 nm, it was impossible to get all maximum spectra of this compound to confirm the assumption. Compound was noticed in fractions of *R. x bohemica* and *R. sachalinensis.*

Compound 5 observed in fractions of *R. x bohemica* and *R. sachalinensis* with quasi-molecular ion at *m/z* 331 $[M - H]^-$ and the most abundant product ion at *m/z* 169 $[M - H\text{-}162]^-$ by cleavage glucosidic bond was tentatively, based on literature [11], described as galloyl glucose, earlier observed in *R. japonica rhizomes*.

Compound 159 showed deprotonated ion peak at m/z 755 $[M - H]^-$, product ion at *m/z* 593 $[M - H\text{-}162]^-$ by cleavage glucosidic bond, the most abundant product ion at *m/z* 575 $[M - H\text{-}162\text{-}18]^-$ due to the loss of glucosyl moiety and H_2O, product ion at *m/z* 431 $[M - H\text{-}162x2]^-$ by cleavage two glucosidic bond. The next fragmentation of the product ion at *m/z* 575 showed that the most abundant fragment ion was *m/z* 431, what together with the rest fragmentation ions and characteristic UV/VIS spectrum (λ_{max} at 269, 333 nm) suggested that peak 159 could be isovitexin or vitexin diglucoside [29, 30]. It was observed only in dichloromethane fraction of *R. x bohemica.* It was noticed for the first time in this species.

Compound 170 because of its lipophilic character and deprotonated ion peak at m/z 277 $[M - H]^-$, product ion at *m/z* 259 $[M - H\text{-}18]^-$ due to the loss of H_2O and the most abundant product ion at *m/z* 233 $[M - H\text{-}44]^-$ due to loss of CO_2 was tentatively assigned as alpha-carboxyethylhydroxychroman [31]. It was observed in dichloromethane fraction of *R. x bohemica* and *R.sachalinesis.*

2.2. Antioxidant activities and polyphenols content

Results of bioactivity screening of all 18 extracts and fractions are presented in Table 2.

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Table 2. Antioxidant activity of studied extracts and fractions

Fraction	Radical Scavenging activity DPPH (EC50 µg/mL)			Reducing power AAE (%) 37 °C			Reducing power AAE (%) 90 °C			LA-Peroxidation (IC50 µg/mL)		
	R.j	*R.s*	*R.b*	*R.j*	*R.s*	*R.b*	*R.j*	*R.s*	*R.b*	*R.j*	*R.s*	*R.b*
Acetone	9.6 ± 0.5	8.7 ± 0.4	12.6 ± 0.7	6.5 ± 0.3	6.0 ± 0.3	6.4 ± 0.2	28.5 ± 1.1	27.9 ± 1.0	21.4 ± 1.6	80.3 ± 2.8	71.6 ± 2.6	68.9 ± 1.6
Dichloromethane	202.1 ± 5.6	56.5 ± 3.9	63.3 ± 2.9	2.6 ± 0.2	1.8 ± 0.1	1.6 ± 0.06	11.2 ± 0.1	12.2 ± 0.6	10.8 ± 0.4	401.8 ± 12.7	112.2 ± 2.5	153.6 ± 6.0
Diethyl ether	9.3 ± 0.4	10.2 ± 0.8	8.8 ± 0.3	10.2 ± 0.5	8.3 ± 0.4	10.9 ± 0.4	35.0 ± 1.6	32.6 ± 1.2	35.4 ± 1.1	63.8 ± 2.6	67.3 ± 1.4	52.1 ± 2.6
Ethyl acetate	**6.5 ± 0.4**	**4.7 ± 0.3**	**6.2 ± 0.1**	**13.9 ± 0.3**	**16.2 ± 0.2**	**16.6 ± 0.2**	**38.8 ± 1.3**	**44.7 ± 1.3**	**36.5 ± 1.7**	**45.7 ± 1.9**	**32.3 ± 1.7**	**40.6 ± 1.4**
Butanol	9.1 ± 0.3	6.9 ± 0.2	8.1 ± 0.3	6.6 ± 0.2	8.2 ± 0.2	8.1 ± 0.2	29.0 ± 1.1	29.4 ± 0.8	25.7 ± 1.2	93.2 ± 3.5	66.2 ± 2.6	113.4 ± 4.2
Water	58.0 ± 2.5	35.0 ± 0.5	57.3 ± 2.3	0.6 ± 0.02	1.5 ± 0.05	0.1 ± 0.01	13.6 ± 0.4	16.9 ± 0.4	12.8 ± 0.2	650.7 ± 10.6	635.6 ± 17.8	690.1 ± 9.0

Radical Scavenging activity DPPH for ascorbic acid (as control) EC 50=8.6 ± 0.4 µg/mL; Reducing power AAE (%) for quercetin (as control) at 37 °C=30.7 ± 1.2 AAE (%) and at 90 °C=52.0 ± 2.7 AAE (%), LA-Peroxidation for quercetin (as control) IC50=19.6 ± 1.1 µg/mL. *R.j-Reynoutria japonica*, *R.s-Reynoutria sachalinensis*, *R.b-Reynoutria bohemica*. Data were expressed as mean ± SD, performed in at least three independent experiments, assayed in triplicate.

All studied acetone extracts demonstrated high ability to scavenge the 2,2′-diphenylpicrylhydrazyl radical, comparable to ascorbic acid. Fractionation of extracts allowed to obtained fractions like ethyl acetate with even stronger properties of scavenging the stable radical.

High ability to scavenge stable radical was associated with high amount of polyphenols, especially tannins in studied extract and fractions (Table 3), what was demonstrated by Spearman Rank Order Correlation in Table 4.

Table 3. Total polyphenols and tannins content in studied extracts and fractions. Data were expressed as mean ± SD, performed in at least three independent experiments, assayed in triplicate.

Fraction	TPC Total polyphenols [GAE] mg/g fraction			Tannins content [GAE] mg/g fraction		
	R.j	*R.s*	*R.b*	*R.j*	*R.s*	*R.b*
Acetone	324.1 ± 9.8	317.7 ± 14.1	487.7 ± 11.9	233.3 ± 6.4	264.0 ± 7.0	360.0 ± 6.5
Dichloromethane	96.4 ± 5.6	22.7 ± 0.9	81.1 ± 2.7	61.0 ± 2.9	13.0 ± 0.4	60.3 ± 2.7
Diethyl ether	469.1 ± 3.0	355.1 ± 17.1	615.4 ± 6.7	338.6 ± 17.2	241.6 ± 11.3	509.3 ± 19.8
Ethyl acetate	**583.4 ± 6.5**	**640.7 ± 11.0**	**642.9 ± 8.9**	**484.3 ± 19.1**	**528.3 ± 16.9**	**510.5 ± 15.8**
Butanol	307.1 ± 6.9	352.7 ± 7.0	286.1 ±6.0	258.0 ± 9.6	315.0 ± 7.4	243.0 ± 10.4
Water	28.7 ± 1.5	65.4 ± 4.5	29.7 ± 2.2	23.6 ± 1.1	46.6 ± 2.0	29.3 ± 0.6

Table 4. Spearman Rank Order Correlation. Marked correlations are significant at p<0.05

Variable	LA-Peroxidation EC50	DPPH EC50	Reducing power AAE 37°C	Reducing power AAE 90°C	Total polyphenols	Tannins	DMACA	HCL-Butanol
LA-Peroxidation EC50	1,000	**0,751**	**-0,904**	**-0,874**	**-0,823**	**-0,804**	**-0,938**	**-0,300**
DPPH EC50	**0,751**	1,000	**-0,843**	**-0,869**	**-0,663**	**-0,742**	**-0,757**	**-0,736**
Reducing power AAE 37°C	**-0,904**	**-0,843**	1,000	**0,899**	**0,781**	**0,819**	**0,877**	**0,400**
Reducing power AAE 90°C	**-0,874**	**-0,869**	**0,899**	1,000	**0,795**	**0,810**	**0,917**	**0,411**
Total polyphenols	**-0,823**	**-0,663**	**0,781**	**0,795**	1,000	**0,939**	**0,779**	0,259
Tannins	**-0,804**	**-0,742**	**0,819**	**0,810**	**0,939**	1,000	**0,738**	**0,378**
DMACA	**-0,938**	**-0,757**	**0,877**	**0,917**	**0,779**	**0,738**	1,000	**0,272**
HCL-Butanol	**-0,300**	**-0,736**	**0,400**	**0,411**	0,259	**0,378**	**0,272**	1,000

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These results are in accordance with above presented phytochemistry of extracts and fractions, where the most antioxidant active ethyl acetate fractions contained numerous polyphenols including procyanidins [Figure 1,2,3, Table 1]. Ethyl acetate fractions, which were the richest in polyphenols and tannins, exhibited also the highest capacity to reduce metal ions (phosphomolybdenum reduction assay) and to prevent the oxidation of linoleic acid. Diethyl ether and butanol fractions of studied species exhibited slightly weaker antioxidant activity, however they also contained significantly lower content of total polyphenols and tannins [except *R.bohemica* diethyl ether fraction, where the differences were not significant with ethyl acetate fraction]. Because the results indicated the big impact of tannins on antioxidant activity, what was according with phytochemical analysis, we decided to check the amount of procyanidins in studied extracts and fractions using acid butanol method (Bate-Smith method) [40] and DMACA-HCl assay. Results presented on Figure 8 revealed that ethyl acetate and butanol fractions contained the highest amount of proanthocyanidins, whereas *R. sachalinensis* ethyl acetate and butanol fractions contained significantly higher amount proanthocyanidins than others.

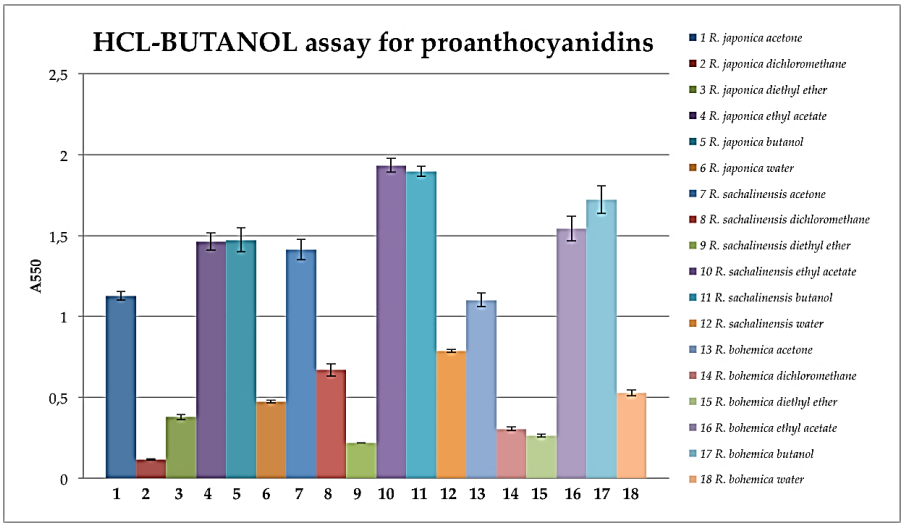

Figure 8. Proanthocyanidins quantified spectrometrically for absorbance at 550 nm in extracts and fractions. Data were expressed as mean ± SD, performed in at least three independent experiments, assayed in triplicate.

R. sachalinensis contained also the highest amount of proanthocyanidins through the acetone extracts and results indicated that in fractionation process fewer of them remained in the diethyl ether fraction and more passed to ethyl acetate and butanol fraction compare to other species. The content of proanthocyanidins in the butanol fractions is very similar to the content in the ethyl acetate fractions of studied species, despite the fact that the Folin-Ciocalteu assay showed significantly less amounts of tannins in the butanol fractions compare to ethyl acetate fractions. It is important to mention that the acid butanol method we used to measure the amount of proanthocyanidins involves depolymerization of the polymer of proanthocyanidins in acid and conversion of the monomers to anthocyanidins, which were spectrophotometrically quantified. Based on our results, we can assumed that in the butanol fractions are more procyanidins with higher degree of polymerization than in the ethyl acetate fraction. This assumption agrees with phytochemistry analysis in which compounds putatively identified as procyanidin heptamer and octamer were noticed mainly in butanol fractions of studied species.

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The results from DMACA assay indicated that in diethyl ether and ethyl acetate fractions are significantly more flavanols than in butanol fractions (Figure 9).

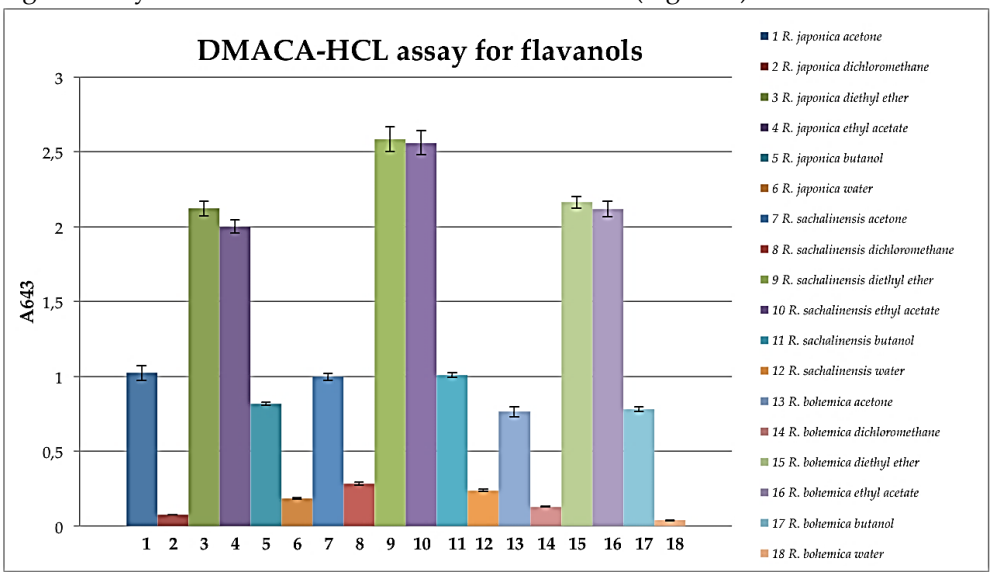


Figure 9. Flavanols quantified spectrometrically for absorbance at 643 nm in extracts and fractions. Data were expressed as mean ± SD, performed in at least three independent experiments, assayed in triplicate.

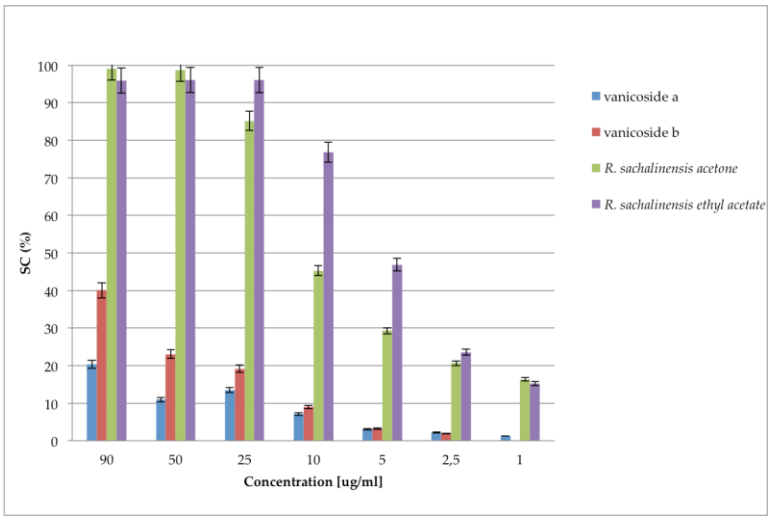


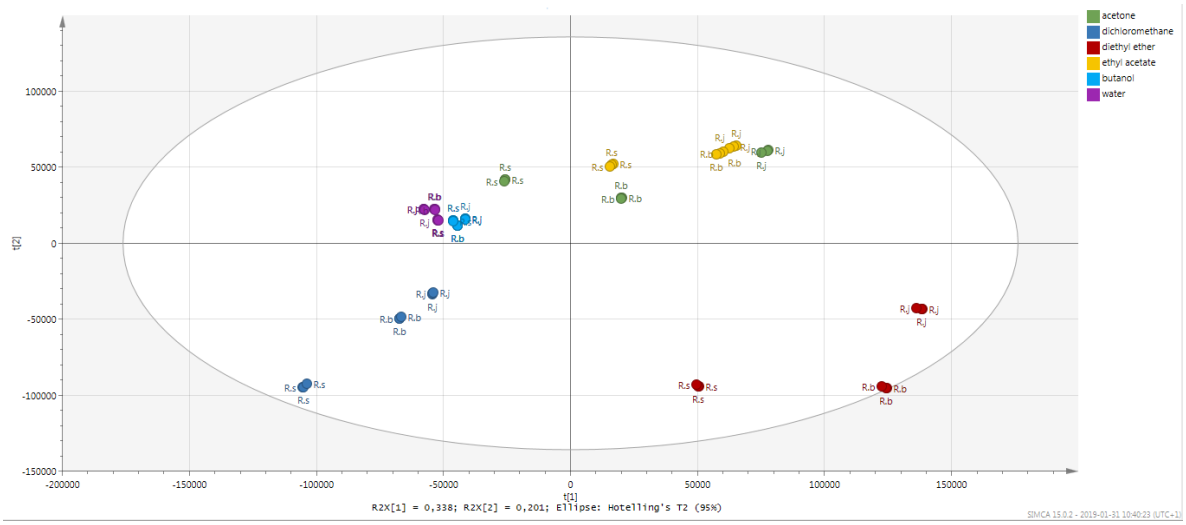
Figure 10. DPPH free radical scavenging activity of vanicoside A, vanicoside B, *R. sachalinensis* acetone extract and *R. sachalinensis* ethyl acetate fraction with range of concentrations. SC% percentage of scavenging activity on DPPH radical. The absorbance at 517 nm was measured after 30 minute.

The 4-dimethylaminocinnamaldehyde (DMACA) reacts with *m*-diphenols to form coloured carbonium ion in acid and this reaction is utilized for assay of flavanols, because the A-rings of flavanols have *m*-diphenol functionality [41]. The DMACA reaction affects the C8 position of the A-ring and reacts only with the terminal units of a proanthocyanidins. In this assay, it does not matter how many monomers a proanthocyanidin molecule is made of, but it has the meaning of how many free C8 position it has. The results agree with the assumption that in the ethyl acetate fractions are more molecules of proanthocyanidins than in butanol, but they are made up of fewer monomers.

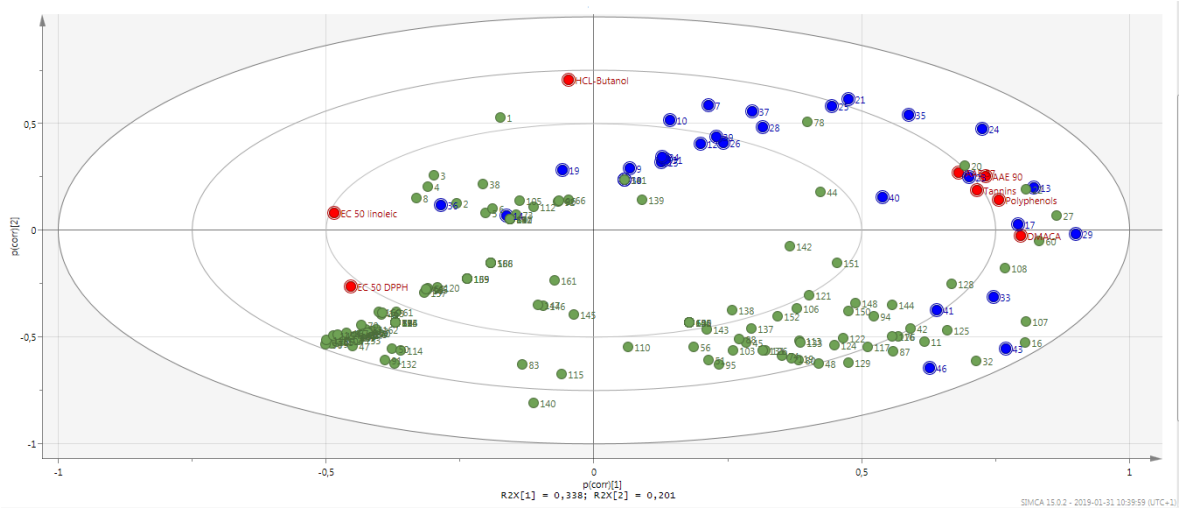
High results of DMACA assay in diethyl ether fractions may be due to a high content of flavanols other than procyanidins, such as catechin, epicatechin or epicatechin-3-O-gallate what is in accordance with chromatographic analysis of this fractions.

In order to observe relationships between the individual compounds present in the fractions and antioxidant activity, we used chemometric analyses. The principal component analysis (PCA) allowed exploratory analyses of the data which included the results of antioxidant tests and the LC-MS data (peak area of compounds), summarizing the multidimensional data in an intelligible way to detect the underlying characteristics and structures of the data (Figure 11).

A)



B)



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C)

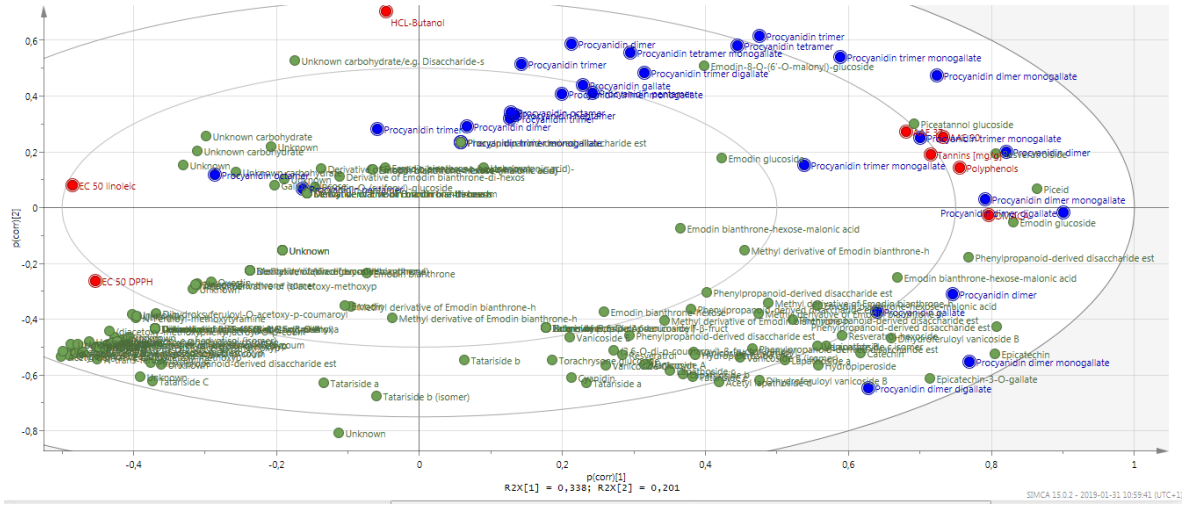

Figure 10. Principal components analysis (PCA) plots indicating the general grouping of the variables in the data sets of extracts (green-acetone) and fractions (blue-dichloromethane, red-diethyl ether, yellow-ethyl acetate, blue-butanol, purple-water) from R.j-*Reynoutria japonica*, R.b-*Reynoutria bohemica*, R.s-*Reynoutria sachalinensis* in three independent experiments. A) The PCA score plot of the LC-MS data and antioxidant assay illustrates the general clustering of the variables. The scores plot was computed using the first two principal components (PC1 vs. PC2). The circle in the score plot represents Hoteling's T2 with 95% confidence interval. R2X(cum) = 0.911, Q2 (cum) = 0.693 for 7 components. B) Loading plot of PCA results obtained from LC-MS data and antioxidant assay. Numbers represent the compounds listed in table1. Blue points represents procyanidins, red –antioxidant tests, green-all compounds without procyanidins. C) Enlarged image of Loading plot of PCA with named compounds.

The visualization of the PCA scores plot shows similarities/dissimilarities between (explained by principal component 1 (PC1)) and within (explained by PC2) the sample clusters. On the PCA score plot all the most antioxidant activity ethyl acetate and diethyl ether fractions as well as *R.j* and *R.b* acetone extracts were located at the right side of the plot. According to loading plot for this differentiation are responsible compounds located most to the right plot, as procyanidins (mainly 13-procyanidin dimer, 17- procyanidin dimer monogallate, 29- procyanidin dimer digallate), stilbenes (mainly 20- piceatannol glucoside, 22- resveratrolside, 27- piceid), emodin glucoside (60), as well as almost all performed assays (without HCl-Butanol). Dissimilarities between ethyl acetate fractions and diethyl ether fractions distributed in the third and fourth quadrant are explained by PC2. According loading plot the biggest impact on created ethyl acetate and acetone cluster in the third quadrant had procyanidins and HCl-Butanol, whereas for diethyl ether cluster formation relevant were phenylpropanoid disaccharide esters , as well catechin (11), epicatechin (16), epicatechin-3-O-gallate (32) and some procyanidins (33, , 41, 43, 46). PCA score plot reveals the difference between *R. sachalinensis* and more similar to each other *R.japonica* and *R.bohemica.* According loading plot, in case of acetone extract, ethyl acetate and diethyl ether fractions, dissimilarities are the results of smaller contribution of PC1, what is accordance with phytochemistry analysis, where, among others, no stilbenes were observed in *R. sachalinensis* extract and fractions. Moreover, loading plot revealed high correlation of performed assays (except HCl-Butanol) to each other, what agrees with the results in the table 4. Located on the left side of plot results from DPPH assay and linoleic acid peroxidation assay are due to the usage of EC50 as an activity measure (i.e., a lower value of the parameter means

a higher activity). Considering the location of AAE 37 and AAE 90 on the loading plot, it can be suggested that there were correlation with procyanidins and some stilbenes - compounds relatively close located to the AAE 37 and AAE 90 points. In the case of DPPH assay, strong correlation is seen mainly with procyanidins, located in the third quadrant of loading plot, close to the line extension running from point EC50 DPPH through point 0. Similarly in the case of EC50 linoleic, where the correlation seems to be strong also with some of the phenylpropanoid disaccharide esters found in fourth quadrant of loading plot. These assumptions are consistent with the results presented in Table 5, which shows the strength of correlation of compounds with antioxidant assays.

Table 5. Correlation between the peak area of detected compounds (established by using mass spectral deconvolution) and activity of extracts/fractions (1/EC50 DPPH , Reducing power AAE 37, 90 (%), 1/EC50 of LA peroxidation) was described with the statistical methods-correlation matrix. In the table are presents only peaks with positive correlation, significant at p <,050.

Nr.	Identification	EC50linoleic	EC50 DPPH	AAE 37	AAE 90
9	Procyanidin dimer	0,563	0,552	0,458	0,484
10	Procyanidin trimer	0,63	0,68	0,62	0,572
11	Catechin	0,611	0,305	0,373	0,502
12	Procyanidin trimer monogallate	0,635	0,646	0,665	0,601
13	Procyanidin dimer	0,554	0,536	0,664	0,645
15	Procyanidin trimer	0,555	0,571	0,536	0,527
17	Procyanidin dimer monogallate	0,763	0,655	0,762	0,795
18	Procyanidin trimer monogallate	0,494	0,504	0,446	0,445
20	Piceatannol glucoside	0,432	0,389	0,588	0,446
21	Procyanidin trimer	0,48	0,512	0,6	0,501
22	Resveratrolside	0,342	0,353	0,499	0,491
23	Procyanidin trimer monogallate	0,781	0,697	0,806	0,783
24	Procyanidin dimer monogallate	0,687	0,684	0,758	0,734
25	Procyanidin tetramer	0,481	0,526	0,608	0,518
26	Procyanidin pentamer	0,35	0,438	0,584	0,387
27	Piceid	0,34	0,319	0,48	0,466
28	Procyanidin trimer digallate	0,592	0,598	0,717	0,585
29	Procyanidin dimer digallate	0,477	0,414	0,592	0,583
30	Procyanidin trimer monogallate	0,494	0,504	0,446	0,445
35	Procyanidin trimer monogallate	0,746	0,719	0,764	0,721
37	Procyanidin tetramer monogallate	0,682	0,701	0,729	0,643
39	Procyanidin gallate	0,666	0,669	0,724	0,618
40	Procyanidin trimer monogallate	0,716	0,561	0,753	0,636
78	Emodin-8-O-(6′-O-malonyl)-glucoside	0,37	0,349	0,496	0,316
87	Hydropiperoside	0,541	0,212	0,264	0,395
106	Phenylpropanoid-derived disaccharide esters	0,659	0,391	0,424	0,509
107	Phenylpropanoid-derived disaccharide esters	0,511	0,366	0,458	0,561
108	Phenylpropanoid-derived disaccharide esters	0,704	0,477	0,631	0,719
113	Vanicoside B (isomer)	0,501	0,166	0,198	0,338
116	Vanicoside B	0,618	0,315	0,349	0,473
117	Lapathoside a	0,537	0,209	0,263	0,394
121	Phenylpropanoid-derived disaccharide esters	0,579	0,41	0,407	0,511
122	Phenylpropanoid-derived disaccharide esters	0,556	0,289	0,358	0,447

124	Vanicoside B (isomer)	0,564	0,217	0,284	0,403
125	Dihydroferuloyl vanicoside B	0,624	0,341	0,39	0,54
141	Phenylpropanoid-derived disaccharide esters	0,494	0,504	0,446	0,445

The presented statistical analyzes showed that the high antioxidant activity of fractions and extracts was significantly influenced by procyanidins. Interestingly, stilbenes occurring in a significant amount in the *R. japonica* and *R. bohemica* extract and fractions and phenylpropanoid disaccharide esters, especialy vanicoside A and B, occurring in a significant amount in the *R. sachalinensis* extract and fractions turned out to have less influence on antioxidant activity of studied samples. Considering that the most antioxidant activity *R. sachalinensis* ethyl acetate fraction contained almost only procyanidins and phenylpropanoid disaccharide esters, especially high amount of vanicoside A and B we decided to check DPPH free radical scavenging activity of isolated vanicosides A and B to find out to what extent they affect the fraction activity. Results from DPPH free radical scavenging activity of vanicoside A, vanicoside B, presented on Figure 10, revealed significantly weaker activity of the tested compounds in relation to the acetone and ethyl acetate *R. sachalinensis* fraction. Thus other compounds had to influence the strong fraction activity.

Fan et al. [23] measured free radical scavenging activity of four phenylpropanoid-derived disaccharide ester obtained from stem of *R. sachalinensis*, which scavenging increased as follows: vanicoside B < hydropiperoside < lapathoside C < lapathoside D, whereas 95 ug/ml of vanicoside B demonstrated scavenging about 32% of DPPH (what was similar to our result) and 95 ug/ml of lapathoside D scavenging about 75% of DPPH. Taking the above results into account, even the strongest scavenger of phenylpropanoid-derived disaccharide esters does not explain much stronger activity of extracts and fractions of *R. sachalinensis*.

Meanwhile, according literature, strong antioxidant activity of *R. japonica rhizomes* is often associated with high amount of stilbenes, mainly resveratrol [6, 42, 43]. However, there are some evidences that other compounds are co-responsible for high antioxidant activity of rhizomes of *Reynoutria japonica*. As shown by Pan et al. [9], ethanol extract from *Polygon cuspidati rhizoma* was stronger than resveratrol in DPPH and hydroxyl radical scavenging, metal reducing capacity, and preventing of polyunsaturated lipids peroxidation. Also, in the study of Lee et al. [7] no correlation was observed between the content of resveratrol or emodin and antioxidant activity. These results suggest the importance other polyphenols or another group of compounds for determination of antioxidant properties of *R. japonica* rhizomes. Research of Lachowicz et al. [44] indicates a significant influence of procyanidins on antioxidant activity; flavan-3-ols derivatives such as catechins and procyanidins as well trans-piceid and trans-resveratrolside had greater radical scavenging capacity than other compounds observed in *R. japonica* and *R. sachalinensis* extracts.

DPPH scavenging activity and inhibition of lipid peroxidation of proanthocyanidins was investigated in numerous studies [45, 46]. Proanthocyanidins are strong DPPH scavengers which e.g. DPPH IC 50 values for procyanidin A2 and procyanidin B2 equal 2.29 and 3.14 μg/ml, respectively [47]. Scavenging activity of proanthocyanidins increases with the number of hydroxyl groups especially if they are in ortho position on benzene.

Furthermore, polymerization up to trimers increases, but further polymerization decreases scavenging activity. Higher scavenging activity was found for galloylated procyanidins [45, 46]. Among various type of polyphenols, dimeric procyanidins were the most active in scavenging of ABTS and hypochlorous acid and in FRAP test, followed by flavanols, hydroxycinnamic acids, simple phenolic acids [48]. Taking the above into account it is very likely that procyanidins including many procyanidins gallate derivatives in the ethyl acetate fractions from studied *Reynoutria rhizomes* were largely responsible for strong radical scavenging activity. Proanthocyanidins are also good inhibitors of lipid peroxidation, with potency similar or higher than trolox and vitamin E [45].

Total antioxidant capacity expressed as antioxidant activity coefficient of ascorbic acid (AAC) was based on the reduction of Mo(VI)to Mo(V) at acidic pH by the extracts and fractions and the formation of a green phosphate/Mo(V) complex [49]. It appears that unlike ascorbic acid, the compounds (including proanthocyanidins) in fractions reduced the Mo ions only at a higher (90°C) temperature (Table 2). The result may be due to the degradation of high polymerized procyanidins at high temperatures and the formation of less polymerized, more active dimer procyanidins. This assumption confirms the study of Luo et al. [50] which developed method for degradation of grape proanthocyanidin polymers into oligomers by sulphurous acid in high temperature (60-80°C) which resulted in many individual procyanidins dimers and trimers. It was also observed that high polymeric procyanidins exhibited lower values of their half-life times in higher temperature than dimeric procyanidins [51].

Rhizomes of *R. japonica* are known as good source of stilbenes [52-55] and antraquinones [53-56]. The European [3] and Chinese Pharmacopeias [57] require determining the content of two compounds emodin and piceid in rhizome of *R. japonica.* However, results of our study suggest that also procyanidins should be considered as compounds affecting the total antioxidant potential of the raw material.

3. Materials and Methods

3.1. Plant material

Rhizomes of studied plants were collected during the last week of September from the synanthropic habitats in disturbed areas in the city of Wrocław (Poland): *R. japonica* (51°07.404' N 17°04.146' E), *R. sachalinensis* (51°06.190' N 17°08.635' E), *R. x bohemica* (51°05.666' N 17°01.746' E). All raw materials were collected during the plants were in the beginning of the last principal growth phase. Species were identified by Klemens Jakubowski (MSc Botany) from Botanical Garden of Medicinal Plants herbarium, based on morphology of vegetative and generative organs (according to available floras). Voucher specimens were deposited in the Botanical Garden herbarium under AAB1022, AAB1023, AAB1024.

Air-dried and powdered rhizomes of *R. japonica*, *R. sachalinensis* and *R.* x *bohemica* (400 g each species) were extracted in 4 steps with 70% acetone (each extraction in ultrasonic bath, 2h). The solvent was evaporated under reduced pressure. 50 g of raw 70% acetone extracts were suspended in water (500 mL) and partitioned between dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), ethyl acetate (AcOEt) and finally butanol (n-BuOH) affording 0.97, 1.05, 5.11, 18.91 g of each dried fraction for *R.sachalinensis*, 2.29, 3.09, 6.80, 13.54 for *R.japonica* and 1.42, 1.835, 8.68, 14.3 g for *R.* x *bohemica* and for all -water residue fraction.

3.2. Reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), thiobarbituric acid (TBA), and hide powder were purchased from Sigma-Aldrich (Steinheim, Germany). Linoleic and gallic acid were purchased from Fluka AG, and trichloroacetic acid from Ubichem UK. Vanicoside A and vanicoside B were earlier isolated according procedure described in previous article [5]. All other reagents and solvents were obtained from Avantor-POCh, (Gliwice, Poland).

3.3. DPPH scavenging assay

The ability to scavenge the DPPH free radical was monitored according to a modified method of [58]. Briefly, DPPH solution (0.3 mM) was prepared in methanol. The extract and fractions were dissolved in a mixture of methanol and water (9:1, v/v) to obtain stock solution (1 mg/mL). Then each stock solution was diluted to obtain final concentrations of 1-250 µg/mL in the assay mixture. DPPH solution (125 µL) and 125 µL of the test extract and fractions at different concentrations were added to a 96- well plate. The absorbance at 517 nm was measured 30 min after mixing using a microplate reader (µQUANT, BioTek, USA). The percentage of scavenged DPPH was then calculated according to Eq1:

$$\%DPPH=((Abt-Abr)/Ab0)\times100$$

where Abt is the absorbance of DPPH solution with the test extracts, Ab0 is the absorbance of DPPH solution with a mixture of methanol and water (9:1, v/v) and Abr is the absorbance of the test extract solution with the addition of methanol. The antiradical activity of extracts was expressed as an EC_{50} value.

3.4. Phosphomolybdenum reduction assay

The antioxidant capacity of the extract and fractions was assessed as described by Prieto et al. [49], with modifications. Extract and fractions were dissolved in a mixture of methanol and water (9:1 v/v) to obtain stock solution (5 mg/mL). Then each stock solution was diluted to obtain final

concentrations of 10-500 µg/mL in the assay mixture. The extract and fractions were combined with the reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulfuric acid (600 mM). The reaction mixture was incubated in a water bath at either 37°C or 90°C for 90 min. The absorbance of the colored complex was measured at 695 nm. The antioxidant activity was compared with that of ascorbic acid in the same concentration range and was expressed as antioxidant activity coefficient of ascorbic acid (AAC).

3.5. Inhibition of linoleic acid peroxidation

The procedure of Wozniak et al. [59] using Fenton reaction- induced lipid peroxidation, has been adapted for this assay. The extract and fractions dissolved in water, achieved a concentration range of 10-500 µg/mL in the assay mixture. Each fraction (150 µL) was mixed with 500 µL phosphate buffer (0.1M, pH 7.4), and 550 µL linoleic acid emulsion (linoleic acid mixed with Tween 80, 3:1, w/w); next 1.12 g emulsion was mixed with 50 mL 0.1 M phosphate buffer (pH 7.4)), and 150 µL 10 mM ascorbic acid. The peroxidation was started with the addition of 150 µL 10 mM $FeSO_4$. The reaction mixture was incubated for 90 min. at 37°C. Thereafter, 1.5 mL of 10% ice cold trichloroacetic acid was added and 1.5 mL of 1% thiobarbituric acid in 50 mM NaOH. The samples were heated in a water bath at 90°C for 10 min. After cooling the samples, 2 mL of *n*-BuOH was added and mixed well. The absorbance was read at 532 nm after transferring 300 µL of BuOH phase from samples to the 96-well plate. The percentage of linoleic acid peroxidation inhibition was calculated as in [59] using appropriate controls. The inhibition of linoleic acid peroxidation of extracts was expressed as an IC_{50} value.

3.6. Total polyphenols and tannins content

Total phenolic content was determined with the Folin-Ciocalteu reagent according to a procedure described previously [60]. Tannin compounds were measured by parallel experiments with extracts vortexed for 1 h with 10 mg mL^{-1} hide powder. The results were expressed as gallic acid equivalents according to the standard gallic acid calibration curve. Total tannins were calculated by subtraction of polyphenols non-absorbed by hide powder from the total phenol content.

3.7. *HCl-Butanol Assay*

Quantification of proanthocyanidins (i.e. procyanidins and delphinidins) in the *Reynoutria* extracts and fractions was performed in three replicates using the acid butanol method (Bate-Smith method) [40]. (Proanthocyanidins contained in 1 ml of *Reynoutria* extracts or fractions (at 1mg ml^{-1}) were oxidatively cleaved to anthocyanidins (i.e. cyanidins and delphinidins) at 95°C for 50 min by adding 6 ml of acid- butanol reagent (butanol/ 12 N HCl; 95/5; v/v) and 200 µl of 2% (w/v) $NH_4Fe^{III}(SO_4)_2$ (in 2 mol L^{-1} HCl). The reaction mixture was cooled and anthocyanidins quantified spectrometrically for absorbance at 550 nm. Blank spectra were obtained for each extract before boiling.

3.8. DMACA-HCl assay for flavanols

770 µl of *Reynoutria* extracts or fractions (at 0,1mg ml^{-1}) were mixed with 385 µl methanol and 192 µl

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of a DMACA reagent, left at room temperature for 20 min and the absorbance at 643 nm was measured [61]. The DMACA reagent was prepared immediately before use, containing 2% (w/v) DMACA in a cold mixture of methanol and 6 M HCl (1 :1, v/v).

3.9. HPLC-MS apparatus

The HPLC analyses were performed using Ultimate 3000 series system (Dionex, Idstein, Germany) equipped with dual low-pressure gradient pump with vacuum degasser, an autosampler, a column compartment, a diode array detector, an Amazon SL ion trap mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) and Corona Ultra RS charged aerosol detector (Thermo Scientific, Belle-fonte, PA, USA).

3.10. HPLC-DAD–MS³conditions

Separation was carried out with Kinetex XB C18 analytical column (150 mm × 2.1 mm × 1.7 μm), (Phenomenex (Torrance, CA, USA). Column temperature was maintained at 25°C. The identification of constituents found in plant materials was based on DAD and negative mode MS spectra. The multi-step gradient was used as follows: 0–50 min 15–70% B, 50–55 min 70–95% B, 55–60 min 95% B. The mobile phase "A" (0.1% HCOOH in water), mobile phase "B" (0.1% HCOOH in MeCN), the flow rate was 0.3 ml/min during analysis. 4 μl of each sample with concentration 5 mg/ml was introduced by the autosampler to the column. The column was equilibrated for 10 min between injections. UV–vis spectra were recorded in the range of 200–450 nm. The eluate was introduced into mass spectrometer without splitting. The ion trap AmazonSL mass spectrometer was equipped with ESI interface. The parameters for ESI source were: nebulizer pressure 40 psi; dry gas flow 9 l/min; dry temperature 300°C; and capillary voltage 4.5 kV. Analysis was carried out using scan from m/z 70 to 2200. Compounds were analyzed in negative ion mode. The parameters for Dissect: Internal S/N threshold -5; Max.number of overlapping compounds-3; Spectrum type-auto; Cut-off intensity -0,1%.

3.11. Statistical analysis

Each of the antioxidant tests and analysis of total polyphenols and tannins was made in three independent experiments, assayed in triplicate. Significant differences ($p \leq 0.05$) between mean values were evaluated by one-way ANOVA and Duncan's multiple range test using Statistica 13.1 (Statsoft, Poland); results are given in Supplementary Materials. Spearman's rank order correlation were calculated using Statistica 13.1 Correlation between the peak area of detected compounds (established by using mass spectral deconvolution) and activity of extracts/fractions ($1/EC_{50}$ DPPH , Reducing power AAE 37, 90 (%), 1/EC50 of LA peroxidation) was described with the statistical methods-correlation matrix using Statistica 13.1. Mass spectral deconvolution - the dissect command in Data Analysis TM software (Bruker Daltonics, Billerica, MA) was used to automatically find peak area of compounds on an LC-MS chromatogram trace. The Dissect algorithm utilises fuzzy logic algorithms, which allow a peak separation process to be run without the need for user interaction or any prior information. The parameters for Dissect algorithm: Internal S/N threshold -5; Max. number of overlapping compounds-3; Spectrum type-auto; Cut-off intensity -0,1%.

The Principal Component Analysis (PCA) by involved of the LC-MS data (peak area of detected compounds established by using mass spectral deconvolution) and antioxidant assays was

performed using Simca-P software (version 15.0.2, Umetrics, Umea, Sweden). Pareto (Par) scaling method with centered and normalized in units of standard deviation were applied to PCA.

4. Conclusions

Fractionation of *Reynoutria* extracts allowed to evaluate compounds present in studied raw materials even in small amounts. HPLC/UV/ESI-MS analysis revealed 171 compounds, a total number of 134 constituents were annotated unambiguously (20) or tentatively (114).

Many of identified compounds were noticed for the first time in the studied materials. The rhizomes of all species are a rich source of proanthocyanidins. We confirmed the presence of procyanidins with high degree of polymerization, up to decamers in the rhizomes of *R. japonica* and brought new data on the presence of these compounds in other *Reynoutria* species. A procyanidin trimer digallate was described for the first time in the studied plants. Moreover, we suggest a presence of new, for these species, dianthrone glycosides (emodin bianthrone, emodin bianthrone-hexose, emodin bianthrone-di-hexose, emodin bianthrone-hexose-malonic acid, emodin bianthrone-hexose-(malonic acid)-hexose and their methyl or undefined derivatives) that, however, need to be confirmed by isolation and structure elucidation. Fractionation has also allowed to observe the numerous and previously unrecorded phenylpropanoid disaccharide esters (tatariside e, tatariside a, tatariside c, lapathoside b, hydropiperoside b, vanicoside e their isomers and undefined derivatives of phenylpropanoid disaccharide esters) and hydroxycinnamic acid derivatives (dihydroksyferuloyl – O – acetoxy – p – coumaroyl – O - caffeoylquinic acid, (diacetoxy – methoxyphenyl) acroyl-O-p-coumaroyl-O-caffeoylquinic acid and its acetyl derivatives), mainly in *R. sachalinensis*. Furthemore, compounds tentatively annotated as lignin oligomers (trimer lignin β-O-4-linked S unit with syringaresinol [S-(β-O-4')-S-(β-β')-S], tetramer lignin, S-(8-O- 4')-S-(8-O-4')-S-(8-8')-S, derivative of lignin- S(8–8)S, hedyotisol and its isomers) were observed for the first time in dichloromethane fractions from studied species. Other compounds that have been observed for the first time are: N-Feruloyl-methoxytyramine, isovitexin or vitexin diglucoside and slightly suggested: alpha-carboxyethylhydroxychroman and cyanidin.

The rhizomes of all *Reynoutria* species exhibited strong antioxidant activity. The ethyl acetate fractions, rich in proanthocyanidins, also in galloylated form were most active in all antioxidant tests. Statistical analysis demonstrated that proanthocyanidins should be taken into account as important contributors to the total antioxidant capacity.

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