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EXPLORING AN INVASIVE PLANT *SOLIDAGO CANADENSIS* AS THE POTENTIAL SOURCE OF TRITERPENOIDS

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ABSTRACT

Canadian goldenrod (*Solidago canadensis* L.) is a perennial herb introduced from North America and widely distributed in most European countries, often regarded as an invasive plant threatening the native species, simultaneously being a hard-to-control weed in agriculture. Nevertheless, due to its application in traditional herbal medicine (as *Solidaginis herba*), *S. canadensis* also constitutes a cheap and readily available medicinal raw material. The content of such metabolites as flavonoids, polysaccharides, diterpenes and triterpenoid saponins in this plant is well characterized, whereas the data on the occurrence of triterpenoids and steroids in a free (not conjugated) form are scarce. Thus, the present study was aimed toward the gas chromatography-mass spectrometry (GC-MS) analysis of the extracts obtained from inflorescences (branched panicles) and leaves of *S. canadensis*. Diethyl ether extracts were obtained from dried and powdered samples of *S. canadensis* leaves and inflorescences divided into flowers and stems. Analysis was made by gas chromatography-mass spectrometry method (GC-MS). In both inflorescences and leaves, the significant contents of triterpenoids belonging to lupane-, oleanane- and ursane- groups were found, i.e., lupeol acetate, β-amyrin, and α-amyrin accompanied by its ketone, α-amyrenone. Flowers were the richest source of triterpenoids (approx. 1.2 mg/g of dry weight), whereas the contents in leaves (0.91 mg/g d.w.) and inflorescence stems (0.69 mg/g d.w.) were lower. In all analyzed extracts one compound belonging to plant Δ^7-sterols, spinasterol, was found, present mainly as an ester conjugate.

Keywords: *Canada goldenrod, invasive plants, Solidago canadiensis, steroids, triterpenoids*

INTRODUCTION

Solidago canadensis L. (family Asteraceae, synonyms: Canadian goldenrod, field goldenrod) is an erect rhizomatous perennial plant distributed naturally in North America. In its native range, *S. canadensis* occupies forest edges, roadsides and abandoned fields. Flowering occurs from July to October, the yellow flowers are arranged into small heads on branched pyramidal shaped inflorescences. After its introduction as an ornamental plant, this species (and the closely related *S.*

gigantea) has spread throughout almost all Europe, being among the most successful invasive plants. It is found also in Asia, Africa and Australia. In an invaded area, *S. canadensis* exists in the similar habitats as in the natural range, rapidly colonizing abandoned fields and unmanaged urban sites. As a successive intruder, it is threatening the native species, exerting a negative impact on biodiversity. One of this plant's few benefits is its late blooming period in the second half of summer, when there is a deficiency of bee forage species, therefore, *S. candensis* is valued by beekeepers as nectar- and pollen-providing plant (Guzikowa and Maycock 1986, Weber 1997, Bielecka and Królak 2019).

A developing bioeconomic approach leads to search for new high added-value bioproducts that can be obtained from available local natural resources, including invasive species. In this context of bioeconomy, *S. canadensis* has been suggested for the use in biomass production and biorefinery, however, its traditional applications as a medicinal plant might suggest also other possibilities (Zihare and Blumberga 2017). Historically, the flowers and aerial parts of *S. canadensis* were used in Amerindian medicine to treat urinary tract, tuberculosis, diabetes, hemorrhoids, internal bleeding, asthma, arthritis, ulcers and other skin disorders (Bradette-Hébert *et al*. 2008, Kołodziej *et al*. 2011, Baki *et al*. 2019). In Europe, *Solidago* species were widely used as *Solidaginis herba* to treat urinary tract infections and inflammations, as well as to prevent formation or facilitate elimination of kidney stones and urinary gravel (Kelly *et al*. 2020). Extracts obtained from *S. canadensis* flowers or aerial parts were reported to exhibit several biological activities including antibacterial, antimycotic, antitumor, analgesic, antioxidant, cytotoxic, spasmolytic, sedative and hypotensive properties. Phytochemical characterization of these extracts led to identification of carotenoids, flavonoids, phenolic acids (e.g., hydroxycinnamates), polysaccharides, diterpenes, sesquiterpenes, triterpenoid saponins, tannins, alkaloids, polyacetylenes and essential oils (Baki *et al*. 2019, Shelepova *et al*. 2019, Kelly *et al*. 2020). The aim of the present work was the qualitative and quantitative determination of one of the less characterized group of compounds, i.e., steroids and triterpenoids (occurring in non-glycosidic form) in the aerial parts of *S. canadensis*, i.e., leaves and inflorescences divided into flowers and stems, analyzed separately.

MATERIAL AND METHODS

Aerial parts of randomly chosen plants of *Solidago canadensis* were collected in early September 2021 from the urban habitat in the Pole Mokotowskie park in Warsaw, Poland. Selected not damaged inflorescences and leaves were air-dried at room temperature. Prior to the extraction, inflorescences were separated into flowers and stems. The samples of leaves (3.23 g), flowers (3.20 g) and stems (1.13 g) were homogenized in a laboratory mortar. The ground plant material was placed in thimbles and extracted using a Soxhlet apparatus for 8 h with diethyl ether. The obtained extracts were evaporated to dryness under reduced pressure on a rotary evaporator.

Obtained diethyl ether extracts were fractionated by adsorption preparative thin-layer chromatography (TLC) on 20 cm × 20 cm glass plates coated manually with silica gel 60H (Merck). The solvent system chloroform:methanol 97:3 (v/v) was applied for developing. Three fractions were obtained as described earlier (Dashbaldan *et al*. 2020): free (non-esterified) steroids and triterpenoids, triterpenoid acids and steroid/triterpenoid esters. Fractions were eluted from the gel in diethyl ether. Subsequently, fractions containing free neutral triterpenes and sterols (R_F 0.3-0.9) were directly analyzed by GC-MS, fractions containing triterpene acids (R_F 0.2-0.3) were methylated with diazomethane, whereas fractions containing esters (R_F 0.9-1) were subjected to alkaline hydrolysis. For methylation, nitrosomethylurea (2.06 g) was added to a mixture of 20 mL of diethyl ether and 6 mL of 50% aqueous KOH, and the organic layer was then separated from the aqueous layer. Samples containing triterpenoid acids were dissolved in 2 mL of the obtained solution of diazomethane in diethyl ether, and held at 2 °C for 24 h. For alkaline hydrolysis, the ester fraction was treated with 10% NaOH in 80% MeOH at 80 °C for 3 h. Subsequently, the obtained mixtures were extracted with diethyl ether, and the obtained extracts were fractionated by preparative TLC as described above.

An Agilent Technologies 7890A gas chromatograph (Perlan Technologies, Warszawa, Poland) equipped with a 5975C mass spectrum detector was used for qualitative and quantitative analyses. Samples dissolved in diethyl ether:methanol (5:1, v/v) were applied (in a volume of 1-4 µL) using 1:10 split injection. The column used was a 30 m x 0.25 mm i.d., 0.25-µm, HP-5MS UI (Agilent Technologies, Santa Clara, CA). Helium was used as the carrier gas at a flow rate of 1 mL/min. The separation was made with the following temperature program: initial temperature of 160°C held for 2 min, then increased to 280°C at 5°C/min, and the final temperature of 280°C held for a further 44 min. The other employed parameters were as follows: inlet and FID (flame ionization detector, part of 7890A chromatograph) temperature 290°C; MS transfer line temperature 275°C; quadrupole temperature 150°C; ion source temperature 230°C; EI 70 eV; *m/z* range 33-500; FID gas (H_2) flow 30 mL·min^{-1} (hydrogen generator HydroGen PH300, Peak Scientific, Inchinnan, UK); and air flow 400 mL·min^{-1}. Individual compounds were identified by comparing their mass spectra with spectral libraries (Wiley 9th ED. and NIST 2008 Lib. SW Version 2010), previously reported data and the results of earlier experiments, as well as by comparison of their retention times and corresponding mass spectra with those of authentic standards, where available. Quantitation was conducted with a FID detector and performed using an external standard method based on calibration curves determined for authentic standards of ursolic acid methyl ester, α-amyrin and stigmasterol (Rogowska *et al*. 2022).

RESULTS AND DISCUSSION

The diethyl ether extracts were obtained from the dried plant material with yield ranging from 2% to 4%, depending on the plant part (the highest yield, 4%, was obtained from the flowers; 2% from the stems; and 2.4% from the leaves). GC-MS analysis revealed the presence of triterpenoids belonging to three of the most common carbon skeleton groups found in higher plants, i.e., lupane-, oleanane- and ursane-type: lupeol acetate, β-amyrin and oleanolic acid, as well as α-amyrin accompanied by its ketone, α-amyrenone. Both amyrins were also found in the ester fraction analyzed after alkaline hydrolysis. The fraction of steroids occurring in a free form was composed only of one sterol, identified as belonging to a group of Δ^7-sterols, spinasterol (stigmasta-7,22-dien-3-ol) and one steroid ketone, tremulone (stigmasta-3,5-dien-7-one). Spinasterol was also identified in the ester fraction analyzed after hydrolysis.

The quantitative determination of identified compounds is presented in Table 1. The amount of triterpenoids occurring both in free and esterified forms was the highest in the extract of *S. canadensis* flowers (reaching 1.2 mg/g of dry weight), whereas the contents in leaves (0.91 mg/g d.w.) and inflorescence stems (0.69 mg/g d.w.) were lower. The predominating compound in all analyzed extracts was α-amyrin, constituting more than 50% of analyzed triterpenoids (with the highest proportion in the flower extract, reaching almost 60%). Free forms of triterpenoids were more abundant than their ester forms, approx. 8-fold in the flower and leaf extracts, and even more, 12-fold, in the inflorescence stem extract.

The content of a free form of spinasterol was relatively low in all analyzed samples, the highest amount was found in the flower extract (approx. 0.2 mg/g d.w.). Sterols are important constituents of plant cell membranes, regulating their fluidity and permeability (Rogowska and Szakiel 2020), therefore, such low content of a free form of spinasterol could suggest more abundant occurrence of sterol conjugated forms. Indeed, significant amounts of spinasterol released after alkaline hydrolysis of the ester fractions were found in all analyzed extracts. This might suggest the occurrence of sterols also in other forms, mainly as glycosides, however, due to their higher polarity, these forms could not be extracted by diethyl ether and therefore they were not present in extracts obtained in the present study. Regarding the results of other reports (Janson *et al*. 2009), the sterol conjugates seemed to be the prevailing form of these compounds in Solidago species, e.g., *S. altissima*, where the sterol esters and glycosides constituted 85% of the total sterol profile. The present study on *S. canadensis* revealed clearly only the presence of spinasterol, however, it cannot be ruled out that the other related compounds (as 22-dihydrospinasterol or other Δ^5 and Δ^7-sterols, reported for leaf extracts of *S. altissima*) were also present in analyzed samples, however, in very small amounts below the limit of MS identification.

Table 1. The content of free and ester forms of triterpenoids and steroids in extracts obtained from various parts of *S. canadensis* aerial part: leaves, flowers, and the inflorescence stems.

Compound	Plant part		
	leaves	flowers	inflorescence stems
	Content (µg/g d.w.)		
Triterpenoids			
free forms:			
α-amyrin	434.77	640.98	322.86
α-amyrenone	66.40	87.62	7.61
β-amyrin	229.68	240.7	270.14
lupeol acetate	16.41	112.54	29.87
oleanolic acid	66.52	14.91	6.01
Sum of free forms	*813.78*	*1096.75*	*636.49*
ester forms:			
α-amyrin	70.05	105.65	32.94
β-amyrin	32.51	48.78	18.07
Sum of ester forms	*102.56*	*154.43*	*51.01*
Steroids			
free forms			
spinasterol	69.90	214.57	12.91
tremulone	59.20	108.43	35.44
Sum of free forms	*129.10*	*323.0*	*48.35*
ester forms:			
spinasterol	114.64	340.24	42.93

The phytochemical characteristics of many alien invasive species have not yet been fully studied in terms of bioeconomy. The preliminary studies on *S. canadensis* demonstrated that this plant can constitute a valuable raw material for many sectors of the industry, with the possibility of its wider applications in the future as a new source of functional ingredients for food, nutraceuticals, cosmeteuticals, and medicines (Mietlińska *et al*. 2019, Shelepova *et al*. 2019). Other studies pointed to this plant as potentially valuable as a bioaccumulator and phytoremediator of heavy metals (Bielecka and Królak 2019). Any of the potential application can be helpful for the balanced and optimized management proposal, involving not just the trials of mechanical elimination but the simultaneous practical utilization of obtained *S. canadensis* biomass (Zihare and Blumberga 2017, Baranová *et al*. 2022).

CONCLUSIONS

This report supplements the phytochemical characteristics of an invasive plant *S. canadensis* as a potential source of bioactive ingredients, demonstrating the occurrence of significant amounts of triterpenoid alcohols, α- and β-amyrins, particularly in the flower extract.

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