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Article

Dereplication of Natural Extracts Diluted in Propylene Glycol, 1,3-Propanediol and Glycerin. Comparison of Leontopodium alpinum Cass. (Edelweiss) Extracts as a Case Study

Acquisitions required the recording of 1024 scans and 8 dummy scans; P1 = 14.25 µs.

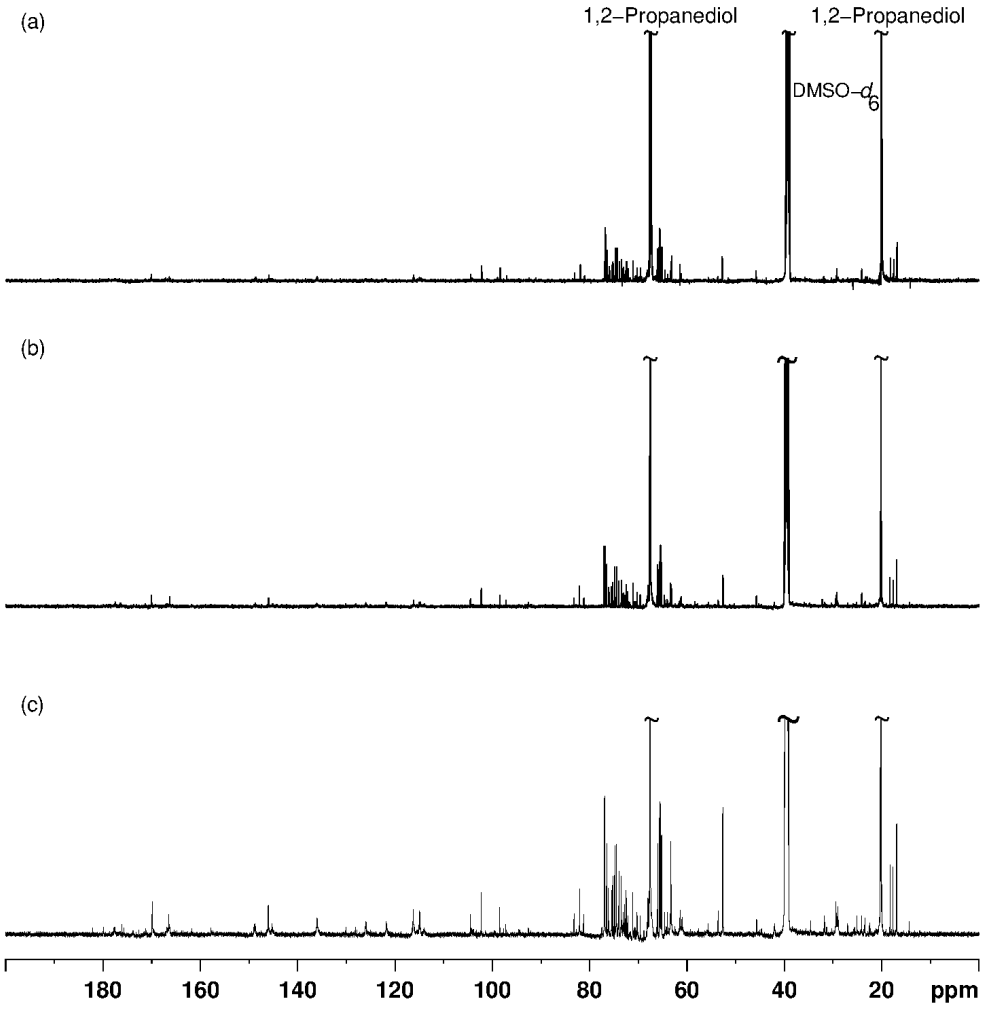

Figure S1. Confirmation of the non-degradation of the edelweiss extract metabolites diluted in 1,2-propanediol/water 1:1 (w/w) by ^{13}C NMR with 1,2-propanediol signals presaturation; (a) ^{13}C NMR spectrum of crude extract; (b) ^{13}C NMR spectrum of extract after a single evaporation run; (c) ^{13}C NMR spectrim of extract after 2 evaporation runs.

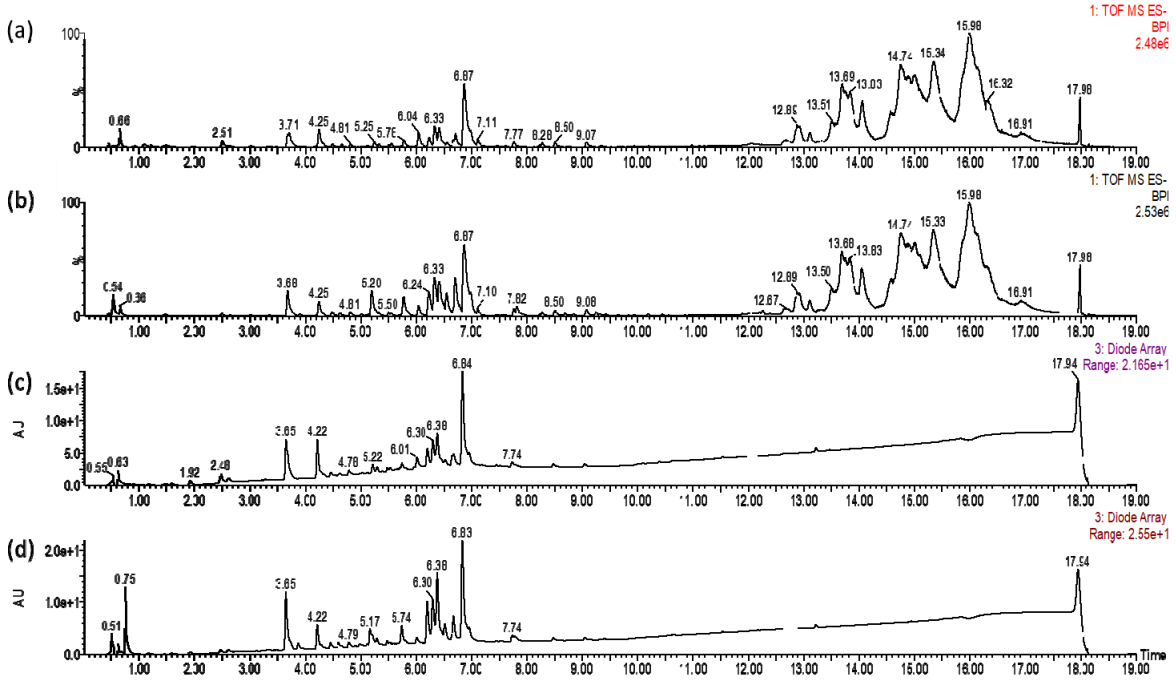

Figure 2. Confirmation of the non-degradation of the edelweiss extract metabolites diluted in 1,3-propanediol/water 1:1 (w/w) by LC-MS and LC-DAD; (a)(c) chromatograms of crude extract obtained in LC-MS ESI$^-$ and LC-DAD, respectively; (b)(d) chromatograms of extract after preparation sample by evaporation obtained in LC-MS ESI$^-$ and LC-DAD, respectively. No significant differences were observed in the two edelweiss extracts due to the solvent evaporation.

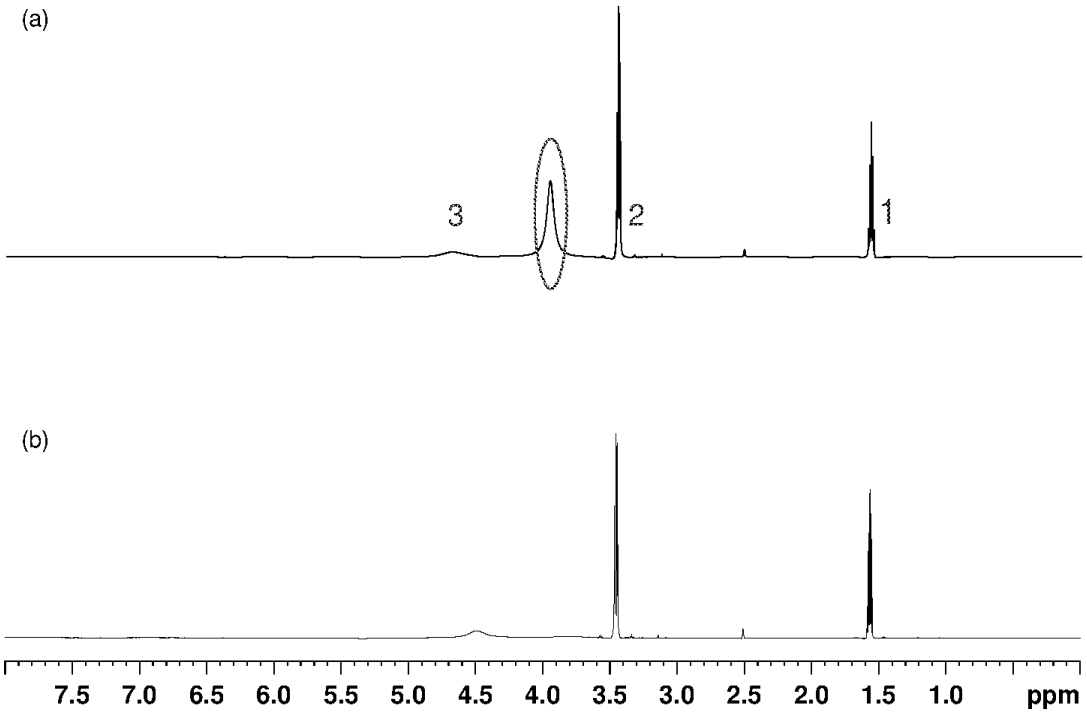

Figure S3. Comparison of ^{1}H NMR spectra of edelweiss extract diluted in propanediol/water (a) before and (b) after a single evaporation run of 15h at 40°C. ***The water signal is surrounded in the spectrum (a) and does not appear in the spectrum (b). 1: CH_2 central group of propanediol; 2: pair of CH_2 in α position of the OH groups; 3: OH groups.***

Σ_1: EtOAc/CH_3CN/water 3:3:4 (v/v/v)

- Σ_2: M*t*BE/CH_3CN/water 3:3:4 (v/v/v)
- Σ_3: *n*-Heptane/EtOAc/MeOH/water 1:1:1:1 (v/v/v/v)
- Σ_4: *n*-Heptane/ EtOAc/ MeOH/water 8:2:8:2 (v/v/v/v)
- Σ_5: EtOAc/ MeOH/ water 4:2:4 (v/v/v)
- Σ_7: Toluene/CH_3CN/ water 2:4:4 (v/v/v)
- Σ_8: *n*-Heptane/Toluene/CH_3CN 5:1:4 (v/v/v)
- Σ_9: CH_3CN/n-BuOH/n-Heptane 35:15:50 (v/v/v)
- Σ_{10}: 2-Methyltetrahydrofuran/CH_3CN/water 4:1:5 (v/v/v)

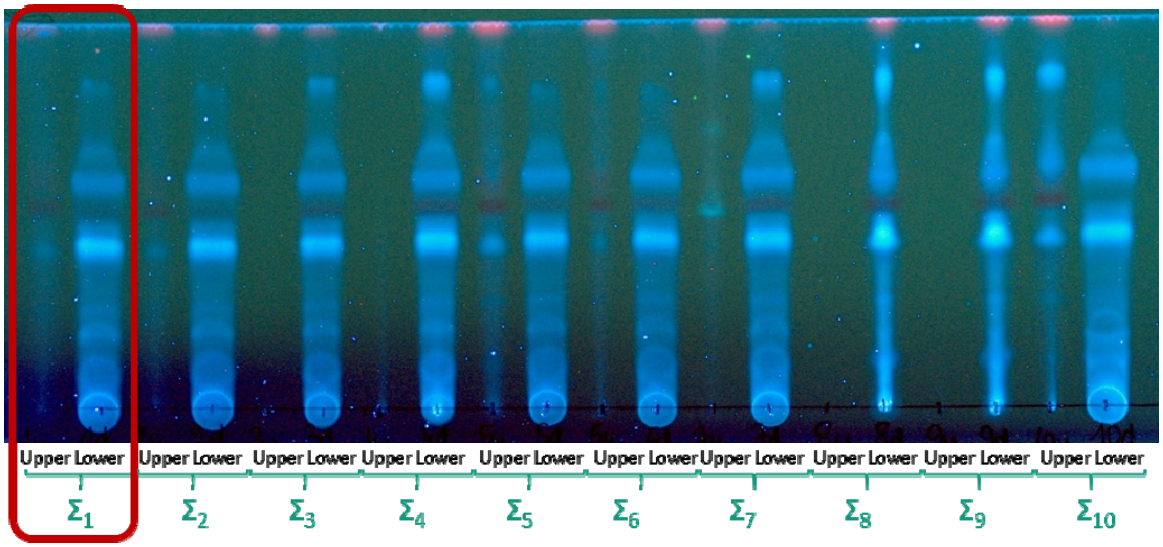

Figure S4. TLC summarizing shake flask assays to observe the partitioning of compounds in edelweiss extract diluted in 1,2-propanediol in 10 biphasic systems.

1: luteolin; 2: caffeic acid; 3: leontopodic acid; 4: luteolin-3'-O-β-D-glucoside; 5: luteolin-4'-O-β-D-glucoside, luteolin-7-O-β-D-glucoside; 6: chlorogenic acid; 7: 6-hydroxy-luteolin-7-O-β-D-glucoside; 8: glycerin.

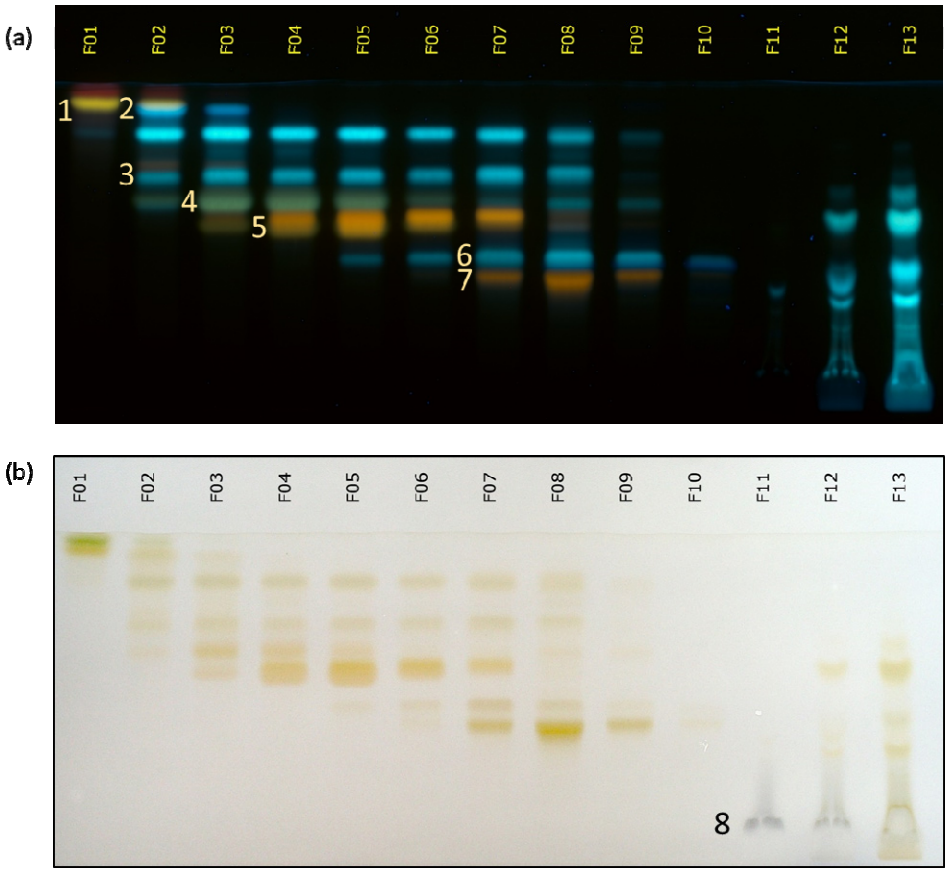

Figure S5. Summary of CPC fractionation on edelweiss extract diluted in glycerin with EtOAc/CH_3CN/water 3:3:4 (v/v/v) solvent system. *Elution solvents: EtOAc/CH_3COOH/HCOOH/water 100:11:11:26 (v/v/v/v); Derivatization: Neu reagent; Visualization 366 nm.*

Table s1. Evaporation of edelweiss extract 5% wt. diluted in 1,2-propanediol/water 1:1. RSD: relative standard deviation.

Replica	Initial masses (g)	After 1st program run				Global Mean (%)	RSD (%)	After 2nd program run				Global Mean (%)	RSD (%)
		Final masses (g)	% dry extract	Means (%)	RSD (%)			Final masses (g)	% dry extract	Means (%)	RSD (%)		
1.1	**1.0016**	**0.5735**	**8.7323**					0.1851	27.0556				
1.2	1.0013	0.5640	8.8768					0.1614	31.0192				
1.3	1.0147	0.5822	8.7143	**8.76**	0.93			0.1914	26.5073	29.62	8.89		
1.4	1.0073	0.5721	8.8035					0.1629	30.9178				
1.5	0.9930	0.5747	8.6393					0.1487	33.3894				
1.6	1.0126	0.5772	8.7717					0.1755	28.8490				
2.1	1.0126	0.5707	8.8716					0.1805	28.0499				
2.2	1.0073	0.576	8.7439					0.1651	30.5057				
2.3	1.0022	0.5711	8.7743	8.82	0.74	8.80	0.79	0.2316	21.6364	26.65	12.06	27.38	11.37
2.4	1.0223	0.5811	8.7962					0.2127	24.0315				
2.5	0.9878	0.5536	8.9216					0.1776	27.8097				
2.6	1.0361	0.5883	8.8059					0.1858	27.8821				
3.1	0.9901	0.5563	8.8990					0.1869	26.4874				
3.2	0.9831	0.5551	8.8552					0.2007	24.4918				
3.3	1.0045	0.5722	8.7775	8.82	0.53			0.2179	23.0496	25.88	9.72		
3.4	1.0098	0.5721	8.8254					0.1683	30.0000				
3.5	0.9866	0.5601	8.8074					0.1822	27.0746				
3.6	0.9933	0.5657	8.7794					0.2055	24.1679				

Program used: from atmospheric pressure to full vacuum in 1h; then 8h of full vacuum; temperature set at 30°C.

Table S2. Evaporation of edelweiss extract 5% wt. diluted in 1,3-propanediol/water 1:1. RSD: relative standard deviation.

Replica	Initial masses (g)	After 1st program run				Global Mean (%)	RSD (%)	After 2nd program run				Global Mean (%)	RSD (%)
		Final masses (g)	% dry extract	Means (%)	RSD (%)			Final masses (g)	% dry extract	Means (%)	RSD (%)		
1.1	**1.0168**	**0.5941**	**8.5575**					0.5241	9.7004				
1.2	1.0078	0.5919	8.5133					0.5194	9.7016				
1.3	1.0309	0.6063	8.5016	**8.53**	0.35			0.5325	9.6798	9.67	0.38		
1.4	1.0099	0.5947	8.4908					0.5226	9.6623				
1.5	1.0316	0.6045	8.5327					0.5370	9.6052				
1.6	1.0287	0.6007	8.5625					0.5331	9.6483				
2.1	0.9988	0.5855	8.5295					0.5163	9.6727				
2.2	0.9940	0.583	8.5249					0.5111	9.7241				
2.3	1.0351	0.6084	8.5067	8.58	1.62	8.57	1.50	0.5316	9.7357	9.69	0.34	9.66	0.51
2.4	1.0099	0.5936	8.5066					0.5224	9.6660				
2.5	1.0143	0.5944	8.5321					0.5253	9.6545				
2.6	1.0595	0.5980	8.8587					0.5459	9.7042				
3.1	0.9993	0.5864	8.5206					0.5179	9.6476				
3.2	1.015	0.595	8.5294					0.5312	9.5538				
3.3	1.0002	0.5842	8.5604	8.61	2.08			0.5205	9.6081	9.61	0.36		
3.4	1.0327	0.6065	8.5136					0.5364	9.6262				
3.5	1.0033	0.5591	8.9725					0.5235	9.5826				
3.6	1.0663	0.6232	8.5550					0.5536	9.6306				

Program used: from atmospheric pressure to full vacuum in 1h; then 14h of full vacuum; temperature set at 40°C.

Table S3. Details of pooling after CPC fractionations.

CPC fractions	Extract diluted in propylene glycol / water 1:1 (w/w)				Extract diluted in propanediol / water 1:1 (w/w)				Extract diluted in glycerin / water 1:1 (w/w)	
	Concentrated by evaporation		Crude extract		Concentrated by evaporation		Crude extract			
	Grouped collection tube	Mass of fractions (g)	Grouped collection tube	Mass of fractions (g)	Grouped collection tube	Mass of fractions (g)	Grouped collection tube	Mass of fractions (g)	Grouped collection tube	Mass of fractions (g)
F_{01}	11-14	0.029	10-12	0.035	14-17	0.024	10-11	0.037	12-15	0.043
F_{02}	15-17	0.044	13-16	0.043	18	0.051	12-15	0.061	16-18	0.039
F_{03}	18-20	0.443	17-25	0.049	19-20	3.752	16-28	0.040	19-21	0.028
F_{04}	21-25	3.816	26-32	0.039	21-22	7.376	29-38	0.039	22-25	0.047
F_{05}	26-32	0.194	33-40	0.032	23-25	6.47	39-50	0.056	26-28	0.026
F_{06}	33-40	0.096	41-50	0.233	26-30	0.687	51-58	0.118	29-34	0.035
F_{07}	41-57	0.119	51-71	15.139	31-40	0.157	59-76	13.251	35-42	0.029
F_{08}	58	0.817	72-90	0.230	41-57	0.080	77-97	0.184	43-68	0.048
F_{09}	59-65	0.301	91-118	0.128	58-66	0.671	98-119	0.076	69-75	0.024
F_{10}	-	-	119-135	0.039	-	-	120-130	0.020	76-77	0.018
F_{11}	-	-	136	0.166	-	-	131-132	0.143	78	1.008
F_{12}	-	-	137-140	0.949	-	-	133-140	1.061	79-82	17.3
F_{13}	-	-	-	-	-	-	-	-	83-90	2.159
Total masses		5.859		17.082		19.268				20.804
Recovery		89 %		93 %		89 %		95 %		110 %

Table S4. Summary of compounds identified in *L. alpinum* by NMR (solvent DMSO-d_6 which the central peak was calibrated at 39.8 ppm) and LC-UV-HRMS ESI^-; R_t: retention time; n.e.: not exhaustive. Loliolide, betaine and choline which are not observed in LC-HRMS ESI^- are detected in ESI^+ (data not shown).

Molecule	Chemical group	Formula	Exact mass	δ ^{13}C	R_t (min)	ESI^- (m/z)	λ (nm)
Linoleic acid	Fatty acid	$C_{18}H_{32}O_2$	280,2402	174.9 ; 130.1 ; 130.0 ; 128.1 ; 128.0 ; 34.0 ; 31.7 ; 29.5 ; 24.9 ; 20.4 ; 14.3 ; *n.e.*	14.67	279.2329	-
Linolenic acid	Fatty acid	$C_{18}H_{30}O_2$	278.2246	174.9 ; 130.1 ; 130.0 ; 128.1 ; 128.0 ; 127.4 ; 34.0 ; 31.7 ; 29.5 ; 24.9 ; 20.4 ; 14.3 ; *n.e.*	14.15	277.2166	-
Suberic acid	Organic acid	$C_8H_{14}O_4$	174.0892	174.9 ; 34.0 ; 29.0 ; 24.9	4.93	173.0814	-
Loliolide	Lactone	$C_{11}H_{16}O_3$	196.1099	183.6 ; 171.5 ; 112.4 ; 87.0 ; 65.2 ; 47.0 ; 45.6 ; 36.0 ; 30.7 ; 27.0 ; 26.5	-	-	-
Luteolin	Flavonoid	$C_{15}H_{10}O_6$	286.0477	182.1 ; 164.6 ; 164.3 ; 161.7 ; 157.6 ; 150.1 ; 146.0 ; 121.8 ; 119.0 ; 116.4 ; 113.8 ; 104.1 ; 103.2 ; 99.3 ; 94.2	7.83	285.0404	266-347
Luteolin-4'-*O*-β-D-glucoside	Flavonoid	$C_{21}H_{20}O_{11}$	448.1006	182.1 ; 164.7 ; 163.6 ; 161.8 ; 157.8 ; 149.0 ; 147.3 ; 125.1 ; 119.0 ; 116.3 ; 113.9 ; 104.3 ; 104.2 ; 101.5 ; 99.3 ; 94.4 ; 77.6 ; 76.2 ; 73.6 ; 70.1 ; 61.0	6,42	447.0933 285.0404	237-336
Luteolin-3'-*O*-β-D-glucoside = Dracocephaloside	Flavonoid	$C_{21}H_{20}O_{11}$	448.1006	182.3 ; 164.6 ; 163.8 ; 161.7 ; 157.7 ; 151.1 ; 146.0 ; 122.3 ; 122.2 ; 116.8 ; 114.7 ; 104.3 ; 104.0 ; 102.2 ; 99.2 ; 94.5 ; 77.7 ; 76.3 ; 73.5 ; 70.4 ; 61.3	6,72	447.0934 285.0403	267-338
Luteolin-7-*O*-β-D-glucoside = Cynaroside	Flavonoid	$C_{21}H_{20}O_{11}$	448.1006	182.8 ; 163.6 ; 163.4 ; 161.6 ; 157.4 ; 151.7 ; 145.4 ; 122.4 ; 122.2 ; 116.9 ; 114.7 ; 106.3 ; 103.7 ; 100.4 ; 99.9 ; 95.2 ; 77.6 ; 76.9 ; 73.6 ; 69.9 ; 61.2	5.79	447.0930 285.0401	249-254
6-Hydroxy-luteolin-7-*O*-β-*D*-glucoside	Flavonoid	$C_{21}H_{20}O_{12}$	464.0955	182.7 ; 164.7 ; 151.7 ; 150.2 ; 149.2 ; 146.8 ; 146.2 ; 130.9 ; 122.2 ; 119.4 ; 116.4 ; 113.8 ; 106.2 ; 103.0 ; 94.4 ; 101.4 ; 77.7 ; 76.2 ; 73.7 ; 70.1 ; 61.0	5.21	463.0876 301.0345	243-281
3,5-Dicaffeoylquinic acid	Hydroxycinnamic acid	$C_{25}H_{24}O_{12}$	516.1268	175.0 ; 166.6 ; 148.9 ; 145.9 ; 145.5 ; 126.0 ; 121.8 ; 116.0 ; 115.2 ; 115.0 ; 73.0 ; 70.8 ; 67.6 ; 35.0	6,25	515.1190 353.0873 191.0559 179.0344 161.0240	327

Molecule	Chemical group	Formula	Exact mass	δ ^{13}C	R_t (min)	ESI^- (m/z)	λ (nm)
Leontopodic acid A	Hydroxycinnamic acid	$C_{37}H_{34}O_{19}$	782.1694	170.6 ; 168.8 ; 168.7 ; 166.5-165.8 ; 149.1-148.9 ; 147.1 ; 146.6-145.9 ; 126.1-125.6 ; 122.1-121.7 ; 115-116 ; 113.6-113.3 ; 71.3 ; 70.3 ; 69.6 ; 63.8 ; 44.4 ; 23.6	6.93	781.1613 695.1249 619.1288 309.0605 179.0341 161.0237	328
Leontopodic acid B	Hydroxycinnamic acid	$C_{33}H_{28}O_{17}$	696.1326	168.8 ; 168.7 ; 166.5-165.8 ; 149.1-148.9 ; 147.1 ; 146.6-145.9 ; 126.1-125.6 ; 122.1-121.7 ; 115-116 ; 113.6-113.3 ; 71.3 ; 70.3 ; 69.6	6.38	695.1245 533.0925 179.0345 161.0240	327
Chlorogenic acid	Hydroxycinnamic acid	$C_{16}H_{18}O_9$	354.0951	175.6 ; 166.3 ; 148.7 ; 146.0 ; 145.4 ; 126.0 ; 121.8 ; 116.2 ; 115.1 ; 114.7 ; 74.0 ; 71.3 ; 70.7 ; 68.6 ; 37.6 ; 36.9	3.68	353.0873 191.0555	325
Caffeic acid	Hydroxycinnamic acid	$C_9H_8O_4$	180.0423	168.4 ; 148.9 ; 145.9 ; 145.6 ; 125.9 ; 121.8 ; 116.1 ; 115.0 ; 114.6	3.89	179.0347 135.0446	322
Succinic acid	Organic acid	$C_4H_6O_4$	118.0266	174.1 ; 29.3	0.77	117.0192	212
Fumaric acid	Organic acid	$C_4H_4O_4$	116.0110	166.9 ; 134.7	-	-	-
Lactic acid	Organic acid	$C_3H_6O_3$	90.0317	176.8 ; 66.1 ; 20.9	-	-	-
Malic acid	Organic acid	$C_4H_6O_5$	134.0215	177.2 ; 172.7 ; 66.1 ; 42.7	0.58	133.0138	-
Betaine	Betaine	$C_5H_{12}NO_2$	118.0868	165.0 ; 66.5 ; 52.4 ; 52.4 ; 52.4	-	-	-
Choline	Betaine	$C_5H_{14}NO$	104.1075	67.4 ; 55.5 ; 53.6 ; 53.6 ; 53.6	-	-	-
α-D-Glucose	Monosaccharide	$C_6H_{12}O_6$	180.0634	92.6 ; 73.4 ; 72.7 ; 72.3 ; 71.0 ; 61.1	0.55	179.0557	-
β-D-Glucose	Monosaccharide	$C_6H_{12}O_6$	180.0634	97.2 ; 77.1 ; 77.0 ; 75.5 ; 70.9 ; 61.5	0.55	179.0557	-
α-D-Fructofuranose	Monosaccharide	$C_6H_{12}O_6$	180.0634	104.5 ; 83.4 ; 81.3 ; 76.2 ; 64.5 ; 61.6	0.55	179.0557	-
β-D-Fructofuranose	Monosaccharide	$C_6H_{12}O_6$	180.0634	102.5 ; 82.3 ; 76.2 ; 75.8 ; 63.6 ; 63.3	0.55	179.0557	-
β-D-Fructopyranose	Monosaccharide	$C_6H_{12}O_6$	180.0634	98.5 ; 70.2 ; 69.5 ; 68.2 ; 64.7 ; 63.5	0.55	179.0557	-
Saccharose	Disaccharide	$C_{12}H_{22}O_{11}$	342.1162	104.4 ; 92.1 ; 82.9 ; 77.4 ; 74.6 ; 73.2 ; 73.1 ; 71.9 ; 70.2 ; 62.5 ; 62.4 ; 60.8	0.52	341.1078	-