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Supplementary material

1. Folin Ciocalteu assay

One mL of Folin Ciocalteu reagent was added to 0.25 mL e extract. After 3 mL, 0.75 mL of sodium carbonate (1% w/w) were added. The solution was vortexed and incubated for 2 h protected from light. A calibration curve of gallic acid was constructed and total phenolic content was calculated according to the following equation:

$A=0.0311x-0.0016$ (R^2: 0.998)

2. Total flavonoid content

Equal volumes (1 mL), of the extract and a methanolic solution of aluminum trichloride (2%) were mixed. For the preparation of the blank solution 1 mL of the sample was mixed with methanol (1:1). Samples were left at room temperature for 15 min and the absorbance was measured at 415 nm. A calibration curve of quercetin was constructed and total flavonoid content was calculated according to the following equation:

$A=0.0341x-0.0140$ (R^2: 0.997)

For the calculation of the results the absorbance generated by the blank solution was subtracted from that derived from the sample.

3. Antioxidant activity assays

The phosphomolybdenum assay was used to evaluate the total antioxidant activity of the received extracts. A reagent solution of sodium phosphate (28 mM), ammonium molybdate (4 M) and sulfuric acid (28 mM) was prepared. Two mL of this solution were added to 0.2 mL of each extract. Absorbance was read at 695 nm after incubation for 90 min at 95 °C. Results were expressed as IC50 values.

For the ferrous ion chelating activity, 0.5 mL of FeCl2 (2 mM) were mixed with 2 mL of the sample. To initiate the reaction 0.2 mL of ferrozine at the concentration of 5 mM were added.

A blank solution was prepared using 2 mL of the sample, 0.05 mL of FeCl2 and 0.2 mL of water. Absorbance was measured at 562 nm after incubation at room temperature for 10 min.

FRAP antioxidant capacity was estimated by adding 0.1 mL of the sample in 2 mL of a premix FRAP reagent prepared as follows: acetate buffer (0.3 M, pH: 3.6), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) (10 mM) in 40 mM HCl and ferric chloride (20 mM) in a ratio of 10:1:1 (v/v/v). Incubation at room temperature took place for 30 min and then the absorbance was read at 593 nm.

For the CURAC assay, 0.5 mL of the sample were added to a premix contained CuCl2 (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH4Ac buffer (1 mL, 1 M, pH: 7.0). Three mL of the premix solution and 0.5 mL of the sample were mixed in order to prepare a blank solution, which in this case did not contain the CuCl2. Samples were incubated for 30 min at room temperature and then the absorbance was measured at 450 nm.

For the DPPH assay, 4 mL of DPPH methanolic solution (0.004 % w/v) and 1 mL of the sample were added in a test tube, vortexed and left at room temperature in the dark for 30 min Absorbance was measured at 517 nm.

As for the ABTS assay, a stock solution of ABTS•+ radical was prepared by dissolving the ABTS reagent (7 mM) in distilled water. Potassium persulfate (2.45 mM), was then added and the mixture was left at room temperature protected from light for 12-16 h.

The ABTS stock solution was diluted with methanol so as the absorbance of the working solution measured at 734 nm was 0.700 ± 0.02. One mL of the sample was added to 2 mL of ABTS working solution. The mixture was vortexed and incubated for 7 min at room temperature in the dark.

4. Enzyme inhibitory activity

The α-amylase inhibitory assay was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method. Twenty-five μL of the sample were mixed with 50 μL of an α-amylase solution in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96 well microplate. Incubation for 10 min at 37 °C took place and after 50 μL of aqueous starch solution (0.05% w/v) were added. The mixture was incubated as previously described. To stop the reaction, 25 μL of HCl at the concentration of 1 M and 100 μL iodine-potassium iodide solution were added to the mixture.

A blank solution was prepared by mixing all the reaction reagents apart from α-amylase solution. Absorbance was measured at 630 nm. For the calculation of the results the absorbance of the blank solution was subtracted from that of the sample.

Dopachrome method, slightly modificated, was used to evaluate the tyrosinase inhibitory activity. L-DOPA was used as substrate. Twenty-five μL of the sample were mixed with 40 μL of tyrosinase solution and 100 μL of phosphate buffer (pH 6.8) in a 96 well microplate. Incubation for 15 min at 25 °C took place. Then 40 μL of L-DOPA solution (10 mM), were added. To prepare the blank solution, reaction reagents were added to the sample, except from the enzyme solution. Absorbance was read at 492 nm after incubation of 10 min at 25 °C.

5. Liquid Chromatography–Electrospray Tandem Mass Spectrometry (LC–ESI–MS/MS) analysis

For higher sensitivity and resolution of the target compounds, three different mobile phases were tested, namely: (i) A: 0.1% formic acid (v/v) and B: methanol, (ii) A: ammonium acetate (5 mM) and B: acidified acetonitrile with acetic acid (0.1%), and (iii) A: ammonium formate (10 mM) with 0.1% formic acid and B: acidified acetonitrile with 0.1% formic acid. Better chromatographic results and higher resolution of the isomers presented, was achieved when analyzing the extract with the first mobile phase i.e: 0.1% (v/v) formic acid (solvent A)/ methanol (solvent B).

The following gradient program~~me~~ was applied: 0.00 min 2% B eluent, 3.00 min 2% B eluent, 6.00 min 25% B eluent, 10.00 min 50% B eluent, 14.00 min 95% B eluent, 17.00 min 95% B and 17.50 min 2% B eluent. The column temperature was maintained at 25 °C. The flow rate was set at 4 mL/min and the injection volume was 2.0 μL.

Mass spectra were acquired–in the negative and positive multiple reaction monitoring mode (MRM). The operating parameters were the following: capillary voltage of -3.5 kV, gas temperature of 300 °C and gas flow of 11 L/min. The nebulizer pressure was 40 psi.

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Table S1. Correlation between phenolic compounds and assays [x].

Assays	Phosphomolybdenum	DPPH	ABTS	CUPRAC	FRAP	Ferrous ion chelating	Tyrosinase	α-Amylase
DPPH	-0.734							
ABTS	-0.810	0.993						
CUPRAC	0.998 [y]	-0.688	-0.770					
FRAP	-0.458	0.940	0.892	-0.399				
Ferrous ion chelating	-0.978	0.861	0.915	-0.962	0.635			
Tyrosinase	0.841	-0.251	-0.364	0.875	0.095	-0.708		
α-Amylase	0.982	-0.850	-0.907	0.967	-0.619	-0.999 [y]	0.722	
Total flavonoid	0.786	-0.157	-0.274	0.825	0.190	-0.638	0.995	0.653
Total phenolic	0.844	-0.984	-0.998 [y]	0.807	-0.864	-0.938	0.420	0.931
Chlorogenic acid	-0.300	0.868	0.802	-0.237	0.985	0.494	0.263	-0.477
Luteolin 7-glucoside	0.179	0.536	0.432	0.243	0.793	0.032	0.683	-0.012
Hesperidin	-0.016	0.690	0.599	0.049	0.896	0.226	0.527	-0.207
Rosmarinic acid	0.065	0.629	0.532	0.130	0.857	0.146	0.594	-0.126
Apigenin 7-glucoside	0.544	0.171	0.052	0.597	0.497	-0.354	0.911	0.373
Luteolin	0.735	-0.080	-0.198	0.778	0.266	-0.576	0.985	0.592

[x] Data show the Pearson Correlation Coefficients between the parameters.

[y] Significant at $p < 0.05$.

Table 2. Concentration (μg/g extract) and analytical characteristics of *Z. taurica* subsp. *cleonioides* extracts[x].

No.	Compound	Ethyl acetate	Methanol	Water	Linear equation	R^2	LoD (μg/L)	LoQ (μg/L)
1	Verbascoside	40.26 ± 1.33[a]	3.17 ± 0.28[c]	7.44 ± 0.04[b]	y=8.59x−28.05	0.9988	0.82	2.75
2	Vanillin	49.73 ± 2.02[a]	12.92 ± 0.27[b]	nd	y=2.02x+135.49	0.9926	15.23	50.77
3	Vanillic acid	53.97 ± 0.70[a]	28.44 ± 2.86[b]	12.24 ± 0.23[c]	y=0.49x−1.61	0.9968	2.56	8.54
4	Syringic acid	17.90 ± 0.18[a]	20.06 ± 0.96[a]	10.55 ± 0.13[b]	y=0.74x−1.54	0.9975	3.75	12.50
5	Sinapic acid	3.96 ± 0.15[a]	3.28 ± 0.69[a]	0.59 ± 0.13[b]	y=2.09x−6.79	0.9974	2.64	8.78
6	Rosmarinic acid	634.30 ± 3.22[c]	3375.67 ± 38.02[a]	884.14 ± 28.88[b]	y=9.82x−17.98	0.9989	0.57	1.89
7	Quercetin	10.21 ± 0.11[a]	2.24 ± 0.01[c]	7.67 ± 0.14[b]	y=14.68x−18.25	0.9997	1.23	4.10
8	Protocatechuic acid	34.51 ± 0.21[c]	110.16 ± 2.92[a]	55.31 ± 0.05[b]	y=5.65x−9.99	0.9990	1.17	3.88
9	p-Coumaric acid	30.49 ± 1.56[a]	33.16 ± 0.59[a]	25.24 ± 0.87[b]	y=17.51x+53.73	0.9997	1.93	6.44
10	Luteolin-7-glucoside	23.09 ± 0.44[b]	597.46 ± 12.85[a]	1.26 ± 0.07[b]	y=45.25x+156.48	0.9996	0.45	1.51
11	Luteolin	214.09 ± 3.43[b]	327.88 ± 0.55[a]	16.36 ± 0.04[c]	y=8.96x+26.80	0.9992	1.34	4.46
12	Kaempferol	nd	nd	0.60 ± 0.05	y=0.82x−3.06	0.9959	3.30	10.99
13	Hyperoside	39.11 ± 0.34[b]	199.17 ± 5.96[a]	13.25 ± 0.12[c]	y=16.32x−1.26	0.9998	0.99	3.31
14	Hesperidin	38.92 ± 3.55[c]	711.57 ± 44.79[a]	156.09 ± 3.68[b]	y=5.98x+0.42	0.9993	1.73	5.77
15	Gallic acid	nd	10.49 ± 0.14[a]	2.59 ± 0.08[b]	y=4.82x−26.48	0.9988	1.46	4.88
16	Ferulic acid	61.96 ± 1.60[a]	29.53 ± 1.15[b]	15.32 ± 0.49[c]	y=3.32x−4.30	0.9992	1.43	4.76
17	Eriodictyol	17.78 ± 0.44[a]	2.01 ± 0.07[b]	0.32 ± 0.02[c]	y=14.24x−0.50	0.9998	0.80	2.68
18	Chlorogenic acid	515.22 ± 51.35[c]	3225.10 ± 16.44[a]	1703.14 ± 298.91[b]	y=12.14x+32.34	0.9995	0.55	1.82
19	Caffeic acid	96.77 ± 3.33[b]	175.14 ± 9.14[a]	77.18 ± 0.65[b]	y=11.09x+16.73	0.9997	3.15	10.50
20	Apigenin-7-glucoside	109.45 ± 1.25[b]	260.64 ± 3.61[a]	1.82 ± 0.09[c]	y=21.33x−31.69	0.9983	0.41	1.35
21	Apigenin	29.33 ± 1.64[a]	19.96 ± 0.81[b]	5.85 ± 0.05[c]	y=11.29x+38.05	0.9987	0.96	3.20
22	4-Hydroxybenzoic acid	24.82 ± 0.75[a]	25.39 ± 0.18[a]	5.25 ± 0.16[b]	y=7.62x+22.79	0.9996	1.72	5.72
23	3,4-Dihydroxyphenylacetic acid	nd	2.69 ± 0.05[a]	2.39 ± 0.01[b]	y=5.13x−12.39	0.9990	1.35	4.51

x Within each column, means sharing the different superscripts show comparison between the samples by Tukey's test at $p < 0.05$.

nd, not detected.

LoD and LoQ, limit of detection and limit of quantification, respectively.

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