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Antioxidant activity of ethanolic and aqueous extracts of *Uncaria tomentosa* (Willd.) DC.

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Abstract

The antioxidant properties of aqueous and ethanolic extracts of the *Uncaria tomentosa* bark were evaluated. The analysis included trolox equivalent antioxidant capacity (TEAC), peroxyl radical-trapping capacity (PRTC), superoxide radical scavenging activity (SOD) and quantitation of total tannins (TT) and total phenolic compounds (TPC). The obtained results indicate high antioxidant capacity of the studied materials in comparison to the other extracts of fruits, vegetables, cereals and medicinal plants. Higher antioxidant activity and total phenolic compounds of the alcoholic preparations – TEAC = 0.57 mmol of Trolox/g, PRTC = 0.52 mmol of Trolox/g and SOD = 0.39 U/mg than of the aqueous preparation – TEAC = 0.34 mmol of Trolox/g, PRTC = 0.19 mmol of Trolox/g and SOD = 0.10 U/mg were observed. These results might suggest higher medical suitability of alcoholic extracts. However, the highly elevated level of tannins in alcoholic extracts may cause undesirable gastric effects.

Keywords: *Uncaria tomentosa*; Cat's claw; Antioxidant activity; Free radicals; Phenolic compounds; Tannins

1. Introduction

Uncaria tomentosa (Willd.) DC. commonly known as cat's claw or uña de gato, is indigenous to tropical areas of South and Central America liana classified into *Rubiaceae* family (Reinhard, 1999). For at least 2000 years, among many Peruvian tribes, especially Ashaninka, this species has been deeply believed to possess magical healing power and has been extensively used for the treatment of asthma, cancer, cirrhosis, fevers, gastritis, diabetes, rheumatism, dysentery, inflammation of the urinary tract and many other diseases (Keplinger et al., 1999; Falkiewicz et al., 2001; Heitzman et al., 2005). Recently, medical preparations from *Uncaria tomentosa* have become very popular in Europe and America, particularly as an anticancer remedy (De Jong et al., 1999). In majority of the latest studies, high biological activity of cat's claw is attributed to unique tetracyclic and pentacyclic oxindole alkaloids (Phillipson et al., 1978; Laus and Keplinger, 1994; Laus, 1998). However, due to wide spectrum of the uña de gato activity, synergistic participation of other chemical compounds in the healing process must be taken into account (Falkiewicz et al., 2001).

Phenolic constituents, such as flavonoids, phenolic acids, diterpenes and tannins are especially worthy of notice due to their high antioxidative activity (Shahidi et al., 1992; Rice-Evans et al., 1996). Many investigations indicate that these compounds are of great value in preventing the onset and/or progression of many human diseases (Halliwell and Gutteridge, 1989; Halliwell et al., 1992; Willet, 1994; Tsao and Akhtar, 2005). This effect has been explained by the restoration of redox equilibrium disturbed by different factors (e.g. diet, alcohol, some drugs) and, in consequence, by diminishing of damages in cellular structures (Ames et al., 1993).

Therefore, over the past few years, a number of medicinal plants have been extensively investigated for the presence and activity of polyphenols and other antioxidants (Pietta, 1998; Pietta et al., 1998; Sindambiwe et al., 1999; Halvorsen et al., 2002; Naik et al., 2003; Pegg et al., 2005). This study was designed for the evaluation of possible beneficial antioxidative potency of the *Uncaria tomentosa* extracts by employing

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different methods and techniques. The results we report comprise determination of total antioxidant status (TAS), peroxyl radical-trapping capacity (PRTC), superoxide scavenging activity (SOD) and quantitation of tannins and total phenolic compounds (TPC).

2. Materials and methods

2.1. Plant material and chemicals

The analysis was performed on the bark of the *Uncaria tomentosa* originated from Peru and supplied by A–Z Medica, Gdańsk, Poland. The voucher material is deposited at the Laboratory of Phytochemistry, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland.

The standards of oxindole alkaloids were purchased from ChromaDex (Santa Ana, USA). Ammonium hydroxide, sodium carbonate, sulfuric acid, hydrochloric acid, methanol, ethanol, ethylacetate were supported by POCh (Gliwice, Poland). Ferric chloride was supplied from FLUKA (Germany). Acetonitrile (HPLC grade) was purchased from ACROS-ORGANIC (Belgium). Bovine serum albumin (BSA, Fraction V), sodium dodecyl sulphate (SDS) and triethanolamine (TEA) were obtained from Sigma–Aldrich (Germany). Folin-Ciocalteu reagent was provided by MERCK (Germany). 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) ($ABTS^{\bullet+}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and superoxide dismutase were supplied with Randox Kits No. NX2332 and SD125 (Randox Laboratories Ltd., UK). 2,2′-Azobis(2-amidopropane) hydrochloride (ABAP) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2.2. HPLC-fingerprint analysis of alkaloids in the plant material

To 100 mg of the bark, 15 mL of 2% sulphuric acid solution was added and sonified for 15 min in an ultrasonic bath (Bandelin Sonorex RK 103H). The mixture was then centrifuged 3000 rpm for 10 min and extracted three times with 10 mL of ethylacetate. The aqueous phase was separated and adjusted to pH 10 with 10% NH_4OH and then extracted three times with 10 mL of ethylacetate each. The organic extracts were combined, evaporated to dryness and the residue dissolved in 1 mL of methanol. The qualitative and quantitative content of alkaloids was determined by the HPLC fingerprint analysis [HPLC: L-7100 Intelligent Pump (Merck-Hitachi), L-7200 Autosampler (Merck-Hitachi), L-7450 Diode Array Detector (Merck-Hitachi)]; Software: D-7000 Chromatography Data Station Software version 4.0; Column: LiChrospher® 100 RP-18 (250 mm × 4 mm, Merck); Precolumn: LiChrospher® 100 RP-18 (4 mm × 4 mm, Merck); Solvents: A—phosphate buffer solution (10 mM, pH 6.6), B—methanol: acetonitrile (1:1); Gradient: (60% A and 40% B) to (30% A and 70% B); Time: 30 min; Washing: 20% solvent A and 80% B; Temperature: 21 °C; Flow rate: 1.0 mL/min.; Detection: 245 nm] (Sheng et al., 2000; Stuppner et al., 1992).

2.3. Preparation of samples

2.3.1. Ethanol extract (EX_{ET})

An amount of 0.5 g of the bark was extracted in 5 mL of 50% ethanol for 3 h at 37 °C. Then, the extract was centrifuged (MLW K70D) for 15 min at 4000 rpm. Supernatant was evaporated on Speed-Vac and next exsiccated with P_2O_5.

2.3.2. Aqueous extract (EX_{AQ})

An amount of 0.5 g of the bark was extracted in 5 mL of 0.1 M, pH 7.4 phosphate buffer for 3 h in a tight dark glass ware. The extract was then centrifuged for 15 min at 4000 rpm. Supernatant was evaporated on Speed-Vac and exsiccated with P_2O_5.

The dry preparations (10 mg) were dissolved in 10 mL of adequate solvent (EX_{ET} in 50% ethanol; EX_{AQ} in 0.1 M of phosphate buffer).

2.4. Quantitative determination of tannins

Quantitative determination of tannins was carried out with the protein precipitation method (Hagerman and Butler, 1978). The standard protein solution (1 mg/1 mL) was prepared by dissolving bovine serum albumin (BSA) in 0.20 M of acetate buffer, pH 5.0, containing 0.17 M of sodium chloride. An amount of 2 mL of BSA (1 mg/mL) was added to 1 mL of the sample in a 5 mL glass centrifuge tube. The solutions were carefully mixed and left at room temperature for about 15 min and, then, they were centrifuged for 15 min (Sigma K 300D). The supernatant was decanted and discarded. The surface of the pellet and the walls of the tube were washed with buffer without disturbing the pellet and centrifuged once again. The precipitate was dissolved in 4 mL of the sodium dodecyl sulphate (SDS)–triethanolamine (TEA) solution (1% SDS and 5% (v/v) TEA in distilled water). An amount of 1 mL of the ferric chloride reagent (0.01 M of ferric chloride in 0.01 M of hydrochloric acid) was added and mixed immediately.

Approximately 15–30 min after the addition of the ferric chloride reagent, the absorbance at 510 nm (A_{510}) was measured on Zeiss Spectrophotometer (slit width, 0.03 mm; path length, 1.0 cm; zeroed against air).

2.5. Total phenolic compounds (TPC)

TPC in the EX_{ET} and EX_{PB} samples were determined according to the method of Shahidi and Naczk (1995). An amount of 0.25 mL aliquot of the prepared samples were mixed with 0.25 mL Folin-Ciocalteu reagent (previously diluted with water 1:1 (v/v)) and 0.5 mL of saturated sodium carbonate (Na_2CO_3) solution and 4 mL of deionized water. The mixtures were intensively shaken, left at room temperature for 25 min and centrifuged at 5000 rpm for 10 min. The supernatant absorbance was measured at 725 nm using spectrophotometer (Schimadzu UV-160 1PC). The results were expressed as D-catechin equivalents.

2.6. Trolox equivalent antioxidant capacity (TEAC)

The relative abilities of antioxidants to scavenge radical cation 2,2′-azinobis-[3-ethylbenzothiazoline-6-sulfonic acid]