Supporting Information

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Secondary Metabolites of Ethanol Extracts of *Pinus sylvestris* Cones from Eastern Anatolia and Their Antioxidant, Cholinesterase and α-Glycosidase Activities

Meryem Topal *

Gumushane University, Vocational School of Health Services, 29100-Gumushane, Türkiye

Table of Contents	Page

S.1: Antioxidant Capacity Assays

S.1.1: Fe^{3+} Reduction Capacity

The total reduction procedure was performed according to the method of Oyaizu as described previously [1]. For this, a stock solution was first prepared at a concentration of 1 mg/mL. This stock was transferred to the test tubes in different concentrations to the solution. The volume was supplemented with 1 mL of distilled water. Then, 2.5 mL phosphate buffer (0.2 M, pH 6.6) and 2.5 mL, $K_3Fe[CN]_6$ (1%). pH was added to each tube and the mixture was incubated for 20 min at 50°C. After that, 2.5 mL of 10% (TCA) was added to the reaction mixture. 2.5 mL of the supernatant of the solution were added, followed by the addition of 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (0.1%), and the absorbance at 700 nm.

S.1.2 : Cu^{2+} Reduction Capacity (Cuprac Method)

P. sylvestris cones were made by a slight modification of the Cu^{2+} reduction activity method [2]. 0.25 mL of $CuCl_2$ solution (0.01 M), 0.25 mL of ethanolic neocuproine solution (7.5 mM) and 0.25 mL of CH_3COONH_4 buffer solution (1.0 M) were added to the tubes of *P. sylvestris* cones prepared in different concentrations. After half an hour, absorbance values were measured at 450 nm.

S.1.3: DPPH Free Radical Scavenging Activity

DPPH free radical scavenging activity was performed according to Blois method as described in previous studies [3]. $DPPH^{\cdot}$ solution (1 mM) was used as the free radical. 1 mg/mL stock solution prepared previously was used as a sample. Stock solutions were transferred to the test tubes at a concentration of 10, 20 and 30 µg/µL respectively, and the total volume was completed with ethanol to be 3 mL. Then an aliquot of stock DPPH solution (1 mL) was added to each sample tube. After incubation in the dark, absorbance was measured at 517 nm. The descending absorbance gave the remaining free radical scavenging activity.

S.1.4: ABTS Radical Scavenging Activity

ABTS radical scavenging activity was determined according to the method of Re et al. as explained in detail [4]. Firstly, 7 mM ABTS solution was prepared. ABTS radicals were produced by adding 2.45 nM persulfate solution to this solution. 1 mL of ABTS radical solution was added to different concentrations of *P. sylvestris* cones to be investigated for ABTS radical scavenging activity. The absorbances were recorded at 734 nm.

S.1.5: Determination of Total Phenolic Compounds

Total phenolic compounds in *P. sylvestris* cones were determined by using Folin-Ciocalteu regent [5]. Gallic acid was preferred as a phenolic compound standard. For this, a standard graphic was prepared from gallic acid. Stock solution was used from the cone extracts prepared in ethyl alcohol. The stocks were taken from the solution and pipetted into meters. The final volume was completed to 23 mL with distilled water. Folin-Ciocalteu reactant and Na_2CO_3 (2%) were added to the mixture. Samples were allowed to mix at room temperature. At the end of this time, the absorbance of the samples was

read at 760 nm. Absorbance values of cone extracts corresponding to gallic acid equivalents (GAE) content were determined using the equations derived from the standard graph [5].

S.1.6 : Determination of Total Flavonoids

Quercetin was used as a standard for total flavonoid concentration determination. The total flavonoid content of cone extracts was determined by using the method of Gulcin et al. [6]. For this, 1000 µg extract was added to a meter. Then, the extracts transferred to the test tube were vortexed by diluting with ethanol solution containing CH_3COOK and 10% $Al(NO_3)_3$ solutions prepared in 1 M water. It was subjected to incubation at room temperature. The absorbance was recorded at 415 nm.

S.1.7 : Enzyme Activity Studies

BChE (C2490-1KU; Sigma-Aldrich Chemie) was obtained from equine serum. (C2888-500UN; Sigma-Aldrich Chemie) was obtained from electric eel (*Electrophorus electricus*). AChE, and BChE inhibition activities were carried out according to the Ellman's method [7]. 5,5'-Dithiobis(2-nitrobenzoic acid) (DTNB), acetylcholine iodide (AChI) and Butyrylthiocholine iodide (BChI) were used as substrates of AChE/BChE activities. Tris-HCl buffer (1.0 M, pH 8.0) and sample solutions with different concentrations were dissolved in pure water. To the resulting solution, 30 μL were added to AChE/BChE solution. The mixture was incubated for 10 min at 25°C. Then, DTNB (0.5 mM, 100 μL) was supplemented. After this procedure, the reaction mixture was added 100 mL AChI/BChI (10/10 mM) and activity studies were complete [8]. Absorbances at 412 nm were evaluated in both enzymes.

Table S1. The mass paramaters and linear regression equation of applied method for determination of phenolics by LC-HRMS

Compound	*m/z*	Linear regression equation	R^2
(-) Epigallocatechin	305.0666	y=0.06961-0.01052	0.99
(-) Epigallocatechin gallate	457.0776	y=0.02363-0.00321	0.99
(+)-Catechin	289.0717	y=0.18920-0.01504	0.99
Fumaric acid	115.0036	y=0.01536-0.00009	0.99
(-) Epicatechin	291.0863	y=0.03726-0.00397	0.99
Verbascoside	623.1981	y=0.01903-0.00329	0.99
Caffeic acid	179.0349	y=0.12530-0.01410	0.99
Luteolin-7-O-rutinoside	593.1511	y=0.03827-0.00299	0.99
Hesperidin	609.1824	y=0.00552-0.00008	0.99
(+) *trans* Taxifolin	303.0510	y=0.08903-0.00311	0.99
Hyperoside	463.0882	y=0.01739-0.00277	0.99
Rosmarinic acid	359.0772	y=0.07493-0.00456	0.99
Nepetin-7-O-glucoside	477.1038	y=0.06021-0.00343	0.99
Apigenin 7-O-glucoside	433.1129	y=0.02039-0.00044	0.99
Dihydrokaempferol	287.0561	y=0.10830+0.00435	0.99
Quercitrin	447.0932	y=0.09150-0.00486	0.99
Myricetin	317.0302	y=0.09298-0.03409	0.98
Herniarin	177.0546	y=1.55500+0.15820	0.99
Naringenin	271.06120	y=0.08118-0.00290	0.99
Nepetin	315.0510	y=0.84250-0.00138	0.99
Rhamnocitrin	299.0561	y=0.01249-0.00401	0.99
Hispidulin	299.0561	y=0.05143-0.00035	0.99
Kaempferol	301.0353	y=0.28170-0.03338	0.99
Luteolin	285.0404	y=1.39800-0.08994	0.99
Apigenin	271.0601	y=0.51310+0.01576	0.99
Isosakuranetin	285.0768	y=0.01938-0.00057	0.99
Eupatilin	343.0823	y=0.02342-0.00285	0.99
Chrysin	253.0506	y=0.18300-0.02227	0.99
Acacetin	283.0612	y=0.13960-0.03039	0.99
Quillaic acid	485.3272	y=0.04231-0.01048	0.99
Hederagenin	473.3625	y=0.00370+0.00108	0.99

S5: References

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