SUPPLEMENTARY MATERIAL

Polarography as a technique of choice for the evaluation of total antioxidant activity: The case study of selected *Coprinus comatus* extracts and quinic acid, their antidiabetic ingredient

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Abstract

This study was focused on *in vitro* screening of the total antioxidant activity of the selected extracts of the mushroom *Coprinus comatus* and quinic acid, one of their antidiabetic ingredients, by an uncommon electrochemical assay. Indeed, direct current (DC) polarographic HydroxoPerhydroxo Mercury(II) Complex (HPMC) assay based on decrease of anodic limiting current originating from HPMC formation in alkaline solutions of hydrogen peroxide at potential of mercury dissolution, observed upon gradual addition of antioxidants, was applied herein for the estimation of the natural products' antioxidativity. Quinic acid was found to exhibit most promising antioxidant potential (4.0±0.2 $\%\mu L^{-1}$) being ≈ 2-fold more active than the screened *C. comatus* extract samples. Actually, such a finding puts some light on the antioxidativity of cyclic polyols, well understimated class of organic compounds, compared to aromatic (poly)phenolics. As a low cost, easy-to handle and accurate this polarographic assay may be thoroughly recommended for much broader use.

Keywords: cyclic polyols, quinic acid, antioxidativity, polarography, HPMC assay

Experimental

Chemicals

While quinic acid was obtained from Sigma-Aldrich Chem (Steinheim, Germany), HPLC gradient grade methanol and p.a. formic acid were purchased from J. T. Baker (Deventer, The Netherlands) and Merck (Darmstadt, Germany), respectively. Finally, deionised water was produced using a Millipore water purification system (Elga LabWater, UK).

Biological material

C. comatus was collected in autumn 2014 (during the month of November) on the lawn, near the cultivable land in Sremski Karlovci (North Serbia). Upon determination, it was deposited as a voucher species (Voucher N° 12-00704) in the BUNS Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Novi Sad. Mycelia was isolated from the FB under sterile condition and preserved in a fungal culture collection of the Laboratory of Microbiology (FUNGICULT – Fungi Culture of Basidiomycota /FCB/ N° 0056).

Submerged cultivation

Upon the growth of mycelia for 10 days (26 °C, Malt agar, Torlak, Serbia), five plugs were transferred to 100 mL of fermentation medium (per L: 5 g peptone, 35 g glucose, 5 g yeast extract, 1 g K_2HPO_4, 0.5 g $MgSO_4 \times 7H_2O$ and 0.05 g vitamin B_1) at initial pH 6.5. After 14 days cultivation in Erlenmeyer flasks on a horizontal orbital shaker (120 rpm min^{-1}, 26 °C) (New Brunswick Scientific, Edison, USA), the cultures were filtrated (Fioroni Filter N° 114, France) while both M (biomass) and extracellular F were collected separately and lyophilised to dryness (CHRIST Bio ALPHA, LDplus, Freeze Dryer, Germany).

Preparation of extracts

In brief, the dry lyophilised biomasses of FB, M and F were measured and extracted with 250 mL of 80 % EtOH (72 h, 120 rpm min^{-1}, 25 °C). The yield was ranged from 7 (FB) to 11 (F) %. The samples were filtrated and evaporated to dryness on rotary evaporator at 50 °C (Büchi R-210, Switzerland). The residues obtained after evaporation were dissolved in 5 % DMSO, aiming to reach 100 µg mL^{-1}.

LC-MS/MS identification and quantification of quinic acid

All examined extract samples (conc. 2 mg mL^{-1}) and working standard (1.5 - 25×10^3 ng mL^{-1}) were analysed using Agilent Technologies 1200 Series HPLC coupled with Agilent Technologies 6410A QqQ ESIMS. QA was separated on Zorbax Eclipse XDB-C18 (50 mm × 4.6 mm, 1.8 µm) column (inject. vol. 5 µL; 50 °C; flow 1 mL min^{-1}) in gradient mode (0 min 30% B, 6 min 70% B, 9 min 100% B, 12 min 100% B, post time 3 min). The ion source parameters were as follows: nebulisation gas 40 psi, drying flow 9 L min^{-1}, temp. 350 °C, capillary voltage 4 kV, NI. Dynamic MRM with the optimised quinic acid-specific parameters (retention time, precursor ion, product ion, fragmentor voltage, collision voltage) was used. Peak area was determined using Agilent MassHunter Workstation software – Qualitative Analysis (ver. B.04.00.), while calibration curve was plotted in the OriginLabs Origin Pro (ver. 8.0) software (Northampton, MA, USA). Limit of detection (LoD) was estimated as the lowest concentration resulting in a well-defined peak (Orčić et al. 2014).

Determination of antioxidant activity

Direct current (DC) polarographic HydroxoPerhydroxo Mercury(II) Complex (HPMC) assay based on decrease of anodic limiting current originating from HPMC formation in alkaline solutions of hydrogen peroxide at potential of mercury dissolution, observed upon gradual addition of antioxidants, was performed, as previously described in literature (Sužnjević et al. 2012). Dependence of decrease of anodic limiting current of HPMC on volume of gradually added complex samples (extracts) or amount of individual compound (QA) was followed and plotted. The slope of the starting linear part of that plot was considered as a measure of antioxidant activity. The activity was expressed as

percentage of peak height decrease per volume of the complex samples (fungal extracts) added (% mL^{-1}) or amount of pure compound (% μmoL^{-1}).

Statistical analysis

The aforementioned polarographic assay was performed in triplicate, while the results are expressed as mean ± SD.

References

Orčić D, Francišković M, Bekvalac K, et al. 2014. Quantitative determination of plant phenolics in *Urtica dioica* extracts by high performance liquid chromatography coupled with tandem mass spectrometric detection. Food Chem. 143:48–53.

Sužnjević DŽ, Pastor FT, Gorjanović SŽ. 2011. Polarographic study of hydrogen peroxide anodic current and its application to antioxidant activity determination. Talanta 85:1398–1403.