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Assessment of rosehips based on the content of their biologically active compounds

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ABSTRACT

In this study, an in-depth analysis of the unique set of rosehip samples from 71 *Rosa* genotypes was conducted with the aim to identify the most suitable ones for applications in the food and pharmaceutical industries based on the content of biologically active compounds. In the first part of our experiments, the antioxidant activity was determined by 2,2-diphenyl-1-picrylhydrazyl assay and the genotypes with the highest values were selected for the follow-up analysis. In the second part of experiments, the major classes of biologically active compounds in rosehips such as carotenoids, tocopherols, flavonoids, and triterpenoic acids were further quantified using liquid chromatography-based techniques. Large variation was observed among all the analyzed compounds with intraspecific variation often hiding interspecific or intersectional differences. The compounds studied herein thus do not provide a sharp tool for chemotaxonomic resolution of the genus *Rosa*. High intraspecific variation indicates the necessity to screen and utilize individual rose genotypes rather than representatives of the species when searching for sources of biologically active compounds. In the final stage of the study, 10 genotypes were selected for further cultivation and use, based on the highest concentrations of the analyzed biologically active compounds.

1. Introduction

Consumers' growing interest in herbal food supplements and nutraceuticals has accelerated the search for raw materials rich in biologically active compounds. Rosehips are the aggregate fruits of shrubs belonging to the *Rosa* genus of the Rosaceae family that are widely used by both food and pharmaceutical industries. The genus comprises nearly 200 species with complex taxonomy [1,2]. Roses are widespread in temperate to subtropical habitats of Europe, Asia, Middle East, and North America [3,4]. Rosehips are found in varied sizes and colors from yellow-orange to dark red and sometimes even black, depending on the pattern of pigments such as carotenoids, flavonoids, or anthocyanins.

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Rosehips contain a large range of important dietary antioxidants. The high antioxidant activity is mainly attributed to ascorbic acid that typically ranges from 3 g/kg to 40 g/kg [5], which is fairly more than any other commonly available fruits or vegetables [6]. Apart from ascorbic acid, carotenoids represent mainly lycopene, β-carotene, and only traces of lutein and zeaxanthin [7]. Tocopherols detected in rosehips include α- and γ-tocopherols [8,9], and polyphenolic compounds include flavonoids and proanthocynidins [10,11]. In rosehips, flavonoids comprise glycoside derivatives of quercetin, including quercitrin (quercetin-3-O-rhamnoside), isoquercitrin (quercetin-3-O-glucoside), and hyperoside (quercetin-3-O-galactoside) [10,12–14], and some aglycones, including catechin, quercetin, taxifolin, and eriodictyol [10]. Triterpenoic acids present in rosehips are primarily known for their immunomodulatory properties [15]. Ursolic and oleanolic acids have shown hepatoprotective, anti-inflammatory, antitumor, and antihyperlipidemic effects in *in vitro* and *in vivo* experiments [15–18], while betulinic acid is well known for other biological activities such as inhibition of human immunodeficiency virus and antibacterial, antimalarial, anti-inflammatory, anthelmintic, and antioxidant properties [15,19]. Other significant groups of biologically active compounds found in rosehips are galactolipids with their anti-inflammatory, antioxidant [20], antiviral, and antitumor activities [21]. Unsaturated fatty acids found in rosehip seeds, mainly linoleic and α-linolenic acids, have been considered responsible for the inhibitory effects on cyclooxygenase 1 and 2 in *in vitro* experiments [22].

Rosehips being rich sources of biologically active compounds, analytical studies were conducted to explore the health-promoting compounds, which focused mainly on samples from a particular region or were restricted to a particular variety/species with limited chemical analysis [4,23–26]. The increasing importance of rosehips as food supplements triggered the need to analyze and find the best species/genotype for the future. The novelty of this study was to critically assess the unique set of 71 rose genotypes that were all grown in the same conditions (to erase the environmental effects), and observe the influence of various genotypes and sections with regard to the content of health-promoting compounds occurring in rosehips. Previously, no study was carried out in such detail to compare and analysis the biologically active compounds occurring in them. For this, the total antioxidant activity was determined, followed by an analysis of selected biologically active compounds using liquid chromatography (LC)-based techniques. In the end, best rose genotypes were selected based on the highest content of biologically active compounds for future agricultural purposes and later use in commercial applications of rosehips.

2. Methods

2.1. Chemicals and reagents

Methanol [high-performance liquid chromatography (HPLC) grade], n-hexane (chromatography grade), and ethanol (≥ 99.5%) were purchased from Merck (Darmstadt, Germany). Formic acid (~98%), ethyl acetate, and Pestanal were purchased from Fluka Analytical (Steinheim, Germany). Deionized water was prepared with a Milli-Q purification system from Millipore (Eschborn, Germany). All the other chemicals such as ammonium formate (≥ 99.0%), acetonitrile, Chromasolv (HPLC grade, ≥ 99.9%), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and *tert*-butyl-hydroxytoluene (*t*-BHT) were purchased from Sigma-Aldrich (Steinheim, Germany). Analytical standards used as references, including L-ascorbic acid (≥ 99%), β-carotene (≥ 97%), lycopene (≥ 90%), α-tocopherol (≥ 95.5%), γ-tocopherol (≥ 95.5%), δ-tocopherol (≥ 90%), quercetin (≥ 95%), catechin (≥ 98%), rutin (≥ 95%), betulinic acid (≥ 98%), oleanolic acid (≥ 97%), and ursolic acid (≥ 90%), were also purchased from Sigma-Aldrich.

2.2. Sample material

Rosehips belonging to 71 different genotypes (both pure species and hybrids), coming from the rose collection of the Institute of Botany, Academy of Sciences of the Czech Republic (49°59′34.994″N, 14°34′8.266″E, Průhonice, Czech Republic), were used for the analysis. The selected genotypes belonged to seven rose sections: Bracteatae, Caninae, Carolinae, Cinnamomeae, Pimpinellifoliae, Rosa, and Synstylae (Table 1). If not stated otherwise, nomenclature and section affiliation followed that of Bruneau et al [1], and Wissemann and Ritz [2]. The ripened rosehips were harvested in the beginning of October 2012 before the drop of the minimum temperature below 0°C. Fruits were dried to a constant weight at 35°C (7–10 days) and then stored at room temperature prior to analysis. Prior to extraction, samples were deseeded manually by breaking the hips and further crushed in a mortar and pestle to a fine size.

A unique set of 71 rosehip samples from different genotypes was organized systematically for the analysis of their biologically active compounds. Primarily, antioxidant activity (DPPH assay) was determined in all samples.

2.3. DPPH radical scavenging activity

Each sample was extracted by shaking 0.5 g crushed rosehip shell with 40 mL deionized water (Milli-Q purification system; Millipore) for 1 hour on a rotary shaker. For the determination of the antioxidant activity, 2 mL of methanolic solution containing DPPH (2,2-diphenyl-1-picrylhydrazyl) radicals (5.2 mg/L) was added to 1 mL of the prepared extracts, reference standards, and control samples. A linear calibration plot was plotted using freshly prepared aqueous solution of L-ascorbic acid, with concentration ranging from 1.08 mg/L to 6.45 mg/L, and deionized water was used as a blank. The reaction mixture was kept in dark for 60 minutes before the measurement, and the reduction of DPPH free radicals was measured at 517 nm wavelength using the Spectrophotometer Cary 100 Bio (Agilent Technologies, Palo Alto, CA, USA). Linear equations from reference standards were used to calculate the concentration of antioxidants, expressed as ascorbic acid equivalents. The method was validated using linear equation, and the relative standard deviation (RSD) was obtained to be 3.6%.