SUPPLEMENTARY MATERIAL

Potentilla erecta (L.) rhizomes as a source of phenolic acids

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Abstract

The aim of this study was to evaluate the content of major phenolic acids from *Potentilla erecta* rhiozomes. Water and ethanol-water mixture was used for extraction of these compounds. The extracts were also evaluated for the quantification of total phenolic content and the antioxidant capacity. The contents of phenolic acids and resulting antioxidant activities are dependent on the nature of extracting solvent due to the presence of different antioxidant compounds. Results showed that *P. erecta* rhiozomes contained high amount of gallic and *p*-HBA acids. The contents of chlorogenic and protocatechuic acids in the extracts of *Potentilla* species have not been reported yet. The results suggested that the extracts could be used as the active cosmetics ingredients and nutraceuticals.

Keywords: Potentilla erecta; phenolic acids; antioxidant

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Experimental

Plant material and its extraction

The sample of *P. erecta* rhiozomes was collected during September of 2015 in the east part of Poland (the Podlasie plain). was from 12-years old pine forest (N 52°24', E 23°16'). The voucher specimen was authenticated by Dr Catherina Fyalkowska from Forest Ecology Department and deposited in Forest Research Institute, Poland (No. PEI002015). The collected plant material was dried at 40^{o}C for 24h, then ground (Pulverisette 15) and sieved (0.5 mm).

The dry plant material (700 mg) was mixed with 25 mL of water or ethanol-water solution (60:40, v/v) for 20 min at room temperature. Then, the extracts were filtered through Whatman No.1filter paper. Three independent extractions using appropriate solvent were carried out.

Chromatographic analysis

Chromatographic analysis was performed using a Shimadzu HPLC system equipment with a binary pump, degasser, autosampler and connected to 3200 QTRAP Mass spectrometer (Applied Biosystem/MDS SCIEX). A MS system (3200 QTRAP, Applied Biosystem/MDS SCIEX) was equipped with an electrospray ionization source (ESI) operated in negative-ion mode and a quadrupole mass analyser in a scan mode from 50 to 1500 m/z. Nitrogen was used as curtain and auxiliary gas at 0.3 MPa.

Compounds were separated on SeQuant^{TM} ZIC-HILIC column (100 x 2.1 mm, 3.5 µm) from Merck (Darmstadt, Germany) at 30 °C. with mobile phase containing 5 mM ammonium formate (eluent A) and ACN (eluent B). The gradient profile was as follows: 0-4 min 98% B, 6-7 min 90% B, 8-8.4 min 80% B, 8.4-12 min 50% B, and 13-28 min 98% B. The mobile phase was delivered at 0.2 mL/min. formic acid (2 mmol/L, pH 2.8) as eluent A and acetonitrile as eluent B (Sentkowska et al., 2013). The mobile phase was delivered at 0.2 mL min^{-1}. Compounds were identified by comparing retention time and *m/z* values obtained by MS and MS^2 with the mass spectra from standards tested under the same conditions. Quantification of compounds was done from the calibration curves obtained in Multiple Reaction Mode (MRM).

Determination of total phenolics

Total phenolic content of extracts was assessed by using the Folin–Ciocalteu (FC) phenol reagent (Singleton et al., 1999). 0.1 mL of extract was mixed with 0.1 mL of FC reagent and

0.9 mL of water. After 5 min, 1 mL of 7% (w/v) Na_2CO_3 and 0.4 mL of water were added. The extracts were mixed and allowed to stand for 30 min before measuring the absorbance on a spectrophotometer (PerkinElmer, UV–visible Lambada Bio 20) at 765 nm. A mixture of water and reagents was used as a blank. Total phenolic content was expressed in mg of gallic acid (GA) per gram. Each analysis was done in three repetitions.

The scavenging ability on 1,1-diphenyl-2-pirylhydrazyl (DPPH) radicals

The DPPH assay was applied to estimate the radical-scavenging ability of the fruit extracts (Pyrzynska and Pekal, 2013). 0.1 mL of a given extract was mixed with 2.4 mL DPPH solution (9×10^{-5} M) in methanol . After 30 min absorbance was measured at 539 nm against the blank. Trolox, a vitamin E analogue, solution was used to calibrate the standard curve. The mean ± SD results of triplicate analyses were expressed in mmol of trolox (TR) per gram of dry sample.

Cupric reducing antioxidant capacity

For assessing cupric reducing ability (CUPRAC), the assay described by Apak et al. (2004) was adapted. 1 mL of $CuCl_2$ solution (0.01 mol L^{-1}) was mixed with 1 mL of neocuproine alcoholic solution (7.5×10^{-3} mol L^{-1}) and 1 mL of 1 mol L^{-1} CH_3COONH_4, followed by adding 0.5 mL of juice sample and 0.6 mL of water. The tube containing sample and reagents was incubated in a water bath at a temperature of 50 ^oC for 20 min, after which was cooled under running water. Absorbance against the blank reagent was measured at 450 nm. The results were expressed as trolox equivalent (TR) in mmol g^{-1}.

Statistical analysis

The results were expressed as mean ± standard deviation for at least three independent determinations. Statistical comparison of the means was performed using one-way ANOVA, followed by Turkey's test and differences at $p < 0.05$ were considered significant.

References

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