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Phytochemical composition and *in vitro* functional properties of three wild rose hips and their traditional preserves

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ABSTRACT

The aim of the present study was investigation of the phenolic profile, ascorbic acid content, antioxidant, anti-acetylcholinesterase, anti-inflammatory and cytotoxic activity of rose hips and the preserves (purée and jam) of three insufficiently examined *Rosa* species: *Rosa dumalis* Bechst., *R. dumetorum* Thuill. and *R. sempervirens* L. The liquid chromatography–tandem mass spectrometry analysis resulted in quantification of 14 of the 45 phenolic compounds examined, with ellagic acid as the most dominant. Notable antioxidant activity of all three species was confirmed through several assays. Moderate inhibition of acetylcholinesterase by extracts of all investigated *Rosa* species was observed. Several extracts of examined *Rosa* species demonstrated inhibition potency towards production of some monitored eicosanoids in cyclooxygenase-1 and 12-lipoxygenase pathways. Two *R. sempervirens* extracts exerted cytotoxic activity against HeLa and HT-29 cell lines, but were inactive towards MRC-5 and MCF7. The results support the potential of these rose hips as food with health-promoting properties.

1. Introduction

Rose hips, the pseudo-fruit of *Rosa* species (genus *Rosa* L., family Rosaceae) have a long history of utilization in diet and medicine (Nybom & Werlemark, 2017). This wild fruit has been traditionally used fresh or dried for the production of tasty tea, juices, jams, jelly, marmalades, bakery products and national dishes, as well as providing the ingredients of probiotic drinks, candies and beverages (Nybom & Werlemark, 2017). Furthermore, several rose hips extracts have been commercialised as dietary supplements and cosmetics (Nybom & Werlemark, 2017). Rose hips are a well-known herbal remedy due to their healing properties in cold, flu, gastrointestinal, kidney and lower urinary tract disorders, diabetes, arthritis, lung ailments and for enhancing immunity (Deliorman Orhan, Hartevioğlu, Küpeli, & Yesilada, 2007; Nybom & Werlemark, 2017). Moreover, the beneficial effects of rose hips and preparations based on rose hip powder in osteoarthritis have been confirmed in various *in vivo* studies (Nybom & Werlemark, 2017; Patel, 2017; Schwager, Hoeller, Wolfram, & Richard, 2011). It was suggested that this effect involves anti-inflammatory and antioxidant mechanisms of action, already known for *R. canina* hips (Barros, Carvalho, Morais, & Ferreira, 2010; Deliorman Orhan et al., 2007; Demir, Yildiz, Alpaslan, & Hayaloglu, 2014; Gao, Björk, Trajkovski, & Uggla, 2000; Guimarães et al., 2014; Jäger, Eldeen, & van Staden, 2007; Lattanzio et al., 2011; Nađpal et al., 2016; Wenzig et al., 2008). Pharmacological studies revealed that rose hips of the most recognized *Rosa* species, *R. canina*, also possess anti-ulcerogenic, antinociceptive, anti-obesity, anti-diabetic and anti-proliferative activity (Cunja, Mikulic-Petkovsek, Zupan, Stampar, & Schmitzer, 2015; Guimarães et al., 2014; Lattanzio et al., 2011; Nybom & Werlemark, 2017). This wide range of biological activities could be a consequence of their rich phytochemical composition, which includes not only a high content of ascorbic acid, but also

Abbreviations: 12-HETE, 12(*S*)-hydroxy-(5*Z*,8*Z*,10*E*,14*Z*)-eicosatetraenoic acid; 12-HHT, 12(*S*)-hydroxy-(5*Z*,8*E*,10*E*)-heptadecatrienoic acid; 12-LOX, 12-lipoxygenase; AAC, ascorbic acid content; AAE, ascorbic acid equivalents; AChE, acetylcholinesterase; Ame, amentoflavone; BHT, butylated hydroxytoluene; Cat, catechin; COX-1, cyclooxygenase-1; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; dw, dry weight; EA, ellagic acid; Ecat, epicatechin; FA, ferulic acid, FRAP, reducing power assay; GA, gallic, acid; GAE, gallic acid equivalents; HeLa, cervix epitheloid carcinoma cell line; HT-29, colon adenocarinoma cell line; Hyp, hyperoside; J, jam extract; KTOG, kaempferol–3–*O*–glucoside; loq, limit of quantification; LP, lipid peroxidation; MCF7, breast adenocarcinoma cell line; MD, methanol extract of air-dried rose hips; MDA, malondialdehyde; MF, methanol extract of fresh rose hips; MRC-5, human fetal lung cell line; P, purée extract; PA, protocatechuic acid; pCoA, *p*–coumaric acid; phBA, *p*–hydroxybenzoic acid; PG, propyl gallate; PGB_2, prostaglandin B_2; PGE_2, prostaglandin E_2; QE, quercetin equivalents; Que, quercitrin; QTOG, quercetin–3–*O*–glucoside; Rd, *Rosa dumalis*; Rde, *R. dumetorum*; Rs, *R. sempervirens*; Sec, secoisolariciresinol; SRB, sulforhodamine B; TFC, total flavonoid content; TPC, total phenolic content; TXB_2, thromboxane B_2; WD, water extract of air-dried rose hips; WF, water extract of fresh rose hips

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other bioactive compounds and nutrients such as phenolics, carotenoids, tocopherols, terpenes, galactolipids, fatty acids, organic acids, sugars, proteins and minerals (Barros et al., 2010; Cunja et al., 2016; Demir et al., 2014; Ercisli, 2007; Nowak, 2006a).

Rose hips of *Rosa dumalis* and *R. dumetorum* are indigenous to Europe and commonly applied in food manufacturing (Barros et al., 2010; Demir et al., 2014; Ercisli, 2007). A limited number of studies showed that *R. dumalis* rose hips have a considerable content of phenolics, fatty acids, triterpenoic acids, carotenoids, vitamins C and E (Andersson, Rumpunen, Johansson, & Olsson, 2011; Adamczak, Buchwald, Zieliński, & Mielcarek, 2012; Bhave, Schulzova, Chmelarova, Mrnka, & Hajslova, 2017; Demir et al., 2014; Elmastaş, Demir, Genç, Dölek, & Güneş, 2017; Ercisli, 2007; Nowak, 2006a, 2005), as well as notable antioxidant potential (Bhave et al., 2017; Demir et al., 2014; Gao et al., 2000). However, there are only a few reports on the content of phenolics and fatty acids in *R. dumetorum* rose hips (Nowak, 2006a, 2006b, 2005), whereas their biological activities are completely unknown. On the other hand, to the best of our knowledge, there are no previous studies of the chemical composition and biological potential of the Mediterranean *R. sempervirens* rose hips.

Growing interest in the utilisation of rose hips in the pharmaceutical and food industries requires detailed characterisation of their chemical composition and biological activities, as it was suggested that content of bioactive compounds differ significantly between *Rosa* species (Bhave et al., 2017; Cunja et al., 2016; Ercisli, 2007; Nowak, 2006a; Patel, 2017). Also, Nybom and Werlemark (2017) emphasized that for the field production and breeding of rose hips for medical and food properties it is crucial to acknowledge, collect and analyse potentially valuable wild species. Therefore, the present study investigated and compared the content of 45 of the most common plant phenolics, together with the total phenolics, flavonoid and ascorbic acid contents in wild rose hips of two insufficiently investigated (*R. dumalis* Bechst. and *R. dumetorum* Thuill., section *Caninae*) and one unexamined *Rosa* species (*R. sempervirens* L., section *Synstylae*). In order properly to evaluate the potential of these rose hips for use in the development of rose-hip based health-promoting food products, antioxidant activity using six *in vitro* assays, as well as anti-acetylcholinesterase (AChE), anti-inflammatory and cytotoxic activities of these three rose hips were examined and compared for the first time. With the common type of rose hip food and pharmaceutical products in mind, the study included not only water and methanol extracts of fresh and air-dried rose hips, but also preserves such as purée and jam, prepared according to traditional recipes.

2. Material and methods

2.1. Chemicals and reagents

All standards of phenolic compounds were purchased from Sigma–Aldrich Chem (Steinheim, Germany), Fluka Chemie GmbH (Buchs, Switzerland) or from ChromaDex (Santa Ana, USA). All other reagents used in this study were of analytical grade.

2.2. Plant material and extract preparation

Plant material of *Rosa dumalis* Bechst. 1842 was collected in October 2012, on the Fruška gora hills, while *R. dumetorum* Thuill. rose hips were collected in November 2013, in the Deliblato Sands area (Republic of Serbia). Plant material of *R. sempervirens* L. 1753 was collected in May 2014, in Čanj (Montenegro). The specimen vouchers (*R. dumalis*, No. 2-1586; *R. dumetorum,* No. 2-1594; *R. sempervirens,* No. 2-1578) were prepared and identified by Goran Anačkov, PhD, and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), University of Novi Sad, Faculty of Sciences, Republic of Serbia.

Six types of extracts of each *Rosa* species were prepared according to previously published procedure (Nađpal et al., 2016): water extracts of fresh (WF) and air-dried (WD) rose hips with seeds, methanol extracts of fresh (MF) and air-dried (MD) rose hips with seeds, and extracts of traditionally prepared preserves, purée (P) and jam (J). Briefly, for the preparation of water and methanol extracts, chopped fresh or air-dried rose hips with seeds of each species (weighing 30 g) were extracted by maceration either with boiling water (for 1 h) or methanol/water (4:1; for 3 days) constantly shaken at 120 rpm/min at room temperature. The plant material was removed by filtration, water or solvent were evaporated to dryness under reduced pressure at 40 °C. Removal of non-polar compounds was performed by extraction with petroleum ether (fraction 40–60 °C), after dissolving crude residue in distilled water. Extracts were evaporated to dryness under reduced pressure and re-constituted in distilled water to a final concentration of 300 mg/mL. The following extract yields were obtained: 20.48%, 20.27%, 30.85% and 17.85% for WF, MF, WD and MD extracts of *R. dumalis* hips respectively, or 13.91%, 12.39%, 32.16% and 23.98% for WF, MF, WD and MD extracts of *R. dumetorum* hips respectively, and 17.72%, 17.55%, 32.92% and 20.62% for WF, MF, WD and MD extracts of *R. sempervirens* hips respectively.

Purée and jam were prepared consistent with Serbian traditional recipes previously described by Nađpal et al. (2016). For preparation of extracts of purée and jam, aliquots of purée and jam (40 mL and 10 g respectively) were concentrated to dryness under reduced pressure at 40 °C. The removal of insoluble particles was performed by filtration, after dissolving crude residue in distilled water. Extracts of purée and jam were evaporated to dryness under reduced pressure and reconstituted in distilled water to a final concentration of 200 mg/mL and 300 mg/mL respectively. The following extract yields were obtained: 22.17% and 47.39% for purée and jam of *Rosa dumalis* respectively, or 9.23% and 41.46% for purée and jam of *R. dumetorum,* respectively and 14.13% and 32.51% for purée and jam of *R. sempervirens* respectively.

2.3. LC–MS/MS analysis of the selected phenolics

Quantification of 44 phenolics in extracts of *Rosa dumalis, R. dumetorum* and *R. sempervirens* rose hips was carried out in conditions described by Orčić et al. (2014), using Agilent Technologies 1200 Series HPLC coupled with Agilent Technologies 6410A Triple Quad tandem mass spectrometer with electrospray ion source and controlled by Agilent Technologies MassHunter Workstation software-Data Acquisition (ver. B.03.01).

Additionally, LC–MS/MS analysis of ellagic acid content in the extracts was performed according to previously published procedure, quantification was done in negative ionization multiple reactions monitoring (MRM) mode (fragmentor 152 V, precursor ion m/z = 301, collision energy 0 V, product ion m/z = 301, retention time 2.23 min; Šibul et al., 2016). For all compounds, peak areas were determined using Agilent Technologies MassHunter Workstation software-Qualitative Analysis (ver. B.03.01). Calibration curves were plotted and concentrations of phenolic compounds in extracts were calculated using the OriginLabs Origin Pro (ver. 8.0). Content of phenolic compounds were expressed as μg/g of dry weight (dw).

2.4. Determination of total phenolic and flavonoid content

Determination of total phenolic content was done by using Folin-Ciocalteau reagent (using three extract concentrations in a range of 0.125–0.5 mg/mL, three replicates per concentration) as described by Beara et al. (2014). Gallic acid was used as standard and total phenol content was expressed as mg of gallic acid equivalents (GAE) per g of dw. Quantitative determination of flavonoid content in rose hip extracts was conducted according to the aluminum chloride method (using three extract concentrations in a range of 20.0–80.0 mg/mL, three replicates per concentration). Total flavonoid content was expressed as mg of quercetin equivalents (QE) per g of dw, calculated according to