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In vitro antioxidant and anti-inflammatory activities of extracts from *Potentilla recta* and its main ellagitannin, agrimoniin

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ABSTRACT

Ethnopharmacological relevance: Potentilla recta is one of the numerous cinquefoil species growing in Poland. It is used in traditional medicine e.g. in the treatment of skin inflammation.
Aim of the study: The purpose of the present study is to evaluate antioxidant and anti-inflammatory activities of extracts and subfractions of the *P. recta* herb (obtained by using solvents of different polarity) in *in vitro* systems as well as to examine their chemical composition.
Materials and methods: Antioxidant activities of the extracts, subfractions and agrimoniin were evaluated using DPPH and three other radicals ($O_2^{\bullet -}$, H_2O_2, and HClO) generated in cell-free systems. Anti-hyaluronidase activity was measured by using the turbidimetric method. Inhibition of lipoxidase activity was measured spectrophotometrically, using linoleic acid as a substrate. The composition of the most active subfraction was determined using the HPLC-DAD-MS^n method.
Results: All tested samples showed scavenging activity against all the examined reactive species in a concentration-dependent manner. The highest scavenging activity against DPPH, H_2O_2 and HClO was observed in the ethyl acetate subfraction (PRE3) (SC50 ± SEM [μg/mL]: 25.39 ± 2.49, 1.79 ± 0.25 and 8.52 ± 1.16 respectively). It was only in the xanthine/xanthine oxidase system that the antioxidation potential of the diethyl ether subfraction (PRE2) (SC50 ± SEM [μg/mL]: 6.59 ± 1.33) was higher than that of the subfraction PRE3 (SC50 ± SEM [μg/mL]: 8.57 ± 1.37). Also, in the studies of lipoxidase and hyaluronidase inhibition activity the strongest effect was observed for PRE3, with IC50 [μg/mL] = 86.31 ± 5.46, and 12.99 ± 1.31, respectively. The chromatographic method (HPTLC-DPPH) revealed that the principal substance responsible for the activity, is a tannin like compound. Isolated agrimoniin showed significant reactive oxygen species scavenging activity and significant enzyme inhibition activity (including xanthine oxidase inhibition activity). Agrimoniin exerted the strongest scavenging activity against H_2O_2 (SC50 ± SEM [μM]: 0.20 ± 0.01). This compound also significantly inhibited the enzymatic activity of lipoxidase (IC50 [μM] = 36.47 ± 1.29), and, particularly, of hyaluronidase (IC50 [μM] = 2.65 ± 0.40).
Conclusions: The strong scavenging activity against H_2O_2, and the inhibition of the enzymatic activity of lipoxidase, and particularly, hyaluronidase observed for the tested subfractions and agrimoniin, partly explain the beneficial effects of *P. recta* in treatment of skin inflammation.

Abbreviations: ADC2, automatic developing chamber; ASA, ascorbic acid; DPPH, 2-diphenyl-1-picrylhydrazyl radical; DTNB, 5, 5-dithiobis-(2-nitrobenzoic acid); ESI, electrospray ionization; $FeCl_3$, ferric chloride (III); H_2O_2, hydrogen peroxide; HClO, hypochlorous acid; HHDP, hexahydroxydiphenyl unit; HPLC-DAD-MS, high-performance liquid chromatography with diode array detector and mass spectrometer; HPR, heparin; HPTLC, high-performance thin layer chromatography; HRP, horseradish peroxidase; HYAL, hyaluronidase; IC50, half maximal inhibitory concentration; LA, linoleic acid; LOX, lipoxidase; LTB4, leukotriene B4; $NaBH_4$, sodium borohydride; NaClO, sodium hypochlorite; NBT, nitrobluetetrazolium; NMR, nuclear magnetic resonance; $O_2^{\bullet}-$, superoxide anion; PBS, phosphate-buffered saline; PRE, aqueous extract; PRE1, hydroethanolic extract; PRE2, diethyl ether subfraction; PRE3, ethyl acetate subfraction; PRE4, n-butanolic subfraction; Q, quercetin; ROS, reactive oxygen species; SC50, half maximal scavenging concentration; SEM, standard error of measurement; TNB, 5-thio-2-nitrobenzoic acid; UV-Vis, ultraviolet – visible light

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1. Introduction

Ethnic medicine has come to be an irreplaceable source of knowledge of medicinal plants and their curative qualities. These plants have also provided clues for future scientific investigations, which usually confirm the legitimacy of their usage (Fabricant and Farnsworth, 2001; Tomczyk and Latté, 2009). In Europe, herbal preparations obtained from *Potentilla* sp. are used in a variety of ways. They can be taken orally to treat inflammations of the gastrointestinal tract, such as diarrhea. Topically, these herbal drugs can be used to treat skin inflammation and to act as wound healing agents (Tomczyk and Latté, 2009). Sulfur cinquefoil *Potentilla recta* L. (Rosaceae) is a long-lived invasive perennial plant from Eurasia that has become one of the most serious invaders of natural area grasslands in North America and Canada. In traditional medicine *P. recta* is used as an astringent, styptic, stomachic, anti-inflammatory, cleansing, and antipyretic and tonic agent (Tosun et al., 2006; Popović et al., 2012). American herbalist Matthew Wood (2008), who has systematically studied ancient and traditional herbal literature, and applies the acquired knowledge in his practice, presents some information about *P. recta* as an analgesic and injury remedy. Polish naturalist and entomologist Krzysztof Kluk (1739–1796) also recommended sulfur cinquefoil as a medicinal plant in his work "Dictionary of Plants" (Kluk, 1985). Modern pharmacological studies of the plant have so far focused on antimicrobial activities, as well as the inhibitory action on human aldose reductase, and hematological activity, including not only antiplatelet aggregating or anti-coagulating activities but also anticariogenic properties and androgenous action (Komar et al., 1981; Enomoto et al., 2004; Tosun et al., 2006; Tomczyk et al., 2008, 2011). *P. recta* has been reported to contain 2-pyrone-4,6-dicarboxylic acid (Wilkes and Glasl, 2001). More recently, some polyphenolic compounds including a neolignan glycoside and flavonol derivatives: tiliroside, apigenin 7-O-β-glucoside, and luteolin 7-O-β-glucoside have been isolated from the aerial parts of *P. recta* (Şöhretoğlu and Kırmızıbekmez, 2011; Tomczyk, 2011).

Taking into consideration the traditional claims and reported activities for *Potentilla* species, the present study was planned to investigate the antioxidant and anti-inflammatory properties of *P. recta*. The antioxidant activity was evaluated by the examining scavenging of synthetic radical ($DPPH^{\bullet}$) and three radicals ($O_2^{\bullet -}$, H_2O_2, and HClO) generated in cell-free systems. Reactive oxygen species (ROS) fall easily and unpredictably in reactions with compounds inside the cells – lipids, proteins as well as nucleic acids – which results in their oxidation, rearrangement, decomposition and formation of harmful metabolites leading to disorders of their biological activity. It should be noted that they also cause degradation of high-molecular-weight hyaluronan, an anti-inflammatory extracellular matrix component (Stern et al., 2006). Since other factors responsible for hyaluronan degradation, are hyaluronidases, to examine the anti-inflammatory activity of the extracts and subfractions, inhibition of hyaluronidase (HYAL) activity was tested. Other enzymes involved in the inflammatory process are lipoxidases (LOX). These enzymes are involved in the formation of pro-inflammatory eicosanoids (LTB4) from fatty acids. Therefore, the inhibition of lipoxidase activity was also determined. In the second part of the study, composition of the most active extract/subfraction was carried out using the HPLC-MS^n method, and an indication of compound/s responsible for the demonstrated antioxidant and anti-inflammatory action was made.

2. Materials and methods

2.1. Chemicals

Bovine testis hyaluronidase (BTH), 2-diphenyl-1-picrylhydrazyl radical (DPPH), 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB), horseradish peroxidase (HRP), hydrogen peroxide (H_2O_2), linoleic acid (LA), lipoxidase from glycine max (LOX), luminol, nitrobluetetrazolium (NBT), xanthine, and xanthine oxidase were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). $NaBH_4$ was purchased from Matheson Coleman & Bell (Ohio, USA). Ethanol 96° p.a., ferric chloride (III) ($FeCl_3$) and sodium hypochlorite (NaClO) were purchased from POCH (Gliwice, Poland). Hyaluronic acid was purchased from Fluka (Steinheim, Germany). Heparin natricum was purchased from WZF Polfa S.A. Warsaw (Warsaw, Poland).

Standards for HPLC and HPTLC analyses were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), Serva Feinbiochemica (Heidelberg, Germany) and Carl Roth GmbH (Karlsruhe, Germany). Apigenin 7-glucoside, luteolin 7-glucoside and ellagic acid 3,3′-di-O-methyl ether 4-xyloside (purity > 96%) were isolated from aerial parts of *P. recta* (Tomczyk, 2011). Quercetin 3-glucuronide (purity > 96%) was isolated from willow herb (*Epilobium angustifolium* L.) and was obtained from Dr. A.K. Kiss (Department of Pharmacognosy and Molecular Basis of Phytotherapy, Medical University of Warsaw) (Kiss et al., 2004).

Phosphate-buffered saline (PBS) was purchased from Biomed (Lublin, Poland). Solvents used for the HPLC analysis were HPLC grade; solvents for HPTLC and CC were analytical grade.

2.2. Plant material and extracts/subfractions preparation

2.2.1. Plant material

Aerial parts of *P. recta* were collected during June–July 2005–2009 from the Medicinal Plants Garden of the Medical University of Białystok, Poland. Collected plant materials were air-dried under shade at room temperature and then ground with an electric grinder into fine powders which were stored in airtight containers at room temperature.

2.2.2. Preparation of extracts and subfractions

The extracts and subfractions were prepared as described previously (Tomczyk et al., 2011).

2.2.2.1. Preparation of extracts. Powdered plant material (2.0 g) was separately extracted with water (2 × 150 mL) – PRE or 50% ethanol (2 × 150 mL) – PRE1 in an ultrasonicator bath (Sonic-5, POLSONIC, Poland) at controlled temperature (40 ± 2 °C) for time 45 min. Supernatants were filtered through a funnel with glass wool, which was washed with 5 mL of solvent and concentrated to dryness under vacuum (Büchi System, Switzerland) at controlled temperature (40 ± 2 °C) and subjected to lyophilization using LABCONCO vacuum concentrator until a constant weight was obtained.

2.2.2.2. Preparation of subfractions. Accurately weighed 2.0 g quantities of plant material were separately extracted with methanol (3 × 50 mL) and once with 50 mL of 80% (v/v) methanol in an ultrasonicator bath (Sonic-5, POLSONIC, Poland) at controlled temperature (40 ± 2 °C) for time 45 min. After solvent evaporation under reduced pressure from each sample, the methanolic extracts were diluted with water and successively partitioned between chloroform, diethyl ether (PRE2), ethyl acetate (PRE3) and n-butanol (PRE4). All these extracts were concentrated to dryness under vacuum controlled temperature (Büchi System, Switzerland) (temperature: 40 ± 2 °C) and subjected to lyophilization using LABCONCO vacuum concentrator until a constant weight were obtained.

2.3. Isolation of agrimoniin

The ethyl acetate subfraction (PRE3) (520 mg) was subjected to Diaion HP-20 (Supelco, Bellefonte, PA, USA) column and eluted