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Phytochemical composition and *in vitro* pharmacological activity of two rose hip (*Rosa canina* L.) preparations

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Abstract

The aim of the present study was to compare powdered rose hip with and without fruits (Rosae pseudofructus cum/sine fructibus, *Rosa canina* L., Rosaceae) with regard to their phytochemical profile and their *in vitro* anti-inflammatory and radical-scavenging properties. The two powders were subsequently extracted with solvents of increasing polarity and tested for inhibition of cyclooxygenase (COX-1, COX-2) and of 5-LOX-mediated leukotriene B_4 (LTB_4) formation as well as for DPPH-radical-scavenging capacity. While the water and methanol extracts were inactive in the COX-1, COX-2 and LTB_4 inhibition assays, the *n*-hexane and the dichloromethane extracts inhibited all three enzymes. In the active extracts, the triterpenoic acids ursolic acid, oleanolic acid and betulinic acid were identified, although only in minute amounts. Furthermore, oleic, linoleic and α-linolenic acid were identified apart from several saturated fatty acids. Even though unsaturated fatty acids are known to be good inhibitors of COX-1, COX-2 and LT formation, no clear correlation between their concentration in the extracts and their activity was found. We suggest that other, yet unidentified, lipophilic constituents might play a more important role for the observed *in vitro* inhibitory activity on arachidonic acid metabolism. Some of the extracts also showed considerable DPPH radical scavenging activity, the methanolic extracts being most potent. The radical scavenging activity of the extracts correlated very well with their total phenolic content, while ascorbic acid contributes only little to the radical-scavenging activity due to its low concentration present in the extracts.

In summary, extracts derived from powdered rose hip without fruits were more effective in all assays carried out compared with extracts derived from powdered rose hip with fruits.

Keywords: *Rosa canina* L.; Rose hip; Rosae pseudofructus; Cyclooxygenase; Lipoxygenase; Anti-inflammatory; Radical-scavenging; DPPH; Triterpenoic acid

Introduction

Rose hip, the pseudo-fruit of *Rosa canina* L. (Rosaceae), consists of an urn-shaped receptacle with numerous achenes inside. Fresh rose hip is rich in vitamin C and is widely used for food production. Both fresh and dried rose

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hips are frequently used as an ingredient of fruit and herbal teas. Concerning the use as a herbal drug, the plant part officinal in the European pharmacopoeia is the rose hip without fruits (Ph. Eur., 2005).

Rose hip with or without fruits (Rosae pseudofructus cum/sine fructibus) is traditionally used for the prevention and therapy of common cold and other infections, as diuretic agent, for the treatment of various inflammatory diseases and as a vitamin C source. So far, for none of these indications clinical effectiveness has been demonstrated except for osteoarthritis (Blumenthal et al., 1998; Chrubasik et al., 2006). A rose hip and fruit powder, which is marketed as a food supplement in several European countries, has been shown to reduce osteoarthritis symptoms in clinical trials (Warholm et al., 2003; Rein et al., 2004). An anti-inflammatory mode of action has been suggested to explain the observed effects. This suggestion is corroborated by experiments in mice (Deliorman Orhan et al., 2007) and by *in vitro* data. A rose hip and fruit powder as well as a galactolipid isolated from this material have been shown *in vitro* to reduce chemotaxis of peripheral blood polymorphonuclear leucocytes and monocytes. These cells are involved in inflammatory processes and play a role for tissue damage in inflammatory diseases (Kharazmi and Winther, 1999; Larsen et al., 2003). A recent study also revealed an inhibitory effect of rose hip with fruit extracts on cyclooxygenase (COX)-1 and -2 *in vitro* (Jäger et al., 2007), and unsaturated fatty acids were suggested as active principle (Jäger et al., 2008).

Apart from the anti-inflammatory activity, an antioxidant mode of action might contribute to the observed clinical effects of rose hip preparations. Daels-Rakotoarison et al. (2002) found that a rose hip acetone/water extract was able to scavenge reactive oxygen species. These radicals can lead to cell and tissue injury by reaction with biological materials, in cellular and acellular test systems.

The aim of this study was to compare two rose hip powders – one prepared from rose hip with fruits, and the other prepared from rose hip without fruits – with regard to their impact on arachidonic acid metabolism, their radical-scavenging potential and their phytochemical profiles.

Materials and methods

General experimental procedures

GC–MS measurements were performed on a HP 6890 GC–MS system (Agilent Technologies, Waldbronn, Germany) equipped with a J&W Scientific DB 225 column (30 m, i.d. 0.25 mm, film 0.25 μm; Agilent Technologies). Helium (0.8 ml/min) was used as a carrier gas, injector and detector temperatures were 220 °C, and the following temperature programme was used: 0–0.5 min 40 °C; 40–195 °C (25 °C/min); 195–202 °C (0.8 °C/min). Analytical HPLC measurements were performed on an Agilent 1100 series HPLC system (Agilent Technologies). LC–MS experiments were performed on a Thermo Finnigan Surveyor liquid chromatograph interfaced with a LCQ™ Deca XP^{PLUS} mass detector. NMR spectra were recorded with a Varian® UnityInova (600 MHz) spectrometer using the parameters described by Seebacher et al. (2003). Pyridine-d_5 was used as solvent and TMS as an internal standard; the experimental temperature was 40 °C.

Plant material and extraction

Two different starting materials were used for extract preparation: Rose hip fine powder, batch 119372 (Supplier Martin Bauer GmbH & Co Kg, Germany), containing rose hip without fruits (Rosae pseudofructus sine fructibus) for the RSF extracts and LitoZin®, batch 5141081 (Green medicine AB, Malmö, kindly provided by Nycomed, Switzerland), containing rose hip and fruits, for the RCF extracts, respectively. Litozin® is marketed as a food supplement in several European countries. Voucher specimens of the used materials are deposited at the herbarium of the Department of Pharmacognosy in Graz (RSF2007/1; RCF2007/1).

Both materials were subsequently soxhlet extracted with *n*-hexane, dichloromethane (DCM) and methanol. After methanol extraction the dried material was mixed with 10 fold amount of boiling water and stirred at room temperature for 1 h. After filtration, this step was repeated and the combined filtrates were concentrated and lyophilised. The organic extracts were concentrated under reduced pressure, and remaining solvent was removed under N_2. The following extract yields were obtained: RSF *n*-hexane 0.9%, DCM 0.7%, methanol 21.0%, water 21.0%; RCF *n*-hexane 5.1%, DCM 0.5%, methanol 24.0%, water 11.2%.

In vitro assays for COX-1, COX-2 and LT formation inhibitory activity

COX-1 and COX-2 inhibition assays were performed in a 96-well-plate format with purified prostaglandin H synthase (PGHS)-1 from ram seminal vesicles for COX-1 and purified PGHS-2 from sheep placental cotyledons for COX-2 (both Cayman Chemical Company, Ann Arbor, USA) as previously described (Fiebich et al., 2005; Reininger and Bauer, 2006). The concentration of PGE_2, the main arachidonic acid metabolite in this reaction, was determined by a competitive PGE_2 EIA kit (Assay Designs Inc., Ann Arbor, MI, USA). Indomethacin (ICN, Aurora, USA; IC_{50} COX-1 0.9 μM) and NS-398 (Cayman