Supporting Information to:

Plant Polyphenols against UV-C-Induced Cellular Death

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**VB Extraction and Isolation**

The parent plant cell line was used for the industrial fermentors. Cell cultures obtained at the end of the expansion process were collected, homogenized, separated by centrifugation, and the solid residue discarded. The VB in the supernatant was recovered by solid phase extraction on XAD4 resin, followed by elution with an 80/20 ethanol/water (v/v) mixture. Then, the eluted VB was concentrated under reduced pressure and lyophilized. The yield of VB was approx. 3 g/L of the plant cell culture liquid suspension. The final cell extract, a pale yellow powder, contained VB to more than 80% (w/w), together with minor admixture (> 10% w/w) of other caffeic acid derivatives including isoverbascoside. Further purification of VB was performed by repeated column chromatography on C18 silica gel and Sephadex LH20 and subsequent crystallization obtaining a final product with VB content above 97% (w/w).

**LC-DAD analysis**

LC-DAD analysis was performed using a HPLC system (Agilent, series 1100 DAD; Hewlett-Packard) consisting of an auto sampler, high pressure mixing pump, and the column C18(2) Phenomenex 4.6 x 150 mm. The gradient system was: Phase A – H2O/0.01 N H3PO4; Phase B – acetonitrile/0.01 N H3PO4; elution gradient: from 0 % to 10% B in 10 min, then from 10% to 20% B in 10 min, then from 20% to 55% B in 20 min, then to 100% A in 5 min. Flow rate 0.8 mL/min and the injection volume 25 µL. The retention time for the two major phenylpropanoids was in the range: 20.3 – 20.6 min for verbascoside and 21.3 – 21.6 min for isoverbascoside. DAD: UV maximum absorbance at 330 nm.

**Assays for superoxide scavenging activity of polyphenols**

Superoxide-mediated reduction of nitroblue tetrazolium (NBT) by photochemically reduced riboflavin was carried out according to [1] at room temperature (22°C) under fluorescent light (20 W, 20 cm).

**Assays for lipid peroxidation inhibition by polyphenols**

Rat liver microsomes (1.2-1.3 mg protein/mL) were incubated at 37° for 5 min with 0.3 mM NADPH, 20 mM NaCl and 10 µM FeSO4 in phosphate buffer (0.05M, pH 7.4). The thiobarbituric acid reactive substances (TBARS) content was determined by measuring the absorbance at 532 nm [2].

Assay for metal chelating activity of polyphenols

Chelating properties of VB, quercetin and rutin were compared using the method of competitive replacement. The formation of complexes was assessed spectrophotometrically in terms of bathochromic shifts in band I. Ethylenediaminetetraacetic acid (EDTA) was used as a competitive standard.

References


Fig. 1S Effect of prolonged UV-C irradiation (20 min, 1.5 mW/cm², dose 1.8 J/cm²) on HaCaT (A, B) and MCF 7 (C, D) cells. Representative fluorescence micrographs of control (A, C) and irradiated cells stained with acridine orange/ethidium bromide as described in Materials and Methods.
Fig. 2S Protective effect of plant polyphenols on the loss of membrane integrity of HaCaT cells that follows prolonged UV-C exposure (20 min, 1.5 mW/cm², dose 1.8 J/cm²). Representative fluorescence micrographs of the cells exposed to UV-C alone (A) or with quercetin, 25 µM (B), rutin, 100 µM (C), verbascosice, 200 µM (D). Cells were stained with acridine orange/ethidium bromide as described in Materials and Methods.
Fig. 3S Protective effect of plant polyphenols on the loss of membrane integrity of MCF 7 cells that follows prolonged UV-C exposure (20 min, 1.5 mW/cm², dose 1.8 J/cm²). Representative fluorescence micrographs of the cells exposed to UV-C alone (A) or with quercetin, 25 µM (B), rutin, 100 µM (C), verbascosice, 200 µM (D). Cells were stained with acridine orange/ethidium bromide as described in Materials and Methods.
Fig. 4S Effect of short UV-C irradiation (1 min, 1.5 mW/cm², dose 0.09 J/cm²) on HaCaT (A, B) and MCF 7 (C, D) cells. Representative fluorescence micrographs of control cells (A, C) and cells 24 h after irradiation (B, D) stained with Hoechst 33342 as described in Materials and Methods.