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Assessment of phenolic compound accumulation in two widespread goldenrods

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ABSTRACT

This research investigated the accumulation of phenolic compounds in two alien goldenrods, *Solidago canadensis* and *S. gigantea*, to assess their inter- and intra-specific chemical diversity. Five compounds of pharmacological interest, chlorogenic acid, rutin, hyperoside, quercitrin, and isoquercitrin, were detected in metanolic extracts from the leaves and inflorescences of goldenrods and were quantified using the HPLC-PAD method. Differences in the compound accumulations between the species, plant parts and accessions were tested using multivariate statistical analyses, including HCA and PCA. *S. canadensis* and *S. gigantea* plants had highly different chemical compositions. *S. gigantea* had significantly higher accumulations of all investigated compounds except for rutin; the rutin content was much greater in *S. canadensis*. The leaves of both species had greater accumulations of chlorogenic acid and quercitrin, whereas the inflorescences of *S. gigantea* had the greatest accumulations of rutin, isoquercitrin and hyperoside. The statistical analysis indicated that the intraspecific chemical diversity of goldenrods is relatively weak, resulting in wild populations producing a fairly homogenous raw material. Invasive goldenrods are negatively valued due to the threat they pose to the local vegetation; the results of our study indicate that they can be of value as potential sources of phenolic compound production.

1. Introduction

Solidago canadensis L. (Canada goldenrod) and *S. gigantea* Aiton (Late goldenrod) were introduced to Europe as ornamentals from North America in the mid-18th century and began to spread during the 19th century (Wagenitz, 1979). Both species are considered most aggressive invaders, which is defined by the European and Mediterranean Plant Protection Organization as invasive species having a high potential for spread and posing an important threat to the environment and biodiversity in the region (EPPO). The distribution of invasive goldenrods in Lithuania is closely related to political and economic changes that occurred while restoring independence in the 1990s. At that time, abandoned and poorly managed agricultural areas contributed to the rapid spread and high numbers of goldenrods.

Invasive goldenrods are negatively valued because they reduce the abundance of native plants. On the other hand, goldenrods are considered to be medicinal plants. The raw material known as *Herba Solidaginis* includes herbs of *S. canadensis*, *S. gigantea* and *S. virgaurea* (Skrzypczak et al., 1999). Goldenrods have been traditionally used to treat inflammations of the urinary tract. Preparations from goldenrods have a well-defined diuretic, spasmolytic and hypotensive effect together with anti-inflammatory, bacteriostatic and analgesic properties (Demir et al., 2009). In addition to the above indications, preliminary studies of *Solidago* species have shown that plants of this genus contain a high-molecular-weight polysaccharide-protein complex that has strong cytotoxic activity against prostate cancer cells (Gross et al., 2002) and an antitussive effect (Sutovskáa et al., 2013). There are also antitumor activities in the saponines fraction of *Solidago* species (Lendl and Reznicek, 2007) and antimicrobial, sedative, cytotoxic and hypotensive effects in the essential oils of *Solidago* species (Chanotiya and Yadav, 2008; Huang et al., 2012; Kołodziej et al., 2011; Mishra et al., 2011). The toxicity and contraindications for goldenrods preparations have not been reported, and the information that is available is based largely on studies conducted on the native European goldenrod (*S. virgaurea* L.).

Flavonoids and phenolic acids constitute one of the most important groups of pharmacologically active substances acting against oxidative damage; therefore, they limit the risk of various

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degenerative diseases (Andersen and Markham, 2010; Demir et al., 2009; Hendrich, 2006; Pandey and Rizvi, 2009). The effect of goldenrod preparations in urinary therapy is highly related to the biological action of flavonoids; they inhibit the enzyme neutral endopeptidase, which is responsible for the interaction of the atrial natriuretic peptide with the glomerulus, and, thus, they regulate the formation of urine via the excretion of sodium ions (Melzig, 2004). However, despite the importance of flavonoids, investigations of these compounds in Solidago species are scarce. Studies on *S. vigaurea* and *S. graminifolia* (L.) Elliot in Poland (Roslon et al., 2014; Thiem et al., 2001), *S. canadensis* in Hungary (Apati, 2003), *S. chilensis* (Güntner et al., 1999) and *S. microglossa* (Sabir et al., 2012) in Brazil have been conducted.

The objectives of the present work were to identify and quantify the concentrations of the principal phenolic compounds in two widespread invasive species of Solidago, to assess the inter- and intra-specific chemical diversity of goldenrods and to determine the importance of Solidago raw material in herbal preparations.

2. Materials and methods

2.1. Plant material collection and identification

Sixty accessions of *S. canadensis* and 33 accessions of *S. gigantea* were collected from different wild populations. The harvested plant material consisted of the three tops of two to three shoots of the same clonal plant in the flowering phase. Collected plants were dissected into inflorescence and leaf parts and dried at 25 °C for chemical analysis.

The botanical identification of the species was carried out according to the morphological description of Central European Solidago species (Wagenitz, 1979). The aim of the present study was not to clarify the taxonomy of goldenrods; the species studied were *S. canadensis* and *S. gigantea*. Plants that had green or reddish stems; sparse to dense hair in at least the upper half of their stems; abaxial faces that were pubescent and hairy along the main nerves; and adaxial glabrous or slightly scabrous leaves were identified as *S. canadensis*. *S. gigantea* plants were distinguished by their glabrous purplish or sometimes green wax stems, and leaves that were glabrous on both sides or slightly hairy along the main nerves of their adaxial side. The florets of *S. gigantea* had achenes with brownish-white pappus, whereas the pappus of *S. canadensis* was silvery/whitish.

The herbarium vouchers of the accessions were deposited at the Herbarium of the Institute of Botany, Vilnius, Lithuania (BILAS).

2.2. Preparation of extracts

Air-dried plant material was mechanically ground with a laboratory mill to obtain a homogenous drug powder. All the samples, sixty of *S. canadensis* and 33 of *S. gigantea*, of approximately 0.1 g (weighed with 0.0001 g precision) were extracted in 10 ml of a methanol and water mixture (70:30, v/v) by ultra-sonication at 25 °C for 50 min. The methanol was chosen as extraction solvent because it mixed well with the mobile phase and results the high extraction efficiency. Before it was tested various methanol/water mixtures and the highest peak areas of compounds were obtained using 70% methanol (data not displayed). The prepared extracts were passed through a 0.22 μm filter and stored at 4 °C until analysis.

2.3. HPLC conditions and analysis

A Waters Alliance 2695 (Waters, Milford, USA) separation module system equipped with Waters 2487 UV/vis and Waters 996 PDA diode-array detector (DAD) was used for HPLC analysis. The data were analysed using the Empower Software chromatographic manager system (Waters Corporation, Milford, USA). The separation of the compounds was carried out on a YMC-Pack ODS-A column (3.0 μm, 150 mm × 4.6 mm i.d.) with a YMC guard cartridge ODS-A (3.0 μm, 10 mm × 4.0 mm). The modified and optimised gradient elution was used for detection of the compounds according to Apati et al. (2002). The mobile phase consisted of eluent A (0.05% trifluoroacetic acid in water) and eluent B (100% acetonitrile). The elution programme was fixed as follows: 5% B at 0–5 min, 12% B at 5–50 min, 30% B at 50–51 min, 90% B at 51–56 min, and 5% B at 56–57 min. The flow rate was 1.0 mL/min, and the column temperature was 25 °C. The volume of extract injected was 10 μL. Detection was monitored at a wavelength range of 210–550 nm. Peaks were identified by comparing their UV-vis spectra and retention times to those of authentic reference standards. The samples were analysed twice. The chromatograms of *S. canadensis* and *S. gigantea* flower extracts are shown in Fig. 1.

2.4. Quantification and validation

The quantification and validation was followed in accordace with the methodical revision of natural products presented by Wolfender (2009). Standard stock solutions with a concentration of 0.1 mg/g for rutin, hyperoside, isoquercitrin and quercitrin and 0.2 mg/g for chlorogenic acid were freshly prepared in 70% methanol and diluted to six different concentrations. Three injections per concentration were performed to determine linearity. The chromatogram peak areas were 324 nm for chlorogenic acid and 355 nm for rutin. Isoquercitrin, hyperoside and quercitrin were plotted against the known concentrations of their associated standard solutions to establish calibration equations. A linear regression equation was calculated by the least squares method. The regression coefficients of all calibration curves were $R^2 > 0.999$, confirming the linearity of the concentration ranges.

The sensitivity of the method was evaluated by determining the limit of detection (LOD) and limit of quantitation (LOQ). LOD and LOQ were calculated as the concentrations that gave signal-to-noise ratios of 3 and 10, respectively.

A standard mixture of rutin, quercitrin and isoquercitrin each at a concentration of 0.0053 mg/ml and chlorogenic acid and hyperoside at concentrations of 0.0106 mg/ml and 0.0526 mg/ml, respectively, was used for intra-day and inter-day precision testing. The precision of the method was demonstrated by performing five replicate non-consecutive injections of the standard mixture on the same day on 4 different days. The results are reported in terms of RSD. The retention time, linear range, regression equation and correlation coefficient of each analyte, the LOD and LOQ values, and the intra-day and inter-day precision are summarised in Table 1.

2.5. Chemicals

Acetonitrile and methanol were of HPLC grade and supplied by Roth GmbH (Karlsruhe, Germany). Water was filtered through the Millipore HPLC grade water preparation cartridge (Millipore, Bedford, USA). The reference substances, chlorogenic acid (purity ≥ 95.33%), rutin trihydrate (purity 97.11%), and isoquercetin (purity ≥ 94.16%), were purchased from HWI ANALYTIK GmbH (Germany); quercitrin (purity ≥ 98.0%) was obtained from Roth GmbH (Karlsruhe, Germany).

2.6. Data analysis

Multivariate mathematical approaches using the SAS Version 4.3 software package were carried out to reveal inter- and intra-specific similarities and differences for the phenolic compound accumulations in the evaluated Solidago species. A hierarchical