SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to:

Flavonoids and biological activities of various extracts from *Rosa sempervirens* leaves

Leyla Bitis, Ali Sen, Nurten Ozsoy, Seher Birteksoz-Tan, Sukran Kultur and Gulay Melikoglu

Biotechnol Biotechnol Equip. 2017, 31

SUPPLEMENTAL MATERIAL

Materials and methods

**Determination of total phenolic compounds**

Phenolic compounds in the extracts of *R. sempervirens* leaves were determined by a colorimetric assay, based on procedure described by Gao et al. [1]. Aliquots (0.1 mL) of the extracts were transferred into test-tubes and diluted with 2 mL of distilled water. After addition of 0.2 mL of Folin–Ciocalteu reagent and 1 mL of 15% aqueous sodium carbonate solution, the tubes were vortexed and the absorbance of the resulting blue colored mixture was recorded after 2 h at 760 nm (UV-1800, Shimadzu, Kyoto, Japan) against a blank containing 0.1 mL of extraction solvent. Gallic acid (0.95–15.20 µg/mL) was used for calibration of a standard curve. The results were expressed as gallic acid equivalents (GAE)/g extract. The data are presented as the average of triplicate analyses.

**Determination of total flavonoid compounds**

The total flavonoids (TF) content of the extracts of *R. sempervirens* leaves was measured using the AlCl3 colourimetric assay [2]. Briefly, 250 µL of extracts in various concentrations were mixed with 1250 µL of distilled water and 75 µL of 5% NaNO2. After 6 min, 150 µL of 10% AlCl3 was added. Five minutes later, 0.5 mL of 1 mol/L NaOH was added and the solution was then diluted to 2.5 mL with distilled water. The solution was mixed well and the absorbance was measured against the reagent blank at 510 nm with a spectrophotometer (UV-1800, Shimadzu, Kyoto, Japan). The standard curve for total flavonoids was made using a catechin standard solution (7-112 µg/mL) under the same procedure as earlier described. The total flavonoids were expressed as milligrams of catechin equivalents per gram of extract. The data are presented as the average of triplicate analyses.

**DPPH radical-scavenging activity**

The DPPH free-radical-scavenging activity of the extracts from *R. sempervirens* leaves was measured according to the procedure described by Brand-Williams et al. [3]. Extract solution (0.1 mL) in ethanol at different concentrations or ascorbic acid was added to 3.9 mL of 6 x 10^{-5} mol/L methanolic solution of DPPH. The mixture was shaken vigorously and allowed to stand in the dark at room temperature for 30 min. The decrease in absorbance of the resulting solution was then measured spectrophotometrically at 517 nm (UV-1800, Shimadzu, Kyoto, Japan) against methanol. All measurements were made in triplicate and averaged. Two controls were used for this test, a negative control (containing all reagents except the test sample) and positive controls (using the reference antioxidant, ascorbic acid) for comparison. The ability to scavenge DPPH radical was calculated by the following equation: DPPH radical-scavenging activity (%) = [1–(As/Ac)]x100, where As is the absorbance of the sample at 517 nm and Ac is the absorbance of the control at 517 nm.

**Total radical-antioxidant potential (TRAP) assay**

The total radical antioxidant potential of the extract was measured using the Trolox equivalent antioxidant coefficient (TEAC) assay as described by Cai et al. [4] with minor modifications. The TEAC value is based on the ability of the antioxidant molecules to quench the long-lived ABTS\(^{+}\), a blue-green
chromophore with characteristic absorption at 734 nm, compared with that of Trolox, a water-soluble vitamin E analogue. ABTS was dissolved in water to a concentration of 7 mmol/L. ABTS cation radical (ABTS•+) was produced by reacting ABTS•+ stock solution with 2.45 mmol/L potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 hours before use. At the beginning of the analysis day, an ABTS•+ working solution was obtained by dilution of the stock solution in ethanol to an absorbance of 0.70 (± 0.02) at 734 nm (UV-1800, Shimadzu, Kyoto, Japan). After addition of 3.9 mL of ABTS•+ solution to 0.1 mL of extract from R. sempervirens leaves at different concentrations or Trolox standards (626–39.13 µg/mL) in ethanol, the decrease in absorbance at 734 nm was monitored exactly 6 min after the initial mixing. Appropriate ethanol blanks were run in each assay. All determinations were carried out in triplicate. The ability to scavenge ABTS•+ radical was calculated by the following equation: ABTS•+ radical scavenging activity (%) = \[1 - \left(\frac{A_s}{A_c}\right)\] x 100, where \(A_s\) is the absorbance of the sample at 734 nm and \(A_c\) is the absorbance of the control at 734 nm. The total antioxidant capacity value in a sample was assessed as Trolox Equivalent Antioxidant Capacity (TEAC, mmol/L Trolox equivalents (TE)/g extract). Also, the ABTS•+ radical-scavenging activity was calculated as IC50 values for extracts.

References
[1]. Gao X, Ohlander M, Jeppsson N, et al. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (Hippophae rhamnoides L.) during maturation. J Agric Food Chem. 2000;48:1485–1490.
[2]. Zhang R, Zeng Q, Deng Y, et al. Phenolic profiles and antioxidant activity of litchipulp of different cultivars cultivated in Southern China. Food Chem. 2013;136:1169–1176.
[3]. Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol. 1995;28:25–30.
[4]. Cai Y, Luo Q, Sun M, et al. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci. 2004;74:2157–2184.