

Supplementary Materials (S1)

Article

Ziziphora taurica subsp. *taurica*: Analytical Characterization and Biological Activities

Michał Tomczyk ^{1*}, Olcay Ceylan ², Marcello Locatelli ³, Angela Tartaglia ³, Vincenzo Ferrone ³, Cengiz Sarikurkcu ⁴

¹ Department of Pharmacognosy, Faculty of Pharmacy, Medical Univeristy of Bialystok, Poland

² Department of Biology; Science Faculty, University of Mugla Sitki Kocman, Mugla, Turkey

³ Departemnt of Pharmacy; University of Chieti–Pescara “G. d’Annunzio”; Chieti; Italy

⁴ Department of Analytical Chemistry; Süleyman Demirel University; Faculty of Pharmacy; Isparta; Turkey

* Correspondence: michal.tomczyk@umb.edu.pl (M.T.) Tel.: +48-85-746-5694

For total phenolic content, sample solution (0.25 mL) was mixed with diluted Folin-Ciocalteu reagent (1 mL, 1:9) and shaken vigorously. After 3 min, Na₂CO₃ solution (0.75 mL, 1%) was added and the sample absorbance was read at 760 nm after 2 h incubation at room temperature. Total phenolic content was expressed as equivalents of gallic acid.

For total flavonoid content, sample solution (1 mL) was mixed with the same volume of aluminium trichloride (2%) in methanol. Similarly, a blank was prepared by adding sample solution (1 mL) to methanol (1 mL) without AlCl₃. The sample and blank absorbance were read at 415 nm after 10 min incubation at room temperature. Absorbance of the blank was subtracted from that of the sample. Total flavonoid content was expressed as equivalents of quercetin.

Total antioxidant activity of the samples was evaluated by phosphomolybdenum method. Sample solution (0.2 mL) was combined with 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample absorbance was read at 695 nm after 90 min incubation at 95 °C.

For 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, sample solution (1 mL) was added to a 4 mL of 0.004% methanol solution of DPPH. Sample absorbance was read at 517 nm after 30 min incubation at room temperature in dark.

For ABTS cation radical scavenging activity, briefly, ABTS⁺ radical cation was produced directly by reacting 7 mM ABTS solution with 2.45 mM potassium persulfate and allowing the mixture to stand for 12-16 h in dark at the room temperature. Prior to beginning the assay, ABTS solution was diluted with methanol to obtain an absorbance of 0.700 ± 0.02 at 734 nm. Sample solution (1 mL) was added to ABTS solution (2 mL) and mixed. Sample absorbance was read at 734 nm after 7 min incubation at room temperature.

For metal chelating activity on ferrous ions, briefly, sample solution (2 mL) was added to FeCl₂ solution (0.05 mL, 2 mM). The reaction was initiated by the addition of 5 mM ferrozine (0.2 mL). Similarly, a blank was prepared by adding

sample solution (2 mL) to FeCl₂ solution (0.05 mL, 2 mM) and water (0.2 mL) without ferrozine. Then, the sample and blank absorbance were read at 562 nm after 10 min incubation at room temperature.

For cupric ion reducing activity (CUPRAC), sample solution (0.5 mL) was added to a premixed reaction mixture containing CuCl₂ (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM) and NH₄Ac buffer (1 mL, 1 M, pH 7.0). Similarly, a blank was prepared by adding sample solution (0.5 mL) to a premixed reaction mixture (3 mL) without CuCl₂. Then, the sample and blank absorbance were read at 450 nm after 30 min incubation at room temperature.

For ferric reducing antioxidant power (FRAP), sample solution (0.1 mL) was added to a premixed FRAP reagent (2 mL) containing 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*). Then, the sample absorbance was read at 593 nm after 30 min incubation at room temperature.

Inhibitory activity on α -amylase was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method. Sample solution (25 μ L) was mixed with α -amylase solution (50 μ L) in phosphate buffer (pH 6.9 with 6 mM sodium chloride) in a 96-well micro plate and incubated for 10 min at 37 °C. After pre-incubation, the reaction was initiated by the addition of starch solution (50 μ L, 0.05%). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme solution (α -amylase). The reaction mixture was incubated 10 min at 37 °C. The reaction was then stopped with the addition of HCl (25 μ L, 1 M). This was followed by the addition of iodine-potassium iodide solution (100 μ L). The sample and blank absorbance were read at 630 nm. Absorbance of the blank was subtracted from that of the sample.

Tyrosinase inhibitory activity was measured using a modified dopachrome method with L-DOPA as substrate. Sample solution (25 μ L) was mixed with tyrosinase solution (40 μ L) and phosphate buffer (100 μ L, pH 6.8) in a 96-well microplate and incubated for 15 min at 25°C. The reaction was then initiated with the addition of L-DOPA (40 μ L). Similarly, a blank was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase) solution. The sample and blank absorbance were read at 492 nm after 10 min incubation at 25 °C.