Supporting Information

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Microwave-Assisted Extraction of Polyphenolics from Some Selected Medicinal Herbs Grown in Turkey

Sefa Baki[1], Ayşe Nur Tufan[1], Mehmet Altun[1*], Fevzi Özgökçe[2], Kubilay Güçlü[1] and Mustafa Özyürek[1*]

[1]*Istanbul University, Faculty of Engineering, Department of Chemistry, Avcilar, 34320 Istanbul, Türkiye*

[2]*Yuzuncuyıl University, Faculty of Science & Art, Department of Biology, Van, Türkiye*

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Procedures of Antioxidant Activity/Capacity Assays

[*]Corresponding author: E-Mail: **maltun@istanbul.edu.tr**; Phone:+90-212-4737070 *Fax:*+90-212-4737180

1. Total Phenolic Contents (TPC)

TPC of the medicinal herb extracts were evaluated by using the FC method (Singleton *et al.*, 1999). The solutions used in the assay includes; Lowry A: 2% aqueous Na_2CO_3 in 0.1 M NaOH; Lowry B: 0.5% $CuSO_4$ aqueous solution in 1% $NaKC_4H_4O_6$ solution; Lowry C: a mixture of 50 mL Lowry A and 1 mL Lowry B solutions. The FC reagent was diluted with distilled water at a ratio of 1:3 before use. The microwave-assisted extract (0.5 mL) was added to 1.5 mL of distilled water and 2.5 mL of Lowry C solution in a test tube. Then, 0.25 mL of FC reagent was added and mixed, thirty minutes later the absorbance of the reaction solution was measured at 750 nm. The absorption measurements were recorded in matched quartz cuvettes using a Varian CARY Bio 100 UV−vis spectrophotometer (Mulgrave, Victoria, Australia). The assays were carried out in five replicates and the results expressed as trolox equivalent (μmol TR/g of sample) unit ($\mathcal{E}_{TR} = 4.65x10^3\ M^{-1}cm^{-1}$).

2. CUPRAC Assay

TAC of the microwave-assisted extracts were determined by the spectrophotometric CUPRAC method described by Apak *et al.* (2004) which is utilizing the copper(II)-neocuproine (Cu(II)-Nc) reagent as the chromogenic oxidizing agent. 1 milliliter of $CuCl_2$, 1 mL of Nc solution, and 1 mL of NH_4Ac solution were added to x mL of the analyte, followed by (1.1−x) mL of H_2O. The absorbance of the final solution at 450 nm was read against a reagent blank after 30 min. All spectrophotometric TAC analyses were carried out in five replicates and performed at room temperature. TAC values of medicinal herbs were expressed using trolox equivalent (mmol TE/g of sample) unit (Eq. 1):

$$\text{TAC-CUPRAC} = (A_f/\varepsilon_{TR})\,(V_f/V_s)\,r\,(V_i/m) \qquad \text{(Eq. 1)}$$

where V_i = initial volume of medicinal herb prepared from (m) grams of dry matter,

(r) is the dilution prior to analysis,

V_s is the sample volume taken for analysis from the diluted extract,

A_f is the $A_{450\ nm}$ developed after 30 min CUPRAC reaction, and

V_f is the final CUPRAC reaction volume (4.1 mL).

3. Electrochemical DPV-Based CUPRAC Assay

TAC of the herbs were also determined by the electrochemical DPV-based CUPRAC described by Tufan *et al.* (2014). This electroanalytical method based on the reduction of $Cu(Nc)_2^{2+}$ to $Cu(Nc)_2^{+}$ by antioxidants and electrochemical detection of the remaining Cu(II)-Nc, the difference being related to TAC of the samples. DPV was performed electrochemically *via* a potentiostat model Reference 600 (Potentiostat/Galvanostat/ ZRA, Gamry) in a three-electrode configuration, employing a GC electrode (Gamry, 3 mm disk, 7 mM OD) as the working electrode, Ag/AgCl (5 mol/L KCl) as the reference electrode, and platinum wire as the counter electrode, $Cu(Nc)_2^{2+}$ reagent is comprises of 1:2 molar ratio of metal to ligand (*i.e.*, 2 mL of 2 mM Cu(II) + 2 mL of 4 mM Nc) in 6 mL of 0.1 M acetic acid/acetate buffer solution (supporting electrolyte, pH 4.76). The required volume of samples was added into mixture solution of the supporting electrolyte and Cu(II)−Nc chelate in the electrochemical cell. The reaction mixture was left to react for 1 min on a stirrer at room temperature. The TAC values

of medicinal herbs were determined by DPV experiments, and the results were expressed as trolox equivalent (μmol TR/g of sample) unit (Eq. 1).

4. ABTS Assay

The spectrophotometric analysis of $ABTS^{\bullet+}$ radical scavenging activity was determined according to method of Re *et al.* (1999). ABTS radical cation ($ABTS^{\bullet+}$) was produced by reacting ABTS stock solution with potassium persulfate ($K_2S_2O_8$) and allowing the mixture to stand in the dark for 24 hours before use. Initial absorbance of 1 mL of diluted ABTS solution at 734 nm is recorded, the sample extract is added, and the decrease in absorbance is measured after thirty minutes of reaction time. The assays were carried out in five replicates and the results were expressed as micromoles of trolox equivalents per gram of sample (μmol TE/g sample) ($\varepsilon_{TR} = 2.6x10^4\ M^{-1}cm^{-1}$) (Eq. 1).

5. Free Radical Scavenging (FRS) Activity

Radical scavenging abilities of the samples were evaluated using DPPH assay as described previously (Sanchez-Moreno *et al.*, 1998). The herb extracts (0.35 mL) was mixed with 2.65 mL of methanol and 1 mL of 0.1 mM DPPH solution in a tube. Initial $DPPH^{\bullet}$ absorbance was recorded, an aliquot of the test sample was added, the mixture was incubated for 30 min, and the final absorbance was recorded. The free radical scavenging (FRS) activity was calculated as the percentage of DPPH decolorization using the following equation: FRS activity (%) = $[(A_{DPPH} - A_S)/A_{DPPH}]$ x 100, where A_{DPPH} is the absorbance of DPPH solution without any extract of, A_S is the absorbance of the solution when the sample extract was added to solution mixture. The assays were carried out in five replicates and the results expressed as mean value ± standard deviation. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the curve of FRS inhibition percentage against extract concentration.

6. Hydroxyl Radical Scavenging (HRS) Activity

HRS activity of microwave-assisted extracts were evaluated according to the method of Özyürek *et al.* (2008). In this method, 1.5 mL of phosphate buffer (pH 7.0), 0.5 mL of 10 mM sodium salicylate, 0.25 mL of 20 mM Na_2–EDTA, 0.25 mL of 20 mM $FeCl_2$ solution, 1.9 mL H_2O, 0.1 mL herb extract, and 0.5 mL of 10 mM H_2O_2 were added to a test tube quickly in this order. The mixture was incubated for 10 min in a water bath kept at 37 °C. 0.5 mL of 268 U mL^{-1} catalase solution was added to stop the reaction, and mixed for 30 s. Final solutions (0.5 mL of the incubation solution) were exposed to the HRS-CUPRAC method. The HRS activity (%) of methanolic extract was determined by the following equation: HRS activity (%) = $[(A_0 - A)/A_0]$ x 100, where A and A_0 are the CUPRAC absorbances of the system in the presence and absence of scavenger, respectively. The assays were conducted in five replicates and the results expressed as mean value ± standard deviation. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the curve of HRS inhibition percentage against methanolic extract concentration.

7. Hydrogen Peroxide Scavenging (HPS) Activity

HPS activity of the microwave-assisted extracts were evaluated using the HPS–CUPRAC method which was described by Ozyurek *et al.* (2010). 0.7 mL of phosphate buffer (pH 7.4), 0.4 mL of 1 mM H_2O_2, 0.4 mL of 0.1 mM $CuCl_2.2H_2O$ were added a test tube in this order (hydrogen peroxide incubation solution, used as reference). 0.5 mL of phosphate buffer (pH 7.4), 0.4 mL of 1.0 mM H_2O_2, 0.2 mL scavenger sample solution, and 0.4 mL of 0.1 mM $CuCl_2.2H_2O$ solution were added to other two test tubes rapidly in this order. The mixtures were incubated for 30 min in a water bath kept at 37°C. 0.4 mL H_2O was added to reference and scavenger solution-I and 0.4 mL of 268 U mL^{-1} catalase solution was added to scavenger solution-II, and vortexed. To 1.0 mL of the final incubation solutions, the HPS–CUPRAC method was applied as follows: 1mL Cu(II) + 1mL Nc + 2 mL NH_4Ac buffer + 1 mL final incubation solution. The absorbance at 450 nm was recorded. The HPS activity (%) of herb extracts were calculated using the following formula: HPS activity (%) = $[(A_0 - (A_1 - A_2)) / A_0] \times 100$, where A_0 is the CUPRAC absorbance of reference hydrogen peroxide incubation solution, A_1 and A_2 are the CUPRAC absorbances of scavenger solutions-I and -II, respectively. The assays were conducted in five replicates and the results expressed as mean value ± standard deviation. The extract concentration providing 50% inhibition (EC_{50}) was calculated from the curve of HPS inhibition percentage against extract concentration.

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A. N. Tufan, S. Baki, K. Güçlü, M. Özyürek, R. Apak (2014). A novel differential pulse voltammetric (DPV) method for measuring the antioxidant capacity of polyphenols-reducing cupric neocuproine complex, *J. Agric. Food Chem.* **62**, 7111–7117.

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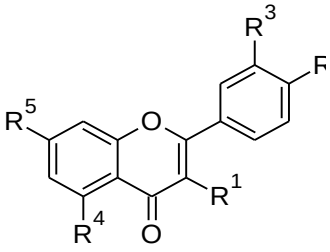
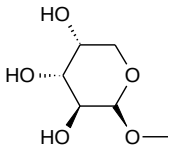
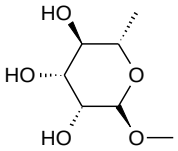
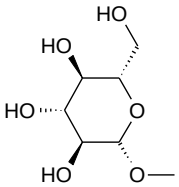
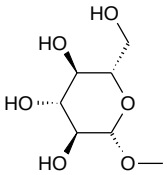
C. Sanchez-Moreno, J. A. Larrauri, F. Saura-Calixto (1998). A procedure to measure the antiradical efficiency of polyphenols, *J. Sci. Food Agric.* **76**, 270–276.

M. Özyürek, B. Bektaşoğlu, K. Güçlü, R. Apak, (2008). Hydroxyl radical scavenging assay of phenolics and flavonoids with a modified cupric reducing antioxidant capacity (CUPRAC) method using catalase for hydrogen peroxide degradation, *Anal. Chim. Acta* 616, 196–206.

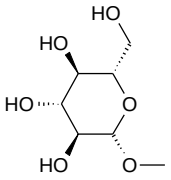
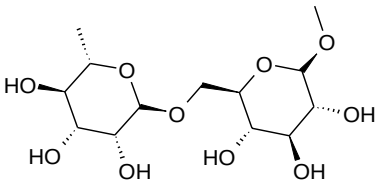
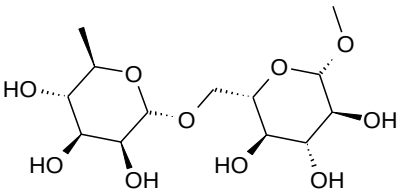
M. Özyürek, B. Bektaşoğlu, K. Güçlü, N. Güngör, R. Apak (2010). A novel hydrogen peroxide scavenging assay of phenolics and flavonoids using cupric reducing antioxidant capacity (CUPRAC) methodology, *J. Food Comp. Anal.* **23**, 689–698.

S1. Structure of the determined flavonoids and derivatives.

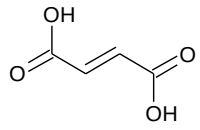


	R^1	R^2	R^3	R^4	R^5
Quercetin	OH	OH	OH	OH	OH
Kaempferol	OH	OH	H	OH	OH
Apigenin	H	OH	H	OH	OH
Luteolin	H	OH	OH	OH	OH
Isorhamnetin	OH	OH	OCH_3	OH	OH
Quercetin-3-*O*-arabinoside		OH	OH	OH	OH
Quercitrin		OH	OH	OH	OH
Luteolin-7-*O*-glucoside	H	OH	OH	OH	
Luteolin-5-*O*-glucoside	H	OH	OH		OH

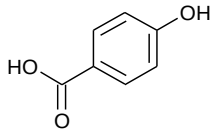
S1 (continued)

Isoquercetin		OH	OH	OH	OH
Kaempferol-3-*O*-rutinoside		OH	H	OH	OH
Rutin		OH	OH	OH	OH

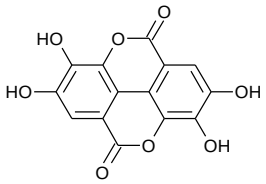
S2. Structure of the determined hydroxycinnamic acids, benzoic acids and others.



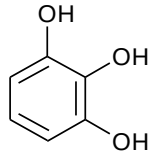
Fumaric acid



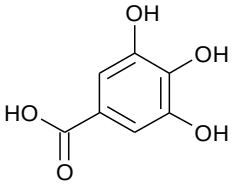
***p*-Hydroxybenzoic acid**



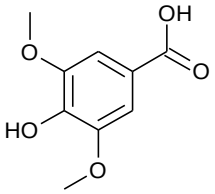
Ellagic acid



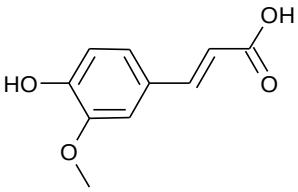
Pyrogallol



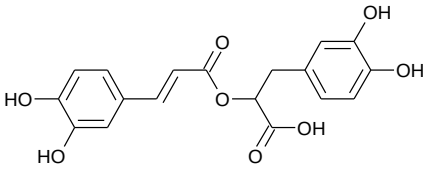
Gallic acid



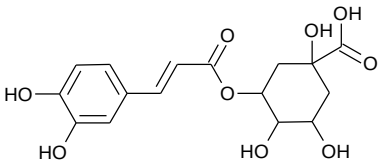
Syringic acid



t-Ferulic acid



Rosmarinic acid



Chlorogenic acid