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Supplementary material

Polyphenolic Compounds of Crataegus Berry, Leaf and Flower Extracts Affect Viability and Invasive Potential of Human Glioblastoma Cells

Natalia Żurek [1,a], Olena Karatsai [2,a], Maria Jolanta Rędowicz [1,2,*,b] and Ireneusz Kapusta [1,b]

[1] Institute of Food Technology and Nutrition, University of Rzeszow, 4 Zelwerowicza St., 35-601 Rzeszow, Poland; nzurek@ur.edu.pl, ikapusta@ur.edu.pl, j.redowicz@nencki.edu.pl
[2] Nencki Institute of Experimental Biology, Polish Academy of Sciences, 3 Pasteur St., 02-093 Warsaw, Poland; o.karatsai@nencki.edu.pl, j.redowicz@nencki.edu.pl
* Correspondence: ikapusta@ur.edu.pl; Tel.: +48-17-785-5238
[a] These authors contributed equally to this article
[b] These authors share senior authorship

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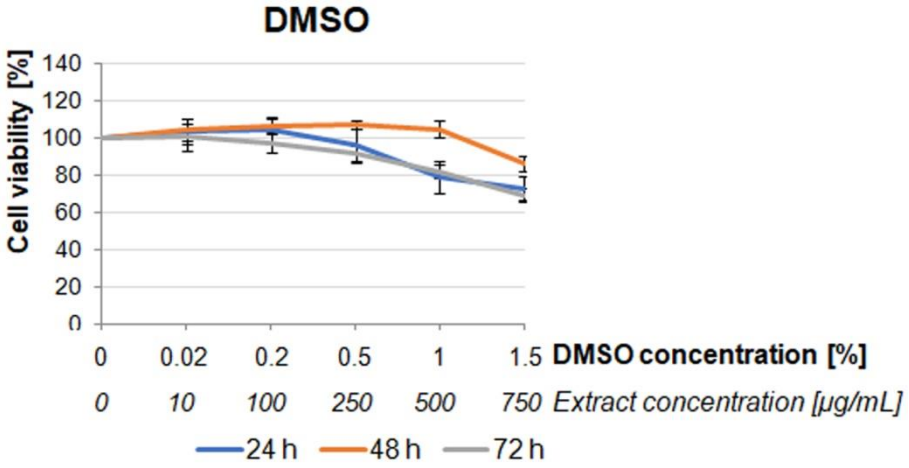

Figure S1. DMSO effect on the viability of U87MG human glioblastoma cells. Cells were treated up to 72 hours with different concentrations of DMSO (0.02-1.5%) deppending on the flower extracts concentration (see lower raw). The viability

was assayed by the MTS test. The number of viable control (non-treated) cells of each time point served as 100%. Graphs represent mean values ± SD from three independent experiments.

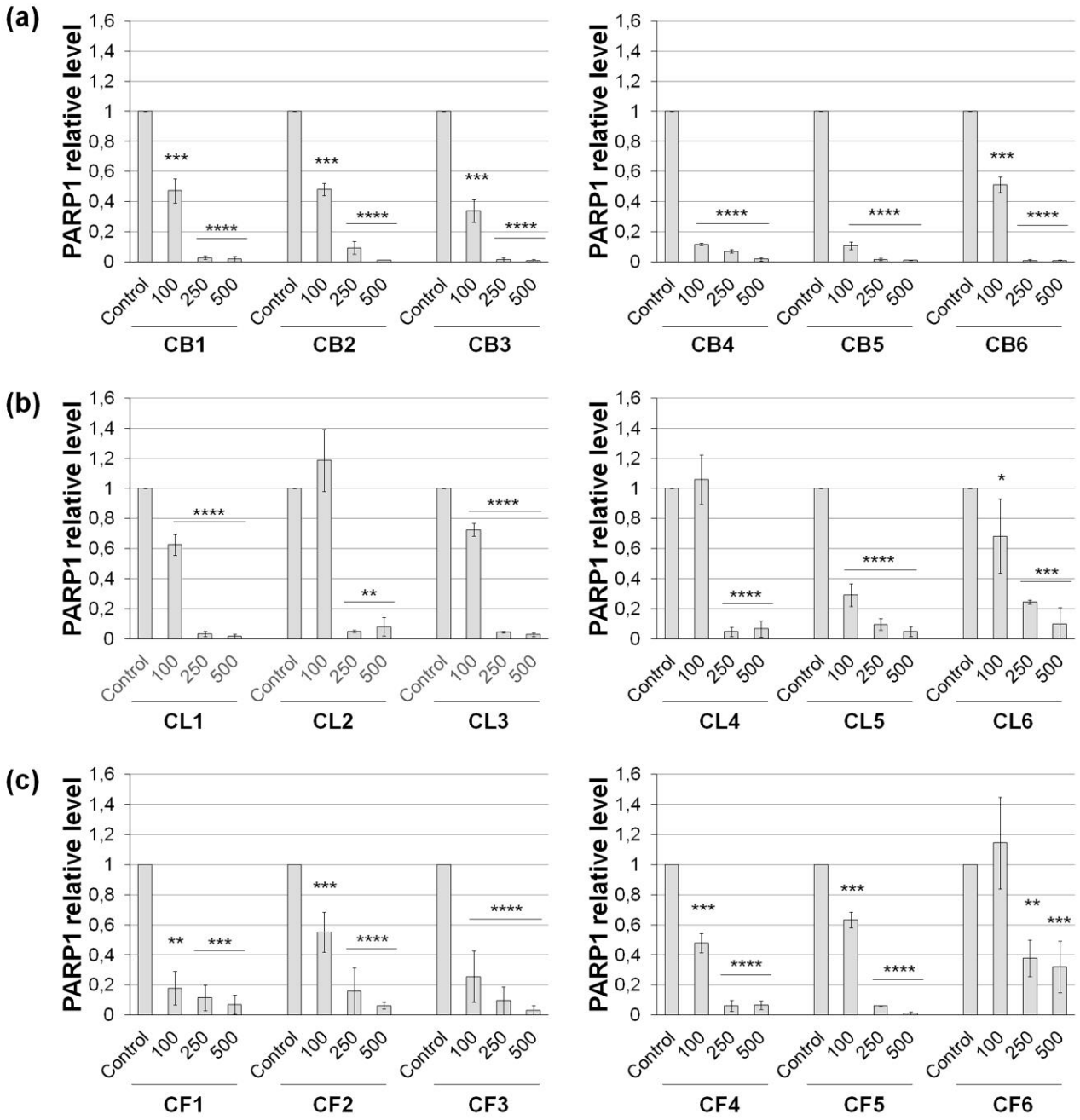

Figure S2. Densitometric analysis of the PARP1 level. Cells were incubated with *Crataegus* berry (**a**), leaf (**b**) and flower (**c**) extracts in 100, 250, and 500 μg/mL concentrations for 48 h. Non-treated cells served as a control. Graphs represent mean value ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ relative to control.

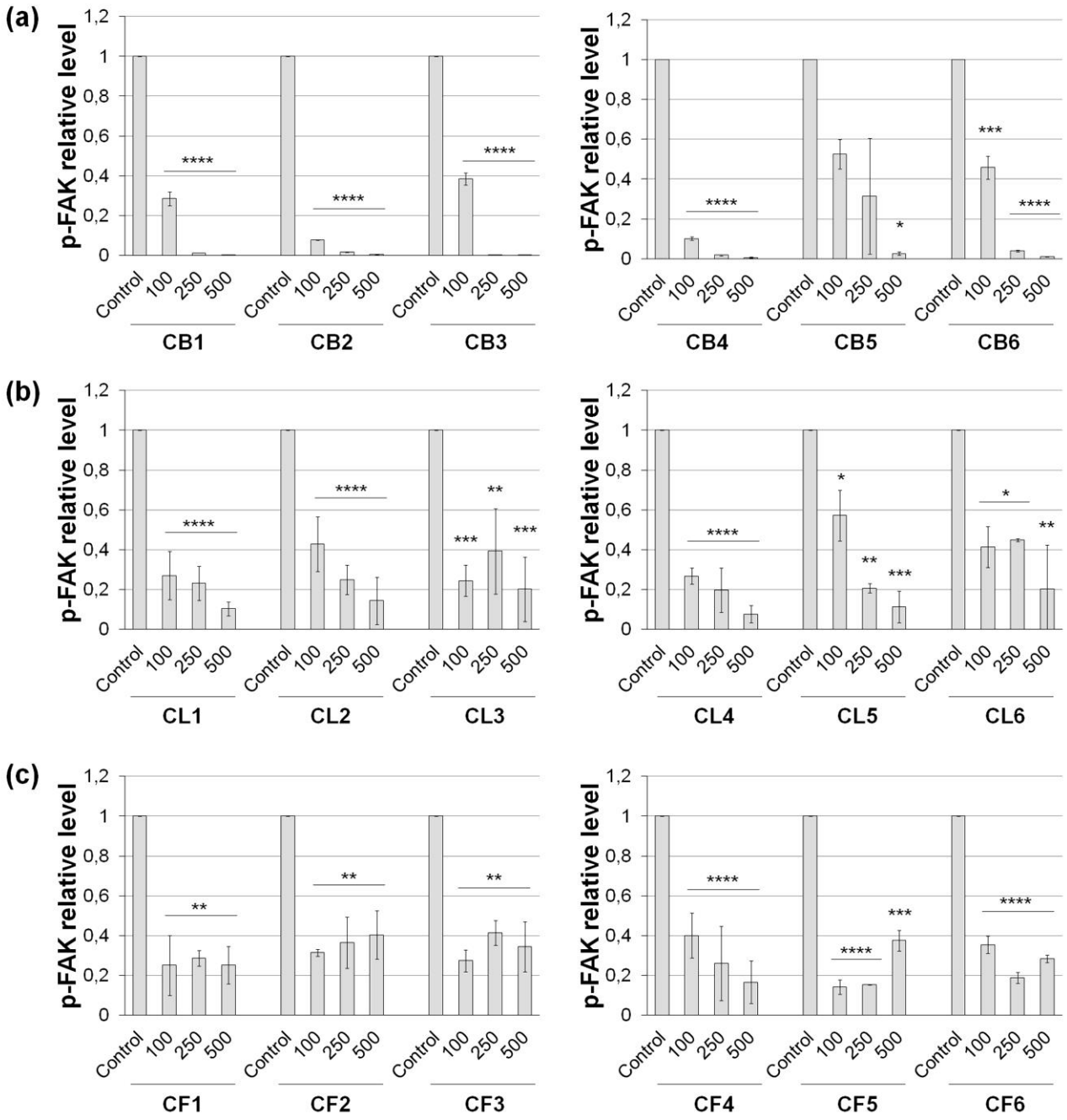

Figure S3. Densitometric analysis of the level of phosphorylated (active) form of FAK (p-FAK). Cells were incubated with *Crataegus* berry (**a**), leaf (**b**) and flower (**c**) extracts in 100, 250, and 500 μg/mL concentrations for 48 h. Non-treated cells served as a control. Graphs represent mean value ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ relative to control.

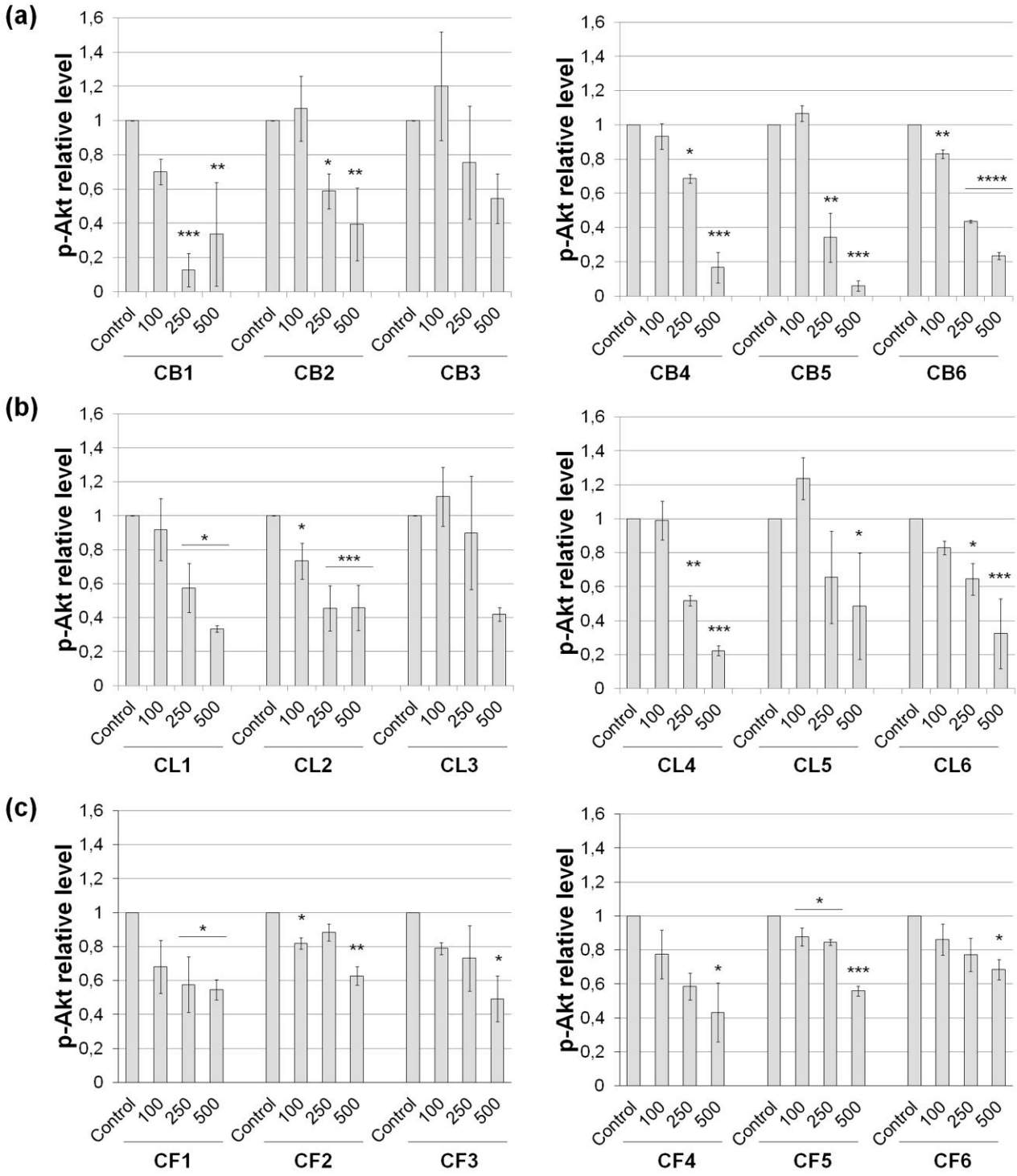

Figure S4. Densitometric analysis of the level of phosphorylated (active) form of Akt (p-Akt). Cells were incubated with *Crataegus* berry (**a**), leaf (**b**) and flower (**c**) extracts in 100, 250, and 500 μg/mL concentrations for 48 h. Non-treated cells served as a control. Graphs represent mean value ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ relative to control.

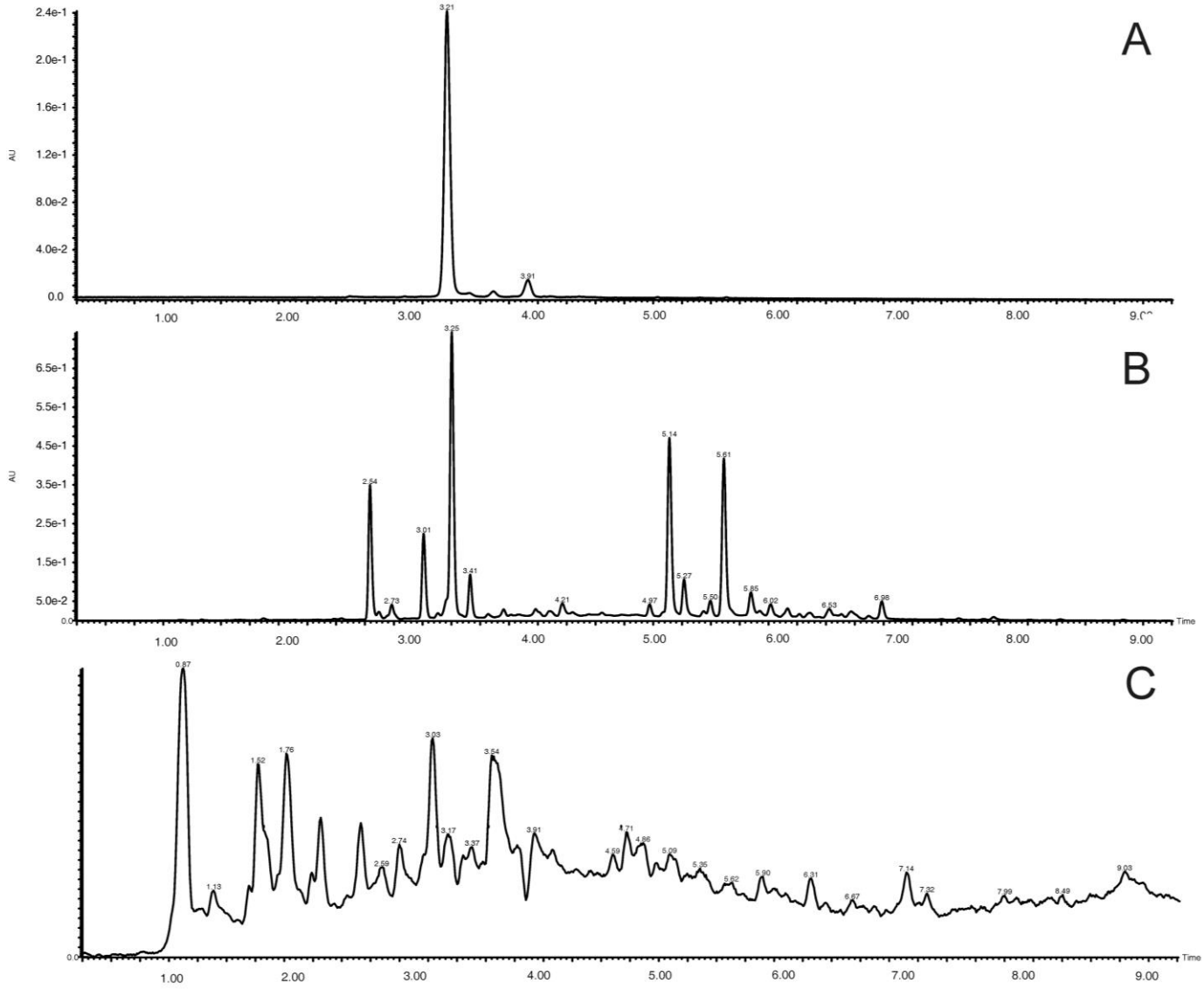

Figure S5. UPLC chromatogram of *Crataegus monogyna* berries (CB1). A, PDA chromatogram extracted at 520 nm for anthocyanins visualization, B, PDA chromatogram extracted at 350 nm for other phenolics, C – total ion current.

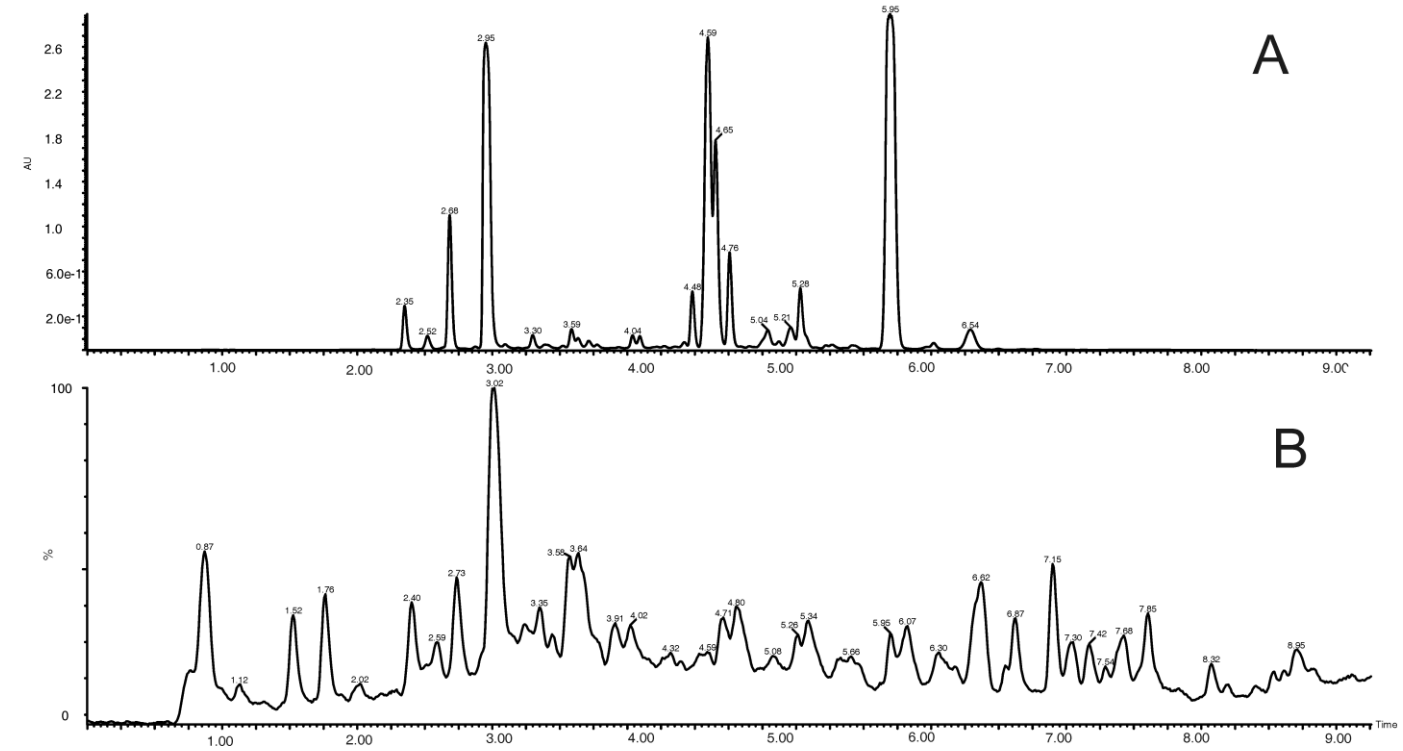

Figure S6. UPLC chromatogram of *Crataegus monogyna* leaves (CL1). A, PDA chromatogram extracted at 350 nm, B, total ion current.

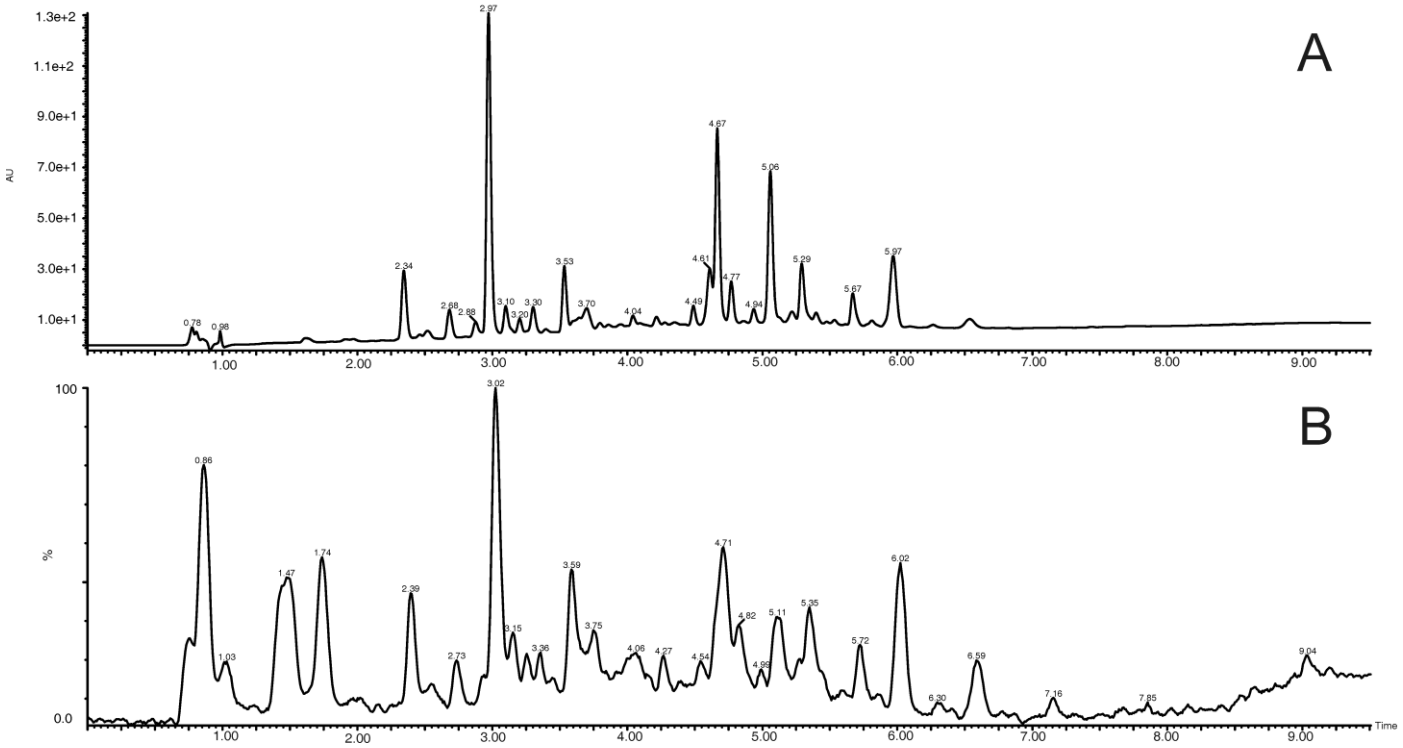

Figure S7. UPLC chromatogram of *Crataegus monogyna* flowers (CF1). A, PDA chromatogram extracted at 350 nm, B, total ion current.

Table S1. EC50 (μg/mL) values for the examined *Crataegus* extracts on the viability of U87MG human glioblastoma cells.

Samples	EC_{50}, μg/mL		
	Time, h		
	24	48	72
CB1	200.26 ± 9.52	189.33 ± 4.87	181.87 ± 6.03
CB2	198.55 ± 6.34	198.83 ± 4.32	183.89 ± 3.02
CB3	168.65 ± 11.71	189.00 ± 15.09	209.49 ± 10.41
CB4	180.88 ± 8.57	173.70 ± 5.50	180.44 ± 8.31
CB5	227.88 ± 9.12	256.79 ± 9.11	335.42 ± 7.00
CB6	356.82 ± 5.90	344.35 ± 7.61	395.09 ± 6.09
CL1	218.79 ± 7.77	233.44 ± 2.70	214.41 ± 3.43
CL2	450.96 ± 10.78	451.59 ± 15.02	403.36 ± 22.66
CL3	263.12 ± 24.97	338.37 ± 6.58	310.73 ± 9.36
CL4	185.84 ± 5.89	116.51 ± 22.21	184.27 ± 1.22
CL5	174.01 ± 19.09	170.08 ± 2.69	207.07 ± 10.58
CL6	236.67 ± 5.58	338.26 ± 11.88	379.43 ± 17.37
CF1	343.02 ± 46.48	426.44 ± 12.95	460.29 ± 10.53
CF2	462.38 ± 8.09	553.58 ± 13.47	551.79 ± 25.92
CF3	429.91 ± 13.24	569.93 ± 17.74	559.15 ± 15.34
CF4	329.95 ± 26.88	391.24 ± 8.53	440.99 ± 13.47
CF5	636.48 ± 20.69	565.96 ± 17.66	523.63 ± 28.60
CF6	629.78 ± 11.09	543.19 ± 30.67	440.29 ± 13.59

Values are expressed as mean ± SD from three independent experiments. Abbreviations: CB, *Crataegus* berry; CL, *Crataegus* leaf; CF, *Crataegus* flower extracts.

Table S2. Content of polyphenolic compounds in berries, leaves and flowers of the different *Crataegus* species.

	Compounds		Species of *Crataegus*					
			1	2	3	4	5	6
Berries			*Anthocyanins*					
	1	Cyanidin 3-*O*-glucoside	7.37 ± 0.07d	6.42 ± 0.01b	5.75 ± 0.01a	11.21 ± 0.05e	12.79 ± 0.27f	6.96 ± 0.02c
	2	Pelargonidin 3-*O*-rutinoside	0.15 ± 0.02b	0.12 ± 0.01a	0.11 ± 0.01a	0.22 ± 0.01c	0.22 ± 0.00c	0.16 ± 0.00b
	3	Cyanidin 3-*O*-arabinoside	0.13 ± 0.00b	0.14 ± 0.01b	0.11 ± 0.00a	0.24 ± 0.01c	0.25 ± 0.01c	0.26 ± 0.00d
	4	Peonidin 3-*O*-glucoside	0.41 ± 0.00c	0.42 ± 0.02c	0.29 ± 0.00b	0.25 ± 0.00ab	0.25 ± 0.04ab	0.21 ± 0.00a
		Sum	8.07 ± 0.09d	7.09 ± 0.13b	6.26 ± 0.02a	11.91 ± 0.07e	13.51 ± 0.32f	7.59 ± 0.03c
			Flavan-3-ols					
	5	Procyanidin trimer	1439.52 ± 21.62a	1763.48 ± 18.20c	1599.89 ± 4.70b	2000.45 ± 21.32de	2048.89 ± 10.93e	1877.19 ± 1.29cd
	6	Procyanidin dimer	1717.54 ± 43.39a	2294.92 ± 13.81c	2009.59 ± 19.49b	2578.00 ± 17.93d	2762.86 ± 7.34e	2636.03 ± 20.84de

7	(+)-Catechin	213.06 ± 30.29c	88.35 ± 5.30a	61.40 ± 1.93a	1073.04 ± 40.66d	1451.14 ± 1.46e	153.14 ± 7.14b
8	(-)-Epicatechin	1399.75 ± 51.77a	1793.75 ± 5.68b	1419.88 ± 16.08a	1800.44 ± 10.24b	1839.19 ± 17.83b	2092.24 ± 13.66c
9	Procyanidin tetramer	114.36 ± 1.41a	162.62 ± 5.71b	145.14 ± 6.67b	215.74 ± 7.61c	213.73 ± 4.76c	140.16 ± 2.94ab
10	Cinchonain	29.10 ± 0.66b	61.47 ± 7.29c	0.33 ± 0.38a	4.27 ± 0.23a	4.49 ± 0.08a	1.32 ± 0.40a
	Sum	4913.36 ± 149.14a	6164.60 ± 56.00c	5236.21 ± 49.26b	7671.94 ± 97.99e	8320.29 ± 42.41f	6900.07 ± 57.91d
	Hydrolyzable Tannins						
11	Ellagic acid pentoside	52.31 ± 4.22ab	78.27 ± 4.54b	540.44 ± 19.81c	25.18 ± 1.46a	56.50 ± 2.75ab	679.17 ± 23.19d
12	Punicalin isomer I	293.34 ± 13.08a	367.42 ± 11.62b	357.38 ± 3.79b	408.15 ± 18.88c	426.93 ± 0.08c	406.85 ± 21.33c
13	Punicalin isomer II	39.31 ± 4.54a	22.52 ± 1.75a	60.82 ± 4.05a	42.49 ± 2.62a	113.56 ± 0.65b	57.17 ± 38.78a
14	2-*O*-galloylpunicalin	52.73 ± 2.80a	59.47 ± 8.50a	86.05 ± 3.72b	135.17 ± 8.86c	136.76 ± 0.85c	56.38 ± 16.98a
15	Eucalbanin A	13.34 ± 0.70a	19.75 ± 0.06b	21.81 ± 1.44b	22.41 ± 1.06b	15.37 ± 0.80a	22.65 ± 2.95b
	Sum	451.04 ± 25.35a	547.43 ± 26.47b	1066.50 ± 32.82e	633.41 ± 32.88c	749.13 ± 5.14d	1222.22 ± 103.25f
	Phenolic acids						
16	Quinic acid	25.91 ± 1.16a	28.18 ± 2.38a	31.50 ± 6.13a	28.43 ± 2.95a		
17	Coumaroylquinic acid	118.21 ± 1.18a	124.46 ± 5.87a	113.45 ± 2.33a	194.09 ± 3.42b		
18	Protocatechuic acid glucoside	54.25 ± 1.05c	181.48 ± 9.46f	39.29 ± 4.06b	100.26 ± 3.69d		
19	4-*O*-caffeoylquinic acid	737.15 ± 23.08d	457.81 ± 4.47c	1043.00 ± 2.21e	1955.17 ± 7.62f		
22	3-*O*-caffeoylquinic acid	745.73 ± 38.11c	867.99 ± 2.97d	319.66 ± 1.09a	1200.17 ± 9.13e		
24	3,4-*O*-dicaffeoylquinic acid	101.86 ± 8.38d	42.11 ± 1.47c	13.35 ± 0.29a	23.81 ± 5.83ab		
	Sum	1783.12 ± 72.95d	1702.03 ± 26.62c	1560.25 ± 16.12b	3501.93 ± 32.64e		
	Flavonols						
25	1,2,3,4-tetra-*O*-galloyl-glucoside	8.68 ± 0.48c	5.99 ± 0.38b	9.72 ± 0.19c	6.00 ± 0.53b		
26	1,3,4,6-tetra-*O*-galloyl-glucoside	18.70 ± 1.70b	23.52 ± 3.27bc	9.58 ± 3.50a	34.82 ± 2.46d		
27	2,3,4,6-tetra-*O*-galloyl-glucoside	87.88 ± 2.88b	95.78 ± 0.13b	59.04 ± 2.78a	144.69 ± 6.69d		
31	Naringenin 7-*O*-glucoside	34.97 ± 1.02b	8.39 ± 0.63a	35.77 ± 0.82b	29.42 ± 4.71b		
32	Quercetin 3-*O*-glucoside	1062.82 ± 51.49c	662.74 ± 20.55a	1322.16 ± 3.80d	1409.36 ± 23.48e		
33	Quercetin 3-*O*-galactoside	345.65 ± 18.51d	191.83 ± 5.26b	455.82 ± 6.39e	297.54 ± 6.92c		
35	Myricetin 3-*O*-rhamnoside	114.94 ± 2.48c	523.56 ± 4.34e	56.89 ± 0.71a	117.30 ± 4.49c		
37	Cratenacin	78.92 ± 6.67c	3.27 ± 0.79a	nd	72.67 ± 2.95c		
	Sum	1752.57 ± 85.23c	1515.09 ± 35.35b	1948.98 ± 18.18d	2111.81 ± 52.23e		
	Total (mg/100 g d.b.)	**8980.77 ± 332.76**a	**10000.07 ± 144.57**b	**9874.49 ± 116.40**b	**14038.21 ± 215.81**e		
	Flavan-3-ols						
5	Procyanidin trimer	78.78 ± 1.34c	57.75 ± 4.24ab	44.74 ± 12.91ab	62.93 ± 1.63bc		

	6	Procyanidin dimer	904.13 ± 4.15d	759.88 ± 1.32c	1475.91 ± 1.03f	719.50 ± 1.04b	9
	7	(+)-Catechin	167.68 ± 7.26c	168.51 ± 5.71c	93.74 ± 9.88a	89.44 ± 1.00a	1
		Sum	1150.59 ± 12.75e	986.14 ± 11.28d	1614.39 ± 23.82f	871.88 ± 3.66b	9
	Hydrolyzable Tannins						
	12	Punicalin isomer I	75.05 ± 0.11d	51.03 ± 4.82b	48.16 ± 0.04b	70.20 ± 0.56d	3
		Sum	75.05 ± 0.11d	51.03 ± 4.82b	48.16 ± 0.04b	70.20 ± 0.56d	3
	Phenolic acids						
	20	Unidentified caffeic derivative	441.64 ± 7.42d	264.98 ± 2.83b	188.07 ± 3.82a	180.78 ± 3.06a	2
	21	3-*O*-*p*-coumaroylquinic acid	357.04 ± 5.87b	179.17 ± 9.79a	197.64 ± 33.50a	223.34 ± 3.79a	1
	22	3-*O*-caffeoylquinic acid	1463.94 ± 6.23e	1247.22 ± 8.88d	1151.22 ± 12.52c	1026.81 ± 6.46b	9
	23	Unidentified cumaric derivative	131.93 ± 1.88f	103.63 ± 0.37e	79.37 ± 0.50b	25.35 ± 0.53a	1
	24	3,4-*O*-dicaffeoylquinic acid	197.09 ± 7.27e	127.37 ± 12.40c	53.98 ± 0.17a	91.05 ± 9.16b	1
		Sum	2591.63 ± 28.66d	1922.37 ± 34.27c	1670.28 ± 50.51b	1547.32 ± 23.01a	1
	Flavonols						
	28	Apigenin 8-C-glucoside (vitexin)	48.57 ± 3.57c	38.20 ± 3.18b	17.42 ± 2.64a	35.10 ± 4.36b	1
	29	Luteolin 6,8-C-diglucoside	50.81 ± 0.18bc	79.51 ± 1.75d	94.14 ± 0.03e	52.44 ± 3.30c	1
	32	Quercetin 3-*O*-glucoside	674.60 ± 0.84f	479.28 ± 3.48c	413.87 ± 1.93a	598.10 ± 2.05e	5
	37	Cratenacin	1672.30 ± 2.03f	1315.66 ± 1.90b	104.89 ± 7.34a	1659.41 ± 8.81e	1
		Sum	2446.28 ± 6.63f	1912.65 ± 10.31c	630.32 ± 11.95a	2345.05 ± 18.52e	2
		Total (mg/100 g d.b.)	**6263.56 ± 48.04**d	**4872.18 ± 55.86**c	**3963.15 ± 86.27**a	**4834.46 ± 45.19**c	4
Flowers	*Flavan-3-ols*						
	6	Procyanidin dimer	192.06 ± 5.27cd	171.94 ± 1.79a	187.02 ± 2.85bc	275.68 ± 2.22e	1
	7	(+)-Catechin	221.65 ± 3.62d	147.20 ± 5.60b	160.24 ± 2.39c	165.86 ± 1.02c	1
		Sum	413.71 ± 8.89c	319.15 ± 7.40a	347.27 ± 5.25b	441.54 ± 3.25d	3
	Phenolic acids						
	20	Unidentified caffeic derivative	284.61 ± 7.72d	68.56 ± 2.63ab	75.16 ± 1.77b	78.61 ± 1.68b	6
	21	3-*O*-*p*-coumaroylquinic acid	551.02 ± 11.27c	113.29 ± 3.59a	106.53 ± 0.85a	294.61 ± 19.07b	3
	22	3-*O*-caffeoylquinic acid	2588.40 ± 47.41d	988.45 ± 1.31a	1077.11 ± 8.51a	1366.45 ± 6.08b	1
	23	Unidentified cumaric derivative	169.77 ± 2.47e	51.74 ± 0.01b	57.89 ± 1.18c	103.55 ± 2.85d	4
	24	3,4-*O*-di-caffeoylquinic acid	458.56 ± 16.07e	140.15 ± 0.30b	165.82 ± 23.03b	89.87 ± 0.44a	2
		Sum	4052.36 ± 84.94e	1362.20 ± 7.85a	1482.51 ± 35.25a	1933.07 ± 30.13b	2
	Flavonols						
	30	Quercetin 3-*O*-rutinoside (rutin)	35.45 ± 2.00b	36.66 ± 0.55b	40.41 ± 0.95c	56.75 ± 0.35d	5
	32	Quercetin 3-*O*-glucoside	2023.70 ± 11.10d	496.88 ± 13.09a	513.48 ± 11.27a	849.00 ± 28.40b	1
	33	Quercetin 3-*O*-galactoside	284.52 ± 1.30b	80.44 ± 2.16a	87.25 ± 2.05a	107.84 ± 6.87a	3
	34	Quercetin 3-*O*-acetyl hexoside	135.95 ± 7.72b	398.37 ± 1.08c	470.60 ± 18.59d	485.67 ± 21.97d	4
	36	Apigenin 6-C-glucoside-8-C-arabinoside	250.39 ± 7.80d	77.67 ± 0.24a	73.23 ± 4.33a	92.45 ± 5.94b	1
	37	Cratenacin	521.92 ± 8.01f	257.45 ± 1.02a	284.17 ± 2.60b	389.44 ± 6.30c	5
		Sum	3251.93 ± 37.93d	1347.48 ± 18.14a	1469.14 ± 39.79a	1981.15 ± 69.85b	2

Total (mg/100 g d.b.)	**7718.00 ± 131.76**f	**3028.82 ± 33.39**a	**3298.92 ± 80.38**b	**4355.76 ± 103.22**c

Values are given in mg/100 g d.b. (dry basis) and are expressed as mean ± SD. Statistical significance was analyzed with Duncan's test. Values marked with different letters (between species) in the same row indicate statistically significant differences ($p < 0.05$), for more details see Materials and Methods; nd, not detected.

Table S3. Extraction yield of particular samples (in %).

Samples	Extraction yield (%)		
	Berries	**Leaves**	**Flowers**
C1	6.27	10.05	9.34
C2	5.73	8.34	8.98
C3	5.25	7.69	7.43
C4	5.69	8.61	8.40
C5	7.40	9.38	5.83
C6	6.32	9.47	5.93

Table 4. Calibration curve parameters of the method developed for each standard.

No	Compound	Linearity range [μg/mL]	Regresion equation	R2	LOD [μg/mL]	LOQ [μg/mL]
			Anthocyanins			
1	Cyanidin 3-*O*-glucoside	25 - 250	$y = 1.66x10^{-5} – 8.92$	0.998	0.015	0.045
			Flavan-3-ols			
2	(+)catechin	25 - 250	$y = 1.4x10^{-5} – 6.36$	0.999	0.013	0.038
			Hydrolyzable tannins			
3	Ellagic acid	25 - 250	$y = 2.98x10^{-5} +0.30$	0.999	0.027	0.081
			Phenolic acids			
4	3-*O*-caffeoylquinic acid	25 - 250	$y = 6.16x10^{-5} – 3.40$	0.997	0.055	0.168
5	*p*-coumaric acid	25 - 250	$y = 8.19x10^{-5} – 0.06$	0.999	0.074	0.22
6	Protocatechuic acid	25 - 250	$y = 7.8x10^{-5} – 0.04$	0.998	0.07	0.213
			Flavonols			
7	Apigenin 8-C-glucoside	25 - 250	$y = 1.33x10^{-5} – 4.02$	0.999	0.012	0.036
8	Quercetin 3-*O*-rutinoside	25 - 250	$y = 4.85x10^{-5} +0.78$	0.999	0.048	0.132
9	Naringenin 7-*O*-glucoside	25 - 250	$y = 4.78x10^{-5} – 7.86$	0.999	0.043	0.13
10	Luteolin 7-*O*-glucoside	25 - 250	$y = 4.52x10^{-5} – 0.06$	0.998	0.047	0.123
11	Myricetin 3-*O*-glucoside	25 - 250	$y = 2.98x10^{-5} – 1.6$	0.989	0.027	0.081