LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

Mindaugas Marksa

STUDY OF THE DISTRIBUTION OF PHENOLIC AND TERPENE COMPOUNDS IN NATIVE, INVASIVE, AND HYBRID *SOLIDAGO* L. SPECIES USING CHROMATOGRAPHIC TECHNIQUES

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LIETUVOS SVEIKATOS MOKSLŲ UNIVERSITETAS

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FENOLINIŲ IR TERPENINIŲ JUNGINIŲ PASISKIRSTYMO VIETINĖSE, INVAZINĖSE IR HIBRIDINĖSE *SOLIDAGO* L. RŪŠYSE TYRIMAS, NAUDOJANT CHROMATOGRAFINIUS METODUS

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The Years of anxious searching in the dark, with their intense longing, their alterations of confidence and exhaustion and the final emergence into the light – only those who have experienced it can understand it.

Albert Einstein

Metų metai nerimastingų paieškų tamsoje, kupini didelio ilgesio, svyruojančio pasitikėjimo savimi ir išsekimo, ir galiausiai – išsiveržimas į šviesą. Tik tie, kurie tai patyrė, gali tai suprasti.

Albertas Einšteinas

LIST OF ABBREVIATIONS

ABTS	–	2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	–	One-way analysis of variance
DPPH	–	2,2-diphenyl-1-picrylhydrazyl
EO(s)	–	Essential oil(s)
GC	–	Gas chromatography
GC-MS	–	Gas chromatography-mass spectrometry
HCA	–	Hierarchical cluster analysis
HPLC	–	High-performance liquid chromatography
HPLC-MS	–	High-performance liquid chromatography-mass spectrometry
LOD	–	Limit of detection
LOQ	–	Limit of quantification
LRI	–	Linear retention indices
PCA	–	Principal component analysis
PDA	–	photodiode array detector
QTOF	–	quadrupole time of flight
TEAC	–	Trolox equivalent antioxidant capacity
UPLC	–	Ultra high-performance liquid chromatography
UTI	–	Urinary tract infection

INTRODUCTION

Analytical sciences are applied to investigate the phytochemical substances that accumulate in plants organs and are just the reason why plants can have one or another effect on various functions of the human body [1]. Through extensive ethnopharmacological investigations, the use of plant-origin materials has been substantiated by science-based evidenced research, revealing the great potential for phytotherapeutic applications. However, even with these advances, pursuing human well-being challenges new uncertainties to be solved [2–4]. Today medicinal plants and their materials are applied in approximately 70–95% of the world's developed countries. Interest in a healthy lifestyle, healthy diet, and functional, natural, and therapeutic products is constantly growing in developed countries [5–7]. Increasing attention is being paid to medicinal herbal preparations or herbal mixtures, which are expected to become an alternative to many synthetic drugs [8]. Such a vision of the future encourages scientists to look for new plants whose accumulated bioactive compounds are characterized by healing properties that positively affect human vital functions.

Therefore, just like a few decades ago, research on the biologically active components of medicinal plants remains relevant today due to the empirical therapeutic efficiency and inexhaustible resources. Even though over 35,000 plants are already used for medicinal purposes in the world, biologically active substances of plant origin are being turned to again in search of new treatment tools and treatment algorithms in medical practice to apply extended therapy and avoid the shortcomings and limitations inherent in chemical medicinal preparations and to reduce the number of unnecessary interventions [9]. Policymakers, healthcare professionals, and the general public are dealing with challenges related to the safety, efficacy, quality, availability, preservation, and regulation of the usage of medicinal plants in various regions of the world [10].

Preparations of medicinal plants can easily be incorporated into preventing chronic and degenerative diseases and improving global health [11]. The quality of herbal preparations affects their effectiveness and safety. The quality indicators of plant raw materials vary due to the influence of internal and external factors. Reliable control of the quality of the medicinal raw material, the production process, and other factors determining the final product’s quality is an essential step in safety assurance [10].

Accumulated scientific knowledge elucidates existing scientific uncertainties. It encourages more detailed qualitative and quantitative studies of plant raw materials, evaluation of the plant's chemical composition, and a

more targeted search for new sources of herbal therapeutic preparations with desired functional properties.

Various scientific groups have studied the plants of the genus *Solidago* L. (Asteraceae Bercht. & J.Presl.), commonly known as goldenrods, for several decades worldwide. By analyzing their chemical composition, they are trying to expand the possibilities of using plant raw materials in medicine [12–14].

Goldenrods have a status of traditional use in various European countries [15]. In traditional medicine, goldenrods are used for their antimicrobial, diuretic, wound healing, and anti-inflammatory properties. Phenolic compounds derived from goldenrods can be used to treat and prevent various systemic disorders of the body in oncology, urology, and infectious diseases [14, 16–19]. A multitude of the abovementioned effects are determined by the rich and diverse phytochemical complex that consists of phenolic compounds, namely anthocyanidins, flavonoids, and phenolic acids, most of which consist of chlorogenic acid, quercetin, quercitrin, rutin, hyperoside [14, 16, 17]. They exhibit antioxidant effects. Therefore, goldenrod is classified in the scientific literature as one of the rich sources of compounds with antioxidant activity [14, 16]. The terpenic profile includes triterpenic compounds of oleanane type, diterpenoid lactones, and essential oil compounds. Essential oil terpenes, including Germacrene D, bornyl acetate, and D-limonene, have also been detected in the essential oil of Canadian goldenrod leaves [20]. Modern chromatographic methods are used to assess qualitatively and quantitatively the chemical composition of plants belonging to the *Solidago* genus – high-performance liquid chromatography (HPLC), gas chromatography (GC), high-performance liquid chromatography-mass spectrometry (HPLC-MS), gas chromatography-mass spectrometry (GC-MS) [14, 21, 22]. An analytical process is created to evaluate a substance's defined characteristics against predetermined acceptance standards for that feature [23].

The genus *Solidago* includes up to 330 species and intraspecific taxons [24]. Distinct species are used as medicinal plants in various parts of the world. *Solidago virgaurea* is native to Eurasia's region, while *S. canadensis* and *S. gigantea* originated from North America. The latter species are considered invasive plants in Lithuania and the rest of Europe [8, 13, 14, 24]. The increase in the population of invasive plants is a critical problem for local vegetation, as it occupies additional growth areas, changes the soil structure, and, therefore, impoverishes and alters native vegetation. Invasive plant research helps to develop tools to control their population dynamics: more detailed studies, such as chemical composition analysis, provide an opportunity to assess the adaptation possibilities of these plants in their non-native environment, comprehensive studies of the chemical composition of hybrid

plants allow us to understand how the genetic expression of plant characteristics changes as they adapt to local natural conditions [12, 13, 25, 26]. The eradication programs can be easily turned into the rational collection of plant raw materials as a source for valuable phytochemical compounds.

The scientific research on goldenrods growing in Lithuania and other regions remains limited, necessitating a comprehensive assessment of their chemical composition. It is essential to determine the interspecies dynamics of the chemical components of *Solidago* plants, their distribution in quantitative and qualitative terms, as well as the distribution between the morphological parts of the plant itself.

Due to the reasons mentioned above, the scientific community's interest in Lithuania and the world in various types of goldenrods is increasing. More and more detailed studies are being conducted to evaluate the antioxidant activity of both complex and individual compounds in the plants of the *Solidago* genus. To carry out such studies, post-column methods are required, which would allow a precise analysis of both the qualitative and quantitative composition of the plant [16, 17, 21, 27–29]. By applying chemometrics bioactivity and the homogeneity, and safety of plant raw materials can be determined, which provides the basis for further steps, such as developing pharmaceutical phytopreparations.

The study concentrated on (1) the phenolic, antioxidant, and essential oil terpene profiles of the inflorescences and leaves of *S. virgaurea*, *S. canadensis*, *S. gigantea*, *S.* × *niederederi* naturally growing in Lithuanian habitats and (2) employed chemometric analysis to clarify chemophenetic peculiarities and establish a relationship between genotype and distributional pattern of phytochemicals. Phenolic compounds may serve as phytochemical markers for the differentiation of species as well as the verification of their origin. To comprehensively evaluate the qualitative traits of plant raw materials and the targeted perspectives of their use, it is essential to understand the peculiarities of the diversification of phytochemical composition in plants and the development of knowledge to predict the importance of these plant raw materials for possible use as the source of antioxidants and food and pharma products.

The aim of the work is to develop integrated analytical tools for evaluating phytochemical composition diversification, antioxidant activity, and quality markers distribution in raw materials of native, invasive, and hybrid *Solidago* L. plants.

The tasks proposed for the achievement of the above-stated aim were as follows:

1. To develop and validate the HPLC-PDA method for identification and quantification of the phenolic compounds' composition diversity in widespread species of *Solidago*.
2. To develop and validate the HPLC post-column assays for the determination and evaluation of fast-acting radical scavengers in goldenrod raw materials.
3. To determine the chemophenetic characteristics of hybrid *Solidago* species for the verification of the species.
4. To determine the variation of volatile profiles of the *Solidago* species growing in mixed populations in Lithuania.
5. To identify and quantify antioxidant profiles of leaves and inflorescences of *Solidago* species from wild populations in Lithuania using UPLC-MS and HPLC post-column methods.

SCIENTIFIC NOVELTY

To our knowledge, comprehensive multivariate assessments based on chemical data were made for the first time towards the inter- and intra-specific phytochemical diversity of native, invasive, and hybrid goldenrods. The newly obtained scientific knowledge serves as the foundation for creating functional products and phytopreparations based on the goldenrod herbal raw material.

During the conducted research, the HPLC method was developed and validated to analyze the qualitative and quantitative composition of the plant raw materials of the *Solidago* L. genus. The influence of the storage temperature of the reaction loop on the post-column reaction of the HPLC was evaluated for the first time. The representatives of the genus *S. canadensis*, *S. gigantea,* and *S. virgaurea*, which grow naturally in Lithuania, have not been studied until now. Qualitative and quantitative studies of phenolic compounds and active compounds with antioxidant properties of *Solidago* L. species growing naturally in the climatic conditions of Lithuania were performed, and the main components and markers of the antioxidant effect of *S. virgaurea* were identified for the first time in the study.

PRACTICAL VALUE

HPLC and HPLC post-column methods developed and validated during the study allow accurate, reliable, and reproducible determinations and are prerequisites for the qualitative and quantitative studies of phytochemical compounds. The optimal factors selected for the methods of antioxidant activity evaluation, namely the length of the reaction loop, temperature, reagent concentration, and flow rate, ensure the accurate separation and reliable quantitative assessment of the compounds. The developed HPLC and HPLC-ABTS methods can be used for routine analyses of *Solidago* species for the evaluation of their active compounds and even for the identification of the species.

The conducted studies of phenolic compounds of the genus *Solidago* L. are essential for the targeted collection of plant material and its pharmacognostic value. The antioxidant activity of phenolic compounds determines the quality and practical importance of plant raw materials in phytotherapy, and their quantitative analysis in different parts of the plant shows the patterns of accumulation and determines the method of preparation of plant raw materials. The research on alien goldenrod contributes to the search for new plant raw materials, expanding the possibilities of their sustainable and optimized use, regulation of the population of invasive plants while turning waste into added value ingredients, and increasing the potential of applying scientific research in practice. Assessment of the dependence of the chemical composition and morphological characteristics of plant raw materials is important in the process of selection and primary quality standardization of raw materials. Determining the chemical composition of plants is essential not only for the evaluation of medicinal raw materials but it can also be important in the chemophenetic studies of goldenrod species, which is especially important when clarifying the very confusing taxonomy of these species.

The evaluation of the complex of bioactive compounds using different HPLC post-column assays showed the fast-acting antioxidant potential of the extracts of the plant raw material of goldenrods. In addition to the flavonoids regulated by the pharmacopoeia, the profile of caffeoylquinic acid, a fast-acting antioxidant, has been established during the study which can also be a quality marker. The determination of antioxidant activity markers can help guarantee the efficacy of plant materials with potential health benefits that may be used in various industries. These studies provide new knowledge about the plant’s antioxidant capabilities and enrich the range of plant raw materials with antioxidant properties.

THE LAYOUT OF THE DISSERTATION

The dissertation is prepared on the basis of five scientific articles. Each article describes the study conducted to fulfil separate tasks proposed for the achievement of the aim of this work. The materials and methods, results, and discussion of each article are presented in three corresponding parts of the thesis. The essential study elements and the progress are presented in Table 1.

The results of all studies conducted contribute to a comprehensive qualitative and quantitative evaluation of goldenrod plant raw materials and the development of knowledge to predict the importance of these plant raw materials for possible use as the source of antioxidants and food and pharma products.

Table 1. *Thesis structure in stages – thesis at a glance*

Phenolic profiles in the leaves and inflorescences of *Solidago* L. species.	
Aim of the study	The aim of the study was to investigate the inter- and intra- specific diversity of phenolic compounds in *Solidago canadensis* and *Solidago gigantea*.
Methods	HPLC-PDA method was applied to evaluate the quality and quantity of phenolic compounds. Multivariate statistical analysis was applied to evaluate differences in the compound accumulation between the species.
Results	Five phenolic compounds (chlorogenic acid, rutin, isoquercitrin, hyperozide, and quercitrin) were detected in the leaves and inflorescences of *S. canadensis* and *S. gigantea*. The two widespread species of goldenrods did not display high intraspecific variation in their phenolic compound accumulations.
Conclusions	The reduced heterogeneity in the chemical composition of goldenrods leads to an increase in the quality and safety of herbal material obtained from the wild. Invasive goldenrods could be appreciated as potentially rich and renewable resources of pharmaceutical raw materials. Furthermore, the gathering of the raw material of goldenrods could help to manage the uncontrollable spreading of invasive plants.
Development of the on-line high performance liquid chromatography coupled radical scavenging assays for antioxidant potential evaluation in goldenrod.	
Aim of the study	The aim of the study was to modify and validate the post-column HPLC-ABTS and HPLC-DPPH methods for evaluating radical scavenging activity of Solidago canadensis extracts.
Methods	A Waters Alliance 2695 (Waters Milford, USA) separation module system equipped with Waters 996 PDA diode-array detector and Waters 2487 UV/VIS detector (Waters Corporation) was used for HPLC post-column analysis.
Results	The developed DPPH and ABTS post-column techniques were applied to the evaluation of methanol extracts of leaves and flowers of *S. canadensis*. Two unknown phenolic compounds together with chlorogenic acid, rutin, and isoquercitrin were detected in these extracts. The results of the t-test demonstrated different antioxidant activities between the goldenrod leaf and flower methanol extracts when using the ABTS and DPPH post-column assay.

Table 1. *Continued*

Development of the on-line high performance liquid chromatography coupled radical scavenging assays for antioxidant potential evaluation in goldenrod.	
Conclusions	HPLC-DPPH and HPLC-ABTS post-column techniques were used in the development and validation of a highly efficient method for evaluating the anti-radical scavenging capacity of *S. canadensis* raw materials. A validated HPLC post-column method is acceptable for the assessment of antioxidant activity in plant materials of similar origin.
Accumulation patterns of phenolic compounds in hybrid *Solidago* L. species.	
Aim of the study	The aim of the study was to determine origin and phenolic compounds profile of the naturally occuring hybrid *Solidago* × *niederederi*.
Methods	HPLC-PAD and multivariate statistical approaches were applied to determine phenolic compounds accumulation and distribution.
Results	All analyzed Solidago species shared the same common phenolic constituents. The principal component analysis (PCA) was performed to detect groupings, similarities, or differences among all analyzed accessions of *Solidago* species. The distant position of *S. gigantea* accessions represented a more significant difference in the chemical composition of phenolic compounds than the other three species (*S.* × *niederederi*, *S. virgaurea*, *S. canadensis*) that formed a separate cluster, representing similarities in their quantitative accumulation of phenolic compounds.
Conclusions	The phytochemical pattern of phenolic compounds provided evidence of hybrid *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*. The hybrid, based on phytochemical phenotypes is close to its parental species.
Consistent patterns of essential oil terpenes in plant organs of *Solidago* L. species.	
Aim of the study	The aim of the study was to determine distribution patterns of volatile compounds in different *Solidago* spp.
Methods	The quantitative analysis of EOs was performed using the GCMS-QP 2010 Ultra system equipped with a Shimazu autoinjector AOC-5000 (Shimadzu, Europa GmbH). Multivariate statistical analysis was performed to identify the differences and similarities between species.
Results	Solidago spp. differed in the presence of terpenes that could be considered species-specific components. The presented results demonstrated similarities and differences in the frequency of distribution and contents of EO constituents among the four *Solidago* spp. There were significant differences detected between monoterpene and sesquiterpene fractions of inflorescences for all species studied. Meanwhile, the EO profiles in leaves revealed no significant differences between the four *Solidago* spp. Tested.
Conclusions	The volatile profiles of *Solidago* spp. varied depending on the species, accessions, and plant parts. The first comparative study of volatile profiles of *Solidago* spp. confirmed the origin of *S.* × *niederederi* as an interspecific taxon between *S. canadensis* and *S. virgaurea* with a higher metabolic contribution of *S. canadensis*. Phytochemical profiling of plant raw materials is an informative tool to correctly identify species of the plant and learn about their potential for further development of new natural products.

Table 1. *Continued*

Interspecific antioxidant profiling of fast-acting radical scavengers in *Solidago* L. species.	
Aim of the study	The aim of the study was to determine antioxidant profiles of leaves and inflorescences of *S. gigantea*, *S. canadensis*, *S. virgaurea*, and *S.* × *niederederi* from wild populations of Lithuania.
Methods	HPLC-PDA-ABTS and HPLC post-column assay was performed. UPLC-QTOF-MS analysis was performed using the Waters Aquity UPLC system with a PDA detector. Tukey multiple comparison tests, as well as the hierarchical analysis and principal component analysis (PCA), were performed to identify significant differences and similarities at $P \leq 0.05$.
Results	Twenty-three phenolic compounds were identified. Fourteen of these compounds demonstrated significant antioxidant activity. For the most part the antioxidant profiles of leaves and inflorescences of Solidago species comprised mainly of caffeoylquinic acid and quercetin derivatives.
Conclusions	Plant materials of *S. canadensis*, *S.* × *niederederi*, and *S. virgaurea* has priority in the antioxidant activity of 3,5-dicaffeoylquinic acid, chlorogenic acid, and rutin against *S. gigantea*. The corresponding compounds could be considered radical scavenging activity markers and, moreover, indicators of plant material quality. The total antioxidant capacity of the compounds detected in the invasive *Solidago* spp. was significantly higher than that of the native species.

Ph.D. CANDIDATE'S CONTRIBUTION

The contribution of the author Mindaugas Marksa is listed below concerning each publication related to the dissertation. Publications A1 to A5 are listed in the List of scientific papers.

A1: Development of extraction conditions, development and validation of HPLC methods, sample preparation, qualitative and quantitative evaluation, contribution to cowriting, and manuscript submission.

A2: Development and validation of HPLC post-column method, sample preparation, qualitative and quantitative evaluation, contribution to cowriting, and manuscript submission.

A3: Sample preparation, qualitative and quantitative evaluation of the samples, cowriting, and manuscript submission.

A4: Method development and validation, sample preparation, formal analysis and investigation, and data curation.

A5: Sample preparation and HPLC post-column analysis, investigation, and data curation.

CO-AUTHORS' CONTRIBUTION

- Jolita Radušienė contributed to the conceptualization and supervision of the studies and coauthored, edited, and approved the final manuscript versions of articles A1, A2, A3, A4, and A5 and provided resources related to the study at the Nature Research Center (Vilnius).
- Liudas Ivanauskas contributed to the conceptualization and supervision of the studies and coauthored and approved the final manuscript versions of articles A1, A2, A4, and A5.
- Birutė Karpavičienė contributed to conceptualizing the studies and coauthored and approved the final manuscript versions of articles A1, A3, and A4. The co-author contributed to the collecting and preparing of plant materials.
- Lina Raudonė contributed to the supervision of the studies and coauthored and approved the final manuscript versions of articles A4 and A5.
- Valdas Jakštas contributed to conceptualizing and supervising the studies and coauthored, edited, and approved the final manuscript versions of articles A1 and A2.
- Rūta Marksienė contributed to the conceptualization and supervision of the studies and coauthored and approved the final manuscript versions of article A2.
- Kristina Zymonė contributed to the conceptualization of the studies and coauthored and approved the final manuscript versions of articles A5.
- Audrius Pukalskas contributed to the conceptualization and supervision of the studies and coauthored and approved the final manuscript versions of article A5.

LIST OF SCIENTIFIC PAPERS

A1: Radušienė, Jolita; Marska, Mindaugas; Ivanauskas, Liudas; Jakštas, Valdas; Karpavičienė, Birutė. Assessment of phenolic compound accumulation in two widespread goldenrods //*Industrial Crops and Products.* Netherlands: Elsevier. ISSN: 0926-6690, 2015, vol. 63, no. 1, p. 158-66. [Impact factor: 3.449, quartile: Q1].

A2: Marksa, Mindaugas; Radušienė, Jolita; Jakštas, Valdas; Ivanauskas, Liudas; Marksienė, Rūta. Development of an HPLC post-column antioxidant assay for Solidago canadensis radical scavengers //*Natural Product Research.* United Kingdom: Taylor & Francis. ISSN: 1478-6427, 2016, vol. 30, iss. 5, p. 536-43. [Impact factor: 1.828, quartile: Q2].

A3: Radušienė, Jolita; Marksa, Mindaugas; Karpavičienė, Birutė. Assessment of Solidago× niederederi origin based on the accumulation of phenolic compounds in plant raw materials //*Weed Science*. USA: Cambridge University Press. ISSN: 0043-1745, 2018, vol. 66, iss. 3, p. 324-30. [Impact factor: 2.0, quartile: Q1].

A4: Radušienė, Jolita; Karpavičienė, Birutė; Marksa, Mindaugas; Ivanauskas, Liudas; Raudonė, Lina. Distribution patterns of essential oil terpenes in native and invasive Solidago species and their comparative assessment //*Plants*. Switzerland: MDPI. ISSN: 2223-7747, 2022, vol. 11, iss. 9, p. 1159. [Impact factor: 4.5, quartile: Q1].

A5: Marksa, Mindaugas; Zymonė, Kristina; Ivanauskas, Liudas; Radušienė, Jolita; Pukalskas, Audrius; Raudonė, Lina. Antioxidant profiles of leaves and inflorescences of native, invasive and hybrid Solidago species //*Industrial Crops and Products*. Netherlands: Science Direct. ISSN: 0926-6690, 2020, vol. 145, p. 112-123. [Impact factor: 5.645, quartile: Q1].

CONFERENCE PRESENTATIONS

1. M. Marksa, L. Ivanauskas, V. Jakštas, J. Radušienė, R. Marksienė. *Modification of postcolumn HPLC method for evaluation of antioxidant activity on Solidago canadensis herbal extracts*. The 4th international conference on pharmaceutical sciences and pharmacy practice dedicated to the 75th anniversary of Lithuanian Pharmacopoeia, November 23, 2013, Kaunas, Lithuania.
2. M. Marksa, L. Ivanauskas, V. Jakštas, J. Radušienė, R. Marksienė, S. Kamandulytė, K. Sendrauskaitė. *Development of HPLC method for evaluation of biologically active compounds in Solidago L. herbal extracts.* 8th International Scientific Conference The Vital Nature Sign, 2014, Kaunas, Lietuva.
3. M. Marksa, L. Ivanauskas, V. Jakštas, J. Radušienė, R. Marksienė, L. Stoškutė. *Modification of ferric reducing antioxidant power (FRAP) post-column assay for evaluation of antioxidant activity in Solidago canadensis*. Young Scientists Meeting "Trends in Natural Products Research", June 23-25, 2014 Olomouc, Czech Republic.
4. M. Marksa, L. Ivanauskas, V. Jakštas, J. Radušienė, R. Marksienė, K. Gaivelytė, J. Mačinskas. *Optimization of cupric reducing antioxidant capacity (CUPRAC) post-column assay for measuring the antioxidant activity of Solidago L.* The 5th International Conference On Pharmaceutical Sciences and Pharmacy Practice dedicated to the 145th Anniversary of prof. Petras Raudonikis, November 22, 2014, Kaunas, Lithuania.
5. J. Radušienė, M. Marksa, L. Ivanauskas, V. Jakštas, B. Karpavičienė. *Sources of phenolic compounds in two widespread species of goldenrods.* 8th Conference on Medicinal and Aromatic Plants of Southeast European Countries (CMAPSEEC), May 19-22, 2014, Durrës, Albania.
6. M. Marksa, J. Mačinskas, J. Radušienė, L. Ivanauskas, V. Jakštas. *Antioxidant activity of Solidago L. using HPLC- Cupric Reducing Antioxidant Capacity (CUPRAC) assay with post-column detection.* 9th International Scientific Conference "The Vital Nature Sign", May 14–16, 2015, Kaunas, Lietuva.
7. M. Auglys, A. Kubilienė, M. Marksa, A. Ževžikovienė. *Phenolic compounds quantitative research in Solidago L. using Thin-layer chromatography and High performance liquid chromatography.* The 6th International Pharmaceutical Conference Science and Practice November 5–6, 2015, Kaunas, Lithuania.

8. G. Navardauskaitė, R. Marksienė, M. Marksa, J. Radušienė. *The Assessment of essential oil compounds found in goldenrod (Solidago L.) plants naturally occurring in Lithuania using the GC-MS method.* The 8th International Conference on Pharmaceutical Sciences and Pharmacy Practice dedicated to the 80th anniversary of the Museum of History of Lithuanian Medicine and Pharmacy, December 15, 2017, Kaunas, Lithuania.
9. G. Pavalkytė, K. Mickutė, A. Kubilienė, I. Sadauskienė, M. Marksa, J. Radušienė. *The Effects of Solidago gigantea L. extracton malondialdehyde (MDA) activity in mice brain*. 9th International Pharmaceutical conference Science and practice 2018, dedicated to the 100th anniversary of independent Lithuania's pharmacy, November 9, 2018, Kaunas, Lithuania.
10. G. Pavalkytė, K. Mickutė, A. Kubilienė, I. Sadauskienė, M. Marksa, J. Radušienė. *The Effect of Solidago gigantea L. extract on malondialdehyde (MDA) level in mice liver*. The 22nd annual international scientific-practical conference „Balt Pharm Forum 2019" „Pharmacists as drug experts: their role in healthcare system": April 13–14, 2019, Kaunas, Lithuania.
11. L. Raudonė, M. Marksa, K. Zymonė, J. Radušienė, L. Ivanauskas. *HPLC-ABTS profiling of phenolics and fast-acting antioxidants in Solidago L.* PSE e-Congress 2020 "Plant Derived Natural Products as Pharmacological and Nutraceutical Tools", September 15–18–22–25, 2020; October 6–9–13–16, 2020.

1. SUMMARY OF MATERIALS AND METHODS

1.1. Plant material preparation

The botanical identification of *Solidago* species was carried out according to the morphological description of Central European *Solidago* species [118] and morphological diagnostic features defined in the previous assay of Karpavičienė and Radušienė, 2016 [30]. Plant samples were collected from different wild populations in Lithuania. The collected plants were dissected into inflorescence and leaf parts and dried at 25 °C, then ground to the homogenous powder and used to make extracts for chemical analysis.

For the determination of phenolic profiles in the leaves and inflorescences of *Solidago* L. species (publication – A1) sixty accessions of *S. canadensis* and 33 accessions of *S. gigantea* were collected.

For the development of the online high-performance liquid chromatography coupled radical scavenging assays for antioxidant potential evaluation in goldenrod (publication – A2), plant material was prepared as follows harvested plant material consisted of the three tops of two to three shoots of the same clone of 5 different plants in the flowering phase.

For the determination of accumulation patterns of phenolic compounds in hybrid *Solidago* L. species (publication – A3) nineteen accessions of *S. virgaurea*, seventeen of *S. canadensis*, eleven of *S. gigantea,* and twenty-four of *S.* × *niederederi* were collected from six mixed wild populations.

To determine consistent patterns of essential oil terpenes in plant organs of *Solidago* L. species (publication – A4) plant material of eighteen accessions of *S. canadensis*, seven of *S. gigantea*, nine of *S.* × *niederederi* Khek, and three accessions of *S. virgaurea* was collected in the flowering phase.

For the interspecific antioxidant profiling of fast-acting radical scavengers in *Solidago* L. species (publication – A5) the sample of the plant material consisted of the three shoots of the same clonal plant.

1.2. Extraction conditions

For the assays described in publications A1, A2, A3, and A5, extraction was carried out as follows. The homogenous powder of air-dried goldenrod leaves and inflorescences was used to make extracts. Methanol and water mixture (70:30, v/v) was used as an extraction solvent. Samples of 0.1 g plant material powder were extracted in 10 mL extraction solvent using an

ultrasonic bath (Witeg Labortechnik GmbH, WUC-A06H, Germany) at 25 °C for 50 min.

For the assay described in publication A4, essential oils were isolated as follows. The plant material from about 30 g of air-dried leaves and inflorescences separately was hydrodistilled for three hours using a Clevenger-type apparatus. Sample preparation for chemical analysis included 1.0 μL of essential oil added into 1.0 mL of n-hexane following previous studies. Essential oil content was calculated as a relative percentage of essential oil for 100 g of dry plant material.

1.3. Physicochemical analysis

HPLC-PDA conditions (A1, A3)

A Waters Alliance 2695 (Waters Milford, USA) separation module system equipped with Waters 996 PDA diode-array detector was used for HPLC analysis. The separation of the compounds was carried out on a YMC-Pack ODS-A column. The mobile phase consisted of eluent A (0.05% trifluoroacetic acid in water) and eluent B (100% acetonitrile). Gradient elution was performed for the separation of compounds. The flow rate – 1 ml/min, injection volume – 10 μL, and column temperature – 25 °C. Peaks of compounds were identified at a wavelength range of 210–550 nm by comparing their UV-Vis spectra and retention times to those of authentic reference standards. The quantification of detected compounds was carried out by the external standard method.

Quantification and validation. Peaks were identified by comparing their UV-Vis spectra and retention times to those of authentic reference standards, determining the limit of detection (LOD) and limit of quantitation (LOQ). Validation of the HPLC-PDA method was determined according to the following parameters: specificity, linearity, precision, the limit of detection (LOD), and the limit of quantification (LOQ).

HPLC-PDA-ABTS/DPPH conditions (A2)

A Waters Alliance 2695 (Waters Milford, USA) separation module system equipped with Waters 996 PDA diode-array detector and Waters 2487 UV/VIS detector (Waters Corporation) was used for HPLC post-column analysis. The separation of the compounds was carried out on a YMC-Pack ODS-A column. The mobile phase consisted of eluent A (0.05% trifluoroacetic acid in water) and eluent B (100% acetonitrile). Gradient elution was performed for the separation of compounds. The flow rate – DPPH 1 mL/min, ABTS 0.5 mL/min, injection volume – 10 μL, column temperature – 25 °C. Post-column coil temperature – 50 °C.

The chromatographic peaks were identified by matching the retention times of the analytes to reference standard compounds and UV absorption spectra within the limits of 210–400 nm. Phenolic acids and flavonoids were detected at 324 and 355 nm wavelengths, respectively.

The HPLC-PDA and HPLC post-column methods were validated according to the following parameters (ICH guidelines): specificity, linearity, precision, LOD, and LOQ. Repeatability was examined by 5 evaluations of 3 analytes (chlorogenic acid, rutin, and isoquercitrin) under the same experimental conditions on the same day.

GC-MS conditions in (A4)

The quantitative analysis of essential oils was performed using the GCMS-QP 2010 Ultra system equipped with a Shimazu autoinjector AOC-5000 (Shimadzu, Europa GmbH). A capillary column Rxi-5MS (30 m × 0.25 mm i.d. × 0.25 film thickness μm) (Restek, Bellefonte, PA, USA) was used. Mass spectra were acquired at an ionization voltage of 70 eV, a scan rate of 2500 *m/z* within the 29–500 *m/z* range, and a scan time of 0.2 s. The chromatographic analysis was run in triplicate.

The compounds' linear retention indices (LRI) were calculated using a homologous series of n-alkanes C8–C26 injected at the beginning of the analysis. The essential oil constituents were identified by comparing the unique mass spectral fragmentation patterns of peaks with the mass spectral data presented in the NIST 14, FFNSC, WR10, and WR10R libraries, as well as comparing the obtained LRI with those presented in NIST 14 datasets and references.

The relative percentage of each identified analyte was calculated as the peak area from the total peak areas of the chromatographic profile as the mean of three runs without correction factors.

HPLC-PDA-ABTS and HPLC post-column assay conditions in (A5)

A Waters Alliance 2695 (Waters Milford, USA) separation module system equipped with Waters 996 PDA diode-array detector and Waters 2487 UV/VIS detector (Waters Corporation) was used for HPLC post-column analysis. The separation of the compounds was carried out on a YMC-Pack ODS-A column. The mobile phase consisted of eluent A (0.05% trifluoroacetic acid in water) and eluent B (100% acetonitrile). Gradient elution was performed for the separation of compounds. The flow rate – is 1 mL/min, injection volume – is 10 μL, and column temperature – is 25 °C. Post-column coil temperature – 50 °C, coil length – 3 m, ABTS flow rate – 0.5 mL/min. After the PDA detection, the mobile phase with the analytes was mixed in a reaction coil with the ABTS solution and recorded at 650 nm wavelength.

UPLC-QTOF-MS conditions in (A5)

The analysis was performed using the Waters Acquity UPLC system with a PDA detector (Waters, Milford, Mass., U.S.A.), and compounds were analyzed by MAXIS 4G QTOF mass spectrometer (BRUKER DALTONIK GmbH, Bremen, Germany) equipped with an ESI ionization source. Waters Acquity HSS T3 column was used. The gradient elution was performed to separate compounds as eluent A – 2% acetic acid and B – acetonitrile.

1.4. Data analysis

Multivariate approaches using the SAS Version 4.3 were performed to process data obtained while analyzing phenolic profiles in the leaves and inflorescences of *Solidago* L. species (A1). One-way analysis of variance – ANOVA (A1, A2, A3, A4, A5), Student's *t-test* (A1), post-hoc Scheffe's multiple comparison test A1, A3), a hierarchical cluster analysis – HCA (A1, A5), and principal components analysis – PCA (A1, A3, A4, A5), post-hoc Tukey multiple comparison tests (A5) were applied to reveal and specify significant differences and similarities at $P \leq 0.05$ for the phenolic compounds and essential oil terpenes in *Solidago* spp.

The statistical package SPSS 17 and 20 (SPSS Inc., Chicago, USA), and Microsoft Office Excel 2010 (Microsoft, USA) were used for statistical processing and assessment of the received data (A2, A5). Multivariate statistical approaches using STATISTICA 10.0 (StatSoft Inc.) were performed to analyze data obtained during the assay of accumulation patterns of phenolic compounds and essential oil terpenes (A3, A4). The relationship between variables was analyzed using Spearman's rank correlation (A3).

2. SUMMARY OF RESULTS

2.1. Phenolic profiles in the leaves and inflorescences of *Solidago* L. species

Five phenolic compounds (chlorogenic acid, rutin, isoquercitrin, hyperoside, and quercitrin) were detected in the leaves and inflorescences of *S. canadensis* and *S. gigantea.* The results of the compound accumulations assay showed considerably higher ($P < 0.05$) values of chlorogenic acid, isoquercitrin, hyperoside, and quercitrin in the leaves and inflorescences of *S. gigantea* than *S. canadensis*. Comparing compound quantities in the two plant parts of *S. canadensis,* the difference was observed for chlorogenic acid and quercitrin, which were – significantly higher in the leaves than the inflorescences ($P < 0.05$). For *S. gigantea,* chlorogenic acid, and quercitrin quantities were higher in the leaves than in the inflorescences. In comparison, the quantities of isoquercitrin, rutin, and hyperoside were significantly higher in the inflorescences ($P < 0.05$).

Intraspecific diversity was comprehensively characterized by applying the chemometric processing techniques, namely hierarchical clustering and principal component analysis. Hierarchical cluster analysis was applied to the group accessions using mean quantities of phenolic compounds as clustering variables. HCA was performed individually for the leaves and flowers. HCA showed a significant difference between compound quantities in plant parts. The clustering of *S. canadensis* accessions divided them into four main clusters, both for leaves and inflorescences. The HCA for *S. gigantea* resulted in clustering the accessions into 3 clusters of leaves and 4 clusters of inflorescences. The identification of significant differences in the phenolic compounds among the clusters was performed. The significant differences among the leaf clusters of *S. canadensis* were detected for rutin, chlorogenic acid, and hyperoside, while for the inflorescences, the significant differences were for rutin and hyperoside. As for *S. gigantea*, significant differences were detected in the mean quantities of the phenolic compounds studied, except for isoquercitrin, among the clusters of both plant parts. The significant differences were found for chlorogenic acid and hyperoside. Inflorescences of *S. gigantea* highly differed in rutin and quercitrin. Differences among clusters of species plant parts were then specified (the results are presented in Table 4 in Article A1).

Principal component analysis was used to separate statistically independent variables for exploration of the relationships among the chemical compounds, thus allowing the identification of the primary predictors among

the investigated accessions. The score plots for the first two PCs explained 69.1 and 70.1% of the total variance of the chemical data of the *S. canadensis* leaves and inflorescences, respectively, showing relatively good separation between the evaluated accessions (the results are presented in Figure 3 in Article A1).

The score plots for the first two PCs, which explain 77.0 and 72.4% of the total variance in the data set of *S. gigantea* leaves and inflorescences, respectively, are shown in Figure 4 in Article A1. The results of the HCA and PCA were similar. On the other hand, graphical visualizations using PCA revealed a relatively simple representation of the structure of the evaluated species based on chemical data.

2.2. Development of the online high–performance liquid chromatography coupled radical scavenging assays for antioxidant potential evaluation in goldenrod

The critical factors impacting post-column implementation are post-column flow rate, the concentration of the derivatization reagents, reaction coil temperature, and its other parameters [31]. At the time, the temperatures used in the post-column reaction had no methodological justification for the chosen temperature limit for radical scavenging assays on plant materials [32, 33]. Temperature changes may negatively affect post-column reactions or the antioxidants themselves [34], therefore in this study, for the first time, the efficiency of the coil temperature and loop length was evaluated after the peak heights of the active compounds were established. The assay was designed to determine how the system temperature and loop length impact the change in peak heights for the HPLC-ABTS and HPLC-DPPH post-column assays. The initial system temperature was 25 °C, which was increased gradually every 10 °C until 55 °C was achieved for both assays. The length of the reaction coil was 3 and 20 m. Aside from temperature, another important parameter affecting the course of the reaction coil is its length and diameter since longer coils determine higher volume retention of the reaction mixture [35]. During the analysis, the need to include a temperature level of 50 °C was distinguished because in both ABTS and DPPH cases, after the temperature reached 55 °C, the decrease in peak heights was recorded. A radical scavenging response baseline noise level was observed during the analysis, ranging from 650 to 890 units (electrical signal micro-volts) using the 3-m loop and from 460 to 890 units using the 20 m coil. In both ABTS and DPPH cases, the lowest noise level was detected at 50 °C temperature, which indicates the system's stability and gives the most accurate antiradical response results of

Herba Solidaginis samples. Therefore, the 3 m loop with a temperature of 50 °C was optimal for evaluating the antioxidant activity of goldenrod raw materials using the ABTS reagent. This differs from the factors established under DPPH reagent conditions of 50 °C and 20 m. After validation of DPPH and ABTS post-column methods, it was confirmed that the methods could be considered appropriate and acceptable for the quantitative evaluation of the antiradical activity of compounds.

The developed DPPH and ABTS post-column assays were applied to evaluate methanol extracts of leaves and flowers of *S. canadensis*. Two unknown phenolic compounds, together with chlorogenic acid, rutin, and isoquercitrin, were detected in these extracts (the results are presented in Figure 1 in Article A2).

The observed Trolox equivalent antioxidant capacity (TEAC) values for detected constituents using the DPPH post-column assay were higher than those of the ABTS method application. The results demonstrated different antioxidant activities between the goldenrod leaf and flower methanol extracts when using the ABTS and DPPH post-column assay.

2.3. Accumulation patterns of phenolic compounds in hybrid *Solidago* L. species

Five phenolic compounds were detected in leaves and inflorescences of *S. gigantea, S. virgaurea*, *S. canadensis,* and *S.* × *niederederi*, namely rutin, chlorogenic acid, isoquercitrin, quercitrin, and hyperoside. All analyzed *Solidago* species shared the same common phenolic constituents. Leaves of goldenrods showed considerably higher mean values for chlorogenic acid than their inflorescences, whereas inflorescences showed significant priority in accumulating isoquercitrin. Significant quantitative differences ($P \leq 0.05$) were determined for mean quantities of all phenolic compounds in leaves and inflorescences among investigated species. The highest values of all studied phenolic compounds, except for rutin content in leaves and inflorescences, were presented in *S. gigantea* compared to the other investigated goldenrods. The highest accumulation of rutin was detected in *S.* × *niederederi* leaves and in *S. canadensis* inflorescences. *S. virgaurea* accumulated the lowest amounts of phenolic compounds, except for isoquercitrin in leaves, when compared with *S. canadensis* and *S.* × *niederederi*. *S.* × *niederederi* demonstrated the highest concentration of chlorogenic acid in leaves; however, the values of other phenolic compounds were average.

PCA was performed to detect groupings, similarities, or differences among all analyzed accessions of *Solidago* species. PCA models represented

the two-dimensional scatter plots that demonstrated differentiation between the chemical data sets of the four *Solidago* species (The results are presented in Figure 2 and Figure 3 in Article A3). PCA was applied as a chemometric technique allowing the description of data sets without preceding knowledge of the structure of this data and providing the visualization of the analytical data resemblance, which opens a possibility to indicate what compounds are the most responsible for the separation of different plant groups. The distant position of *S. gigantea* accessions on score plot PC1 versus score plot PC2 represented a significant difference in the chemical composition of phenolic compounds than found in other species. Meanwhile, the other three species formed a separate cluster, representing similarities in their quantitative accumulation of phenolic compounds. It can be deduced that interspecific chemical diversity among *Solidago* species is genetically based. However, the mode of qualitative inheritance of most chemical compounds is Mendelian with dominance; if both or one of the parents produces a chemical, the hybrids almost always produce it as well [36]. It can be assumed that the genes responsible for synthesizing phenolics are constitutively but differently expressed in the analyzed species [37]. The ploidy level is known to significantly affect the intraspecific variation in concentrations of metabolites in *S. gigantea* [38]. In this context, tetraploid *S. gigantea* demonstrated the highest mean phenolic contents. Meanwhile, diploid *Solidago* species showed relatively lower contents of evaluated phenolic compounds.

The additional PCA showed that based on the analyzed phenolics, *S. virgaurea* and *S. canadensis* were well distinguished from each other. Even though it is not uncommon for interspecific hybrids to be clearly distinguished from parental species based on the evaluation of their biochemical phenotypes [39], *S.* × *niederederi* leaves and inflorescences were in close position with *S. virgaurea,* which makes them a not very clearly defined group. The cluster of *S.* × *niederederi* accessions was less focused and demonstrated greater chemical variation, as some of the samples fell outside the range of the parental species, *S. virgaurea* and *S. canadensis* which corresponds to previous studies [40]. Mean chlorogenic acid content in leaves was significantly higher for *S.* × *niederederi* than the parental species and such results also correspond to previously reported studies [41, 42]. Our research suggested that *S.* × *niederederi* was characterized by the accumulation of phenolic compound contents intermediate or similar to that of *S. virgaurea*, suggesting its hybrid origin.

2.4. Consistent patterns of essential oil terpenes in plant organs of *Solidago* L. species

Inflorescences were superior to leaves in essential oil (EO) content in all *Solidago* spp. The highest yield ($P < 0.05$) of EO was obtained from inflorescences of *S. canadensis*, followed by *S. gigantea*, *S.* × *niederederi*, and *S. virgaurea.* Meanwhile, the leaves of *S. gigantea* accumulated the highest EO content, followed by *S. canadensis* and *S.* × *niederederi* (Table 2.4.1).

Table 2.4.1. *Essential oil yields in the leaves and inflorescences of S. canadensis, S. gigantea, S.* × niederederi*, and S. virgaurea*

Inflorescences of:	**EO Yield**
S. canadensis	0.19–0.26%
S. gigantea	0.16–0.23%
S. × *niederederi*	0.14–0.20%
S. virgaurea	0.15–0.18%
Leaves of:	**EO Yield**
S. gigantea	0.16–0.20%
S. canadensis	0.14–0.18%
S. × *niederederi*	0.13–0.15%
S. virgaurea	0.10–0.15%

Chemical profiles of essential oils

The total EOs composition of four *Solidago* spp. was dominated by monoterpenes (43.9–74.6% in inflorescences, 39.7–69.1% in leaves). Sesquiterpenes constituted 15.0–35.4% of total EO composition in inflorescences and 25.5–38.2% in leaves (The results are presented in Figure 1 in Article A4). The similarities and differences of EO constituent profiles among the four investigated goldenrod species are presented in Table 2.4.2. The results are similar to corresponding studies [43].

Table 2.4.2. *Terpene yields in the leaves and inflorescences of S. canadensis, S. gigantea, S. × niederederi and S. virgaurea*

Solidago gigantea	
Inflorescences	**EO Yield**
monoterpene hydrocarbons	25.4%
oxygenated sesquiterpenes	19.7%
oxygenated monoterpenes	18.5%
sesquiterpene hydrocarbons	15.3%
Leaves	**EO Yield**
oxygenated sesquiterpenes	23.3%
oxygenated monoterpenes	23.1%
Solidago canadensis	
Inflorescences	**EO Yield**
monoterpenoids	46.6.0%
monoterpenes	28.0%
Leaves	**EO Yield**
monoterpenoids	26.4%
sesquiterpenes	24.9%
Solidago × niederederi	
Inflorescences	**EO Yield**
oxygenated monoterpenes	41.5%
monoterpenes	26.4%
sesquiterpenoids	17.0%
sesquiterpenes	6.0%
Leaves	**EO Yield**
oxygenated monoterpenes	35.7%
monoterpenes	17.7%,
sesquiterpenoids	16.9%
sesquiterpenes	15.0%
Solidago virgaurea	
Inflorescences	**EO Yield**
monoterpenes	35.9%
sesquiterpenes	20.7%
Leaves	**EO Yield**
monoterpenes	49.0%
sesquiterpenes	16.9%

Interspecific Differences

The presented results demonstrated similarities and differences in the frequency of distribution and contents of EO constituents among the four *Solidago* spp. Significant differences were detected between monoterpene and sesquiterpene fractions of inflorescences for all species studied (Table 1 in Article A4). Meanwhile, the EO profiles in leaves revealed no significant differences between the four *Solidago* spp. tested (Table 2 in Article A4). Oxygenated monoterpenes predominated in the *S. canadensis* and *S.* × *niederederi* inflorescences, and *S. virgaurea* and *S.* × *niederederi* leaves. The highest proportion of sesquiterpenoids among the species accumulated in the inflorescences and leaves of *S. gigantea*. The common principal constituents in the inflorescence and leaf EOs of all four *Solidago* spp. were *α*-pinene, bornyl acetate, and caryophyllene oxide. The other major compound, *trans*-verbenol, was prevalent in all inflorescences, with the highest levels in *S. canadensis* and *S.* × *niederederi*. Inflorescences of *S. gigantea* accumulated the highest level of germacrene D compared to other species, while both inflorescences and leaves were in priority in proportions of oxygenated sesquiterpenes as epoxyazulene, spathulenol, and isospathulenol.

The inflorescence EOs of all species differed significantly in the mean percentages of oxygenated monoterpenes, such as *α*-campholenal, *trans*-pinocarveol, *cis*-verbenol, pinocarvone, verbenone, and *trans*-carveol, with the highest levels and frequency in *S. canadensis* and *S.* × *niederederi*, followed by *S. virgaurea*. In addition, *S. virgaurea* inflorescence EOs differed from other species in the highest levels and frequency of distribution of *α*- and *β*-copaene, cubebol, *α*-muurolene, and *δ*-cadinene. Consequently, quantitative rather than qualitative differences in EOs were observed between the *Solidago* spp. However, ten constituents common in more than 30% of all studied inflorescence and/or leaf EOs did not differ significantly between *Solidago* spp., among which the most abundant were α- and β-pinene, limonene, bornyl acetate, germacrene D, and caryophyllene oxide.

Multivariate data analysis allowed us to explain the intra- and interspecific diversity of four *Solidago* spp. based on selected variables that differ significantly between species and that represented constituents detected in 30% or more of EO samples. PCA revealed that *Solidago* spp. The phytochemical patterns complemented the evidence of *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*, with the higher contribution of alien species than native ones. In addition, *S.* × *niederederi* EOs indicated higher diversity than other species, suggesting that *S.* × *niederederi* is a continuously evolving taxon.

Solidago spp. differed in the presence of terpenes that could be considered species-specific components. The inflorescence EOs of *S. gigantea* differed from other species by γ-cadinene, γ-muurolene, and spathulenol, and the leaves by camphene, *o*-cymene, epoxyazulene, (*E*)-nerolidol, and spathulenol. The inflorescences of *S. canadensis* and *S.* × *niederederi* exposed differences in the accumulation of oxygenated monoterpenes, such as *α*-campholenal, *trans*-pinocarveol, pinocarvone, verbenone, *cis*-verbenol, *trans*-verbenol, and myrtenal. Furthermore, *S. canadensis* inflorescences were characterized by the prevalence of myrtenol, *trans*-carveol, and carvone, while *S.* × *niederederi* leaves were prominent by *trans*-verbenol. The species-specific constituents in *S. virgaurea* inflorescences were *α*- and *β*-copaene, *α*-cubebene, cubebol, *δ*-cadinene, and *α*-muurolene, and in the leaves – *α*-campholenal, *cis*-verbenol, *trans*-verbenol, pinocarvone, and *trans*-pinocarveol.

2.5. Interspecific antioxidant profiling of fast-acting radical scavengers in *Solidago* L. species

Identification of phenolic compounds was performed using UPLC-QTOF-MS. The results are presented in Table 1 in Article A5. Twenty-three phenolic compounds were identified after applying the negative ionization mode to samples of *Solidago* spp. Fourteen of these compounds demonstrated significant antioxidant activity. Mainly, the antioxidant profiles of leaves and inflorescences of *Solidago* species comprised mostly of caffeoylquinic acid and quercetin derivatives. 3,5-Dicaffeoylquinic acid comprising about 43% of total radical scavenging activity in leaves and 54% in inflorescence extracts, was the predominant radical scavenger in *S. virgaurea*, *S. canadensis* and *S.* × *niederederi* (Table 2 in Article A5). Chlorogenic acid, accounting for 32% of total inflorescence and 66% of total leaf activity, was determined as a predominant radical scavenger in *S. gigantea*. The flavonoid complex was mainly composed of quercetin derivatives. The antioxidant activity of quercetin derivatives was significantly lower than that of caffeoylquinic acids. Quercetin pentoside and hyperoside were the primary antioxidants in *S. gigantean* inflorescences and leaves. Rutin accounted for up to 11% of total radical scavenging activity in other evaluated *Solidago* spp. The amounts of corresponding compounds significantly differed among *Solidago* spp. The hierarchical analysis was applied separately to both leaves and inflorescences based on the mean quantities of Trolox antioxidant equivalents of phenolic compounds as clustering variables.

The clustering of the inflorescences resulted in the grouping into two main clusters (Figure 2 in Article A5), the first of which was composed of all inflorescence samples of *S. gigantea*. The clustering of leaves divided all samples into two clusters as well (Figure 3 in Article A5). The first cluster was composed of *S. gigantean* leaves. Cluster 2 was composed of leaves of the other 3 *Solidago* species. The second cluster was subdivided into well-defined sub-clusters of *S. canadensis*, *S. virgaurea,* and *S.* × *niederederi* leaves.

Hierarchical cluster analysis revealed a similarity of *S. virgaurea, S. canadensis*, and *S.* × *niederederi* in terms of the antioxidant activity of phenolic compounds. The antioxidant activity of *S. gigantean* was highly different from other *Solidago* species.

PCA was performed to detect similarities and differences among the analyzed samples according to statistically independent variables, indicating phenolic compounds' radical scavenging activity. The score plot models for inflorescence (PCA1) and leaf (PCA2) samples have shown relatively good separation of the four *Solidago* species (Figure 6 in Article A5).

PCA allowed us to create a graphical visualization of differences between *Solidago* species based on the antioxidant activity of phenolic compounds. *S. gigantea* leaves and inflorescences composed a separate cluster indicating different radical scavenging activity of phenolic compounds from three other *Solidago* spp. Meanwhile, *S. canadensis*, S. *virgaurea*, and *S.* × *niederederi* demonstrated similarity in the radical scavenging activity of their raw materials.

3. DISCUSSION

Two species of invasive goldenrods, *Solidago canadensis* L. (Canadian goldenrod) and *S. gigantea* Aiton (Giant goldenrod), are spread over all territory of Lithuania, leading to a critical threat to the biodiversity of native plant species. Alien *Solidago* species are considered among the most aggressive worldwide plant invaders, increasing the negative impact on the native flora [44, 45]. On the other hand, goldenrods possess bioactivity that can be exploited for medicinal purposes. The interest in plant-based products has increased significantly in recent years, and research into bioactive agents has evolved into an important occupation for improving human and animal well-being [46]. In this respect, invasive species are of growing interest as a potential resource for acquiring high-value-added products [47, 48].

According to scientific literature, several groups of bioactive compounds were detected in plant extracts of goldenrods, such as tannins, phenols, saponins, essential oils, etc. [12–14]. The plant materials of *S. gigantea* Aiton, *S. canadensis* L., and *S. virgaurea* L. are included in European Pharmacopoeia with therapeutic indications for the treatment of urinary tract and genital disorders, which is related to the biological action of flavonoids [15–17, 50–55, 57]. There are no reported toxicity and contraindications for goldenrod preparations; therefore, most studies were conducted on the native European goldenrod [6]. Phenolic acids and flavonoids are well known due to their strong ability to act against oxidative damage leading to various degenerative diseases [16, 18, 19, 26]. The results of this study revealed that plant of *Solidago* species accumulate a significant amount of caffeoylquinic acids, the chromatographic profiles of which can also be used as markers for species identification. Moreover, the levels of these acids and their antioxidant activity are of importance for the medicinal applications of goldenrods. Despite the importance, research on flavonoid constituents of the *Solidago* species is still limited, and there were no studies conducted on evaluating their chemical heterogeneity or comparative studies among species and their morphological parts, not only in Lithuania but also worldwide.

The research of native, invasive, and hybrid *Solidago* species revealed the remarkable diversity showing species-specific, plant-organ-specific, and activity-related profiles of phytochemical compounds. Our sequentially designed research covered the most important stages of phytochemical chemometric evaluations. The developed and validated HPLC, GC, and post-column assay methods were designed for qualitative and quantitative analysis

of phenolic compounds, essential oil components, and fast-acting antioxidants in the materials of *Solidago* L. species. The validated methods confirm their suitability for qualitative and quantitative analysis of the abovementioned compounds in *Solidago* species.

The results of phenolic profile determination in the leaves and inflorescences of *Solidago* L. species revealed the differences in the phenolic compound composition of two invasive goldenrods, *S. canadensis* and *S. gigantea.* Also, they confirmed the results of corresponding chemical evaluations of invasive species of goldenrods described in scientific literature, revealing that they are a rich source of phenolic compounds [8, 49, 56].

Five main phenolic marker compounds were detected: chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin. These compounds were the main criteria for determining and substantiating the origin of certain *Solidago* species in the later stages of the research. The chemometric analysis revealed significant differences between tested samples in the plant organ and plant genotype dimensions. Multivariate statistical data analyses, in combination with the chemical analysis, provided a relatively simple representation and interpretation of the structural similarities and differences among wild populations of *S. canadensis* and *S. gigantea*. These species' phenolic compound variety is much more restricted than other wild species due to the adaptive attractiveness of the new invasion areas of goldenrods [27–29].

Based on phenolic compound accumulations, the two widespread species of goldenrods did not demonstrate high intraspecific variation. In this regard, previous studies have shown that the chemical diversity of invasive goldenrods is relatively weak compared to the intraspecific morphological diversity of these species [20]. Such results, concerning differences in the morphological and chemical structure of the plants studied, can be interpreted in a way that the flexibility of morphological expression, compared to adaptation, is the main factor leading to the successful spread of invasive species [21]. In this regard, the presented secondary metabolites help protect the plants against fungal parasites, herbivores, and pathogens, UV radiation that that are more important for plant survival and reproduction rather than for invasive spread [58–61]. On the other hand, reduced heterogeneity in the chemical composition of goldenrods leads to an increase in the quality and safety of herbal material obtained from the wild [22, 62].

As mentioned before, phenolic compounds, due to their high antioxidant potential, are the target group compound in research of added value ingredients for food and pharma industries because of their broad spectrum of biological effects, especially the effect against oxidative stress, which is often associated with the origin of various degenerative diseases [1, 18]. Only the chemicals with considerable activity and instant reactions are particularly

included in the antioxidant profiles generated by post-column analysis. This approach is, therefore, suitable for the detection of high-capacity fast-acting antioxidants as well as the identification of antioxidant activity indicators. In this regard, antioxidant activity markers may help guarantee the efficacy of plant materials with potential health benefits that may be used in various industries. The high biomass produced by invasive *Solidago* spp. and the fact that the flowers of goldenrods attract pollinators and are bee honey plants that are not grown under treatment with pesticides makes them a potential source of renewable energy [63, 64].

According to a literature survey, a few published data would provide an assessment of the antioxidant activity of different *Solidago* species [5, 16]. The most reliable and comprehensive evaluation of the antioxidant activity of plant materials is the validation of highly efficient and accurate analytical methods. The HPLC post-column assay combined with different derivatization techniques is a method of choice for the determination of the antiradical scavenging capacity of plant materials [65]. A highly effective technique is obtained when a model oxidation system is created and combined with HPLC separation methods and online post-column detection methods for radical-scavenging compounds, which in this case revealed the importance of caffeoylquinic acid [31, 66, 67]. ABTS and DPPH are extensively used for antioxidant screening in plant extracts [32, 65, 68, 69]. By analyzing the individual components, HPLC assists in identifying the specific antioxidants responsible for the observed activity. Furthermore, the HPLC post-column assays enable the evaluation of the concentration of individual antioxidants and correlate it with their antioxidant activity, providing valuable insights into the relationship between concentration and efficacy.

The main goal in developing the online high-performance liquid chromatography coupled radical scavenging assays for antioxidant potential evaluation in goldenrod was to determine the parameters of the HPLC post-column derivatization technique. This aim was achieved through the assessment of the system temperature influence and the influence of reaction coil parameters on the capacity of the radical-scavenging screening ability in HPLC post-column DPPH and ABTS systems which were further used for the determination and evaluation of the antioxidant activity of goldenrod raw material extracts. When evaluating the results of the antioxidant assay, the quantitative results differed slightly, which is why both methods are suitable for determining antioxidant activity in plants of the *Solidago* genus. These studies showed that, while the length of the coil did not significantly affect the peak height of the individual radical scavengers from the goldenrod, the temperature in the reaction coil did serve as a relevant factor affecting the signal strength of the analytes after reacting with the DPPH and ABTS

radicals. The following characteristics of an optimized assay for determining the individual radical scavengers of raw goldenrod materials are crucial for further routine analysis, ensuring reliable and reproducible results. Validation tests verified that the relevant approach was accurate, sensitive, and targeted to be applied to future analyses of active antioxidant compounds in plant materials of a comparable genetic origin.

Solidago canadensis L. and *Solidago gigantea* Aiton are exceptionally successful invasive species worldwide and have a high potential to spread into new habitats, thus contributing to the global environmental changes that threaten biological diversity and normally functioning ecosystems [44, 70–72]. It is common for invasive species to produce hybrids with native plants, leading to the transfer of adaptations among invasive species. This process increases the invasive potential and can lead to a loss of genetic diversity and robustness of native flora, which altogether leads to a threat of extinction of native species and even evolutionary changes in spontaneous flora. When the hybrids become stable, they are considered new alien species [73–76]. *Solidago* × *niederederi* Khek is a natural hybrid between the *S. virgaurea* L. and alien *S. canadensis.* The new hybrid was first recorded in the early 20th century in Austria. Hybrid identification is complicated due to the exceptionally diverse morphology of *Solidago* species [30, 38, 77, 78]. Therefore, a phytochemical evaluation of a plant provides additional information for species identification after the genetic, morphologic, and other nonmorphological methods [79].

One of the primary objectives of this step of the research was to assess the origin of morphologically identified *S.* × *niederederi based on* phenolic compound accumulation and to reveal the chemophenetic value of some phenolics for the identification of *Solidago* taxa. To the best of our knowledge, our study provides the first phytochemical characterization of *S.* × *niederederi* and a comparison with other *Solidago* species growing in close sympatry with the hybrid. To determine the parental species of hybrid *S.* × *niederederi,* the comprehensive HPLC analysis of phenolic compounds of all goldenrod species collected: *S. gigantea, S. virgaurea*, *S. canadensis,* and *S.* × *niederederi* was performed. Those mentioned above, five key marker phenolic compounds in the leaves and inflorescences of the four plants were evaluated quantitively, and chemometric tools were applied to obtain data for parental species of *S.* × *niederederi* determination. The phytochemical pattern of phenolic compounds provided evidence of hybrid *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*. *Solidago* × *niederederi* is not a completely stabilized taxon in mixed *Solidago* populations leading to the continuous creation of new genotypes and evolutionary novelty. The intermediate chemical composition between spontaneous

S. virgaurea and invasive *S. gigantea* was not detected. Phytochemical profiling of *S.* × *niederederi* is a study that will serve for the further evaluation of the expression and inheritance of phenolic compounds in the second and subsequent hybrid generations. This study also shall contribute to future research on the dynamics of secondary metabolite expression during the hybridization process. *Solidago* is a suitable model genus to study the process of plant invasion, and the present study contributes to further investigations of genetic diversity and the role of secondary metabolite expression in invasive potential during hybridization.

A wide range of secondary metabolites has been detected in *Solidago spp*. raw materials, the most valuable of which are not only the aforementioned phenolic compounds but also essential oils [16, 80, 81]. Essential oils provide a wide range of biological activity and are considered functional ingredients in cosmetics and pharmaceuticals [82]. On the other hand, EOs expose the potential to be a green alternative for pesticides and herbicides or fungicides [43, 82–86]. Furthermore, the antitumor effects of the EOs of *S. canadensis* have been reported [14, 43, 86].

The study of consistent patterns of essential oil terpenes in plant organs of *Solidago* L. species was designed to identify the profiles of volatile constituents and the distribution of their distinctive agents in native and alien *Solidago* spp., together with assessing intraspecific and interspecific diversity of terpenes, as well as confirming the origin of the native hybrid *S.* × *niederederi* on the basis of a multivariate comparative analysis of EOs.

The close relationships between goldenrods are morphologically and phylogenetically reflected in the similarity of their phytochemical profiles [30, 87]. A comprehensive metabolomics approach indicated that the volatiles commonly found in various plant species have a high potential to accumulate in *Solidago* spp. EOs as well. On the other hand, there were compounds such as thymol and carvacrol, which were sporadically found in high levels in only a few accessions of *S.* × *niederederi* and *S. canadensis*. Thymol and carvacrol possess a broad spectrum of biological activity [88-91], giving plant material a high potential. More often, the lower proportion of monoterpenes compared to sesquiterpenes accounted for a higher proportion in *Solidago* spp. EOs. Oxygenated monoterpenes are the main phytotoxic active compounds in different species, which are highlighted as predictors of bioherbicides [91].

Consequently, *S. canadensis* and *S.* × *niederederi*, whose EOs differed from other species in oxygenated monoterpenes, suggested the potential of their raw materials for herbicidal activity. Meanwhile, terpene hydrocarbons were low phytotoxic compounds [88, 92]. On the other hand, recent findings reported that (*E*)-nerolidol and spathulenol, species-specific sesquiterpenoids

in *S. gigantean* EOs, indicated effective allelopathic and insecticidal activities [93–95]. The potential biological activity of EOs is associated with the presence of high-oxygenated compounds, as confirmed by a systematic review of phytotoxicity studies [96]. Consequently, EOs of *S. canadensis* and *S. × niederederi* have great potential in developing new bioproducts. Phytochemical profiling of plant raw materials provides essential information about the potential of these plants in developing new bioproducts.

As stated earlier, alien *Solidago gigantea* L. and *Solidago canadensis* Aiton originating from North America are highly distributed in Europe together with a natural hybrid *Solidago × niederederi* Khek between *S. virgaurea* and *S. canadensis* [30, 72, 97, 98], yet in Europe, the most commonly used and the most studied species is native *Solidago virgaurea* L. (European Medicines Agency, 2008). The qualitative and quantitative composition of phenolic compounds of goldenrods varies significantly and strongly depends on the species, plant part, ontogenetic development, and environmental conditions [5, 16, 53, 54, 99–101]. The phenolic profiles may differ because the latter elucidates the compounds with expressed activity, and their markers do not always correspond to the antioxidant activity markers [102]. Applying combined detection systems is important for detecting minor components in the phytochemical profile, which could possess strong antioxidant activity. HPLC post-column assays are widely used for the coupled determination of phytochemical and antioxidant profiles [102–106].

The study of interspecific antioxidant profiling of fast-acting radical scavengers in *Solidago* L. species was designed to identify and quantify antioxidant profile determination in leaves and inflorescences of four wild *Solidago* species populations in Lithuania and to estimate the potential importance of these plant raw materials as a source of antioxidants. As far as we know, this is the first comprehensive study of *S. canadensis, S. gigantea, S. virgaurea,* and *S.×niederederi* individual antioxidant profiles.

Quercetin and kaempferol derivatives are predominant compounds in phenolic profiles used to determine and standardize the quality of goldenrod extracts [15, 107, 108]. Due to their structure peculiarities, quercetin derivatives are regarded as potent antioxidants [109–111]. *S. gigantea* was distinguished from other *Solidago* spp. by the antioxidant activity of quercetin pentoside, quercitrin, and hyperoside. Rutin being radical scavenging flavonol, is the main antioxidant compound in the other three *Solidago* spp. The findings correspond to previous reports which declared rutin as the main flavonol in extracts of *S. virgaurea* and *S. canadensis* and absent in *S. gigantean* [5, 51, 54, 112]. According to our results, hyperoside was responsible for up to 11% of the total antioxidant activity in *S. gigantean* leaves, while in other species, its impact was less than 2%. Based on our results, hyperoside

could be considered a marker of radical scavenging activity for *S. gigantea*. On the contrary, Woźniak et al. (2018) reported that hyperoside was not detected in the root and above-ground samples of *S. canadensis* and *S. gigantea* [112].

Using UPLC-MS, three kaempferol derivatives (kaempferol deoxyhexose hexoside, kaempferol 3-*O*-arabinoside, and kaempferol 3-*O*-rhamnoside) were identified in the leaves and inflorescences of *Solidago* spp.; however, their antioxidant activities were not significant. Cinnamic acids (caffeoylquinic, coumaroylquinic, and feruloylquinic) and quinic acid esters are common in *Asteraceae* plants with various accumulation patterns [113]. Caffeoylquinic acids are considered potential antioxidants with neuroprotective and cardioprotective functions and anti-inflammatory effects and are potentially helpful in treating metabolic disorders [50, 114, 115]. The present study revealed that 3,5- and 3,4- dicaffeoylquinic acids had higher Trolox equivalent antioxidant values compared to monocafeoylquinic acids. The results are in agreement with Li et al. (2018) and Xu et al. (2012) data which declared that substitution with two caffeoyl groups significantly increased the antioxidant activity of corresponding compounds [116, 117].

Generally, the main goals of this part of the research were to analyze the origin of the hybrid identified for the first time in Lithuania, to check the prospect of different species of *Solidago* for consumption, to select suitable methods for the evaluation of active compounds, and to identify possible markers for ensuring the quality of plant raw materials. The whole research was carried out with leaves and inflorescences of four species of goldenrod (*S. gigantea, S. virgaurea*, *S. canadensis,* and *S.* × *niederederi*) collected in different regions of Lithuania. Extraction conditions were selected, the ESC method was modified for qualitative and quantitative evaluation of phenolic compounds, and conditions were adapted for ESC post-column methods (ABTS and DPPH) to evaluate active compounds' antioxidant activity. The detailed qualitative analysis of phenolic compounds was performed using the UPLC-QTOF-MS method, during which 23 compounds were identified. By applying the GC-MS method, the composition of essential oils was determined, and their percentage ratio was compared among different parts of different species of goldenrod.

After conducting qualitative, quantitative, and antioxidant activity tests of phenolic compounds and essential oils, it was discovered that *S. gigantea* differs from other *Solidago* species in the composition of its active compounds and their quantitative results. *S. gigantea* is richer in its composition of active compounds, and its quantitative and antioxidant activity results are superior to other plants of the species investigated. Statistical tests revealed that *S. virgaurea* and *S. canadensis* are closely related to *S.* × *niederederi*,

leading to the conclusion that the hybrid is derived from these goldenrod species, as they have the greatest similarities in both qualitative and quantitative results. This conclusion was confirmed by the two applied methods (ESC and DC), during which the statistical data confirmed the origin of *S.* × *niederederi*. ESC and ESC post-colony methods were validated, thus confirming their suitability and efficiency for researching plants belonging to the goldenrod genus.

The plants of the genus *Solidago* growing in Lithuania have insignificant interspecific differences in biochemical phenotypes. As a tool to ensure the quality of the goldenrod raw material, specific markers were identified, and their antioxidant activity was evaluated. According to the results of this study, the plant material of the genus *Solidago* growing in Lithuania is homogeneous and can be considered a potential medicinal plant material, which has not been widely used until now, despite its abundant and constantly renewing natural sources.

CONCLUSIONS

1. The HPLC method was developed and validated to identify the five predominant phenolic compounds (chlorogenic acid, rutin, hyperoside, isoquercitrin, quercitrin). Qualitative and quantitative analyses showed that the two species do not show significant interspecific differences.
2. The study modified the post-column HPLC-ABTS and HPLC-DPPH methods to accurately and efficiently assess the antioxidant activity of phenolic compounds accumulated by *Solidago* genus plants. The optimum conditions for the post-column reaction (the most suitable loop temperature is 50 °C (for both methods); for the ABTS method, the loop length is 3 m and for the DPPH method, 20 m) were determined to ensure a strong signal response.
3. Qualitative and quantitative HPLC analyses of four species of goldenrods (*S. virgaurea, S. canadensis, S.* × *niederederi* and *S. gigantea*) showed that the hybrid *S.* × *niederederi* (found in Lithuania for the first time) is the closest species to *S. virgaurea* and *S. canadensis* and therefore, *S.* × *niederederi* can be considered a hybrid of these two species.
4. A comparative study of volatile compound profiles (GC-MS) on four species of goldenrod (*S. virgaurea, S. canadensis, S.* × *niederederi* and *S. gigantea*) confirmed that *S.* × *niederederi* is an interspecies taxon between *S. canadensis* and *S. virgaurea*, with a higher metabolic contribution from *S. canadensis*. The hybrid *S.* × *niederederi* was found to have the highest diversity of essential oils.
5. A detailed determination of the phenolic compounds of the different plant materials from the different species of goldenrod was carried out by UPLC-MS. Leaf and inflorescence samples were analysed and 23 phenolic compounds were identified, 14 of which showed antioxidant activity as assessed by HPLC-ABTS. The identified markers are relevant for the quality assurance of the raw plant material and the evaluation of inter-species differences.

SANTRAUKA

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Analitiniai mokslai taikomi siekiant nustatyti ir įvertinti augaluose ir jų dalyse kaupiamas veikliąsias medžiagas, kurios ir suteikia augalui specifinį poveikį žmogaus gyvybinėms funkcijoms [1]. Tęstiniai etnofarmakologiniai tyrimai ne tik moksliškai pagrindžia augalinės kilmės veikliųjų medžiagų efektus poveikį, bet atskleidžia jų fitoterapinį potencialą. Visgi net ir su šiais pasiekimais, žmogaus gerovės siekimas ir toliau kelia iššūkį naujiems moksliniams neapibrėžtumams [2–4]. Mokslinės literatūros duomenimis, apytiksliai 70–95 proc. išsivysčiusių pasaulio šalių taiko gydymą augalinėmis vaistinėmis medžiagomis. Susidomėjimas sveika gyvensena, sveika mityba, funkciniu maistu, natūraliais maisto bei terapiniais produktais išsivysčiusiose šalyse nuolatos auga [5–7]. Manoma, kad natūralūs vaistiniai augaliniai preparatai ar vaistinių augalų mišiniai galėtų tapti alternatyva daugeliui sintetinių vaistinių preparatų [8]. Tokia ateities vizija yra paskata mokslininkams ieškoti naujų augalų, kurių kaupiami bioaktyvūs junginiai pasižymėtų gydomosiomis ir teigiamai žmogaus gyvybines funkcijas veikiančiomis savybėmis.

Kaip ir prieš kelis dešimtmečius, taip ir šiandien, vaistinių augalų biologiškai aktyvių komponentų tyrimai išlieka aktualūs dėl jų empirinio terapinio efektyvumo bei neišsenkamų resursų. Pasaulyje medicininiais tikslais jau naudojama per 35 000 augalų, tačiau į augalinės kilmės biologiškai aktyvias medžiagas atsigręžiama vis iš naujo, ieškant naujų gydymo priemonių ir gydymo algoritmų medicinos praktikoje, siekiant taikyti išplėstinę terapiją ir išvengti sintetinės kilmės gydomiesiems preparatams būdingų trūkumų ir apribojimų bei norint sumažinti nebūtinųjų intervencijų skaičių [9]. Vaistinių augalų panaudojimo iššūkiai, su kuriais susiduria tiek sveikatos apsaugos politika bei specialistai, tiek pacientai įvairiuose pasaulio regionuose, yra jų saugumas, efektyvumas, kokybė, prieinamumas, laikymas bei vartojimo reguliavimas [10].

Siekiant pagerinti pasaulinius sveikatingumo rodiklius, vaistiniai augaliniai preparatai turėtų būti tikslingai pasitelkiami formuojant prevencinius lėtinių ir degeneracinių ligų mechanizmus [11]. Augalinių preparatų efektyvumą ir saugumą lemia jų kokybė. Atitinkama augalinių vaistinių žaliavų kokybės kontrolė, paruošimo procesas kartu su kitais veiksniais, lemiančiais galutinio produkto kokybę, yra esminiai saugumo užtikrinimui, tačiau, svarbu paminėti, kad augalinių vaistinių žaliavų kokybės rodikliai gali kisti dėl įvairių vidinių ir išorinių veiksnių [10].

Sukauptos mokslinės žinios kelia vis daugiau naujų klausimų ir skatina atlikti išsamesnius augalų cheminės sudėties kokybinius ir kiekybinius tyrimus bei tyrinėti naujus augalinių vaistinių preparatų šaltinius su tikslinėmis pageidaujamomis savybėmis.

Rykštenės (*Solidago* L*., Asteraceae*) genties augalai įvairiuose Europos regionuose priskiriami tradiciniams augaliniams vaistiniams preparatams, o jų cheminė sudėtis pasaulyje jau kelis dešimtmečius tyrinėjama įvairių mokslinių grupių. Gauti duomenys yra naudojami siekiant praplėsti rykštenės augalinių žaliavų panaudojimo galimybes medicinoje [12–14].

Tradicinėje medicinoje rykštenė yra naudojama dėl savo antimikrobinių, diuretinių, žaizdų gijimą skatinančių bei uždegimą slopinančių savybių. Iš rykštenių išgauti fenoliniai junginiai gali būti pritaikyti įvairių sisteminių urologinių, onkologinių ar infekcinių sutrikimų gydymui bei prevencijai [14, 16–19]. Minėtas savybes lemia kompleksinis fitocheminis profilis, kuriame dominuoja fenoliniai junginiai – flavonoidai, antocianinai ir fenolinės rūgštys. Rykštenėje nustatyti didžiausi kiekiai chlorogeno rūgšties, kvercetino, rutino bei hiperozido, kurie pasižymi stipriu antioksidantiniu poveikiu, todėl mokslinėje literatūroje rykštenės genties augalai priskiriami vertingiems antioksidantinio poveikio junginių šaltiniams [14, 16, 17]. Rykštenių terpenų profilį sudaro oleanano tipo triterpeniniai junginiai, diterpenoidų laktonai ir eteriniai aliejai. Kanadinės rykštenės lapų eteriniame aliejuje nustatyta germakreno D, bornil acetato ir D-limoneno [20].

Rykštenės genties augalų cheminės sudėties kokybiniam ir kiekybiniam įvertinimui naudojami šiuolaikiniai chromatografinai metodai – efektyvioji skysčių chromatografija (ESC – angl. *HPLC*), dujų chromatografija (DC – angl. *GC*), efektyvioji skysčių chromatografija-masių spektrometrija (ESC-MS – angl. *HPLC-MS*), dujų chromatografija-masių spektrometrija (DC-MS – angl. *GC-MS*) [14, 21, 22]. Siekiant įvertinti chemines charakteristikas ir jų priimtinumą, kuriami analitiniai metodai [23].

Solidago gentį sudaro apie 330 rūšių ir vidurūšinių taksonų [24]. Kai kurios šių rūšių pasaulyje yra naudojamos kaip vaistiniai augalai. *Solidago virgaurea* yra kilusi iš Eurazijos, tuo tarpu *Solidago canadensis* ir *Solidago gigantea* – iš Šiaurės Amerikos. *Solidago* L. genties augalai Lietuvoje bei likusioje Europoje priskiriami invazinių (arba svetimžemių) augalų grupei [8, 13, 14, 24]. Invazinių augalų populiacijos didėjimas yra svarbi problema vietinei augmenijai, kadangi užimami papildomi augimo plotai, pakeičiama dirvožemio struktūra, todėl skursta ir nyksta vietinė augmenija. Svetimžemių augalų tyrimai padeda sukurti priemones jų populiacijos dinamikai kontroliuoti: išsamesni tyrimai, tokie, kaip cheminės sudėties analizė, suteikia galimybę įvertinti šių augalų prisitaikymo galimybes nevietinėje ir kintančioje aplinkoje, o išsamūs hibridinių augalų cheminės sudėties tyrimai leidžia

suprasti genotipinį plastiškumą ir įvertinti kaip kinta augalo fitocheminės savybės jiems prisitaikant prie vietinės gamtos sąlygų [12, 13, 25, 26]. Remiantis literatūroje aprašytų mokslinių tyrimų rezultatais, invazinių augalų naikinimo programos galėtų būti pakeičiamos vertingų augalinių žaliavų surinkimo programomis ir tvariu panaudojimu.

Rykštenės, augančios Lietuvos ir kituose regionuose, yra mažai tyrinėtos, dėl to, įvertinant jų cheminę kompoziciją, itin svarbu nustatyti Lietuvos klimato sąlygomis augančių rykštenės genties augalų cheminio heterogeniškumo mastą, galimus chemotipus, kiekybinių profilių variabilumą. Atliekama daugiau ir išsamesnių tyrimų, skirtų įvertinti tiek kompleksinę, tiek pavienių junginių antioksidantinę galią rykštenės genties augaluose. Tokiems tyrimams atlikti reikalingos kiek įmanoma tikslesnės pokolonėlinės metodikos, kurios leistų preciziškai išnagrinėti tiek kokybinę, tiek kiekybinę augalo kompoziciją [16, 17, 21, 27–29]. Derinant fizikocheminius ir statistinius analitinius metodus, galima įvertinti ne tik augalinių žaliavų bioaktyvumą, bet ir homogeniškumą bei saugumą – tai yra atspirties taškas tolimesniam augalinių preparatų vystymui.

Pagrindiniai atlikto mokslinio darbo tyrimų etapai yra (1) *S. virgaurea*, *S. canadensis*, *S. gigantea*, *S.* × *niederederi,* natūraliai augančių Lietuvoje, lapų ir žiedynų fenolinių junginių, antioksidantų ir eterinių aliejų terpenų profilių ištyrimas; (2) chemofenetinių kitimų bei ryšio tarp augalo genotipo ir kaupiamų cheminių junginių pasiskirstymo dėsningumų įvertinimas, pasitelkiant chemometrinę analizę.

Identifikuojant augalų rūšis ir nustatant jų kilmę, fenoliniai junginiai gali atlikti fitocheminių žymenų funkciją. Siekiant tiksliai kokybiškai įvertinti augalines žaliavas bei galimas jų panaudojimo sritis, būtina suprasti augalų fitocheminės sudėties įvairavimo tendencijas ir sistemiškai išanalizuoti gautus duomenis, padėsiančius įvertinti šių augalinių žaliavų perspektyvas maisto ir farmacijos pramonėje.

Darbo tikslas – išvystyti ir validuoti analitines matodikas, skirtas įvertinti vietinių, invazinių ir hibridinių *Solidago* L. augalinių žaliavų fitocheminės sudėties įvairavimus, antioksidantinį aktyvumą ir žymenų pasiskirstymo modelius.

Darbo uždaviniai:

1. Išvystyti ir validuoti ESC-PDA metodą, skirtą kiekybiniam ir kokybiniam skirtingų rykštenės rūšių fenolinių junginių kompozicijų įvairumų įvertinimui.
2. Išvystyti ir validuoti ESC pokolonėlinę metodiką, skirtą antioksidantiniu aktyvumu pasižymintiems junginiams nustatyti ir įvertinti rykštenės augalinėje žaliavoje.

3. Nustatyti chemofenetines hibridinės *Solidago* rūšies savybes, reikalingas šios rūšies tiksliam identifikavimui.
4. Įvertinti *Solidago* rūšių, augančių mišriose Lietuvos populiacijose, lakiųjų junginių profilius.
5. Pritaikyti UESC-MS ir ESC pokolonėlinius metodus, kiekybiškai ir kokybiškai įvertinti laukinėse Lietuvos populiacijose augančių *Solidago* rūšių lapų ir žiedynų antioksidantinius profilius.

Praktinė ir teorinė reikšmė

Atlikto mokslinio tyrimo metu sukurtos ir validuotos ESC ir ESC pokolonėlinės metodikos, svarbios fitocheminių junginių kokybiniams ir kiekybiniams tyrimams. Atliktas metodikų validavimas ir įvertintas jų patikimumas užtikriną šių metodikų rezultatų tikslumą, efektyvumą ir atkartojamumą. Pasirinkus optimalius antioksidantinio aktyvumo vertinimo metodų veiksnius – reakcijos kilpos ilgį, temperatūrą, reagento koncentraciją ir srauto greitį užtikrinamas tikslus junginių atskyrimas ir kiekybinis įvertinimas. Tyrimo metu taip pat pritaikytas DC-MS metodas *Solidago* L. genties augalų eterinių aliejų komponentų įvertinimui bei taikytas UESC-MS metodas išsamiam šių augalų fenolinių junginių nustatymui.

Atlikti *Solidago* L. genties fenolinių junginių ir eterinių komponentų tyrimai yra būtini, norint užtikrinti tikslingą augalinės žaliavos rinkimą ir jos farmakognostinę vertę. Fenolinių junginių antioksidantinis aktyvumas lemia augalinės žaliavos kokybę ir praktinę reikšmę fitoterapijoje. Kiekybinis fenolinių junginių ir eterinių komponentų įvertinimas skirtingose augalo dalyse parodo jų kaupimosi dėsningumus ir nulemia augalinės žaliavos ruošimo metodiką. Svetimžemių rykštenių tyrimai prisideda prie naujų augalinių žaliavų paieškos, plečia jų tausaus ir optimalaus naudojimo galimybes, reguliuoja invazinių augalų populiaciją, didina mokslinių tyrimų pritaikymo praktikoje galimybes. Augalinės žaliavos cheminės sudėties ir morfologinių savybių priklausomybės įvertinimas gali būti naudingas žaliavų atrankai ir kokybės standartizavimui. Augalų cheminės sudėties nustatymas svarbus ne tik vertinant rykštenes kaip vaistinę augalinę žaliavą, bet ir atliekant rykštenių chemofenetinius tyrimus ir nagrinėjant sudėtingą šių rūšių taksonomiją.

Rykštenių biologiškai aktyvių junginių rinkinio įvertinimas pasitelkiant skirtingus ESC pokolonėlinius metodus parodė, kad šios augalinės žaliavos ekstraktai pasižymi greitos poveikio reakcijos antioksidantiniu potencialu. Greta farmakopėjoje reglamentuojamų flavonoidų, tyrimo metu buvo nustatytas kafeoilchino rūgščių, pasižyminčių antioksidantiniu aktyvumu, profilis, kuris gali būti vertinamas kaip kokybės rodiklis. Antioksidantinio aktyvumo

žymenų nustatymas gali padėti užtikrinti augalinių medžiagų, naudojamų sveikatinimo ir kitose pramonės šakose, veiksmingumą.

Atlikti tyrimai ne tik suteikia naujų žinių apie rykštenių antioksidantines savybes, bet ir praplečia antioksidantinėmis savybėmis pasižyminčių augalinių žaliavų šaltinius ir jų panaudojimo perspektyvas.

Mokslinis naujumas

Mūsų žiniomis, toks išsamus daugialypis cheminiais duomenimis pagrįstas vietinių, invazinių ir hibridinių rykštenių fitocheminės įvairovės interspecifinės ir intraspecifinės įvairovės vertinimas yra atliktas pirmą kartą. Tyrimo metu įgytos naujos mokslinės žinios yra pamatas siekiant sukurti funkcinius produktus ir fitopreparatus su rykštenių augaline žaliava.

Atlikto mokslinio tyrimo metu buvo parinkta ir validuota ESC metodika, skirta *Solidago* L. genties augalinės žaliavos kokybinei ir kiekybinei sudėčiai analizuoti. Atliekant šį tyrimą, pirmą kartą įvertinta reakcijos kilpai taikomo temperatūrinio režimo įtaka ESC pokolonėlinei reakcijai. Lietuvoje natūraliai augančios rykštenės genties atstovai – *S. canadensis, S. gigantea* ir *S. virgaurea* iki šiol nebuvo tyrinėti. Nebuvo duomenų apie svetimžemių rykštenių populiacijų fitocheminę įvairovę Lietuvoje bei augalinės žaliavos panaudojimo galimybes. Moksliniame darbe įvertinta įvairių veiksnių įtaka *Solidago* L. genties augalų lapų ir žiedų antioksidantinėms savybėms. Atlikti Lietuvos klimatinėmis sąlygomis natūraliai augančių *Solidago* L. rūšių fenolinių junginių ir antioksidantinėmis savybėmis pasižyminčių veikliųjų junginių kokybiniai ir kiekybiniai tyrimai. Tyrimo metu pirmą kartą nustatyti pagrindiniai *S. virgaurea* antioksidantinį poveikį lemiantys fitokomponentai ir žymenys.

Autoriaus indėlis

Autoriaus, Mindaugo Marksos, indėlis pateikiamas pagal susijusias su disertacija publikacijas. Publikacijos A1–A5 pateiktos skyriuje *List of scientific papers*.

A1: Ekstrakcijos sąlygų parinkimas, ESC metodo vystymas ir validacija, mėginių paruošimas, kokybinis ir kiekybinis įvertinimas, bendras rankraščio rašymas ir pateikimas.

A2: ESC pokolonėlinės metodikos vystymas ir validacija, mėginių paruošimas, kokybinis ir kiekybinis įvertinimas, bendras rankraščio rašymas ir pateikimas.

A3: Mėginių paruošimas, kokybinis ir kiekybinis įvertinimas, bendras rankraščio rašymas ir pateikimas.

A4: Metodo vystymas ir validacija, mėginių paruošimas, kokybinis ir kiekybinis įvertinimas ir duomenų palyginimas.
A5: Mėginių paruošimas ir analizė ESC pokolonėlinių metodu, duomenų analizė ir apdorojimas.

Metodai

Augalinė *S. canadensis*, *S. gigantea, S. virgaurea* ir *S. × niederederi* Khek žaliava buvo surinkta iš natūralių rykštenių augaviečių Lietuvoje. Augalai identifikuoti remiantis jų morfologija. Atskirtos ir 25 °C temperatūroje išdžiovintos tyrimams naudojamos augalų dalys, kurios vėliau susmulkintos iki homogeniškų miltelių.

Ekstrakcija vykdyta metanolio ir vandens tirpalu (70:30 v/v): 0,1 g augalinės žaliavos užpilta 10 ml ekstrakcijos tirpalo ir 50 min. patalpinta į ultragarsinę vonelę (Witeg Labortechnik GmbH, WUC-A06H, Vokietija) 25 °C esant temperatūrai.

Tyrimams reikalingi eteriniai aliejai išskirti 30 g augalinės žaliavos hidrodistiliuojant 3 val. Clevenger tipo aparatu. Analitiniai mėginiai paruošti 1,0 µl eterinio aliejaus užpilant 1,0 ml n-heksano.

ESC-PDA sąlygos: analizė atlikta A Waters Alliance 2695 (Waters Milford, JAV) su Waters 996 PDA diodų matricos detektoriumi. Mobili fazė – 0.05% trifluoracto rūgšties vandeninis tirpalas, 100% acetonitrilas. Junginių išskirstymui buvo pritaikytas gradientinis eliuavimas. Tėkmės greitis – 1 ml/min., injekcijos tūris – 10 µl, kolonėlės temperatūra – 25 °C. Veikliųjų junginių smailės identifikuotos 210–550 nm šviesos bangos ilgio intervale, lyginant jų spektrinius duomenis ir sulaikymo laikus su standartų spektriniais duomenimis ir sulaikymo laikais. Nustatytų junginių kiekybinis įvertinimas atliktas išorinio standarto metodu.

Atlikta metodo validacija. Įvertinti validacijos parametrai: specifiškumas, tiesiškumas, preciziškumas, atsikartojamumas, aptikimo riba, nustatymo riba.

ESC-PDA-ABTS/DPPH sąlygos: analizė atlikta Waters Alliance 2695 (Waters Milford, JAV) su Waters 996 PDA diodų matricos detektoriumi, ESC pokolonėlinei analizei naudotas Waters 2487 UV/VIS detektorius (Waters Corporation). Junginių išskirstymas atliktas su YMC-Pack ODS-A kolonėle. Mobili fazė – 0,05 proc. trifluoracto rūgšties vandeninis tirpalas ir 100 proc. acetonitrilas. Junginių išskirstymui buvo pritaikytas gradientinis eliuavimas. Tėkmės greitis – DPPH 1 ml/min., ABTS 0,5 ml/min., injekcijos tūris – 10 µl, kolonėlės temperatūra – 25 °C.

Chromatografinės smailės identifikuotos lyginant analičių sulaikymo laikus ir spektrinius duomenis su atitinkamų standartinių junginių spektriniais duomenimis (šviesos bangos ilgio intervalas 210–400 nm) ir sulaikymo laikais. Fenolinės rūgštys ir flavonoidai nustatyti esant 324 ir 355 nm šviesos bangos ilgiams. ESC-PDA ir ESC pokolonėlinių metodų validacijos parametrai: specifiškumas, tiesiškumas, preciziškumas, atsikartojamumas, aptikimo riba, nustatymo riba.

DC-MS sąlygos: kiekybinė eterinių aliejų analizė atlikta su DCMS-QP 2010 Ultra su Shimazu autoinjektoriumi AOC-5000 (Shimadzu, Europa GmbH). Kapiliarinė kolonėlė RXi-5MS (30 m × 0,25 mm i.d. × 0,25 μm v.d.) (Restek, Bellefonte, PA, JAV). Masių spektrometro jonizacijos įtampa – 70 eV, skenavimo greitis 2500 *m/z*, masių spektro intervalas – 29–500 *m/z*, skenavimo laikas – 0,2 s.

Eterinių aliejų komponentai buvo identifikuoti naudojant masių spektrų junginių bibliotekas NIST 14, FFNSC, WR10, WR10R, ir nustatant junginių Kovats'o indeksus. Junginių kiekis įvertintas procentiškai, lyginant identifikuotų junginių smailių plotą su nustatytu bendru visų junginių smailių plotu.

ESC-PDA-ABTS and ESC pokolonėlinės analizės sąlygos: analizė atlikta Waters Alliance 2695 (Waters Milford, JAV) su Waters 996 PDA diodų matricos detektoriumi, ESC pokolonėlinei analizei naudotas Waters 2487 UV/VIS detektorius (Waters Corporation). Junginių išskirstymas atliktas su YMC-Pack ODS-A kolonėle. Mobili fazė – 0,05 proc. trifluoracto rūgšties vandeninis tirpalas ir 100 proc. acetonitrilas. Tėkmės greitis – 1 ml/min., injekcijos tūris – 10 μl, kolonėlės temperatūra – 25 °C. Po PDA analizės mobili fazė kartu su mėginiu patenka į reakcijos kilpą, kurioje yra maišoma su ABTS darbiniu tirpalu, veiklieji junginiai nustatomi prie 650 nm šviesos bangos ilgio.

UESC-QTOF-MS sąlygos: analizė atlikta Waters Aquity UPLC sistema su PDA detektoriumi (Waters, Milford, Mass., JAV), junginiai analizuoti MAXIS 4G QTOF masių spektrometru (BRUKER DALTONIK GmbH, Bremen, Vokietija) su ESI jonizacijos šaltiniu. Chromatografinė kolonėlė – Waters Aquity HSS T3. Gradientiniam eliuavimui naudota 2 proc. acto rūgštis ir acetonitrilas.

Duomenų statistinė analizė atlikta su SPSS 17 (SPSS Inc., JAV), STATISTICA 10.0 (StatSoft Inc.), Microsoft Office Excel 2010 (Microsoft, JAV). Naudoti metodai – ANOVA, Stjudento *t*-testas, *post-hoc* Scheffe'o testas, hierarchinė klasterių analizė, principinių komponenčių analizė.

Diskusija ir rezultatų apžvalga

Pirmajame atlikto mokslinio tyrimo etape buvo pritaikyta ir validuota ESC metodika, kurios pagalba kiekybiškai ir kokybiškai įvertinti Lietuvoje paplitusių svetimžemių *S. canadensis* ir *S. gigantea* kaupiami fenoliniai junginiai, nustatyti jų intraspecifiniai ir interspecifiniai cheminės įvairovės dėsningumai. *S. canadensis* ir *S. gigantea* lapuose ir žiedynuose nustatyti penki fenoliniai junginiai: chlorogeno rūgštis, rutinas, izokvercitrinas, hiperozidas ir kvercitrinas. Remiantis *t*-testo rezultatais, *S. gigantea* lapuose ir žiedynuose visų šių junginių, išskyrus rutiną, reikšmės yra didesnės nei *S. canadensis*. Lyginant junginių kiekius skirtingose *S. canadensis* augalų dalyse, pastebėtas skirtumas tarp chlorogeno rūgšties ir kvercitrino – lapuose jų buvo žymiai daugiau nei žiedynuose. *S. gigantea* atveju, chlorogeno rūgšties ir kvercitrino kiekiai buvo reikšmingai didesni lapuose, o izokvercitrino, rutino ir hiperozido – žiedynuose.

Hierarchinė klasterinė analizė (HKA – angl. *HCA*) buvo atlikta atskirai lapams ir žiedynams, pasitelkiant vidutinius fenolinių junginių kiekius kaip klasterizacijos kintamuosius. HKA atskleidė reikšmingus junginių kiekių skirtumus tarp augalų dalių grupių. *S. canadensis* suskirstyta į keturias grupes pagal lapų mėginių fitocheminių tyrimų rezultatus ir į keturias grupes – pagal žiedynų mėginių; *S. gigantea* mėginiai suskirstyti į 3 lapų grupes ir 4 žiedynų grupes. Reikšmingi fenolinių junginių skirtumai tarp klasterių buvo nustatyti naudojant ANOVA (angl. *Analysis of Variance*). *S. canadensis* reikšmingiausi skirtumai nustatyti tarp lapų klasterių vertinant rutino, chlorogeno rūgšties ir hiperozido kiekius, o tarp žiedynų klasterių – vertinant rutino ir hiperozido kiekius. Vertinant *S. gigantea* tyrimų rezultatus, nustatyti reikšmingi chlorogeno rūgšties, rutino, hiperozido ir kvercitrino skirtumai tarp abiejų augalų dalių grupių klasterių. Didžiausi reikšmingi skirtumai tarp lapų klasterių nustatyti chlorogeno rūgščiai ir hiperozidui, o tarp žiedų klasterių – rutinui ir kvercitrinui. Pagrindinių komponenčių analizė (PKA – angl. *PCA*) buvo pritaikyta cheminių junginių ryšiams tirti, taip pat siekiant nustatyti pirminius prognozuojančius veiksnius tarp tiriamųjų mėginių. Pirmųjų dviejų *S. canadensis* komponenčių analizių rezultatai paaiškino atitinkamai 69,1 ir 70,1 proc. bendros lapų ir žiedynų cheminių duomenų dispersijos, o pirmųjų dviejų *S. gigantea* komponenčių analizių rezultatai paaiškino atitinkamai 77,0 ir 72,4 proc. bendros lapų ir žiedynų duomenų rinkinio dispersijos. HKA ir PKA rezultatai parodė panašią tendenciją. Grafinės vizualizacijos naudojant PKA atskleidė gana paprastą vertinamų rūšių cheminės struktūros vaizdą (rezultatai pateikiami 3 ir 4 paveiksluose publikacijoje A1).

Šio tyrimo rezultatai atskleidė dviejų invazinių rykštenių – *S. canadensis* ir *S. gigantea* – fenolinių junginių sudėties skirtumus. *S. gigantea* nustatyta

žymiai daugiau visų, išskyrus rutiną, analizuotų junginių, nei *S. canadensis*, tuo tarpu rutino kiekis *S. canadensis* buvo reikšmingai didesnis. Jungtinė daugiamatė statistinių duomenų ir cheminė analizė pateikė gana paprastą struktūrinių panašumų ir skirtumų tarp laukinių *S. canadensis* ir *S. gigantea* populiacijų atvaizdavimą ir interpretavimą.

Dvi paplitusios rykštenių rūšys – *S. canadensis* ir *S. gigantea* – nepasižymėjo dideliais tarprūšiniais skirtumais, vertinant jų kaupiamų fenolinių junginių kiekius. Šiuo atžvilgiu tyrimai parodė, kad invazinių rykštenių cheminė įvairovė yra gana siaura, lyginant su šių rūšių morfologine įvairove [20]. Tokie tiriamų augalų morfologinės ir cheminės struktūros skirtumai gali būti interpretuojami darant išvadą, kad morfologinės raiškos lankstumas (lyginant su prisitaikymu) yra pagrindinis veiksnys, lemiantis sėkmingą invazinių rūšių plitimą [21]. Nustatyti antriniai metabolitai padeda apsaugoti augalus nuo grybelių, parazitų, žolėdžių ir patogenų, UV spindulių, kurie yra svarbesni augalų išlikimui ir dauginimuisi, o ne invaziniam plitimui [58–61]. Kita vertus, siauresnė rykštenių cheminės sudėties įvairovė lemia aukštesnę augalinės žaliavos kokybę ir saugumą [22, 62].

Antrajame atlikto mokslinio tyrimo etape buvo išvystytos DPPH ir ABTS pokolonėlinės metodikos, skirtos rykštenės genties augalų antioksidantiniam aktyvumui įvertinti. Svarbiausi veiksniai, lemiantys pokolonėlinės analizės tikslumą, yra pokolonėlinis tėkmės greitis, derivatizacijos reagentų koncentracija, reakcijos kilpos temperatūra ir kiti jos parametrai [31]. Iki šiol, vykdant antioksidantinius augalinių medžiagų tyrimus, pasirenkami temperatūriniai režimai, taikomi pokolonėlinei reakcijai, neturėjo metodologinio pagrindimo [32, 33]. Temperatūros pokyčiai gali neigiamai paveikti pokolonėlines reakcijas arba pačių antioksidantų stabilumą [34]. Šiame tyrime pirmą kartą buvo įvertinta, kaip sistemos temperatūra ir kilpos ilgis keičia triukšmo ir aukščio santykį ESC-ABTS ir ESC-DPPH pokoloninio tyrimo metu. Pradinė sistemos temperatūra buvo 25 °C, ji buvo palaipsniui didinama po 10 °C, kol abiem minėtiems tyrimams buvo pasiekta 55 °C temperatūra. Reakcijos kilpos ilgis buvo 3 ir 20 m. Be temperatūros, kitas svarbus parametras, turintis įtakos reakcijos eigai, yra kilpos ilgis ir skersmuo, nes ilgesnės kilpos lemia ilgesnį reakcijos laiką [35]. Atliekant analizę, nustatytas poreikis įtraukti 50 °C temperatūrinį režimą, nes tiek ABTS, tiek DPPH atvejais, temperatūrai pasiekus 55 °C, fiksuojamas triukšmo ir aukščio santykio mažėjimas. Visos analizės metu buvo stebimas antioksidantinio atsako triukšmo ir aukščio santykis, kuris svyravo nuo 650 iki 890 vienetų naudojant 3 m kilpą, ir nuo 460 iki 890 vienetų naudojant 20 m kilpą. Tiek ABTS, tiek DPPH atvejais mažiausias triukšmo lygis nustatytas esant 50 °C temperatūrai, o tai rodo sistemos stabilumą ir duoda tiksliausius *Herba Solidaginis* mėginių antioksidantinio atsako rezultatus. Taigi nustatyta, kad 3 m kilpa

esant 50 °C temperatūrai yra tinkamiausios sąlygos vertinant rykštenės antioksidantinį aktyvumą, naudojant ABTS reagentą. Tuo tarpu naudojant DPPH reagentą, nustatytos tinkamiausios sąlygos yra 50 °C temperatūra ir 20 m kilpos ilgis. Patvirtinus DPPH ir ABTS pokolonėlinius metodus, nustatyta, kad metodai yra tinkami kiekybiniam junginių antioksidantiniam aktyvumui įvertinti.

S. canadensis lapų ir žiedynų metanolinių ekstraktų vertinimui pritaikytos sukurtos DPPH ir ABTS pokolonėlinės metodikos. Šiuose ekstraktuose buvo nustatyta chlorogeno rūgštis, rutinas, izokvercitrinas ir du neidentifikuoti fenoliniai junginiai (rezultatai pateikiami 1 paveiksle publikacijoje A2). Įvertintos TEAC (angl. *Trolox equivalent antioxidant capacity*) vertės nustatytiems junginiams taikant DPPH pokolonėlinį metodą buvo didesnės, nei taikant ABTS metodą. Vertinant *t*-testo rezultatus, nustatytas skirtingas rykštenės lapų ir žiedynų metanolinių ekstraktų antioksidantinis aktyvumas, kai buvo taikomi ABTS ir DPPH pokolonėliniai metodai. Panaši antioksidantinio aktyvumo dinamika stebėta visiems junginiams, naudojant ABTS ir DPPH pokolonėlinius metodus.

Trečiajame atlikto mokslinio tyrimo etape įrodyta fenolinių junginių chemotaksonominė vertė, kur remiantis fenolinių junginių profiliais, buvo nustatyta hibridinės *S.* × *niederederi* kilmė. Išanalizavus *S. gigantea*, *S. virgaurea*, *S. canadensis* ir *S.* × *niederederi* lapus ir žiedynus, nustatyti penki fenoliniai junginiai: rutinas, chlorogeno rūgštis, izokvercitrinas, kvercitrinas ir hiperozidas. Visos analizuotos *Solidago* rūšys kaupia tuos pačius fenolinius junginius. Vidutinės chlorogeno rūgšties vertės rykštenių lapuose buvo gerokai didesnės nei jų žiedynuose, o žiedynai kaupė daugiau izokvercitrino. Rezultatai parodė reikšmingus kiekybinius skirtumus ($p \leq 0{,}001$) tarp tirtų rūšių visų fenolinių junginių kiekių lapuose ir žiedynuose. Lyginant su kitomis tirtomis rūšimis, didžiausios visų tirtų fenolinių junginių vertės, išskyrus rutino kiekį lapuose ir žiedynuose, nustatytos S. *gigantea*. Didžiausias rutino kiekis nustatytas *S.* × *niederederi* lapuose ir *S. canadensis* žiedynuose. *S. virgaurea* kaupė mažiausius fenolinių junginių kiekius, išskyrus izokvercitriną lapuose, lyginant su *S. canadensis* ir *S.* × *niederederi*. *S.* × *niederederi* lapuose nustatyta didžiausia chlorogeno rūgšties koncentracija, tačiau kitų fenolinių junginių reikšmės buvo vidutinės.

Siekiant nustatyti visų analizuotų *Solidago* rūšių panašumus ar skirtumus, buvo atlikta pagrindinių komponenčių analizė. PKA modeliai parodė skirtumą tarp keturių *Solidago* rūšių cheminių duomenų rinkinių (rezultatai pristatomi 2 ir 3 paveiksluose publikacijoje A3). Didelė *S. gigantea* mėginių atskirtis komponenčių modeliuose atskleidė reikšmingesnius fenolinių junginių cheminės sudėties skirtumus, nei kitose rūšyse. Tuo tarpu kitos trys rūšys sudarė atskirą klasterį, atspindintį fenolinių junginių kiekybinius

panašumus. Galima daryti išvadą, kad tarprūšinė *Solidago* cheminė įvairovė yra nulemta genetiškai. Daugumos cheminių junginių kokybinio paveldėjimo modelis atitinka Mendelio dominavimo dėsnį: jei abu arba vienas iš tėvinių augalų kaupia cheminę medžiagą, beveik visada ją kaupia ir hibridai [36]. Galima daryti prielaidą, kad už fenolinių junginių sintezę atsakingi genai yra konstituciškai, bet skirtingai išreikšti analizuojamose rūšyse [37]. Yra žinoma, kad ploidiškumo lygis turi reikšmingos įtakos *S. gigantea* specifinei metabolitų koncentracijos įvairovei [38]. Šiame kontekste tetraploidinė *S. gigantea* pasižymi didžiausiu vidutiniu fenolinių junginių kiekiu, kai tuo tarpu diploidinės *Solidago* rūšys pasižymi santykinai mažesniais įvertintų fenolinių junginių kiekiais.

Atlikus papildomą trijų tirtų rūšių PKA, neįskaitant *S. gigantea*, nustatyta, kad remiantis analizuotais fenoliniais junginiais *S. virgaurea* ir *S. canadensis* pasižymėjo aiškiais tarprūšiniais skirtumais. Tarprūšinių hibridų biocheminiai fenotipai neretai skiriasi nuo tėvinių rūšių [39], tačiau *S.* × *niederederi* lapai ir žiedynai šiuo atžvilgiu buvo artimi *S. virgaurea*, todėl jie nėra labai aiškiai apibrėžta grupė. *S.* × *niederederi* lapų ir žiedynų klasteris buvo mažiau sutelktas ir pasižymėjo didesne chemine įvairove, nes kai kurie mėginiai nepateko į tėvinių rūšių, *S. virgaurea* ir *S. canadensis*, ribas. Gauti rezultatai atitinka literatūros duomenis [40]. Lyginant su tėvinėmis rūšimis, vidutinis *S.* × *niederederi* chlorogeno rūgšties kiekis lapuose buvo žymiai didesnis. Gauti rezultatai sutampa su ankstesniais tyrimais, rodančiais, kad hibridinių augalų genetinė variacija yra didesnė nei tėvinių rūšių [41, 42], todėl nustatoma didesnė fenotipinė įvairovė. Mūsų tyrimai parodė, kad *S.* × *niederederi* fenolinių junginių kiekiai buvo iš dalies arba panašus į *S. virgaurea*, o tai rodo hibridinę augalo kilmę.

Kitame atlikto mokslinio tyrimo etape buvo nustatyti vietinių ir invazinių *Solidago* rūšių kaupiamų eterinių aliejų cheminės įvairovės dėsningumai bei įvertintas nustatytų lakiųjų junginių specifiškumas rūšiai. Remiantis gautais rezultatais patvirtinta lakiųjų junginių chemotaksonominė vertė. Atlikus skirtingų rykštenės rūšių eterinių aliejų kiekybinio įvertinimo tyrimus, nustatyta, kad žiedynų mėginiai pasižymi didesniais eterinių aliejų kiekiais, lyginant su lapais. Žiedynuose eterinių aliejų kiekiai svyruoja nuo 0,14 iki 0,26 proc., o lapuose nuo 0,10 iki 0,20 proc. Žiedynuose daugiausiai lakiųjų junginių nustayta *S. canadensis* augalinėje žaliavoje, o lapuose – *S. gigantea*. Suskirsčius eterinių aliejų sudėtinius komponentus į monoterpenus ir seskviterpenus, nustatyta, kad rykštenės rūšies augaluose dominuoja monoterpenai, kurių kiekiai žiedynuose ir lapuose svyruoja nuo 39,7 iki 74,6 proc., o seskviterpenų – nuo 15,00 iki 38,20 proc. (rezultatai pateikiami 1 paveiksle publikacijoje A4).

Daugiamatė duomenų analizė paaiškino keturių *Solidago* rūšių interspecifinę įvairovę. Remiantis PKA rezultatais, *Solidago* rūšių fitocheminiai modeliai patvirtino *S.* × *niederederi* kilmę iš vietinės *S. virgaurea* ir invazinės *S. canadensis*. *S.* × *niederederi* eterinių aliejų (EA – angl. *EO*) rinkinys pademonstravo didesnę įvairovę, nei kitų rūšių, o tai rodo, kad *S.* × *niederederi* yra nuolatos besivystantis taksonas.

Solidago rūšys skyrėsi terpenų sudėtimi, taigi terpenai galėtų būti laikomi konkrečiai rūšiai būdingais komponentais. *S. gigantea* žiedynų EA nuo kitų rūšių skyrėsi γ-kadinenu, γ-muurolenu ir spatulenoliu, o lapai – kamfenu, o-cimenu, epoksiazulenu, (E)-nerolidolu ir spatulenoliu. *S. canadensis* ir *S.* × *niederederi* žiedynai skyrėsi oksigenuotų monoterpenų, tokių, kaip α-kamfolinalis, trans-pinokarveolis, pinokarvonas, verbenonas, cis-verbenolis, trans-verbenolis ir mirtenalis, kaupiamais kiekiais. *S. canadensis* žiedynai specifiškai kaupė mirtenolį, trans-karveolį ir karvoną, o *S.* × *niederederi* lapai – trans-verbenolį. *S. virgaurea* žiedynų specifiniai komponentai buvo α- ir β-kopenas, α-kubebenas, kubebolis, δ-kadinenas ir α-muurolenas, o lapų – α-kamfolinalis, cis-verbenolis, trans-verbenolis, pinokarvonas ir trans-pinokarveolis.

Glaudus rykštenių morfologinis ir filogenetinis ryšys patvirtinamas jų fitocheminių profilių panašumu [30, 87]. Lakiosios medžiagos, plačiai aptinkamos įvairiose augalų rūšyse, dažnai kaupiasi ir *Solidago* rūšių eteriniuose aliejuose. Kita vertus, tokie junginiai kaip timolis ir karvakrolis, dideliais kiekiais buvo nustatyti tik keliuose *S.* × *niederederi* ir *S. canadensis* mėginiuose. Timolis ir karvakrolis pasižymi plačiu biologinio aktyvumo spektru [88–91], todėl jų turtinga augalinė medžiaga turi didelį potencialą. Oksigenuoti monoterpenai yra pagrindiniai fitotoksinai įvairiose augalų rūšyse, todėl yra potencialūs herbicidai [91]. Todėl *S. canadensis* ir *S.* × *niederederi*, kurių EA skyrėsi nuo kitų rūšių oksigenuotų monoterpenų kiekiais, gali būti laikoma potencialia herbicidine žaliava. Tuo tarpu terpenų hidrokarbonatai yra mažai fitotoksiški junginiai [88, 92]. Kita vertus, remiantis naujausių tyrimų rezultatais, (E)-nerolidolis ir spathulenolis, kurie buvo specifiniai seskviterpenoidai *S. gigantea* eteriniuose aliejuose, pasižymi alelopatiniu ir insekticidiniu aktyvumu [93–95]. Potencialus EA biologinis aktyvumas yra siejamas juose esančiais oksigenuotais junginiais, kaip patvirtina sistematinė fitotoksiškumo tyrimų apžvalga [96]. Tai reikštų, kad *S. canadensis* ir *S.* × *niederederi* EA turi didelį potencialą kuriant naujus bioproduktus. Fitocheminis augalinių žaliavų profiliavimas suteikia esminės informacijos apie šių augalų panaudojimo galimybes kuriant naujus bioproduktus.

Paskutiniajame atlikto mokslinio darbo etape ištirti laukinėse Lietuvos augavietėse surinktų *S. gigantea, S. virgaurea*, *S. canadensis* ir *S. × niederederi* lapų ir žiedynų antioksidantiniai profiliai, siekiant įvertinti šios augalinės žaliavos, kaip antioksidantinių medžiagų šaltinio, panaudojimo galimybes.

Fenolinių junginių kokybinė analizė atlikta pasitelkiant ultra-ESC-QTOF-MS metodą. Rezultatai pateikti (rezultatai pateikiami 1 lentelėje publikacijoje A5). Pritaikius rykštenės mėginiams neigiamą jonizacijos režimą, nustatyti 23 fenoliniai junginiai. 14 iš šių junginių pasižymėjo antioksidantiniu aktyvumu. Tirtų rykštenės rūšių lapų ir žiedynų antioksidantiniuose chromatografiniuose profiliuose dominavo kafeoilchino rūgščių ir kvercetino dariniai. Lapų ir žiedynų ekstraktuose 3,5-dikafeoilchino rūgštis nulėmė atitinkamai apytiksliai 43 proc. ir roc. bendro antioksidantinio aktyvumo. 3,5-dikafeoilchino rūgštis nustatyta kaip dominuojantis antioksidantiniu poveikiu pasižymintis junginys *S. virgaurea*, *S. canadensis* ir *S. × niederederi* ekstraktuose. Nustatyta, kad chlorogeno rūgštis lemia 32 proc. bendro antioksidantinio aktyvumo žiedynuose ir 66 proc. lapuose, todėl yra laikoma pagrindiniu *S. gigantea* augalinės žaliavos antioksidantinį aktyvumą lemiančiu junginiu. Kvercetino dariniai sudaro didžiąją dalį flavonoidų komplekso. Kvercetino darinių antioksidantinis aktyvumas reikšmingai mažesnis už kafeoilchino rūgšties. Kvercetino pentozidas ir hiperozidas identifikuoti kaip pagrindiniai antioksidantai *S. gigantea* lapų ir žiedynų mėginiuose, tuo tarpu rutinas lemia 11 proc. bendro antioksidantinio aktyvumo kitose *Solidago* rūšyse. Nustatyti reikšmingi skirtumai tarp *Solidago* rūšių vertinant tų pačių junginių kiekius. Atlikus kiekybinius antioksidantinio aktyvumo tyrimus, taikyta klasterių analizė. Žiedynai suskirstyti į du pagrindinius klasterius, kurių vieną sudarė visi *S. gigantea* žiedynų mėginiai. Lapai taip pat suskirstyti į du klasterius, iš kurių vieną sudarė tik *S. gigantea* lapai, o antrąjį – kitos trys rykštenės rūšys. Antras lapų klasteris suskirstytas į subklasterius – *S. canadensis*, *S. virgaurea* ir *S. × niederederi*. Hierarchinė klasterių analizė atskleidė fenolinių junginių antioksidantinio aktyvumo panašumus tarp *S. virgaurea, S. canadensis* ir *S. × niederederi.* Tuo tarpu *S. gigantea* antioksidantinio aktyvumo rezultatai stipriai išsiskiria, lyginant su kitomis *Solidago* rūšimis.

Siekiant nustatyti visų analizuotų *Solidago* rūšių panašumus ar skirtumus, buvo atlikta pagrindinių komponenčių analizė. PKA rezultatai pademonstravo santykinai gerą visų keturių *Solidago* rūšių atsiskyrimą. Remiantis PKA duomenimis, sukurta grafinė vizualizacija, iliustruojanti skirtumus tarp *Solidago* rūšių fenolinių junginių antioksidantinio aktyvumo (publikacija A5, 6 pav.). Remiantis antioksidantiniu fenolinių junginių aktyvumu, *S. gigantea* lapai ir žiedynai sudarė atskirą klasterį, kai tuo tarpu kitos trys *Solidago* rūšys tarpusavyje pasižymėjo panašiais rezultatais. Rykštenės yra turtingos fenolinių junginių, tačiau šios augalinės žaliavos antioksidantinis aktyvumas iki

šiol nebuvo išsamiai tirtas. Kvercetino ir kemferolio dariniai, kurie dominuoja rykštenės fenolinių junginių profiliuose, naudojami rykštenių ekstraktų kokybės standartizavimui [15, 107, 108].

S. gigantea išsiskiria iš kitų *Solidago* rūšių antioksidantiniu aktyvumu, kurį lemia kvercetino pentozidas, kvercitrinas ir hiperozidas. Kitose trijose *Solidago* rūšyse nustatytas pagrindinis antioksidantas – flavonolis rutinas. Tokie rezultatai atitinka mokslinės literatūros duomenis, kur rutinas taip pat buvo nustatytas kaip pagrindinis flavonolis *S. virgaurea* ir *S. canadensis*, tačiau nenustatytas *S. gigantea* [5, 51, 54, 112]. Remiantis atliktų tyrimų rezultatais, hiperozidas lemia 11 proc. bendro antioksidantinio aktyvumo *S. gigantea* lapuose, o kitose rūšyse – iki 2 proc., tuo tarpu Woźniak et al. (2018) *S. canadensis* ir *S. gigantea augaluose* hiperozido nenustatė [112]. Remiantis šio mokslinio tyrimo rezultatais, hiperozidas gali būti laikomas *S. gigantea* antioksidantinio aktyvumo žymenimi.

Taikant UESC-MS metodą, tirtų rykštenių lapų ir žiedynų mėginiuose taip pat nustatyti trys kemferolio dariniai, tačiau jų antioksidantinis aktyvumas nėra reikšmingas. Tyrimo metu nustatytos cinamono rūgštys ir chino rugščių esteriai yra būdingi *Asteraceae* šeimos augalams [113]. Kafeoilchino rūgštys yra laikomos potencialiais antioksidantais, pasižyminčiais neuroprotekcine ir kardioprotekcine funkcijomis, taip pat priešuždegiminiu poveikiu ir, manoma, kad yra naudingos metabolinių sutrikimų gydymui [50, 114, 115]. Mūsų atlikto tyrimo metu nustatyta, kad 3,5- ir 3,4-dikafeoilchino rūgštys pasižymi didesniu antioksidantiniu aktyvumu, lyginant su monokafeoilchino rūgštimis. Tyrimo metu gauti rezultatai atitinka Li et al. (2018) ir Xu et al. (2012) paskelbtų tyrimų rezultatus, kuriuose nurodoma, kad cheminių junginių papildymas dviem kafeoil- grupėmis reikšmingai padidina jų antioksidantinį aktyvumą [116, 117].

Išvados

1. Tyrimo metu buvo pritaikyta ir validuota ESC metodika, kurios pagalba buvo nustatyti penki vyraujantys fenoliniai junginiai (chlorogeno rūgštis, rutinas, hiperozidas, izokvercitrinas, kvercitrinas). Atlikus kokybinę ir kiekybinę analizę, nustatyta, kad minėtos dvi rykštenės rūšys nepasižymi reikšmingais tarprūšiniais skirtumais.
2. Tyrimo metu modifikuotos pokolonėlines ESC-ABTS ir ESC-DPPH metodikos, siekiant tiksliai ir efektyviai įvertinti *Solidago* genties augalų kaupiamų fenolinių junginių antioksidantinį aktyvumą. Tyrimo metu buvo nustatytos tinkamiausios pokolonėlinės reakcijos sąlygos (abiem metodikoms tinkamiausia kilpos temperatūra – 50 °C; taikant ABTS

metodą, kilpos ilgis 3 metrai, o DPPH – 20 metrų), užtikrinančios stiprų signalo atsaką.

3. Atlikus keturių rykštenės rūšių (*S. virgaurea*, *S. canadensis, S.* × *niederederi* ir *S. gigantea)* ESC kokybinius ir kiekybinius tyrimus, nustatyta, kad hibridas *S.* × *niederederi* (Lietuvoje aptiktas pirmą kartą) yra artimiausias *S. virgaurea* ir *S. canadensis* rūšims, taigi šios dvi rūšys gali būti laikomos hibrido tėvinėmis rūšimis.
4. Atliktas lakiųjų junginių profilių palyginamasis tyrimas (DC-MS) su keturiomis rykštenės rūšimis (*S. virgaurea*, *S. canadensis, S.* × *niederederi* ir *S. gigantea)* patvirtino, kad *S.* × *niederederi* yra tarprūšinis taksonas tarp *S. canadensis* ir *S. virgaurea*, kur didesnis metabolinis indėlis priklauso *S. canadensis*. Šie rezultatai sutapo su ankstesniais ESC tyrimų duomenimis. Nustatyta, kad hibridas *S.* × *niederederi* pasižymi didžiausia eterinių aliejų įvairove.
5. Pritaikius UESC-MS metodą, buvo atliktas išsamus skirtingų rykštenės rūšių augalinės žaliavos fenolinių junginių nustatymas. Atlikta lapų ir žiedynų mėginių analizė, kurios metu buvo nustatyti 23 fenoliniai junginiai, iš kurių 14 pasižymėjo antioksidantiniu aktyvumu, įvertinus juos ESC-ABTS metodu. Šio tyrimo metu nustatyti žymenys svarbūs užtikrinant augalinės žaliavos kokybę bei tarprūšinių skirtumų vertinimui.

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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 (Sutovskåa 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

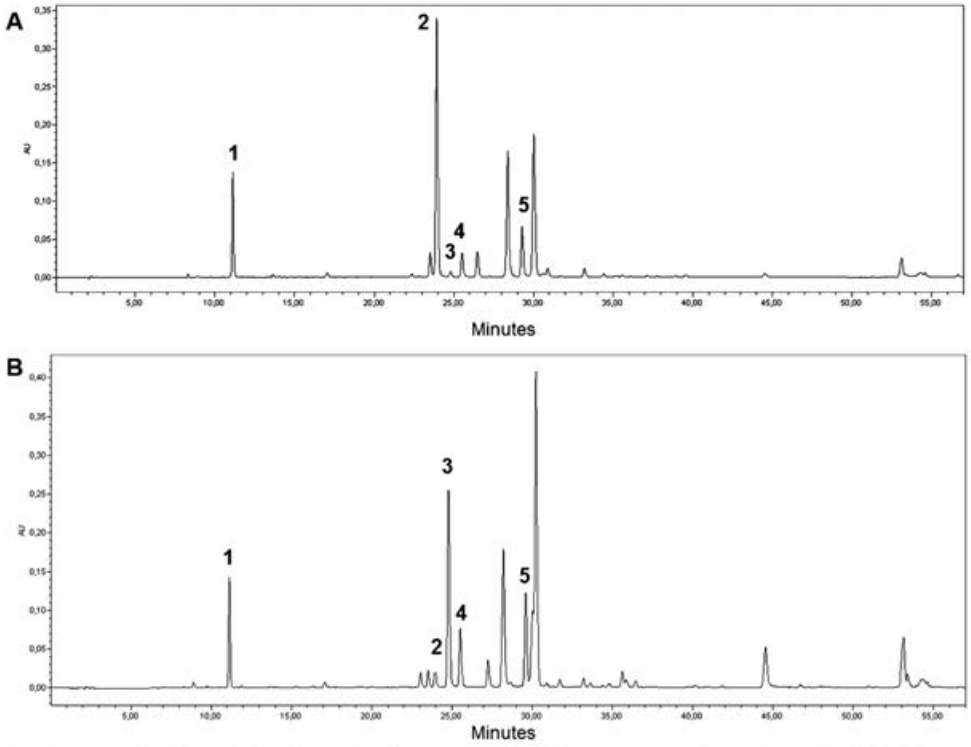

Fig. 1. Chromatograms of *Solidago canadensis* (A) and *S. gigantea* (B) inflorescence extracts detected by HPLC-PAD on YMC-Pack ODS-A column. Peak identified: 1 – chlorogenic acid, 2 – rutin, 3 – hyperoside, 4 – isoqueritrin, 5 – quercitrin.

cluster analysis (HCA) and principal components analysis (PCA) were used to cluster and reduce the data set. The clustering method was based on squared Euclidean distances. PCA was based on the eigenanalysis of the correlation matrix, and factors with eigenvalues greater than 1 were taken into account. One-way analysis of variance (ANOVA) was accomplished to identify significant differences among the groups, and a post hoc Scheffe's multiple comparison test was applied to specify corresponding differences.

3. Results

3.1. Differences between species

In the present study, five phenolic compounds, chlorogenic acid, rutin, isoquercitrin, hyperoside and quercitrin, were detected in the leaves and inflorescences of *S. canadensis* and *S. gigantea*. The *t*-test comparisons of the paired mean quantities of the compound accumulations in the leaves and inflorescences of the two species showed considerably higher values for all investigated compounds except rutin in *S. gigantea* than *S. canadensis*. The rutin contents of both the leaves and inflorescences of *S. canadensis* greatly exceeded those of *S. gigantea*. The most significant differences (at $P < 0.0001$) between the species were observed for chlorogenic acid, rutin and qurecitrin in the leaves. The content of chlorogenic acid did not differ significantly (at $P > 0.05$) in the inflorescences of the two species (Table 2).

The compound quantities in the two plant parts of *S. canadensis* were only highly different for chlorogenic acid and quercitrin, which were both significantly higher in the leaves (20.10 ± 0.40 and 3.50 ± 0.31 mg/g DM, respectively) than the inflorescences

Table 1
Validation parameters of HPLC analysis of *Solidago canadensis* and *S. gigantea* raw material.

Analytes	T_R (min)	Linear range (mg/ml)	Regression equation	R^2	LOD (mg/ml)	LOQ (mg/ml)	Intra-day RSD (%)	Inter-day RSD (%)
Chlorogenic acid	11.26	0.00188–0.2	$Y = 4.26 \times 10^7 + 1.40 \times 10^4$	0.9997	0.001	0.0003	0.8	1.1
Rutin	23.70	0.00094–0.1	$Y = 2.27 \times 10^7 + 4.02 \times 10^3$	09995	0.0003	0.0013	0.9	1.3
Hyperoside	24.78	0.01563–0.1	$Y = 2.05 \times 10^7 + 1.24 \times 10^4$	0.9999	0.0003	0.001	2.4	3.0
Isoqueritrin	25.75	0.00103–0.11	$Y = 6.42 \times 10^7 + 1.13 \times 10^3$	0.9998	0.0001	0.0004	0.4	0.6
Quercitrin	29.22	0.00078–0.1	$Y = 2.02 \times 10^7 + 1.41 \times 10^4$	0.9996	0.0003	0.0009	1.8	2.3

T_R: retention time, R^2: correlation coefficient, LOD: limit of detection, LOQ: limit of quantitation, RSD: relative standard deviation.

Table 2
The pooled-variance *t*-test comparison of mean quantities (mg/g DM) of phenolic compounds between *Solidago canadensis* (1) and *S. gigantea* (2) leaves and inflorescences.

Variables	Leaves					Inflorescences				
	1	2	DF	*t*	*P*	1	2	DF	*t*	*P*
Chlorogenic acid	20.10 ± 0.99	31.31 ± 1.57	91	−6.32	0.0001	8.35 ± 0.40	10.04 ± 1.10	42.0	−1.72	0.09
Rutin	17.47 ± 1.17	0.58 ± 0.08	61.63	14.33	0.0001	18.12 ± 0.99	2.04 ± 0.57	90.23	14.04	0.001
Isoqueritrin	0.54 ± 0.13	0.91 ± 0.11	91	−2.11	0.04	0.61 ± 0.05	1.36 ± 0.07	91	−8.65	0.001
Hyperoside	0.27 ± 0.09	2.60 ± 0.37	37.45	−6.12	0.0001	0.83 ± 0.29	11.72 ± 0.62	91	16.02	0.001
Quercitrin	3.49 ± 0.31	32.87 ± 1.69	35.18	17.12	0.0001	2.64 ± 0.27	17.59 ± 2.05	34.17	−7.22	0.001

DF: degree of freedom, *t*: Student's statistic, *P*: significant level.

(8.35 ± 0.99 and 2.6 ± 0.27 mg/g DM, respectively) (Table 3). Meanwhile, *S. gigantea* showed significant differences between its plant parts for all compound quantities studied. For *S. gigantea*, the quantities of chlorogenic acid and quercitrin were higher in the leaves (31.31 ± 1.57 and 32.87 ± 1.69 mg/g DM, respectively) than the inflorescences (10.03 ± 1.10 and 17.60 ± 02.05 mg/g DM, respectively), while the quantities of rutin, isoquercitrin and hyperoside were significantly higher in the inflorescences (2.04 ± 0.58, 1.36 ± 0.42 and 11.72 ± 0.62 mg/g DM, respectively) than the leaves (0.58 ± 0.09, 0.91 ± 0.66 and 2.60 ± 0.37 mg/g DM, respectively).

3.2. Intraspecific diversity

HCA was applied to the group accessions using mean quantities of phenolic compounds as clustering variables. HCA was performed separately for both the leaves and flowers based on the above data and showed that the quantities of the analysed compounds were significantly different in the two plant parts. The clustering of the *S. canadensis* accessions resulted in grouping both the leaves and inflorescences into four main clusters. The composition of the extracted groups are presented in Fig. 2(A) as the mean contents of the phenolics in order to better recognise the similarities and differences of each group. Furthermore, ANOVA was performed to identify significant differences in the phenolic compounds among the clusters. ANOVA revealed that the mean quantities of all phenolic compounds differed statistically among the clusters of leaves and inflorescences, with the exception of isoquercitrin. The amount of isoquercitrin did not differ significantly between the groups for either plant parts. The most significant differences among the leaf groups were found for rutin ($F = 106.15$; $P < 0.0001$), hyperoside ($F = 74.22$; $P < 0.0001$) and chlorogenic acid ($F = 13.79$; $P < 0.0001$); while, for the flower groups, the most significant differences were for hyperoside ($F = 165.56$; $P < 0.0001$) and rutin ($F = 20.10$; $P < 0.0001$) (Table 4).

A post hoc Scheffe's multiple comparison test enabled us to specify differences among clusters (Fig. 2(A)). The leaves of *S. canadensis* that were grouped into clusters 1 and 2 consisted of most of the accessions that contained compound quantities in the range of their lowest quantities to their mean quantities. The third cluster differed from the others due to its high level of rutin (35.54 ± 1.17 mg/g DM). The accessions forming cluster 4 had the highest quantities of chlorogenic acid (39.1 mg/g DM), quercitrin (10.65 mg/g DM) and hyperoside (5.45 mg/g DM), and, contrary to the third cluster, the lowest content of rutin (0.42 mg/g DM).

The multiple comparisons of the chemical compositions of the inflorescences indicated that the accessions of the first cluster contained the lowest amounts of the phenolic compounds. The plants with the highest contents of chlorogenic acid (18.68 mg/g DM) and quercitrin (11.72 mg/g DM) composed cluster 2, while flowers with the highest quantities of rutin (28.65 ± 0.95 mg/g DM) and hyperoside (12.44 ± 1.22 mg/g DM) composed clusters 3 and 4, respectively.

The HCA for *S. gigantea* divided the accessions of the leaves and inflorescences into three and four clusters, respectively (Fig. 2(B)). ANOVA revealed significant differences in the mean quantities of the phenolic compounds, with the exception of isoquercitrin, among the clusters for both plant parts. The highest differences among the leaf clusters were found for hyperoside ($F = 63.89$; $P < 0.0001$) and chlorogenic acid ($F = 16.90$; $P < 0.0001$), while the inflorescences highly differed in rutin ($F = 144.51$; $P < 0.0001$) and quercitrin ($F = 52.96$; $P < 0.0001$) (Table 4).

The results of the multiple comparison test showed that leaves grouped in the first cluster accumulated from the lowest amount to average amounts of compounds within their range of variations. The accessions composing cluster 2 dominated significantly with the highest content of hyperoside (13.34 mg/g DM), while cluster 3 exhibited maximum levels of quercitrin (52.61 ± 0.8 mg/g DM) and chlorogenic acid (48.7 ± 6.04 mg/g DM). Differences in chemical compositions of the inflorescences revealed a trend similar to that observed in the leaves (Fig. 2(B)). PCA was used to separate statistically independent basic components for exploration of the relationships among the chemical compounds, thus allowing identification of the primary predictors among the investigated accessions. The loadings revealed the characteristic variables of each group of accessions on the graphic of the scores, which represents the data in the space formed by the principal components (Cordella, 2012). On the basis of the HCA results presented above, isoquercitrin was removed from the PCA of both Solidago species; it was found to be a variable that contributed to noise reducing the quality of the model. Plots of the scores for the two PCs showed the similarities and differences among the variables graphically. According to Gergen and Harmanescu (2012), the variables whose unit vectors are close to each other are positively correlated and their influence on the positioning of the accessions is similar, and variables far away from each other are negatively correlated.

Table 3
The pooled-variance *t*-test comparisons of phenolic compounds mean quantities (mg/g DM) between leaves (1) and inflorescences (2) of *Solidago canadensis* and *S. gigantea*.

Variables	*S. canadensis*					*S. gigantea*				
	1	2	DF	*t*	*P*	1	2	DF	*t*	*P*
Chlorogenic acid	20.10 ± 0.40	8.35 ± 0.99	80.53	−10.95	0.001	31.31 ± 1.57	10.03 ± 1.10	59.16	−11.09	0.0001
Rutin	17.47 ± 1.17	18.12 ± 0.98	118	−0.42	0.67	0.58 ± 0.09	2.04 ± 0.58	34.42	2.51	0.02
Isoqueritrin	0.54 ± 0.13	0.61 ± 0.05	78.93	−0.51	0.61	0.91 ± 0.66	1.36 ± 0.42	55.55	3.36	0.001
Hyperoside	0.27 ± 0.09	0.83 ± 0.29	74.29	−1.84	0.07	2.60 ± 0.37	11.72 ± 0.62	53.99	12.69	0.0001
Quercitrin	3.50 ± 0.31	2.6 ± 0.27	118	−2.07	0.04	32.87 ± 1.69	17.60 ± 02.05	64	−5.75	0.0001

DF: degree of freedom, *t*: Student's statistic, *P*: significant level.

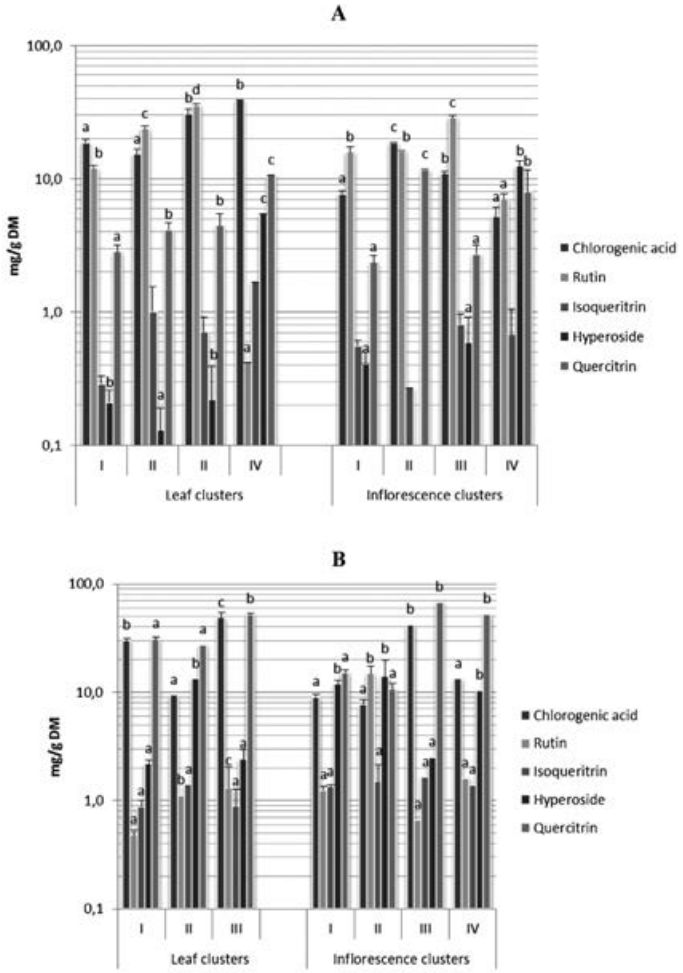

Fig. 2. Mean composition of *Solidago canadensis* (A) and *S. gigantea* (B) leaf and inflorescence groups extracted using hierarchical cluster analysis. The heights of bars (*y*-axes) corresponded mean ± SE quantities (mg/g DM) of compounds. Values marked with the same letter within the leaf or inflorescence groups do not differ significantly at $P < 0.05$ according to Scheffe's test.

Table 4
The results of ANOVA for quantitative differences of phenolic compounds accumulation between leaf and inflorescence groups of *Solidago canadensis* and *S. gigantea* extracted using HCA.

Variables	*S. canadensis*				*S. gigantea*			
	Leaves		Inflorescences		Leaves		Inflorescences	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Chlorogenic acid	13.79	0.0001	10.67	0.0001	16.90	0.0001	34.87	0.0001
Rutin	106.15	0.0001	20.10	0.0001	5.31	0.01	144.51	0.0001
Isoqueritrin	2.31	0.086	1.42	0.25	0.28	0.76	0.24	0.86
Hyperoside	74.22	0.0001	165.56	0.0001	63.89	0.0001	2.98	0.05
Quercitrin	5.58	0.002	22.99	0.0001	10.45	0.001	52.96	0.0001

F: Fisher's statistics, *P*: significant level.

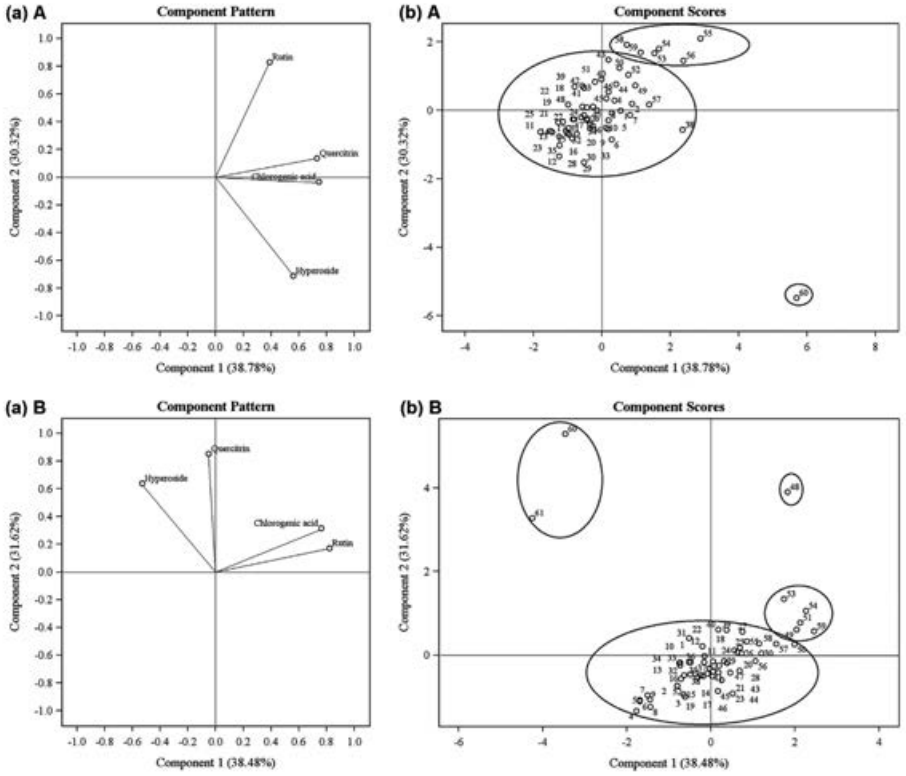

Fig. 3. PCA loading plots of compound variables (a) and score plots of different accessions (b) of *Solidago canadensis* leaves (A) and inflorescences (B).

Samples close to each other in the space of the principal components have similar characteristics. The score plots for the first two PCs explained 69.1 and 70.1% of the total variance of the chemical data of the *S. canadensis* leaves and inflorescences, respectively; relatively good separation between the evaluated accessions is shown in Fig. 3. The PCA loading plots for PC1 vs. PC2 indicated that chlorogenic acid and quercitrin were positively correlated and had the greatest influence on the scores of the leaves (Fig. 3(a)A and (b)A), whereas chlorogenic acid and rutin had effects on the scores of the inflorescences (Fig. 3(a)B and (b)B). The opposing positions of hyperoside and rutin indicate that they are negatively correlated, which suggests opposite values of corresponding compounds in the accessions. The graphics of the PCA scores in Fig. 3(b)A and (b)B showed that the most accession groups for both the leaves and inflorescences are situated near the PC1 and PC2 zero point, which means that they accumulated compounds in the range of the lowest to the mean values. The location of one leaf sample was distanced from all of the others, suggesting that its phenolic compound composition differs significantly from the other samples. Indeed, in this sample, hyperoside, chlorogenic acid and quercitrin had the highest values of all of the samples, and rutin, which was located on the opposite side of the PC1 accumulation had the lowest value of all of the samples. In contrast, samples grouped on the positive side of the PC1 contained the highest amounts of rutin and chlorogenic acid and a low content of hyperoside.

For the evaluation of the inflorescences, the location of one sample in the middle of the chlorogenic acid and quercitrin values may be explained by the high values of these compounds in the corresponding accession (Fig. 3(a)B and (b)B). The group of accessions located on the positive side of the PC1 closer to the position of rutin had the highest quantities of rutin. The position of two samples in the upper left-hand quadrant of the loading plot may be explained by their high hyperoside value and low rutin value, which are co-located in this region of the PC space.

The score plots for the first two PCs, which explain 77.0 and 72.4% of the total variance in the data set of *S. gigantea* leaves and inflorescences, respectively, are shown in Fig. 4. The PCA loading plots of the inflorescences (Fig. 4(a)B) illustrate the highly positive relationship between chlorogenic acid and quercitrin, while quercetrin and rutin were found to be positively correlated in the leaves (Fig. 4(a)A). The opposite location of hyperoside indicates its negative correlation with quercitrin and chlorogenic acid. Using the plots of the variables, the reasons for the locations of the accessions are regards to the variables can be proposed. The majority of the leaf and flower accessions are located near the PC1 and PC2 zero point and were grouped into single clusters (Fig. 4(b)A and (b)B); this

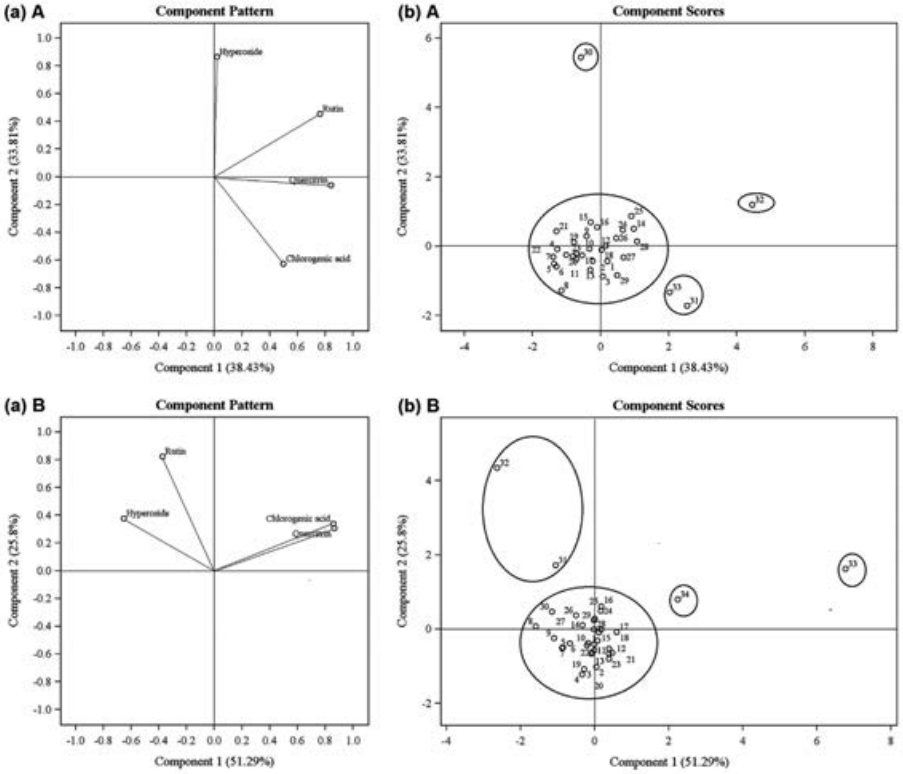

Fig. 4. PCA loading plots of compound variables (a) and score plots of different accessions (b) of *Solidago gigantea* leaves (A) and inflorescences (B).

was similar to the results previously discussed for *S. canadensis*. The main factor influencing the distance of one leaf sample on PC2 was its high content of hyperoside. The location of three accessions to the right of the PC1 zero point may be explained by their high values of quercetrin and chlorogenic acid, which are co-located in the same region of the PC space in Fig. 4(a)A and (b)A. The remote position of another accession on the positive side of PC1 can be explained by its higher content of rutin, which is located on the same plot. Two single accessions of the inflorescences are located on the positive side of PC1 closer to chlorogenic acid and quercitrin, which have the highest concentrations in the first sample and a lower value in the second sample (Fig. 4(a)B and (b)B). The diametrically opposing location of those accessions for the variable hyperoside, suggest a low value of this compound in the corresponding samples. Two samples located on the negative side of PC1 and on the positive side of PC2 are associated with a high concentration of rutin and hyperoside.

The results of the HCA and PCA were similar. Some differences in clustering behaviour between the HCA and PCA were present, as the use of only two PC1 and PC2 axes in combination did not completely explain all data variance. On the other hand, graphical visualisations using PCA revealed a relatively simple representation of the structure of the corresponding species on the basis of chemical data.

4. Discussion and conclusions

The results of this study demonstrated that two invasive species, *Solidago canadensis* and *S. gigantea*, exposed to contain different phenolic compound compositions. Considerably higher values for all detected compounds were found in *S. gigantea* compared to *S. canadensis*, except for rutin; the rutin content was much greater in *S. canadensis*. The raw material of both the leaves and inflorescences of *S. canadensis* are high potential sources of rutin, while leaves of *S. gigantea* are high potential sources of chlorogenic acid and quercitrin. Therefore, the correct identification of plants is highly important for the selection of the appropriate value of the raw material of plants obtained in the wild. Signitificance of right taxonomic identification of plants for drug material deels in different aspects by other authors (Fazal et al., 2013).

The combination of the chemical and multivariate statistical data analyses provides a relatively simple representation of the structure of wild plant materials and allows easy interpretation of the similarities and differences in compound accumulations among wild populations of *S. canadensis* and *S. gigantea*. On the other hand, the use of chemometrics for identification and assessment of the quality plant materials to ensure safety and efficacy of botanical products have been published previously (Patras et al., 2011; Zhu et al., 2014; Truus et al., 2012).

Two wide spread species of goldenrods did not display high intraspecific variation in their phenolic compound accumulations. In this regard, studies have shown that the chemical variation of invasive goldenrods is relatively weak as compared to the intraspecific morphological diversity that has been emphasised in several surveys of these species (Jakobs et al., 2004; Weber, 1997; Weber and Jakobs, 2005). The difference between the morphological and chemical structure of these plant species can be explained by the fact that the plasticity of morphological expression, rather than adaptation, is the key to the successful spread of invasive plants (Lamarque et al., 2013; Lavergne and Molofsky, 2007). Secondary metabolites represent adaptive characters that are important for plant survival and reproductive fitness, rather than for invasive spread. Thus, the most abundant polyphenols help protect the plants against UV radiation, fungal parasites, herbivores and pathogens (Lattanzio, 2013). However, the lack of natural enemies in the new invasion areas of goldenrods has led to a limited phenolic compound variation in these species, which is different from what has been observed previously for other wild species (Bagdonaite et al., 2012; Benetis et al., 2008; Çirak et al., 2012). Reduced heterogeneity in the chemical composition of goldenrods is highly important to increase the quality and safety of herbal material obtained from the wild.

Although most goldenrods within one species have similar quantitative compositions of phenolics in their range of variation, individual plants or small groups have been found that have significantly higher levels of rutin or chlorogenic acid and quercitrin and hyperoside. These plants can be considered to have higher raw material quality sources. It can be assumed that plants with greatly different phenolic compound compositions belong to different inter- or intra-specific taxa or natural hybrids of goldenrods and require additional analysis. In this regard, the research presented here may be useful in understanding the complex and confusing taxonomy of the genus Solidago.

To the best of our knowledge, this research represents the first inter- and intra-specific characterisation of the raw materials of *S. canadensis* and *S. gigantea* based on a multivariate analysis of chemical data. As the published data on phenolic compounds of invasive Solidago species are scarce, a comparison of our results with published data is difficult. Apati (2003) reported the same phenolic compounds in *S. canadensis* herbal extracts; however, they did not quantify their amounts.

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 renewable resources of pharmaceutical raw material. Large fields of goldenrods that accumulate high values of phenolic compounds generate much interest because of the possibility of using those plants to obtain natural phenolic compounds for herbal preparations and pharmaceuticals. Furthermore, the gathering of the raw material of goldenrods could be considered a control measure for these invasive populations with potential social–economic importance.

Acknowledgment

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Development of an HPLC post-column antioxidant assay for *Solidago canadensis* radical scavengers

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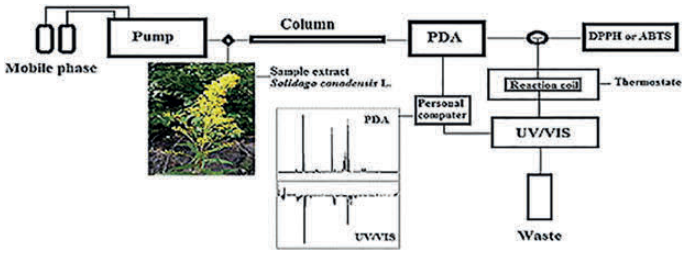

The aim of this work was to modify and validate the post-column high-performance liquid chromatography (HPLC)-ABTS and DPPH methods for evaluating the antioxidant activity of the methanolic extracts of *Solidago canadensis* (Canadian goldenrod) leaves and flowers. Separation of the analytes was performed via the HPLC-PDA method on a YMC analytical column using a gradient elution program. Three compounds with antioxidant properties – chlorogenic acid, rutin and isoquercitrin – and two unidentified antioxidants were established. The research showed that the coil temperature regimes and loop length combinations influence the optimised post-column assay method for detecting the antioxidant activity of goldenrod radical scavengers. Investigations established that the temperature in the reaction coil was a substantial factor contributing to the signal strength of the analytes after reacting with the DPPH and ABTS radicals.

Keywords: HPLC post-column; coil temperature; loop length; DPPH; ABTS; *Solidago canadensis*

1. Introduction

The raw materials of goldenrods, known as *Herba Solidaginis*, display well-defined diuretic, spasmolytic and hypotensive effects along with their anti-inflammatory, bacteriostatic and analgesic properties (Demir et al. 2009; Deepa & Ravichandiran 2010). The toxicity and contraindications for all goldenrod preparations have not been reported; therefore, the information that is available is based largely on studies conducted on the native European

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goldenrod (Gross et al. 2002). Previously reported chemical evaluations of invasive species of goldenrods have indicated that they have high potential to be a source of phenolic compounds (Radusiene et al. 2015). Phenolic compounds are the subject of increasing scientific interest because of their multiple biological effects, including antioxidative activity that is associated with the prevention of various degenerative diseases (Andersen & Markham 2010; Zhang et al. 2011). According to our survey, only a few published reports have provided an assessment of the antioxidant activity of different *Solidago* species (Apáti et al. 2003; Demir et al. 2009).

The most reliable and comprehensive evaluation of the radical-scavenging capacities of plant materials is possible only through the validation of highly efficient analytical methods. One highly promising method for the investigation of antioxidant activity is the HPLC post-column assay with various derivatisation techniques. Online post-column methods are employed in the rapid identification of antioxidants and determination of their activity (Kosar et al. 2004). One highly effective technique developed and modified by different researchers are methods based on a model oxidation system that combines HPLC separation methods with the online post-column detection of radical-scavenging compounds (Ibrahim et al. 2010; Raudonis et al. 2012; Camenzuli et al. 2013). Model oxidation systems of ABTS and DPPH radicals have been extensively used for screening antioxidants from plant extracts, and they possess important advantages when compared with colorimetric methods (Pukalskas et al. 2002; Kosar et al. 2004; Ali et al. 2014).

The objectives of this study were to establish the HPLC post-column derivatisation parameters and to assess the influence of the temperature factor and reaction coil parameters such as loop length modification on the efficacy of screening the radical-scavenging ability using HPLC post-column DPPH and ABTS systems for the determination and evaluation of radical-scavenging analysis in goldenrods raw material extracts.

2. Results and discussion

2.1. *HPLC post-column derivatisation for antioxidant activity assessment*

The main factors affecting post-column detection are the concentration of the derivatisation reagents, post-column flow-rate, tubing parameters and system temperature (Ibrahim et al. 2010).

The development and optimisation of post-column detection methods include the analysis of the relationship between the loop length and the reagents' elution rate with the reaction time. The reaction coil parameters affect the ratio of the peak height and baseline noise for the ABTS and DPPH reagents; derivatisation agents increase the HPLC peak areas (Raudonis et al. 2010). Certain temperatures are used in the post-column reaction, but there is no improvement on the methodological justification for the chosen temperature limit for antioxidant activity assays on plant materials (Oki et al. 2006; Mnatsakanyan et al. 2010). However, the temperature is an important factor because variations in temperature may change the mechanism of action for some antioxidants or affect them in another way (Réblová et al. 2012).

The rate of the post-column reaction is slower when the temperature is insufficient. Lower temperatures induce slower chemical reactions, during which the stable free radicals interact with biologically active materials; therefore, the time of reaction should be prolonged. The efficiency of the coil temperature and loop length was evaluated after the peak heights of the active compounds were established.

2.2. *ABTS and DPPH post-column antioxidant assay*

The influence of the temperature and loop length on the change in peak heights for the ABTS assay is shown in Figure S1. The temperature was increased in increments of 10°C starting from 25°C during the HPLC-post column run cycle. The maximum temperature was achieved

at 55°C. The higher temperature conditions are related to the thermo-destructive changes of antioxidant properties associated with the active compounds and radicals. An additional temperature level of 50°C was introduced after conducting preliminary experiments, during which the positive influence of the 45–55°C temperature regime on the results was observed. The results showed that when the thermostat temperature was increased to 50°C, the antiradical response of the reagent increased as well. A constant difference in the antiradical signal was observed between the use of the two coil lengths, 3 and 20 m, and changing the temperature regime. Analysis of variance (ANOVA) revealed statistically significant differences (at $p < 0.05$).

Aside from temperature, coil parameters such as length and diameter also affected the course of the reaction. Previous research relates the impact of the coil characteristics with the reaction time, such that the longer coils result in a higher volume retention of the reaction mixture (Raudonis et al. 2010). During the post-column reaction, the coils were immobilised in the above-described temperature regimes. After performing repeated injections under the same experimental conditions using the same sample, reagents, instruments, temperature and coil length modulations, and after estimating the mean peak height readings for all three of the indicated active compounds, the reaction coil length and reaction time were determined not to be useful factors for gauging the peak height.

During the entire analysis, an antiradical response base line noise level was observed, which varied from 650 to 890 units (electrical signal micro-volts) using the 3-m loop and from 460 to 890 units using the 20-m coil. In both cases, the lowest noise level was detected at 50°C temperature, which indicates the stability of the system. However, temperature was one of the factors influencing the sensitivity of the method. Therefore, the 3-m loop with a thermostat set at 50°C was the optimal conditions for evaluating the antioxidant activity of goldenrod raw materials using the ABTS reagent.

DPPH post-column antioxidant assay: The application of the DPPH reagent for the resulting antioxidant assay partially corresponds to those obtained when the ABTS reagent (Figure S2) was used. At increasing temperatures, the antiradical response rose unevenly towards the analogous critical analysis point of 50°C. When the temperature reached 55°C, the decrease in peak heights was recorded. For example, in this study, the profile of the established chlorogenic acid graph highly differed in relative signal strength at different temperatures even though no important peak height differences appeared ($p > 0.05$) between 25°C and 50°C (Figure S2). Negative peak height for the 3-m coil was slightly lower (−17,952) than the 20-m coil (−18,221) when using a temperature of 25°C; the same observation occurred at 50°C for the 3- and 20-m reaction coils, with peak height of −18,465 and −19,793, respectively. A significant decrease in the peak height (−1143) was recorded at 35°C. The regime for 35°C had a negative influence on all extract analytes and was, therefore, considered unsuitable for antioxidant assays of goldenrod. For chlorogenic acid, there were no significant differences ($p > 0.05$) between the negative peak height values when using either a 3- or 20-m reaction coil in the post-column assay.

After repeated experiments it can be concluded that under the above-described analysis conditions, the length of the reaction coil (3 and 20 m) did not affect the negative peak values for the standard solutions at low temperatures between 25°C and 35°C (Figure S2). In the post-column DPPH assay, utilising a high temperature (50°C) and a 20-m reaction coil significantly increased the peak heights of the analytes, which represents the best settings for the corresponding analysis. This can be explained by the effect of DPPH reaction kinetics, which makes the time factor more relevant than the extension of the chromatographic elution zone; this differs from the factors established under ABTS reagent conditions.

2.3. *HPLC post-column assay for the analysis of radical scavengers*

HPLC post-column assay validation was performed by assessing the antioxidant negative peak areas of reference standards (Figure S1). Validation of the HPLC post-column assay method for detecting radical scavengers was determined according to intra-day and inter-day precision, limit of detection (LOD) and limit of quantification (LOQ) values and calibration curves (Table S2). The concentration dependence of the signal to the amount of radical scavengers in the extract was substantiated by reference to a Trolox standard calibration curve. The curve was calculated using the concentration of Trolox that is required to produce an equivalent antioxidant potential (μM) in terms of Trolox equivalent antioxidant capacity (TEAC) values (Raudonis et al. 2012). A direct correlation between the amount of Trolox and its negative peak area was determined by the height of the correlation factor. The calibration curves that formed were equivalent to standard Trolox and were expressed by following quadratic equations: R^2 (ABTS) = 0.9991 ($Y = -1.54 \times 10^2 x^2 + 4.16 \times 10^4 x - 2.08 \times 10^4$) and R^2 (DPPH) = 0.9988 ($Y = -3.86 \times 10^1 x^2 + 1.81 \times 10^4 x + 1.01 \times 10^4$).

The precision of the post-column method was demonstrated by performing five replicate non-consecutive injections of the four reference standard solutions (Trolox, chlorogenic acid, rutin and isoquercitrin) on the same day (intra-day) and on three different days (inter-day). The RSD coefficient for repeatability and intermediate precision did not exceed 3.0% for both ABTS and DPPH post-column assay, which shows that the methods can be considered appropriate and acceptable for the quantitative evaluation of the antiradical activity of compounds.

2.4. *Analysis of free radical scavengers of* S. canadensis

In leaf and flower methanol extracts, three phenolics (chlorogenic acid, rutin and isoquercitrin) together with two unknown compounds were established (Figure 1). The antioxidant activities of the identified and non-identified compounds were expressed as TEAC values (Trolox μmol/g) and are presented in Table S3.

The results showed statistically significant differences (at $p < 0.001$) between the antiradical response for ABTS and DPPH post-column assay methods in terms of the TEAC values (μmol/g) for the identified and non-identified compounds, with the exception of isoquercitrin, for which the TEAC values (μmol/g) did not differ significantly. High TEAC values were observed for constituents detected using the DPPH post-column assay versus the ABTS method application. The results of the *t*-test demonstrated different radical scavenger activities between the goldenrod leaf and flower extracts when using the ABTS and DPPH post-column assay (Table S3). The leaves possessed stronger antioxidant properties for chlorogenic acid, rutin, isoquercitrin and unknown compound (**5**) when using the ABTS post-column assay. In case of DPPH, stronger antioxidant activity was observed in leaves for chlorogenic acid and isoquercitrin. The flowers possessed greater antioxidant activity for unknown compounds (**4**) and (**5**) when compared with leaves using the DPPH assay method. Consequently, similar pattern of variation in antioxidant activity can be observed for all compounds using both ABTS and DPPH post-column assay.

3. Experimental

3.1. *Plant material*

Accessions of *S. canadensis* were collected from different wild populations. Voucher specimens of each accession were deposited in the Herbarium of the Institute of Botany/Vilnius, Lithuania/ under the following numbers: EB-35-49, EB-38-62, EB-39-63, EB-07-72 and EB-44-69. The harvested plant material consisted of the three tops of two to three shoots of the same clone of five different plants in the flowering phase. The collected plants were dissected into

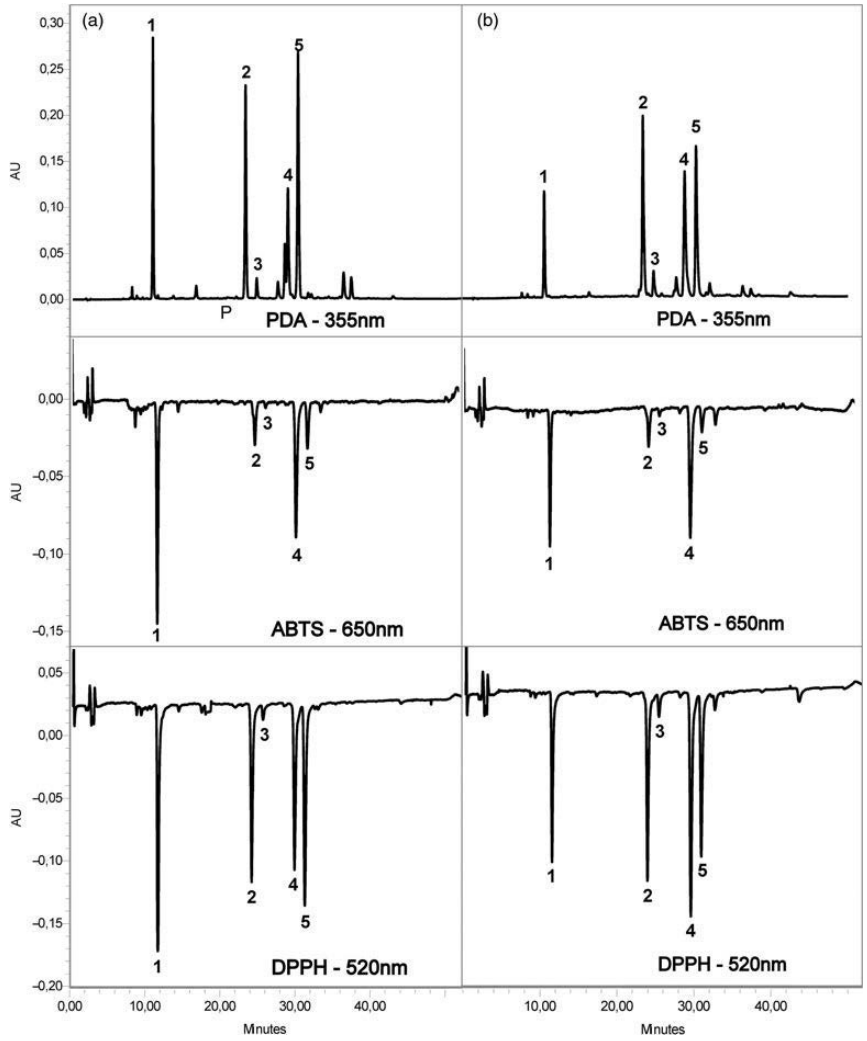

Figure 1. HPLC chromatograms of the *S. canadensis* leaf (a) and flower (b) extracts combined with their respective post-column DPPH and ABTS chromatograms, presented as negative peaks.

inflorescence and leaf parts and then dried at 25°C. The air-dried plant material was mechanically ground with a laboratory mill to obtain a drug powder.

3.2. *Preparation of extracts*

The extracts were prepared using methods reported previously by other researchers (Apati et al. 2002; Papp et al. 2004). The samples of raw material were extracted in a 70% methanol/water mixture (v/v) by ultra-sonication at 25°C for 50 min using a BioSonic UC100 ultrasound bath (Mavay, Mahwah, NJ, USA). Methanol was chosen for extraction because high polarity solvents

are considerably more effective radical scavengers than low polarity solvents (Ali et al. 2014). The extracts were produced at a ratio of 1:100 (w/v) by weighing 0.1 g of material and adding it the solvent up to a total volume of 10 mL. The extracts were filtered through 0.22-μm nylon syringe filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany).

3.3. *HPLC-PDA conditions and validation*

HPLC analysis was conducted with a Waters 2695 chromatographer (Waters Corporation, Milford, CT, USA) and a Waters 996 PDA photodiode matrix detector (Waters Corporation). Separation of the active compounds was carried out on a 150 × 4.6 mm, 3 μm YMC-Pack ODS-A column (YMC Europe Gmbh, Dinslaken, Germany), injection volume – 10 μL, elution flow rate – 1.0 mL/min. The external thermostat for the column was kept at a steady temperature of 25°C during analysis. A gradient elution system was performed using eluent A – 0.05% (v/v) trifluoroacetic acid (TFA) in water and eluent B – 100% acetonitrile. Following that, a linear elution gradient was applied: 0 min – 95% A and 5% B, 5 min – 88% A and 12% B, 50 min – 70% A and 30% B, 51 min – 10% A and 90% B, 56 min – 10% A and 90% B, 57 min – 95% A and 5% B. The identification of chromatographic peaks was performed by matching the retention times of the analytes to reference standard compounds as well as UV absorption spectra within the limits of 210–400 nm. An individual series of standard solutions was prepared in 70% ethanol. The detection of flavonoids and phenolic acids was conducted at 355 and 324 nm wavelengths, respectively.

Validation of the HPLC-PDA method was determined according to the following parameters: specificity, linearity, precision, LOD and LOQ (Table S1).

3.4. *HPLC post-column antioxidant detection method*

After applying the HPLC-PDA detection system, the mobile phase containing the analytes was entered into a reaction coil through a mixing tee where the reagents (ABTS or DPPH solutions) were supplied (split ratio, 1:1) at the same time by a Gilson pump 305 (Middleton, WI, USA). Reaction coils made of TFE (Teflon) of 3 and 20 m length, 0.25 mm i.d. and 1.58 mm o.d. were used (Waters PCR module, Milford, CT, USA). The system with ABTS and DPPH solutions was monitored as follows: temperature range set at 25–55°C and the flow rate of the reagent was set at 0.5 mL/min for ABTS and 1.0 mL/min for DPPH. The ABTS and DPPH solutions were prepared following the methods described by Raudonis et al. (2009, 2012).

The reaction of the antioxidant compounds with the ABTS and DPPH reagents resulted in a colour change that was detected using an additional Waters 2487 UV/VIS detector (Waters Corporation). The detection of ABTS and DPPH in solution was recorded at 650 and 520 nm wavelengths, respectively. The signal strength, which is sensitivity related and reflected by the height of the negative peaks of the active compounds – isoquercitrin, rutin and chlorogenic acid, was chosen as the indicator for selecting analysis conditions.

The post-column antioxidant activity of the extract compounds was assessed by comparing their activity to the standard, Trolox. Calibration curves were prepared from a Trolox ethanol solution at eight dilutions in the range of 0.625–80 μg/mL for the ABTS reagent and at seven dilutions in the range of 0.8–205 μg/mL for DPPH.

3.5. *Reagents and chemicals*

The acetonitrile and methanol were of HPLC grade and supplied by Roth GmbH (Karlsruhe, Germany); the ethanol was produced by Stumbras (Kaunas, Lithuania). 2,2-diphenyl-1-picrylhydrazyl (DPPH, 95%) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

diammonium salt (ABTS, 98%) were purchased from Sigma (Sigma-Aldrich, Steinheim, Germany and St. Louis, MO, USA, respectively). Trolox (98%) and TFA (99%) were received from Fluka Chemika (Buchs, Switzerland). Sodium citrate, citric acid and potassium persulphate (99%) were purchased from Sigma (Sigma-Aldrich, Steinheim, Germany). The reference substances – chlorogenic acid (95.33%), rutin trihydrate (97.11%) and isoquercetin (94.16%) – were purchased from HWI ANALYTIK GmbH (Ruelzheim, Germany). Ultrapure water was purified with a Millipore water cleaning system (Bedford, MA, USA).

3.6. *Data analysis*

The statistical package SPSS 17 (SPSS, Inc., Chicago, IL, USA) was used for statistical processing and evaluation of the received data. One-way ANOVA was used to estimate significant differences among the variables using different reaction coil lengths and temperature regimes. MS Excel was used for the graphical presentation of the results.

4. Conclusions

HPLC coupled with the post-column DPPH and ABTS technique was used for the development and validation of an effective method for screening the antioxidant activities of *S. canadensis* raw materials. These investigations established that the temperature in the reaction coil served as a relevant factor affecting the signal strength of the analytes after reacting with the DPPH and ABTS radicals, while the length of the coil did not have a significant effect on the peak height of the goldenrod individual radical scavengers. Considering the obtained results for the optimal post-column reaction conditions, an optimised assay for measuring the antioxidant activities of goldenrod raw materials exhibits the following factors: a coil temperature regime of 50°C in combination with a coil length of 3 or 20 m.

Validation experiments confirmed sufficient precision, sensitivity and effectiveness of the corresponding method, which could be used effectively for further evaluations of active antioxidant compounds in plant materials of similar origin.

Supplementary material

Supplementary material relating to this paper is available online, alongside Tables S1–S3 and Figures S1–S2.

Disclosure statement

No potential conflict of interest was reported by the authors.

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SUPPLEMENTARY MATERIAL

Development of an HPLC post-column antioxidant assay for Solidago canadensis radical scavengers

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Abstract

The aim of the present work was to modify and validate the post-column HPLC-ABTS and DPPH methods for evaluating the antioxidant activity of the methanolic extracts of *Solidago canadensis* (Canadian goldenrod) leaves and flowers. Separation of the analytes was performed via the HPLC-PDA method on a YMC analytical column using a gradient elution program. Three compounds with antioxidant properties – chlorogenic acid, rutin and isoquercitrin – and two unidentified antioxidants were established. The research showed that the coil temperature regimes and loop length combinations influence the optimized post-column assay method for detecting the antioxidant activity of goldenrod radical scavengers. Investigations established that the temperature in the reaction coil was a substantial factor contributing to the signal strength of the analytes after reacting with the DPPH and ABTS radicals.

Keywords: HPLC post-column; coil temperature; loop length; DPPH; ABTS; *Solidago canadensis.*

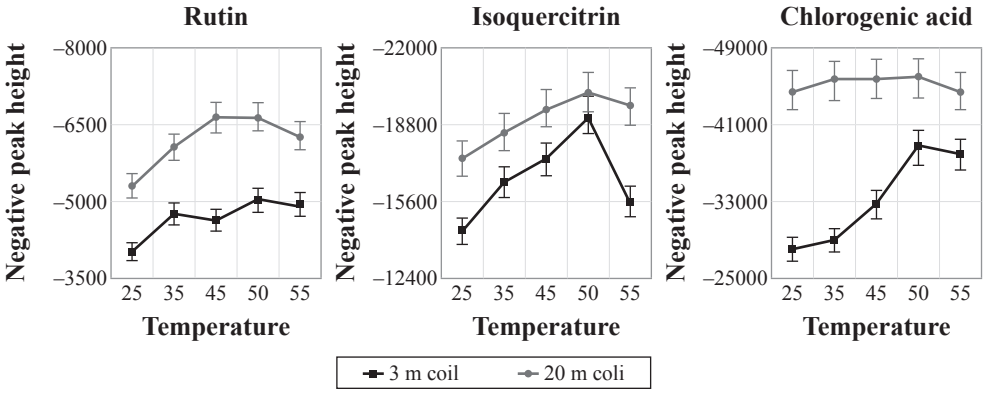

Figure S1. *Coil length and temperature influence for the post-column reaction using the ABTS reagent (according to negative peak heights in micro-volts)*

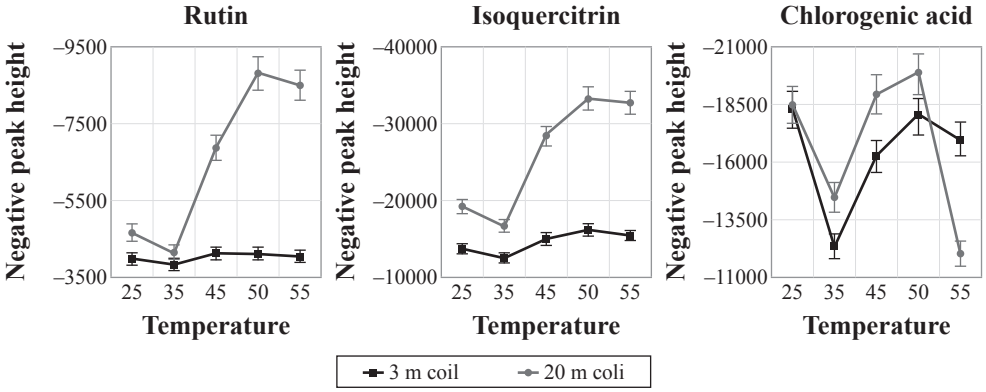

Figure S2. *Coil length and temperature influence for the post-column reaction using the DPPH reagent (according to negative peak heights in micro-volts)*

Table S1. *Analytical characteristics of the HPLC method validation data*

Standards	T_R (min)	Intra-day RSD (%) (n=5)	Inter-day RSD(%) (n=5)	R^2	Regression equation	LOD (µg/mL)	LOQ (µg/mL)
Chlorogenic acid	11.2	0.8	1.10	0.999718	$Y = 4.26 \cdot 10^7 \cdot x + 1.40 \cdot 10^4$	0.0995	0.33
Rutin	23.7	0.9	1.30	0.999865	$Y = 2.27 \cdot 10^7 \cdot x + 4.02 \cdot 10^3$	0.3	1.25
Isoquercitrin	25.5	0.4	0.60	0.999852	$Y = 6.42 \cdot 10^7 \cdot x + 1.13 \cdot 10^3$	0.13	0.44

T_R: retention time, RSD: relative standard deviation, R^2: correlation coefficient, LOD: limit of detection, LOQ: limit of quantitation.

Table S2. *Validation characteristics of the ABTS and DPPH post-column assays*

Parameters	ABTS assay				DPPH assay			
	Trolox	**Chlorogenic acid**	**Rutin**	**Isoquercitrin**	**Trolox**	**Chlorogenic acid**	**Rutin**	**Isoquercitrin**
Linearity range (μg/mL)	0.625–80	1.88–60	0.94–30	1.00–33	0.80–205	1.88–60	0.94–30	1.00–33
Intra-day RSD (%)[a]	0.30	2.00	2.20	1.20	0.30	1.90	1.60	2.30
Inter-day RSD (%)[b]	0.40	2.50	2.90	1.30	1.60	3.00	2.00	2.60
LOD (μg/mL)	0.26	0.78	0.39	0.42	0.87	0.83	0.42	0.44
LOQ (μg/mL)	0.36	2.62	1.75	1.39	1.18	2.77	1.33	1.48

RSD: relative standard deviation, LOD: limit of detection, LOQ: limit of quantitation.

Table S3. *Comparison of the radical scavenging activity of individual compounds expressed as TEAC (μmol/g) between Solidago canadensis leaf (1) and flower (2) extracts using the ABTS and DPPH post-column assay*

Peak No.	Analytes	ABTS assay				DPPH assay				*P**
		1	**2**	**t**	***P***	**1**	**2**	**t**	***P***	
1	Chlorogenic acid	16.9±0.05	15.6±0.18	15.50	0.001	97.65±0.86	40.38±0.2	144.77	0.001	0.001
2	Rutin	10.48±0.24	8.55±0.38	9.51	0.001	18.15±0.25	18.93±0.1	–3.81	0.005	0.001
3	Isoquercitrin	0.75±0.04	0.56±0.02	10.73	0.001	0.84±0.04	0.73±0.02	6.13	0.001	0.03
4	Unknown	57.8±0.31	58.3±0.23	–3.14	0.014	131.77±0.14	153.04±0.23	–178.01	0.001	0.001
5	Unknown	31.92±0.2	22.3±0.16	82.53	0.001	143.78±0.28	144.97±0.07	–9.16	0.001	0.001

Values are expressed as mean quantities with *standard deviation*, t: Student's statistic, *P*: significant level, *P** significant level for comparisons between ABTS and DPPH assays.

A3

Title: Assessment of Solidago x niederederi Origin Based on the Accumulation of Phenolic Compounds in Plant Raw Materials

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Assessment of *Solidago* × *niederederi* Origin Based on the Accumulation of Phenolic Compounds in Plant Raw Materials

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Abstract

This study provides the first phytochemical characterization of the morphologically identified natural hybrid *Solidago* × *niederederi* Khek compared with the native *Solidago virgaurea* and two invasive species, Canada goldenrod (*Solidago canadensis* L.) and giant goldenrod (*Solidago gigantea* Aiton). The phenolic compounds, namely, chlorogenic acid, rutin, isoquercitrin, hyperoside, and quercitrin, were detected in leaves and inflorescences by the high-performance liquid chromatography–photodiode array detector/ultraviolet (PAD/UV) method. All analyzed *Solidago* species contained all of the phenolic compounds investigated. The quantitative phytochemical differentiation among *Solidago* taxa was shown by principal component analysis. The results indicated that *S. gigantea* plants were characterized by significantly different quantities of phenolic compounds compared with three other *Solidago* taxa, which formed a separate cluster in the space of the principal component model, indicating the high similarity of their profiles. An additional multivariate analysis of the three species studied revealed a chemical gradient from *S. canadensis* to *S. virgaurea* with a slightly overlapping zone on the score plots presented by *S.* × *niederederi* and *S. virgaurea* accessions. The results showed that *S.* × *niederederi* was closely related to *S. virgaurea*. This result is suggestive of a hybrid origin with significant contributions from the native species. However, *S.* × *niederederi* was significantly different from its parental species with respect to chlorogenic acid and quercitrin in leaves and rutin with isoquercitrin in inflorescences. Conversely, samples indicating intermediate chemical composition between native *S. virgaurea* and invasive *S. gigantea* were not distinguished. The comparison of phenolic compound accumulation in *Solidago* plants supported the additional identification of the origin of *S.* × *niederederi*.

Introduction

Invasive species are viewed as a significant component of global environmental changes that threaten biological diversity and functioning of ecosystems. The Delivering Alien Invasive Species Inventories for Europe (DAISIE) database contains 2,843 records of plant species alien to Europe, most of which are naturalized in new habitats (Hejda et al. 2009; Pyšek et al. 2012). Canada goldenrod (*Solidago canadensis* L.) and giant goldenrod (*Solidago gigantea* Aiton) are exceptionally successful worldwide invaders from North America and have a great potential to invade new habitats in the future (Weber 2001). Invasive *Solidago* spp. are well adapted to a relatively wide range of habitats with variable soil conditions that are more conducive to their growth and lead to the development of monodominant stands and unification of the landscape over large areas (Fenesi et al. 2015; Karpavičienė et al. 2015; Scharfy et al. 2009). In this way, the spread of alien *Solidago* spp. disturbs spontaneous successions and reduces plant diversity. In addition, alien species invade habitats of native congeners, which affects the origin of new taxa. The hybridization of invasive species with native plants is common and leads to the transfer of adaptations among invasive species. This process increases their invasive potential and can cause a loss of genetic diversity, fitness, threat of extinction of native species, and even evolutionary changes in spontaneous flora (Ellstrand and Schierenbeck 2006; Hovick and Whitney 2014). The spontaneous hybrids becoming stabilized are considered as new alien species (Pyšek et al. 2004).

The natural hybrid *S.* × *niederederi* Khek between the native to Europe *S. virgaurea* and alien *S. canadensis* was first recorded more than a century ago in Austria (Khek 1905). Currently, a comparatively rapid spread of *S.* × *niederederi* in European countries is observed (Jaźwa et al. 2018). The hybrid origin of *S.* × *niederederi* has been estimated using plant morphological characters, achene development, and pollen viability characters (Karpavičienė and Radušienė 2016; Migdałek et al. 2014). The complication of hybrid identification arises due to extremely diverse morphology of *Solidago* species, which causes taxonomic confusion within the genus (Semple et al. 2015). Consequently, alien *Solidago* plants with pubescent stems

and leaves and inflorescences with ascending lower branches may be considered as one complex taxon *S. canadensis* s.l. (Weber 1997). *S. gigantea* appears to be one of the more clearly defined taxa; however, it also refers as a whole complex of varieties (Weber and Jakobs 2005). Furthermore, the European *S. virgaurea* is also highly polymorphic and divided into several closely related taxa (Kiełtyk and Mirek 2014). Taking into consideration the geographical differences in origin and high intraspecific morphological diversity within *Solidago* species, it can be assumed that the identification of hybrids and their parental species solely based on morphological traits can be misleading. Recently, the first genetic identity of hybrids has been confirmed using molecular methods (Pliszko and Zalewska-Gałosz 2016). According to Desjardins (2008), in addition to genetic, classical morphological and other nonmorphological methods, phytochemical evaluation can provide supplementary information on species identification.

The primary objectives of this research were to (1) assess the origin of morphologically identified *S.* × *niederederi* on the basis of phenolic compound accumulation and (2) to reveal chemotaxonomic value of some phenolics for identification of *Solidago* taxa. As far as we know, this study provides the first chemical characterization based on a comparison of the phenolic compound profiles of *S.* × *niederederi* and other *Solidago* species growing in close sympatry with the hybrid.

Materials and Methods

Sample Collection and Preparation

Nineteen accessions of *S. virgaurea*, 17 of *S. canadensis*, 11 of *S. gigantea*, and 24 of *S.* × *niederederi* were collected from 6 mixed wild populations in which all taxa were growing together. The sample of harvested plant material consisted of the three tops of two to three shoots of the same clonal plant in the flowering phase. The botanical identification of *Solidago* taxa was carried out by morphological diagnostic features defined in our previous work (Karpavičienė and Radušienė 2016). The herbarium vouchers of the accessions were deposited at the Herbarium of the Institute of Botany, Vilnius, Lithuania.

Collected plants were dissected into inflorescence and leaf parts and dried at 25 C. Air-dried material was mechanically ground to obtain a homogenous powder, and then samples of approximately 0.1 g were extracted in 10 ml of 70% methanol by ultrasonication at 25 C for 50 min. The solutions were filtered and stored at 4 C until analysis.

Chemicals

Solvents were of high-performance liquid chromatography (HPLC) grade and supplied by Roth GmbH (Karlsruhe, Germany). The reference substances, chlorogenic acid (≥ 95.33%), rutin trihydrate (97.11%), and isoquercitrin (≥ 94.16%), were purchased from HWI Analytik GmbH (Ruelzheim, Germany); quercitrin (≥ 98.0%) was obtained from Roth GmbH. Water was filtered through the Millipore (Billerica, MA, USA) HPLC-grade water-preparation cartridge.

HPLC-PDA Analysis

HPLC analysis was performed using a Waters Alliance 2695 (Waters, Milford, USA) separation module system equipped with a Waters 2487 UV/Vis and Waters 996 PDA photodiode-array detector. The separation of the compounds was carried out on YMC-Pack ODS-A column (3.0 µm, 150 mm by 4.6 mm i.d.) with a guard cartridge (3.0 µm, 10 mm by 4.0 mm). The analytical conditions were performed according to the method described in Radušienė et al. (2015).

Peaks of compounds were identified at a wavelength range of 210–550 nm by comparing their UV-Vis spectra and retention times to those of authentic reference standards. The samples were analyzed twice. The chromatograms of standards and flower extracts are shown in Figure 1. The quantification of detected compounds was carried out by the external standard method. Standard stock solutions for chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin were freshly prepared in 70% methanol and diluted to six different concentrations to establish calibration equations. Three injections per concentration were performed to determine linearity. A linear regression equation for all calibration curves was calculated by the least-squares method. The regression coefficients were $R^2 > 0.999$, confirming the linearity of the concentration ranges.

Data Analysis

Multivariate statistical approaches using the Statistica v. 10.0 (StatSoft) software package were performed. One-way ANOVA and a post hoc Sheffe's multiple-comparison test were used to identify and specify significant differences of phenolic compound quantities among the investigated taxa. The relationship between variables was analyzed using Spearman's rank correlation. Principal component analysis (PCA) was used to detect groupings, similarities, or differences among all analyzed accessions according to statistically independent variables, which represent the phenolic metabolite quantities in *Solidago* species. PCAs were based on eight standardized variables (the concentrations of chlorogenic acid, rutin, isoquercitrin, and quercitrin in leaves and inflorescences). The data sets of phenolic compounds in leaves and inflorescences were combined and used in PCA, yielding more compelling results than separate PCAs for leaves or inflorescences. Each sample had a score along PCA components, which showed its location in the space of the principal component model. The data for hyperoside concentration were eliminated from the PCA, as this variable had little explained variance and contributed to noise, reducing the quality of the model.

Results and Discussion

Interspecific Differences in Phenolics

Phenolic compounds, namely, chlorogenic acid, rutin, isoquercitrin, hyperoside, and quercitrin, were detected in leaves and inflorescences of *S. virgaurea*, *S. canadensis*, *S. gigantea*, and *S.* × *niederederi*. Leaves showed considerably higher mean values for chlorogenic acid compared with inflorescences, whereas inflorescences showed significant accumulation of isoquercitrin. All analyzed *Solidago* species shared the same common phenolic constituents.

ANOVA revealed high significant differences ($P \leq 0.001$) for the mean quantities of all phenolic compounds in leaves and inflorescences among the investigated species. Post hoc multiple comparisons of mean quantities specified that *S. gigantea* significantly differed for all compounds among all species. The highest values of phenolics were found in the leaves and inflorescences of *S. gigantea* compared with other species, except for rutin. Meanwhile, the rutin content in both the leaves and inflorescences of other species greatly exceeded that of *S. gigantea*, showing the highest accumulation in *S.* × *niederederi* leaves and in

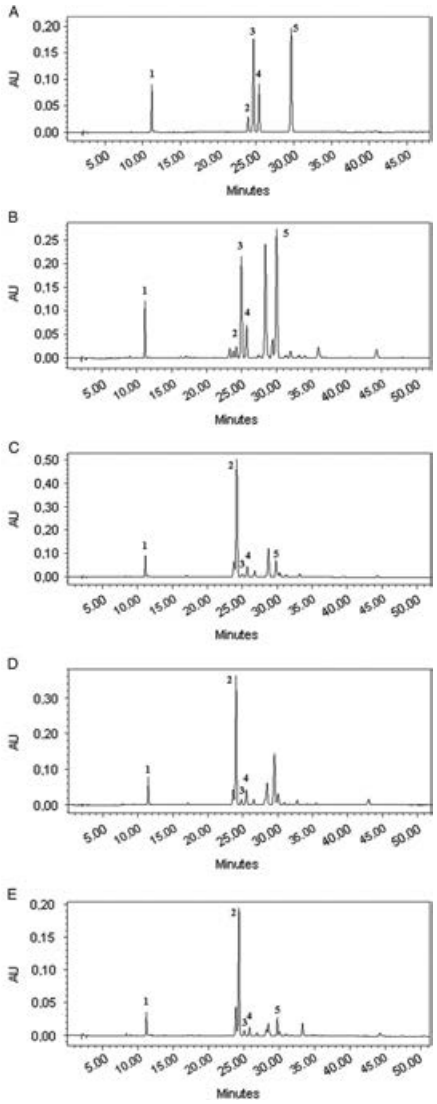

Figure 1. Chromatograms of reference standards (A) and inflorescence extracts of *Solidago* (B), *S. canadensis* (C), *S. virgaurea* (D), and *S.* × *niederederi* (E) detected by HPLC-PAD. Peak identified: 1, chlorogenic acid; 2, rutin; 3, hyperoside; 4, isoquercitrin; 5, quercitrin.

S. canadensis inflorescences. Furthermore, the differences for phenolics were analyzed between *S.* × *niederederi* and putative parental species *S. virgaurea* and *S. canadensis*. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included. Significant differences among the three taxa were observed for chlorogenic acid and quercitrin in the leaves and for chlorogenic acid, rutin, and isoquercitrin in the inflorescences (Tables 1 and 2). The leaves of *S.* × *niederederi* significantly accumulated the highest ($P < 0.01$) level of chlorogenic acid, whereas the flowers of *S. canadensis* showed significant accumulation of this compound in comparison with the other two species. However, the amount of chlorogenic acid in the flowers did not differ significantly ($P > 0.05$) between *S.* × *niederederi* and *S. virgaurea*. The rutin content in the inflorescences of *S. canadensis* greatly exceeded that in the flowers of *S.* × *niederederi* and *S. virgaurea*. The highest amount of quercitrin was accumulated in the leaves and inflorescences of *S. canadensis*, and the mean content of this compound was found in leaves and inflorescences of *S.* × *niederederi*. Meanwhile, quercitrin was detected in minor quantities or not found at all in the flowers of *S. virgaurea*. Species significantly varied for content of isoquercitrin in the inflorescences, the highest levels of which were found in *S. virgaurea* followed by *S.* × *niederederi* and *S. canadensis*. However, the amount of hyperoside did not differ significantly among the species for both plant parts.

Table 1. Differences of phenolic compound accumulation in leaves among four *Solidago* species.[a]

Compounds	*S. virgaurea* ($n = 19$)	*S. canadensis* ($n = 17$)	*S.* × *niederederi* ($n = 24$)	*S. gigantea* ($n = 11$)
	Quantities, mg g^{-1} DM[b]			
Chlorogenic acid	17.65 ± 2.86b	18.78 ± 6.43b	22.42 ± 5.90a	37.38 ± 6.86
Rutin	13.69 ± 3.33a	14.00 ± 6.92a	17.35 ± 7.20a	0.49 ± 0.30
Isoquercitrin	0.08 ± 0.09a	0.11 ± 0.14a	0.13 ± 0.24a	2.50 ± 0.74
Hyperoside	0.28 ± 0.19a	0.41 ± 0.23a	0.52 ± 0.56a	0.93 ± 0.40
Quercitrin	0.07 ± 0.22c	1.08 ± 0.58a	0.33 ± 0.31b	39.48 ± 6.83

[a]Differences among four species for all compounds were significant at $P \leq 0.001$. n = the number of accessions analyzed. Values (mean ± SD) followed by different letters among first three species are significantly ($P \leq 0.05$) different according to Scheffe's test. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included.
[b]Abbreviation: DM, dry mass.

Table 2. Differences of phenolic compound accumulation in inflorescences among four *Solidago* species.[a]

Compounds	*S. virgaurea* ($n = 19$)	*S. canadensis* ($n = 17$)	*S.* × *niederederi* ($n = 24$)	*S. gigantea* ($n = 11$)
	Quantities, mg g^{-1} DM[b]			
Chlorogenic acid	5.80 ± 1.69b	9.34 ± 2.48a	6.67 ± 1.74b	13.72 ± 3.52
Rutin	6.50 ± 1.50c	18.22 ± 6.96a	9.20 ± 3.74b	1.42 ± 0.34
Isoquercitrin	1.98 ± 0.65a	0.34 ± 0.28c	1.25 ± 0.39b	12.78 ± 3.71
Hyperoside	0.74 ± 0.24a	0.66 ± 0.43a	0.78 ± 0.50a	1.59 ± 0.49
Quercitrin	0.00b	0.23 ± 0.39a	0.02 ± 0.08b	18.10 ± 4.91

[a]Differences among four species for all compounds were significant at $P \leq 0.001$. n = the number of accessions analyzed. Values (mean ± SD) followed by different letters among three first species are significantly ($P \leq 0.05$) different according to Scheffe's test. Differences among four species for all compounds were significant at $P \leq 0.001$. *Solidago gigantea* was excluded from the multiple-comparison test because the remaining taxa were not different for most phenolic compounds when *S. gigantea* was included.
[b]Abbreviation: DM, dry mass.

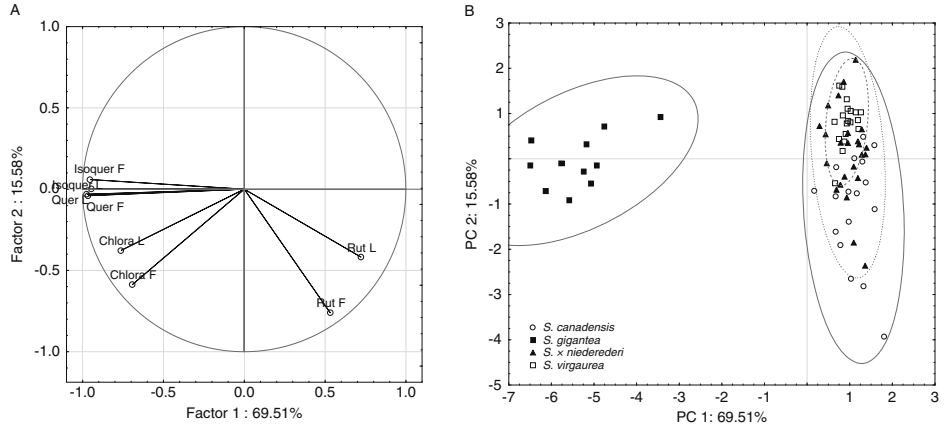

Figure 2. PCA1 representing four *Solidago* taxa for accumulation of phenolic compounds. (A) Loading plots for compound variables contributing to PC1 and PC2. (B) Score plots for the testing accessions with 95% confidence ellipses limit for each species.

Consequently, *S. virgaurea* accumulated the lowest quantities of phenolic compounds compared with *S. canadensis* and *S.* × *niederederi*, with the exception of isoquercitrin in leaves. In contrast, among the three *Solidago* species investigated, for the most part *S. canadensis* contained the highest amounts of the phenolic compounds. *Solidago* × *niederederi* showed average values of phenolics except for having the highest concentration of chlorogenic acid in leaves.

Principal Component Analysis

The first PCA1 model that represented the two-dimensional scatter plots showed differentiation between the sets of chemical data of the four *Solidago* species studied. The score plots for the first two PCs explained 85% of the total variance and represented enough visualization for any possible patterns of the data. PC1 accounted for 69.51% of the observed total variability and was strongly associated with negative loadings of quercitrin (Quer F, Quer L) and isoquercitrin (Isoquer F, Isoquer L) in flowers and leaves and chlorogenic acid (Chlora L) in leaves as well as the positive variable of rutin (Rut L) in leaves. The PC2 model explained 15.58% of the total variance and was characterized by negative loadings of rutin (Rut F) and chlorogenic acid (Chlora F) in flowers (Figure 2A). The PCA1 score plots with 95% confidence limit ellipses for the four species showed an arrangement of accessions into two distinct groups (Figure 2B). Within the right-hand plots, one group was formed by three overlapping ellipses along PC2 covering all accessions of *S. canadensis*, *S. virgaurea* and *S.* × *niederederi*. The location of accessions on the score plots can be explained by the position of variables on the loading plots. Rut F, Rut L, and Chlora F, with higher negative loading on PC2, were found to have high to moderate values in corresponding samples, while Quer F, Quer L, Isoquer F, and Isoquer L, with high negative loading on PC1, were accumulated in low quantities in all accessions of this group. The second group of accessions within the left-hand plots brought together all *S. gigantea* samples, which were closely clustered along negative PC1. In contrast to the first group within the right-hand plots, Quer L, Quer F, Isoquer L, and Isoquer F, scoring high in PC1, were detected as having the highest contents in those accessions; conversely, Rut L and Rut F, scoring low in PC1, were found in minor quantities. Chlora L and Chlora F, loading high to moderately in both PCs, were associated with higher levels of chlorogenic acid, especially in flowers. The results clearly suggested that accessions of *S. gigantea* were highly different with respect to phenolic content compared with the first group, which was formed by accessions of the other three *Solidago* species. The PCA1 model did not clearly separate morphologically different taxa as *S.* × *niederederi*, *S. virgaurea*, and *S. canadensis*. Consequently, the PCA2 model for the three species was used, in order to focus more specifically on differentiation among *S.* × *niederederi*, *S. virgaurea*, and *S. canadensis* accessions.

In the new PCA2, the first two PCs explained more than 56% of the total variance in the data set and presented much better separation between accessions of corresponding species. PC1 accounted for 37% of the observed variance and was best characterized by positive loadings of Chlora F, Rut F, and Quer L variables and negative Isoquer F. PC2 was highly associated with negative loadings of Chlora L and Rut L and moderate with positive Quer F (Figure 3A). Graphical representation of the score plots showed separation of all *S. canadensis* accessions in a separate cluster on the right-hand plot (Figure 3B). The positioning of *S. canadensis* samples coincided with higher loadings of Chlora F, Rut F, Quer L, and Quer F, indicating the greater influence of those variables on the colocated scores. The opposing location of Isoquer F to Chlora F, Rut F Quer L, and Quer F displayed negative correlations, which reflect opposite values of corresponding compounds in the accessions. One sample in the lower right-hand plot was separated from all of the others within the ellipses, suggesting that its composition differs significantly from the other accessions. Indeed, this sample contained the highest values of rutin and chlorogenic acid in both leaves and inflorescences. Meanwhile, the scores of *S.* × *niederederi* and *S. virgaurea* represented two overlapping ellipses. Additionally, *S.* × *niederederi* samples were much more scattered on PC1 versus PC2 along with Rut L and Chlora L loadings, displaying high

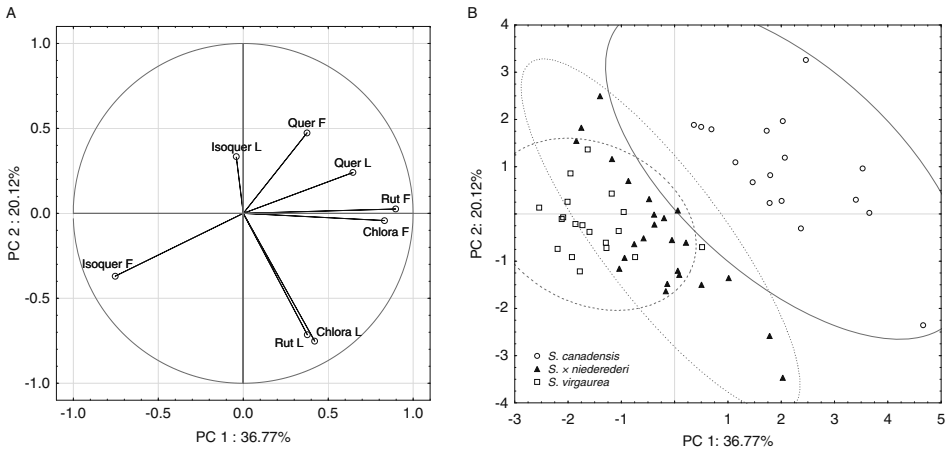

Figure 3. PCA2 representing three *Solidago* taxa for accumulation of phenolic compounds. (A) Loading plots for compound variables contributing to PC1 and PC2. (B) Score plots for the testing accessions with 95% confidence ellipses limit for each species.

variation in rutin and chlorogenic acid content in leaves. For these compounds, *S.* × *niedererderi* was similar to *S. canadensis*. Some of *S.* × *niedererderi* accessions exposed a tight position with *S. virgaurea*, indicating a great similarity in chemical content. Some hybrid samples demonstrated a remote position from all the others, scoring a high negative in PC2 and a low positive in PC1, which suggested higher amounts of chlorogenic acid and rutin in their leaves. The opposing location of some accessions on the upper left-hand score plot associated with low values of Chlora L and Rut L in those samples. Meanwhile, Quer F, scoring moderately in PC2, was found in mean quantities in previous samples. Isoquer F, scoring high in PC1 and moderately in PC2, had the greatest influence on colocated accessions and associated mean content of isoquercitrin in flowers of both species. Moreover, negative correlation of Isoquer F with Chlora F, Rut F, and Quer L suggested low values of these compounds in the corresponding samples. Consequently, the PCA2 model demonstrated that *S. canadensis* accessions formed a separate cluster, indicating a quantitative composition of phenolics highly different from that of from *S.* × *niedererderi* and *S. virgaurea*. Conversely, *S.* × *niedererderi* and *S. virgaurea* accessions did not reveal the separation of two well-defined groups, with high overlapping on the score plots.

In the current research, the main phenolic compounds in *Solidago* spp. plant materials, the quercetin-3-*O*-glycosides: quercitrin, isoquercitrin, rutin, and hyperoside, together with chlorogenic acid, were used to ascertain relationships and differences between *S.* × *niedererderi* and other *Solidago* species growing in mixed populations. In general, the phenolic compounds are the most widely used of all secondary constituents in chemotaxonomic studies, mainly due to their ubiquitous occurrence in plants and their structural variability and chemical stability (Braunberger et al. 2015; Clark et al. 2014; Švehlíková et al. 2002). Furthermore, the phenolics listed above are considered principal bioactive compounds in *Solidago* spp. and are used for plant-derived medical preparations (Melzig 2004; Sabir et al. 2012). The present research on the comparison of four taxa demonstrated that all analyzed *Solidago* spp. exposed the same investigated phenolic constituents, which seems to be characteristic of the whole *Solidago* genus. On the other hand, the data obtained showed the significant quantitative differences in phenolics among corresponding taxa. The highest values of all studied phenolic compounds, with the only exception being rutin content in leaves and flowers, were found in *S. gigantea*. Further, chlorogenic acid was the only compound whose content in the leaves of *S.* × *niedererderi* significantly exceeded that found in parental species, while the content of other compounds was intermediate or did not differ significantly. The accumulation of high amounts of phenolics may provide a competitive advantage via suppression of neighboring plants (Kim and Lee 2011). Moreover, phenolic compounds are known to be particularly important for plant interactions with abiotic and biotic environments and constitute one of the most common defense groups against herbivores (Mallikarjuna et al. 2004; Treutter 2006). Furthermore, chlorogenic acid is known to be associated with plant resistance to insects, fungi, bacteria, and viruses (Leiss et al. 2009; Niggeweg et al. 2004). It should be noted that chlorogenic acid and rutin in the leaves of *S.* × *niedererderi* was highly variable. Moreover, chemical variability provides abundant material for environment-mediated selection and may play a role in superior resistance to herbivores and pathogens. Therefore, this variability may contribute to a higher hybrid fitness and persistence in a different environment (Oberprieler et al. 2011).

The chemistry of hybrids varied not only qualitatively, but also quantitatively (Orians 2000). Generally, hybrids express parental chemicals, but parental compounds are sometimes missing, or novel compounds are present. The survey by Cheng et al. (2011), based on 1,112 secondary metabolites of different hybrids, showed that the frequency of metabolite novelty accounted for 5.5% of all studied compounds. The probability of occurrence of novel compounds increases in polyploids, especially in F_2 and later-generation hybrids (Orians 2000). Accordingly, we have not detected any novel chemicals in *S.* × *niedererderi*, which is diploid and apparently only a first-generation hybrid (Karpavičienė and Radušienė 2016).

PCA has been used here as an exploratory method to describe data sets without a priori knowledge of the data structure and to

allow the visualization of the similarities and differences within analyzed data to indicate the compounds most responsible for separation of different plant groups. In this regard, the position of *S. gigantea* accessions on PC1 versus PC2 plots at a distance from others represented a greater difference in chemical composition as determined by the study of all phenolic compounds. Meanwhile, the other three taxa formed a separate cluster, indicating similarities in their quantitative accumulation of phenolics. It can be assumed that interspecific chemical variability among *Solidago* species should be genetically based. However, the mode of qualitative inheritance of most chemical compounds is Mendelian with dominance; if both or one of the parents produce a chemical, the hybrids almost always produce it as well (Orians 2000). It can be assumed that the genes responsible for the synthesis of phenolics are constitutively, but differently expressed in the analyzed species (Cheng et al. 2011). Although morphologically and phylogenetically *S. canadensis* is closer to *S. gigantea* than to *S. virgaurea* (Semple 2016), invasive *Solidago* spp. differ in their ploidy level. *S. gigantea* is tetraploid ($2n = 36$), whereas *S. canadensis* is diploid ($2n = 18$), as are *S. virgaurea* and *S.* × *niederederi* (Karpavičienė and Radušienė 2016). The ploidy level is known to have a significant effect on the intraspecific variation in concentrations of metabolites in *S. gigantea* (Hull-Sanders et al. 2009). In this context, tetraploid *S. gigantea* showed high mean phenolic content, while diploid *Solidago* spp. exhibited comparatively lower content for the compounds analyzed.

The additional PCA for the three species studied, with the exception of *S. gigantea*, demonstrated that the parental species *S. virgaurea* and *S. canadensis* were well distinguished from each other based on the analyzed phenolics. Although interspecific hybrids can often be clearly distinguished from parental species based on their biochemical phenotypes (Kirk et al. 2012), *S.* × *niederederi* accessions did not show itself to be a clearly defined group compared with the parental species, being in close position with *S. virgaurea*. They clustered less tightly and displayed greater variation in chemical composition, with some of the samples falling outside the range of the parental species. These results are similar to those reported previously by Kirk et al. (2005) for chemical composition of two *Senecio* species and their F_1 hybrids, which did not cluster intermediately to parents on the basis of PCA. According to Orians (2000), parental chemicals in hybrids are mostly either expressed at concentrations similar to one of the parents or at intermediate concentrations. However, *S.* × *niederederi* significantly exceeded the parental species in mean chlorogenic acid content in leaves, but the range of variation of this compound in the hybrid was similar to that of *S. canadensis*. The results agree with other studies showing that hybrid plants have much more genetic variation than the parental species (Ellstrand and Schierenbeck 2006; Zalapa et al. 2010), which leads to higher phenotypic diversity. Overall, in our research, *S.* × *niederederi* was characterized by the accumulation of phenolic compound content intermediate or similar to that of *S. virgaurea*, suggesting its hybrid origin.

The sparse chemical diversity of invasive species can be explained by the assumption that invasive species undergo one to several bottlenecks when they are introduced to new areas, which leads to genetic uniformity in plant populations (Müller-Schärer and Schaffner 2008). In addition, there exist some data about the lack of differentiation in DNA content observed in native *S. virgaurea* (Szymura et al. 2015) that may be associated with low chemical diversity. However, in contrast to low chemical diversity, high intraspecific morphological variations have been observed in the analyzed *Solidago* species (Karpavičienė and Radušienė 2016; Kiełtyk and Mirek 2014; Semple et al. 2015). Based on the hypothesis of Bossdorf et al. (2005), high plasticity of morphotype allows introduced species to naturalize across different environments, revealing the potential for their invasiveness. Therefore, the use of only morphological descriptions to examine taxa with large intraspecific variation may result in wrong conclusions and taxonomic confusion, as has been the case with *Solidago* taxa. Consequently, the phytochemical pattern in the present study complemented the evidence of hybrid *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*. However, it can be assumed that *S.* × *niederederi* is continuously formed in mixed *Solidago* spp. populations, because the phytochemical intermediate of the hybrid is not fixed, suggesting that it is not a completely stabilized hybrid derivative leading to the creation of new genotypes and evolutionary novelty. *Solidago* × *niederederi* has occurred rather frequently in mixed *Solidago* spp. populations; therefore, it has the potential to spread and increase due to viable seed production (Migdałek et al. 2014) as well as through vegetative propagation by clonal growth (Pliszko and Kostrakiewicz-Gierałt 2017). However, accessions exhibiting an intermediate chemical composition between spontaneous *S. virgaurea* and invasive *S. gigantea* were not detected. This can be explained by the differing number of chromosomes of these species, which leads to more complicated hybridization. On the other hand, *S.* × *snarskisii* Gudž. & Žaln., the spontaneous putative hybrid between *S. virgaurea* and *S. gigantea*, has been recently described (Gudžinskas and Žalneravičius 2016). Furthermore, the appearance of a new hybrid indicated the growing impact of invasive *Solidago* spp. on local flora.

Although statistical analysis revealed the presence of phenolic compounds such as the chlorogenic acid and quercitrin in leaves and rutin with isoquercitrin in inflorescences of *S.* × *niederederi* at significantly different quantities from its parental species, high variability of these components and their overlapping ranges between species impact clear identification of hybrids in the wild. Nevertheless, the present study provided the first examination of expression of phenolic compounds in *S.* × *niederederi*. It is important to further assess its inheritance of phenolic compound expression in the second and later generations of the hybrids, which is very likely, because according to Pliszko and Kostrakiewicz-Gierałt (2017), hybrids are able to generate their own offspring by sexual reproduction. On the other hand, the implication of phenolic compounds does not preclude the importance of other secondary metabolites in species differentiation. Additional analysis of these species could show some differences for minor components, which could expose their importance in chemotaxonomy and the evolutionary dynamics of invasion. Overall, *Solidago* is a suitable model genus for the study of the plant invasion process, and the present study provides a basis for further research on the role of genetic diversity and expression of secondary metabolites during hybridization on invasive potential.

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Title: Distribution Patterns of Essential Oil Terpenes in Native and Invasive Solidago Species and Their Comparative Assessment

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Distribution Patterns of Essential Oil Terpenes in Native and Invasive *Solidago* Species and Their Comparative Assessment

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Abstract: The importance of invasive *Solidago* L. species to the environment creates a new approach to controlling their spread through the use of potentially high value raw materials. The aim of this study was to assess the distribution patterns of volatile compounds in the four *Solidago* spp., by identifying common and species-specific compounds with their potentials, and to confirm the origin of the spontaneous hybrid *Solidago* × *niederederi* on the basis of comparative assessment of essential oil (EO) profiles. Plant material in the flowering phase was collected in mixed populations from six different sites. The EOs were isolated separately from the leaf and the inflorescence samples by hydrodistillation for 3 h. The chemical analysis was performed by gas chromatography—mass spectrometry. Multivariate data analysis was employed to explain the interspecies relationships among *Solidago* spp. The results revealed the similarity among *Solidago* spp. EO profiles, which were dominated by monoterpenes and oxygenated compound fractions. *Solidago* spp. differed in species distinctive terpenes and their distribution between accessions and plant parts. Volatile compound patterns confirmed the origin of *Solidago* × *niederederi* between *Solidago canadensis* and *Solidago virgaurea*, with the higher contribution of alien species than native ones. Correct taxonomic identification of species is highly essential for the targeted collection of raw material from the wild for different applications. *Solidago* spp. can be considered to be underutilized sources of bioactive secondary metabolites.

Keywords: invasive species; distinctive terpenes; interspecific diversity; *Solidago* × *niederederi*; underutilized resources

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1. Introduction

The use of plant products has grown remarkably in recent years, and research into natural products such as volatile terpenoids has become an important task for future human and animal well-being [1]. Natural products are generally easy to prepare and, due to their natural origin, are environmentally friendly and not financially challenging. In this respect, invasive species are of growing interest as a potential resource for obtaining high value-added products. *Solidago canadensis* L. (Canadian goldenrod) and *Solidago gigantea* Aiton (Giant goldenrod), native to North America, are considered to be one of the most aggressive plant invaders, which were introduced to Europe as garden ornamentals in the middle of the 18th century and began to spread in the 19th century [2]. Abandoned previously cultivated and disturbed areas contribute to a rapid and successful invasion

of goldenrods. Both species form pure dense stands due to the clonal growth of their re-sprouting rhizome system, which provides a strong competitive ability to eliminate other grassland species while reducing biodiversity [3]. Propagation of the *Solidago* species easily by wind distributed seeds guarantees their distance dispersal and the occupation of new disturbed areas creating homogenized landscape [4]. On the other hand, spontaneous hybridization gives rise to new hybrids, such as sexually reproducing *Solidago* × *niederederi* Khek., which was first recorded in Austria more than a century ago [5]. The hybrid has been described as a new alien species between the invasive *S. canadensis* and native *Solidago virgaurea* L. (European goldenrod), which is spreading rapidly along with the parental species, increasing the negative impact on the native flora [6,7]. In addition, phytotoxicity of alien species has been often invoked as a significant factor negatively influencing the native species and composition of plant communities [8,9]. The fastest and cheapest result to eradicate or limit the invasion of goldenrods is the use of herbicides [10]. However, the application of herbicides has a negative impact on the environment and their use is limited. Based on the importance of goldenrods to the surrounding environment, a new approach has recently been developed so that invasive species can be a potential source of high value-added products, instead of eliminating them by labor consumption and environmentally unfriendly ways [11]. The high biomass produced by exotic goldenrods is a promising source of renewable energy that can be exploited in rural households as an alternative to expensive firewood and that do not compete with crops for food or animal feed [12]. Late-flowering goldenrods attract pollinators and are honey bee plants that are considered to be superior to crops treated with pesticides [13]. *Solidaginis virgaureae herba* is included in the ESCOP Monographs with therapeutic indications for the treatment of urinary tract and genital disorders [14]. The European Medicines Agency in a finalized community monograph of *Solidago virgaurea* confirms traditional use of this plant material for the treatment of minor urinary tract complaints [15]. Furthermore, *S. gigantea*, *S. canadensis*, and their hybrids, as well as *S. virgaurea,* are included in European Pharmacopoeia [16]. A wide range of specialized metabolites have been reported in goldenrod raw materials, of which phenolic compounds and EOs were considered to be the most valuable [17–19]. The comparative evaluation of phenolic compounds in *Solidago* spp. has been presented in our previous studies [20].

Essential oils (EOs), due to the structural diversity in their constituents, expose a wide range of biological effects and are of great interest as a source of functional ingredients for agriculture, food, cosmetics, and pharmaceuticals [21]. Numerous studies have reported the potential use of EOs for integrated weed and pest control as an environmentally friendly approach [22]. Significant antifungal activity of *S. canadensis* EO was found against *Botrytis cinerea*, which reduced fruit rot and successfully controlled gray mold in inoculated strawberries [21]. Elshafie et al. [23] has also demonstrated antimicrobial activities of *S. canadensis* EO in vitro against some other phytopathogens. In this way, EOs of alien *Solidago* spp. are a promising source for the development of organic pesticides and can meet the high demand for their production. In addition, the allelopathic activity of *S. virgaurea* is considered as the herbicidal potential of EOs for environmentally acceptable weed control in organic farming [24]. The EO of *S. canadensis* has been shown to exhibit significant cytotoxic and antiproliferative activities against human tumor cell lines, correlating with the terpene compounds [25,26].

The major constituents of EO usually contribute to the principal role of the biological activity of the mixture, so their efficiency can be predicted to some extent from the complex of components [27]. On the other hand, even minor components have been shown to play a significant role in different biological activities due to their complementary and synergistic effects [28]. Consequently, it is important to know the distribution of phytochemical compounds in species and their populations due to the targeted selection of raw materials for their possible multifunctional use. The study hypothesized that the screening of *Solidago* spp. growing in the same area and in their mixed populations could provide reasonable comparative information on the volatile profiles and their chemotaxonomic relationships

and confirm the origin of the *S.* × *niederederi* taxon. The objectives of the study were: (1) to assess the distribution of volatile constituents in the populations of native and alien *Solidago* spp.; (2) to identify intraspecific and interspecific diversity in *Solidago* spp.; (3) to identify common and species distinctive volatiles; and (4) to confirm the origin of the spontaneous hybrid *S.* × *niederederi* on the basis of a multivariate comparative analysis of volatile profiles.

2. Results

2.1. Essential Oil Content of Solidago Species

Inflorescences took priority over the leaves in EO content in all the evaluated *Solidago* species. The highest yield of EO was obtained from inflorescences of *S. canadensis* (0.19–0.26%), followed by *S. gigantea* (0.16–0.23%), *S.* × *niederederi* (0.14–0.20%), and *S. virgaurea* (0.15–0.18%). Meanwhile, the leaves of *S. gigantea* accumulated the highest EO content (0.16–0.20%), followed by *S. canadensis* (0.14–0.18%), *S.* × *niederederi* (0.13–0.15%), and *S. virgaurea* (0.10–0.15%). Previous studies reported the similar range of EO yield in *S. canadensis* (0.18–0.27%) [23,29] and *S. gigantea* (0.15–0.16%) [29]. Kalemba [30] reported higher levels of EO contents in *S. virgaurea* (0.32–0.38%) than levels found in this study.

2.2. Chemical Profiles of Essential Oils

The EOs of four *Solidago* spp. were dominated by monoterpenes, with an average of 43.9–74.6% of the total composition of EOs in inflorescences and 39.7–69.1% in leaves. The mean percentage of sesquiterpenes ranged from 15.0 to 35.4% in inflorescences and from 25.5 to 38.2% in leaf EOs. Chemical profiles for EOs of *Solidago* spp. varied according to the contents of individual compounds and their distribution among accessions and plant parts. Examples of fingerprint profiles for inflorescence EOs are shown in Figure 1.

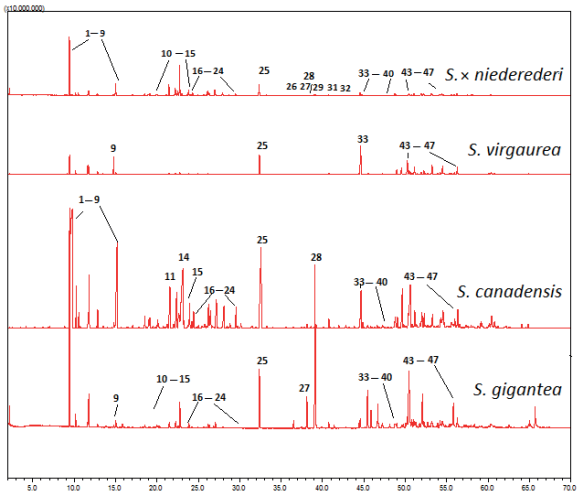


Figure 1. Examples of fingerprint profiles for *S.* × *niederederi*, *S. virgaurea*, *S. canadensis*, and *S. gigantea* inflorescence EOs performed by GC–MS using the GC–MS-QP2010 Ultra Gas system. The peak numbers correspond to the number of EO compounds listed in the Table 1.

Table 1. The frequency of distribution (F, %) of the compounds detected in more than 30% of the inflorescence EO samples in at least one of the four *Solidago* species; their mean relative percentage (M) with SD and significance (*p*) of interspecies differences according to the Kruskal–Wallis two-tailed test.

No	Compounds	LRI Exp.	LRI Ref.	*S. gigantea* (*n* = 7)			*S. canadensis* (*n* = 18)			*S.* × *niederederi* (*n* = 9)			*S. virgaurea* (*n* = 3)			*p*
				F	M	SD	F	M	SD	F	M	SD	F	M	SD	
1	α-pinene	930	930	100	10.7	8.31	100	18.1	13.84	100	17.4	12.84	100	28.6	8.96	0.196
2	camphene	945	948	71.4	1.8	0.77	72.2	1.7	0.69	77.8	1.2	0.34	100	1.0	0.35	0.713
3	thuja-2,4(10)-diene	950	957	28.6	0.6	0.35	66.7	0.5	0.16	44.4	0.7	0.32	66.7	0.6	0.13	0.504
4	sabinene	971	976	71.4	2.5	2.07	66.7	1.1	0.79	66.7	1.3	1.79	33.3	0.6	–	0.258
5	β-pinene	974	980	71.4	3.0	0.90	83.3	2.3	1.40	88.9	2.2	1.71	100	3.6	1.51	0.536
6	β-myrcene	992	991	42.9	1.6	0.34	55.6	0.8	0.60	33.3	0.6	0.15	66.7	1.5	0.23	0.564
7	*p*-cymene	1018	1014	42.9	2.5	1.42	5.6	0.5	–	0	0	–	0	0	–	0.021
8	*o*-cymene	1022	1009–1076	42.9	10.9	6.57	55.6	0.5	0.42	33.3	0.5	0.30	0	0	–	0.338
9	limonene	1023	10270	71.4	1.1	0.19	100	5.2	4.48	88.9	5.8	5.05	100	1.1	0.57	0.032
10	linalool	1090	1098	0	0	–	16.7	0.1	0.07	44.4	0.5	0.10	33.3	0.8	–	0.105
11	[1] **α-campholenal**	1109	1105	85.7	1.3 a	0.58	100	4.1 b	1.45	100	3.6 b	1.61	66.7	3.1	1.35	0.004
12	***trans*-pinocarveol**	1135	1139	100	0.8 a	0.20	88.9	2.9 b	1.18	100	3.0 b	1.28	100	1.6	0.93	0.005
13	*trans*-sabinol	1139	1140	42.9	0.3	0.16	0	0	–	0	0	–	0	0	–	0.004
14	***trans*-verbenol**	1143	1144	100	2.9 a	1.12	100	13.1 b	5.39	100	13.6 b	5.79	100	6.6	3.43	0.001
15	***cis*-verbenol**	1145	1142	100	0.5 a	0.27	100	1.9 b	0.88	100	1.8 b	0.88	66.7	1.2	0.23	0.001
16	**pinocarvone**	1158	1160	85.7	0.4 a	0.04	100	1.6 b	0.50	88.9	1.8 b	0.62	66.7	1.4	0.34	0.003
17	*p*-mentha-1,5-dien-8-ol	1164	1166	0	0	–	5.6	0.4	0.31	44.4	0.7	0.39	0	0	–	0.016
18	**α-phellandrene-8-ol**	1165	1166	0	0 a	–	77.8	0.9 b	0.32	0	0 a	–	0	0	–	<0.001
19	terpinen-4-ol	1174	1175	42.9	1.0	0.51	16.7	0.3	0.03	22.2	0.4	0.06	0	0	–	0.241
20	**myrtenal**	1192	1193	85.7	0.5 a	0.13	100	1.9 b	0.63	88.9	1.7 b	0.57	66.7	1.2	0.24	0.001
21	**myrtenol**	1193	1194	85.7	0.7 a	0.35	100	1.5 b	0.63	88.9	1.3	0.54	33.3	1.1	–	0.014
22	**verbenone**	1205	1205	42.9	0.4 a	0.29	100	2.7 b	1.19	100	2.7 b	1.38	100	1.2	0.67	<0.001
23	***trans*-carveol**	1219	1215	14.3	0.6 a	–	94.4	1.9 b	0.69	88.9	1.6	0.60	33.3	0.8	–	<0.003
24	**carvone**	1241	1242	0	0 a	–	94.4	1.3 b	0.18	77.8	1.1	0.52	0	0	–	<0.001
25	bornyl acetate	1287	1285	100	10.4	4.89	100	11.9	4.74	100	9.3	5.73	100	4.1	2.81	0.098
26	**α-cubebene**	1347	1345	0	0	–	0	0 a	–	0	0	–	100	1.0 b	0.40	<0.001
27	**α-copaene**	1372	1376	0	0	–	11.1	0.2 a	0.01	0	0	–	100	2.6 b	1.34	<0.001
28	**β-cubebene**	1380	1389	0	0 a	–	77.8	4.9 b	3.21	11.1	6.0 a	0.04	0	0	–	<0.001
29	β-elemene	1389	1391	42.9	0.3	0.08	55.6	0.8	0.46	55.6	0.7	0.28	0	0	–	0.243
30	(*E*)-β-caryophyllene	1414	1419	71.4	0.9	0.31	88.9	0.6	0.16	44.4	0.8	0.44	66.7	0.7	0.04	0.425
31	**β-copaene**	1424	1432	14.3	0.4	–	22.2	0.2 a	0.13	33.3	3.6	3.80	100	12.2	8.15	0.006
32	α-*h*umulene	1448	1452	42.9	0.4	0.04	38.9	0.2	0.21	22.2	1.3	1.34	0	0	–	0.588
33	**γ-muurolene**	1475	1477	100	0.8 a	0.23	0	0 b	–	0	0 b	–	33.3	0.7	–	<0.001
34	**germacrene D**	1480	1480	100	11.2 a	4.80	22.2	8.1 b	4.21	88.9	2.5	1.65	100	1.5	0.20	<0.001

Table 1. *Cont.*

No	Compounds	LRI Exp.	LRI Ref.	*S. gigantea* (*n* = 7)			*S. canadensis* (*n* = 18)			*S.* × *niederederi* (*n* = 9)			*S. virgaurea* (*n* = 3)			*p*
				F	M	SD	F	M	SD	F	M	SD	F	M	SD	
35	*β*-selinene	1481	1486	57.1	0.4	0.01	16.7	0.3	0.08	22.2	0.7	0.01	0	0	–	0.155
36	*epi*-cubebol	1489	1493	14.3	0.7	–	0	0	–	11.1	1.0	–	66.7	1.6	1.24	0.007
37	**α-muurolene**	1499	1499	28.6	0.4	0.03	0	0 a	–	22.2	1.1	0.16	100	1.4 b	0.78	<0.001
38	**γ-cadinene**	1507	1513	85.7	0.7 a	0.26	16.7	0.4 b	0.09	0	0 b	–	0	0	–	<0.001
39	**cubebol**	1514	1515	0	0	–	0	0 a	–	22.2	1.5	0.04	100	2.8 b	2.08	<0.001
40	**δ-cadinene**	1520	1524	14.3	0.5	–	0	0 a	–	22.2	1.1	0.16	100	1.3 b	0.90	<0.001
41	epoxyazulene	1561	1554	71.4	6.4	2.98	55.6	1.9	1.22	88.9	1.6	1.35	0	0	–	0.054
42	*(E)-n*erolidol	1565	1564	71.4	0.7	0.28	11.1	0.4	0.13	11.1	0.4	–	0	0	–	0.002
43	**spathulenol**	1575	1576	100	5.0 a	2.97	38.9	0.9 b	0.74	66.7	2.8	3.96	100	1.3	0.20	<0.001
44	caryophyllene oxide	1578	1581	100	3.6	2.44	100	3.6	2.17	100	8.4	8.26	100	8.5	2.48	0.083
45	viridiflorol	1586	1590	0	0	–	0	0	–	0	0	–	33.3	1.2	–	0.010
46	**humulene epoxide II**	1607	1606	0	0	–	0	0 a	–	77.8	4.5 b	8.60	0	0	–	<0.001
47	**isospathulenol**	1627	1630	100	6.1 a	6.47	83.3	2.3	1.52	66.7	2.1	0.73	33.3	1.3 b	–	0.018
	Monoterpene hydrocarbons			100	25.4	16.52	100	28.0	20.88	100	26.4	18.09	100	35.9	11.86	0.608
	Oxygenated monoterpenes			100	18.5 a	6.04	100	46.6 b	15.42	100	41.5 b	16.35	100	19.2	7.60	<0.001
	Sesquiterpene hydrocarbons			100	15.3 b	6.63	100	7.9	6.00	100	6.0	4.85	100	20.7 a	10.64	0.007
	Oxygenated sesquiterpenes			100	19.7 a	8.85	100	7.1 b	3.48	100	17.0	15.54	100	14.7	5.25	<0.001
	Oxygenated diterpenes			14.3	0.1	–	11.1	0.9	0.19	22.2	1.0	0.51	0	0	–	0.479

[1] Compounds in bold were included in the PCA. Compounds are listed in order of their linear retention indices (LRI exp.) calculated using homologous series of *n*-alkanes (C8–C26). LRI ref.—linear retention indices from NIST 14 data basis and reference. The mean values of the compounds marked with the letters (a, b) differ significantly at $p \leq 0.05$ between species according to the Kruskal–Wallis test. *n*—number of distilled accessions per species.

2.2.1. *Solidago gigantea*

Inflorescence EOs were dominated by monoterpene hydrocarbons (25.4%) and oxygenated sesquiterpenes (19.7%) followed by oxygenated monoterpenes (18.5%) and sesquiterpene hydrocarbons (15.3%). Meanwhile, oxygenated sesquiterpenes (23.3%) and oxygenated monoterpenes (23.1%) were the major chemical fractions in leaf EOs.

The principal compounds in all inflorescence and leaf EOs were α-pinene, bornyl acetate, spathulenol, isospathulenol, and caryophyllene oxide. Nevertheless, there were high differences for other compound prevalences and their concentrations between samples and plant parts. Germacrene D was a major common component in inflorescence oils (3.4–18.3%), whereas this compound was detected in less than half of the leaf samples (1.6–12.7%). One of the major components in the inflorescences was *o*-cymene (6.8–18.4%), while among the principal compounds in the leaves was β-cubebene (17.3–19.6%), but these compounds were found in only a few EO samples. Additionally, *trans*-pinocarveol (0.6–1.1%), *cis*-verbenol (0.3–1.0%), *trans*-verbenol (1.2–3.0%), and γ-muurolene (0.5–1.1%) were found in minor concentrations in all inflorescence EOs. Meanwhile, camphene (1.54–3.9%), β-pinene (1.0–3.4%), *o*-cymene (0.8–4.9%), limonene (0.6–1.3%), and γ-cadinene (0.6–1.5%) were common in all leaf EOs.

In agreement with our results, α-pinene, bornyl acetate, germacrene D, and spathulenol have been previously reported to be major components of *S. gigantea* EOs [29,31–33]. In addition, cyclocolorenone, α- and γ-gurjunene, khusinol, and/or ledol and selina-3,11-dien-6-α-ol were also reported as the predominant constituents [31,33], although these sesquiterpenoids were not detected in the *S. gigantea* EOs tested. According to Grul'ová et al. [34], *S. gigantea* EOs were dominated by sesquiterpene hydrocarbons such as δ-cadinene, γ-muurolene, α-cubebene, and γ-cadinene, and two of them, γ-muurolene and γ-cadinene, were common in the inflorescence or leaf EOs studied.

2.2.2. *Solidago canadensis*

Monoterpenoids were predominant in *S. canadensis* EOs, with an average of 46.6.0% in inflorescences and 26.4% in leaves, followed by monoterpenes (28.0%) in inflorescences and sesquiterpenes (24.9%) in leaves. The first or second principal components in most of inflorescence EOs were α-pinene (0.1–36.1), *trans*-verbenol (5.2–21.7%), and bornyl acetate (3.8–19.8%). Among the most abundant constituents in leaf and inflorescence EOs were α-pinene (1.3–21.8) and bornyl acetate (6.5–20.4%). Leaf EOs were dominated by sesquiterpene hydrocarbons as β-cubebene (6.1–33.6%) and germacrene D (4.0–45.2%), and their concentrations exceeded those in inflorescence oils (0.8–13.1% and 1.9–11.4%, respectively). The other main constituent common for all leaf EOs was isospathulenol (0.7–10.2%). In addition, carvacrol was present as a major compound in two leaf EOs (22.83 and 23.7%) and was not detected in the remaining samples. Other components with noteworthy values in both inflorescence and leaf EOs were limonene (0.3–16.2% and 0.8–10.9%, respectively) and caryophyllene oxide (1.6–10.0% and 1.4–10.4%, respectively). The data revealed that *cis*-verbenol (0.6–3.7%), pinocarvone (0.9–2.7%), myrtenal (0.8–3.4%), and verbenone (1.0–5.2%) were detected in all inflorescence EOs in highly variable concentrations. Meanwhile, camphene (0.4–2.2%), β-pinene (0.7–2.7%), β-caryophyllene (0.8–6.9%), *trans*-verbenol (0.5–5.4%), β-elemene (0.6–5.9%), epoxyazulene (0.7–6.47%), and spathulenol (0.4–3.8%) were found in all or most of the leaf EOs.

The presented results are in agreement with previous studies that confirmed α-pinene, limonene, bornyl acetate, germacrene D, β-cubebene, and caryophyllene oxide among the predominant compounds in *S. canadensis* EOs [19,23,25,33,35]. In addition, studies from different countries have shown that γ-cadinene and myrcene [36,37] sabinene [36], cyclocolorenone [29], or thymol [34] were among the major compounds in *S. canadensis* EOs. Meanwhile, our results showed a low concentration or frequency of these compounds in the samples tested.

2.2.3. *Solidago* × *niederederi*

Solidago × *niederederi* inflorescence and leaf EO profiles were characterized by oxygenated monoterpenes (41.5 and 35.7%, respectively), followed by monoterpenes (26.4 and 17.7%, respectively), sesquiterpenoids (17.0 and 16.9%, respectively), and sesquiterpenes (6.0 and 15.0%, respectively). The principal constituents in the inflorescence EOs were α-pinene and *trans*-verbenol, which were the first or second major compounds in seven EOs, accounting for 22.3–31.7% and 13.4–22.9%, respectively. Caryophyllene oxide was among the major constituents in four (7.9–25.3%) and bornyl acetate in three (12.8–21.7%) EOs. Meanwhile, limonene and humulene epoxide II were present in most of the samples, however, dominated only in two (12.2 and 16.3%) and one (28.4%) samples, respectively. All inflorescence EOs contained varied levels of α-campholenal (0.9–6.2%), *trans*-pinocarveol (1.6–5.4%), and verbenone (0.6–5.1%). Other compounds with a mean content of 1 to 5.0% were β-pinene, camphene, *cis*-verbenol, pinocarvone, myrtenal, myrtenol, *trans*-carveol, germacrene D, epoxyazulene, and spathulenol.

Solidago × *niederederi* leaf EOs contained seven compounds in concentrations above 10% in at least one sample and were considered as principal compounds. The major compounds such as α-pinene (1.1–25.8%), *trans*-verbenol (2.2–20.2%), bornyl acetate (4.2–20.2%), and caryophyllene oxide (2.1–38.1%) were found in all leaves in highly different concentrations. Verbenone and germacrene D were present in most EOs, averaging 5.2 and 8.8%, respectively, with the exception of the two EOs in which these compounds were predominant, accounting for 20.2 and 18.0%, respectively. Meanwhile, β-cubebene was detected only in two oils (16.4 and 23.9%) in which it was the first or second major compound. One leaf EO contained noteworthy concentrations of thymol (6.4%) and carvacrol (9.5%). In addition, 21 compounds were detected with a mean content of 1 to 5.0%, the most prominent of which were sesquiterpenes such as β-copaene, β-bisabolene, epoxyazulene, humulene epoxide II, and isospathulenol. Similar results for the composition of the major compounds in *S.* × *niederederi* EO have been recently published [26]. However, the results of only one plant accession were reported, making the comparison insufficient as the prevalence and number of compounds varied among samples.

2.2.4. *Solidago virgaurea*

Solidago virgaurea inflorescence and leaf EOs were dominated by monoterpenes fraction, which accounted for an average of 35.9 and 49.0% of the total EO composition, respectively, followed by sesquiterpenes (20.7 and 16.9%, respectively). The main compounds in inflorescence EOs were α-pinene (18.8–36.3%), β-copaene (5.3–21.2%), and caryophyllene oxide (6.7–11.4%). All leaves were predominated by trans-verbenol (10.2–49.0%), two samples prevailed by α-pinene (22.4 and 23.3%) and caryophyllene oxide (10.1 and 14.7%), and one by verbenone (16.9%). Inflorescence and leaf EOs contained 22 and 29 compounds, respectively, with a mean percentage greater than 1% and less or equal to 5%. Among them, the most prominent were β-pinene, limonene, verbenone, α-campholenal, *trans*-pinocarveol, pinocarvone, bornyl acetate, α-copaene, germacrene D, cubebol, α-muurolene, δ-cadinene, and spathulenol, which were common to all inflorescence and/or leaf EOs.

Similar to our identification, previous studies have confirmed the dominance of monoterpene and sesquiterpene fractions in *S. virgaurea* EOs [30,32]. Monoterpenes such as α-pinene, myrcene, β-pinene, and limonene together with sesquiterpene germacrene D have been reported as the major constituents in *S. virgaurea*. In addition, oxygenated sesquiterpenes, humulene epoxide II, spathulenol, selina-3,11-dien-6-α-ol, and caryophyllene oxide have been also considered as major compounds in *S. virgaurea* EOs [26,33]. Meanwhile, in this study, only α-pinene and caryophyllene oxide were found among the predominant compounds in all EOs tested, and the contents of the other mentioned compounds differed among samples. Meanwhile, selina-3,11-dien-6-α-ol was not detected at all in the presented EOs.

2.3. Interspecific Differences

The results presented revealed similarities and differences in the frequency of distribution and contents of EO constituents among the four *Solidago* spp. Significant differences in monoterpene and sesquiterpene fractions were found between the inflorescences of the species studied (Table 1). Meanwhile, the chemical groups of compounds in leaves did not differ significantly between the four *Solidago* spp. (Table 2).

Oxygenated monoterpenes predominated in the EOs of *S. canadensis* and *S.* × *niederederi* inflorescences and *S. virgaurea* and *S.* × *niederederi* leaves. The highest proportion of sesquiterpenoids among the species was found in *S. gigantea* inflorescences and leaves. Mono- and sesquiterpenes prevailed in the *S. virgaurea* inflorescence EOs; however, no significant differences were found between the species for monoterpenes. The common principal constituents of the inflorescence and leaf EOs of all four *Solidago* species were α-pinene, bornyl acetate, and caryophyllene oxide. Species showed significant differences ($p \leq 0.05$) in the accumulation of bornyl acetate and caryophyllene oxide in leaf EOs, but no differences in α-pinene were observed between species (Tables 1 and 2). The highest mean concentration of bornyl acetate was found in inflorescence and leaf EOs of both *S. gigantea* and *S. canadensis*, while caryophyllene oxide prevailed in *S.* × *niederederi* and *S. virgaurea*. The other major compound *trans*-verbenol was prevalent in all inflorescence EOs with the highest level ($p \leq 0.001$) in *S. canadensis* and *S.* × *niederederi*. Meanwhile, the leaves of *S. virgaurea* and *S.* × *niederederi* had priority over the other two species in accumulation of trans-verbenol. Inflorescences of *S. gigantea* accumulated the highest ($p \leq 0.001$) level of germacrene D compared to other species, but this compound did not differ significantly ($p > 0.05$) in the leaves between species. The inflorescences and leaves of *S. gigantea* were in priority to other species in accumulation of oxygenated sesquiterpenes as epoxyazulene, spathulenol, and isospathulenol. In addition, γ-muurolene, differently other species, was a common compound in all inflorescence EOs of *S. gigantea*, while other monoterpene hydrocarbons such as camphene and o-cymene were common in all leaf EOs of this species.

The inflorescence EOs of all species differed significantly in the mean concentrations of oxygenated monoterpenes, such as α-campholenal, *trans*-pinocarveol, *cis*-verbenol, pinocarvone, verbenone, and *trans*-carveol, with the highest levels and frequency found in *S. canadensis* and *S.* × *niederederi*, followed by *S. virgaurea*. The same compounds, with the exception of *trans*-carveol, differed significantly in leaf EOs, with the highest concentrations in *S. virgaurea* and *S.* × *niederederi* leaves. Meanwhile, *trans*-carveol was detected in small amounts only in *S. canadensis* and *S.* × *niederederi* leaves. In addition, *S. virgaurea* inflorescence EOs differed from other species in the highest levels and frequency of distribution in α- and β-copaene, cubebol, α-muurolene, and δ-cadinene. As a consequence, quantitative rather than qualitative differences were observed between the species EOs. However, 10 compounds common in more than 30% of all studied inflorescence and/or leaf EOs did not differ significantly between *Solidago* spp. (Tables 1 and 2). Among them, the most abundant were α- and β-pinene, limonene, bornyl acetate, germacrene D, and caryophyllene oxide.

Table 2. The frequency of distribution (F, %) of the compounds detected in more than 30% of the leaf EO samples in at least one of the four *Solidago* species; their mean relative percentage (M) with SD and significance (*p*) of interspecies differences according to the Kruskal–Wallis two-tailed test.

No	Compounds	[a] LRI Exp.	[b] LRI Ref.	*S. gigantea* (*n* = 7)			*S. canadensis* (*n* = 18)			*S.* × *niederederi* (*n* = 9)			*S. virgaurea* (*n* = 3)			*p*
				F	M	SD	F	M	SD	F	M	SD	F	M	SD	
1	**α-pinene**	930	930	100	7.8	2.18	100	8.6	5.69	100	11.9	10.37	100	16.0	11.77	0.856
2	**[1] camphene**	945	948	100	2.8 a	0.76	100	1.2 b	0.40	77.8	1.1 b	0.45	66.7	0.5 b	0.07	<0.001
3	thuja-2,4(10)-diene	950	957	0	0	–	11.1	0.2	0.13	33.3	0.6	0.13	33.3	0.3	–	0.187
4	sabinene	971	976	42.9	1.1	0.61	44.4	0.6	0.42	33.3	1.1	1.21	66.7	0.3	0.01	0.926
5	β-pinene	974	980	100	2.2	0.88	94.4	1.4	0.61	88.9	1.9	1.16	66.7	3.5	0.64	0.193
6	β-myrcene	992	988	28.6	1.2	0.12	5.6	1.0	–	22.2	0.8	0.49	33.3	0.4	–	0.372
7	***o*-cymene**	1022	1009–1076	100	2.1 a	1.40	50.0	0.5 b	0.46	22.2	0.4 b	0.06	0	0 b	–	<0.001
8	**limonene**	1023	1027	100	1.1 a	0.16	100	2.5 b	2.17	100	2.2	1.56	66.7	1.1	0.35	0.006
9	**α-campholenal**	1109	1105	14.3	0.6 a	–	77.8	0.8	0.72	77.8	2.6	1.68	100	2.8 b	0.52	0.007
10	***trans*-pinocarveol**	1135	1139	42.9	0.3 a	0.16	66.7	0.9 a	0.50	77.8	1.6	0.75	100	3.6 b	1.83	0.003
11	***trans*-verbenol**	1143	1144	42.9	0.5 a	0.23	100	2.7 b	1.81	100	8.9 b	6.85	100	24.6 b	21.21	<0.001
12	***cis*-verbenol**	1145	1142	0	0 a	–	27.8	0.4	0.27	44.4	1.6	0.39	100	1.5 b	0.82	0.004
13	**pinocarvone**	1158	1160	0	0 a	–	22.2	0.6 a	0.31	66.7	1.1	0.51	100	1.8 b	0.04	<0.001
14	myrtenal	1192	1193	0	0	–	72.2	0.6	0.29	55.6	1.6	0.48	66.7	1.6	0.02	0.024
15	myrtenol	1193	1194	0	0	–	55.6	0.3	0.25	55.6	1.0	0.33	66.7	1.2	0.03	0.038
16	**verbenone**	1205	1206	42.9	0.3 a	0.16	88.9	1.5	1.35	77.8	5.2 b	6.77	100	7.3 b	8.37	0.008
17	*cis*-carveol	1208	1206	0	0	–	5.6	1.1	–	0	0	–	33.3	0.7	–	0.168
18	*trans*-carveol	1219	1215	0	0	–	16.7	0.2	0.07	44.4	0.9	0.21	33.3	0.8	–	0.066
19	**bornyl acetate**	1287	1285	100	22.4 a	5.37	100	18.7 a	7.55	100	14.0	5.53	100	2.6 b	0.46	0.004
20	thymol	1295	1297	0	0	–	5.6	0.5	–	11.1	9.5	–	33.3	3.8	–	0.330
21	carvacrol	1306	1308	0	0	–	11.1	23.3	0.63	11.1	6.4	–	66.7	3.2	0.42	0.079
22	α-copaene	1372	1376	0	0	–	22.2	0.3	0.09	0	0	–	66.7	0.5	0.03	0.059
23	β-bourbonene	1378	1385	85.7	0.9	0.45	50.0	0.5	0.46	22.2	2.0	2.27	33.3	1.8	–	0.056
24	β-cubebene	1380	1389	57.1	14.1	8.41	38.9	21.3	10.25	22.2	20.1	5.25	0	0	–	0.467
25	**β-elemene**	1389	1391	28.6	0.8 a	0.04	83.3	1.7 b	1.45	66.7	0.8	0.57	33.3	0.4	–	0.005
26	(*E*)-β-caryophyllene	1414	1419	85.7	1.0	0.27	61.1	2.5	2.14	66.7	2.0	1.67	66.7	1.5	1.42	0.985
27	β-copaene	1424	1432	14.3	0.5	–	50.0	0.6	0.19	22.2	3.8	4.56	66.7	3.2	0.79	0.161
28	α-humulene	1448	1452	42.9	0.5	0.08	61.1	0.8	0.54	33.3	1.9	1.89	33.3	0.4	–	0.380
29	**γ-muurolene**	1475	1477	100	0.8 a	0.23	0	0 b	–	0	0 b	–	33.3	0.7	–	<0.001
30	germacrene D	1480	1480	42.9	5.9	5.95	50.0	19.6	15.22	66.7	8.8	5.22	66.7	5.2	6.46	0.751
31	β-selinene	1481	1486	14.3	0.6	–	38.9	0.5	0.47	0	0	–	0	0	–	0.103
32	*epi*-cubebol	1489	1493	0	0	–	0	0	–	0	0	–	66.7	1.4	0.31	0.012
33	α-muurolene	1499	1499	0	0	–	0	0	–	0	0	–	33.3	0.3	–	0.222
34	β-bisabolene	1505	1509	0	0	–	11.1	5.0	1.05	11.1	4.7	–	33.3	1.8	–	0.569

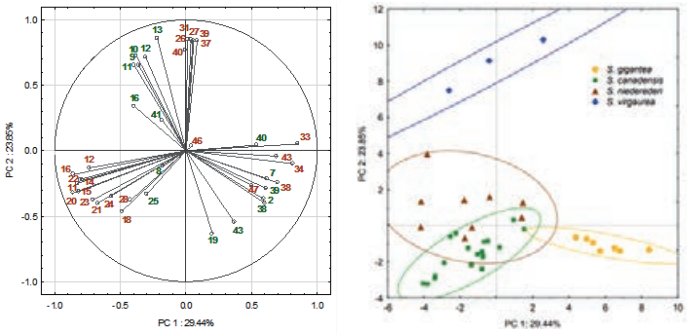
Table 2. *Cont.*

No	Compounds	[a] LRI Exp.	[b] LRI Ref.	*S. gigantea* (*n* = 7)			*S. canadensis* (*n* = 18)			*S.* × *niederederi* (*n* = 9)			*S. virgaurea* (*n* = 3)			*p*
				F	M	SD	F	M	SD	F	M	SD	F	M	SD	
35	γ-cadinene	1507	1513	100	1.0	0.32	5.6	0.7	–	0	0	–	0	0	–	<0.001
36	cubebol	1514	1515	0	0	–	0	0	–	0	0	–	66.7	0.7	0.10	0.014
37	δ-cadinene	1520	1524	0	0	–	5.6	0.7	–	11.1	0.9	–	33.3	0.4	–	0.384
38	**epoxyazulene**	1561	1554	100	6.1 a	3.22	100	2.7	1.43	66.7	2.9 b	2.49	33.3	0.3 b	–	0.001
39	***(E)*-nerolidol**	1565	1564	85.7	0.9 a	0.20	38.9	0.6	0.20	0	0 b	–	0	0	–	0.001
40	**spathulenol**	1575	1576	100	6.7 a	6.25	88.9	1.2 b	0.98	66.7	2.1 b	1.94	100	3.1	1.30	0.003
41	**caryophyllene oxide**	1578	1581	100	3.1 a	0.77	77.8	3.9 a	2.24	100	10.4 b	11.18	100	9.0	6.36	0.004
42	humulene epoxide II	1607	1606	57.1	2.4	1.26	38.9	2.7	1.04	22.2	3.2	0.92	66.7	5.0	1.44	0.276
43	**isospathulenol**	1627	1630	100	6.3 a	3.11	100	4.8 a	2.54	66.7	3.7	2.26	0	0 b	–	0.004
	Monoterpene hydrocarbons			100	16.6	4.08	100	14.4	9.42	100	17.7	10.92	100	20.1	15.28	0.634
	Oxygenated monoterpenes			100	23.1	5.64	100	27.9	11.2	100	35.7	13.07	100	49.0	27.81	0.072
	Sesquiterpene hydrocarbons			100	14.9	7.85	94.4	23.3	15.3	100	15.0	11.32	66.7	8.6	10.93	0.252
	Oxygenated sesquiterpenes			100	23.3	10.03	100	13.3	6.07	100	16.9	12.56	100	16.9	7.63	0.181
	Oxygenated diterpenes			0	0	–	5.6	0.7	0.26	11.1	0.1	0.33	0	0	–	0.375

[1] Compounds in bold were included in the PCA. Compounds are listed in order of their linear retention indices (LRI exp.) calculated using homologous series of *n*-alkanes (C8–C26). LRI ref.—linear retention indices from NIST 14 data basis and reference. The mean values of the compounds marked with the letters (a, b) differ significantly at $p \leq 0.05$ between species according to the Kruskal–Wallis test. *n*—number of distilled accessions per species.

2.4. Principal Component Analysis (PCA)

PCA was employed to explain the phytochemical relationships arising due to inter- and intraspecific differences between the four *Solidago* spp., using the selected EOs compounds. A scree plot criterion was applied to reduce the number of PCs for explaining the variance in the selected variables. A two-dimensional PCA square matrix model explained more than 53.3% of the total variance and was used to visualize the available patterns of *Solidago* spp. EOs profiles (Figure 2). PC3 explained only 6.8% of the total variance and had no significant effect on scores differentiation, so results were not presented. PC1 accounted for 29.4% of the total data set variance and showed high negative correlation with *α*-campholenal, *trans*-pinocarveol, *cis*- and *trans*-verbenol, pinocarvone, myrtenal, myrtenol, verbenone, and trans-carveol and positive with carvone, germacrene D, *γ*-muurolene, *γ*-cadinene, spathulenol, and isospathulenol in inflorescences, and camphene, *o*-cymene, epoxyazulene, and (*E*)-nerolidol in leaves (Figure 2a). PC2 explained 23.6% of the total data set variance and was highly associated with positive loadings of *α*- and *β*-copaene, *α*-cubebene, cubebol, *α*-muurolene, and *δ*-cadinene in inflorescences and with *α*-campholenal, *trans*-pinocarveol, *cis*-verbenol, and pinocarvone in leaves, as well as with negative loadings of bornyl acetate and isospathulenol in leaves.



(**a**) (**b**)

Figure 2. PCA model representing the accumulation of terpenes in inflorescences and leaves of four *Solidago* species: (**a**) loading plot of the variables contributing to PC1 and PC2; (**b**) scores plot for the tested EOs with a 95% ellipses confidence limit for each species. The numbers of the variables correspond to the numbers of EO compounds listed in the Tables 1 and 2. Inflorescence variable numbers were marked in brown; leaf variable numbers were marked in green.

The PCA score plot model showed the arrangement of 40 EOs into two separate and two overlapping ellipses, each with a 95% confidence interval limit (Figure 2b). The group on the right-hand plot combined all *S. gigantea* EOs along the positive PC1. Variables with high PC1 loadings contributed the highest impact on the grouping of *S. gigantea* samples were germacrene D, *γ*-cadinene, *γ*-muurolene, and spathulenol in inflorescences, and camphene, *o*-cymene, epoxyazulene, (*E*)-nerolidol, and spathulenol in leaves. These compounds were shared among all *S. gigantea* EOs in the highest amounts compared to the other species studied. Conversely, variables with high PC2 loadings had a weak contribution on the grouping of EOs and were found in minor quantities in *S. gigantea*.

Solidago virgaurea EOs were clustered into a separate group on the upper positive side of the score plot, in distance from all other samples, indicating differences in their composition. The location of the samples can be explained by the same position of variables, which have a significant positive contribution to PC2. Variables with unit vectors close to each other were positively correlated, and their impact on the position of the samples was similar. Thus, *α*- and *β*-copaene, *α*-cubebene, cubebol, *δ*-cadinene, and *α*-muurolene in

inflorescences, and α-campholenal, *cis*-verbenol, *trans*-verbenol, pinocarvone, and *trans*-pinocarveol in leaves, were common in *S. virgaurea* EOs and found in significantly higher amounts than in other species analyzed. Meanwhile, *S. canadensis* and *S.* × *niederederi* EOs were clustered into two partially overlapping ellipses, mainly in the left-hand score plot, showing the similarity of the volatile compound patterns. The arrangement of inflorescence EOs for both species coincided with a significant correlation of α-campholenal, *trans*-pinocarveol, pinocarvone, verbenone, *cis*-verbenol, *trans*-verbenol, myrtenal with PC1. In addition, the clustering of *S. canadensis* was influenced by myrtenol, *trans*-carveol, and carvone. Variables with high PC2 loading had no significant impact on EOs' arrangement, except for *trans*-verbenol in *S.* × *niederederi* leaves, showing similarity of this taxon to *S. virgaurea*. Meanwhile, a previous study of phenolic compounds showed greater chemical similarity of *S.* × *niederederi* to *S. virgaurea* than to *S. canadensis* [20]. Consequently, the phytochemical patterns complemented the evidence of *S.* × *niederederi* origin between native *S. virgaurea* and invasive *S. canadensis*, with the higher contribution of alien species than that of native ones. In addition, *S.* × *niederederi* EOs were much more scattered on the PCs space, indicating higher diversity than other species, suggesting that *S.* × *niederederi* is a continuously evolving taxon.

Consequently, *Solidago* spp. EOs differed significantly in the presence of terpenes that could be considered as species distinctive components. The inflorescence EOs of *S. gigantea* differed from other species by γ-cadinene, γ-muurolene, and spathulenol, and the leaves by camphene, *o*-cymene, epoxyazulene, (*E*)-nerolidol, and spathulenol. The inflorescences of *S. canadensis* and *S.* × *niederederi* differed significantly from the other species by the accumulation of oxygenated monoterpenes, such as α-campholenal, *trans*-pinocarveol, pinocarvone, verbenone, *cis*-verbenol, *trans*-verbenol, and myrtenal. In addition, *S. canadensis* inflorescence EOs were characterized by the prevalence of myrtenol, *trans*-carveol, and carvone, while *S.* × *niederederi* leaves were prominent by *trans*-verbenol. The species distinctive volatiles in *S. virgaurea* inflorescence EOs were α- and β-copaene, α-cubebene, cubebol, δ-cadinene, and α-muurolene, and in the leaves—α-campholenal, *cis*-verbenol, *trans*-verbenol, pinocarvone, and *trans*-pinocarveol. Consequently, multivariate data analysis allowed for an explanation in the intra- and interspecific diversity in four *Solidago* taxa according to the differences in EO volatiles.

3. Discussion

Alien goldenrods are morphologically and phylogenetically close to each other, but differ in their ploidy level; *S. gigantea* is tetraploid (2n = 36), while *S. canadensis,* and *S.* × *niederederi* together with native *S. virgaurea* are diploids (2n = 18) [3,38]. The close relationships between *Solidago* spp. were reflected in the similarity of their phytochemical profiles. A comprehensive metabolomics approach to the different species indicated that the successful alien species had higher total number and more unique composition of secondary metabolites than their native congeners [39]. A comparison of the current and previous reports showed that our results are in agreement with previous reports for higher proportions of sesquiterpenoids in *S. gigantea* and hydrodrocarbons in *S. virgaurea* EOs [26,29,32,33]. Similar to our identification, the most abundant common compounds detected in the present study were also observed in previous studies on different species. Thus, the volatiles commonly found in various plant species have a high potential to accumulate in *Solidago* spp. EOs as well. On the other hand, there were compounds such as thymol and carvacrol, sporadically high levels of which were found in only a few *S.* × *niederederi* and *S. canadensis* EOs. Populations rich in thymol and carvacrol, compounds with a broad spectrum of biological activity [40–42], can be considered as a source of high potential raw material. In addition, *Solidago* spp. EOs differed significantly in some of the terpenes that can be considered as volatiles with great potential in chemophenetic studies of species. The first comparative study on terpenes as species distinctive compounds confirmed the origin of *S.* × *niederederi* as an interspecific taxon between *S. canadensis* and *S. virgaurea*. According to Orians [43], parental phytochemicals in hybrids tend to mostly express as either interme-

diate or similar to one of the parent's compositions. The composition of *S.* × *niederederi* EOs was close to *S. canadensis*, one of parental species. The chromosome number may provide information about the hybrid origin of the species when it display allopolyploidy, but *S.* × *niederederi* exhibit a homoploid condition compared to its parental species and may backcross toward parental species. In this way, hybridization can increase the invasive capacity of goldenrods through gene introgression and significantly alter the ecosystems in which they grow [44]. However, species–specific compounds and chromosome number are not the main tools for hybrid identification. DNA fingerprinting techniques are the most reliable tools, but the use of additional phytochemical markers can provide insight into the ecological performance of hybrids and their further applications [45].

More often, the lower proportion of monoterpenes compared to sesquiterpenes [46] accounted for a higher proportion in the *Solidago* spp. EOs tested. Oxygenated monoterpenes have been proven to be the main phytotoxic active compounds in different plant EOs and have been highlighted as predictors of potential bioherbicides [47]. In addition, a tendency has been suggested that the monoterpene-rich EOs to promote higher phytotoxicity than sesquiterpene-rich EOs [48]. Comparative studies on the toxic activity of oxygenated monoterpenes revealed that the most active were alcohols, myrtenol and *trans*-pinocarveol, and ketones, verbenone and pinocarvone, which can be classified as predictors of the herbicidal activity of EOs [49–51]. Thus, *S. canadensis* and *S.* × *niederederi*, whose EOs differed from other species in some of oxygenated monoterpenes, suggested the potential of their raw materials for herbicidal activity. Meanwhile, terpene hydrocarbons have been found to be low phytotoxic EOs compounds [40,52]. On the other hand, Lawson et al. [36] reported that monoterpenes such as α- and β-pinene, limonene, or myrcene showed weak antifungal activity. Recent findings reported that (*E*)-nerolidol and spathulenol, which were presented as species–specific sesquiterpenoids in *S. gigantea* EOs, revealed effective allelopathic and insecticidal effects with potential for the developing a new natural pesticide [53–55]. Many findings have demonstrated the biological activity of caryophyllene oxide [53,55–58] that was a common compound in the presented *Solidago* spp. EOs. The potential biological activity of EOs is associated with the presence of high oxygenated compounds, as confirmed by a systematic review of phytotoxicity studies [59]. In this context, *S. canadensis* and *S.* × *niederederi* EOs, are of greatest interest in the development of new and safe bioproducts.

Considering the previous and presented results for *Solidago* spp. EOs, the prevalence of predominant and other compounds varied across different studies, and their comparison is not informative enough. Reports often provide single sample data that are difficult to summarize as a species–specific composition of volatiles. According to Zidorn [60], correct taxonomic identification, geographical location, plant harvesting season, plant parts, and other indirect factors are crucial in phytochemical studies, which often receive little attention. Exogenous factors or environmental regulated factors such as light, precipitation, growing site and soil are often considered to be the most important factors modifying the qualitative/quantitative composition of EOs [61]. Experiments have shown that plants exposed to drought stress increased the concentration of monoterpenes to protect plant cells from ROS damage [62]. Meanwhile, Caser et al. [63] found that drought increased the production of sesquiterpenoids and decreased monoterpenoids. According to Paulsen and Selmar [64], increased terpene synthesis is not supported by carbon allocation theory, but is attributed to changes in biomass production. In addition, differences in the chemical profiles of EOs are often explained in the context of the interaction of the metabolic inversion of the ratio of oxygen-free to oxygen-containing terpenes with the surrounding environment. Sesquiterpenes have been observed to be predominate during the dry season, while higher concentrations of sesquiterpenoids were found during the wet season [65]. The water scarcity increased the production of monoterpenoids and monoterpenes, while the opposite trend was observed for sesquiterpenes [66]. On the other hand, Tsusaka et al. [67], investigating the influence of genetic and environmental factors on sesquiterpenoids in *Atractylodes lancea*, (Thunb.) DC. found that the genotype had a greater effect on EO com-

pounds than the conditions of the plant cultivation year. The volatiles were stable despite the changing growing conditions, but the absolute values of terpenoids were induced by the site of cultivation. Tardugno et al. [68] determined that the composition of *Thymus vulgaris* L. essential oils were highly influenced by the cultivating techniques. Over time, local environment leads to differences in metabolomics and the formation of ecotypes and chemotypes within a species [69].

The intraspecific differences in *Solidago* spp. volatile compounds observed in the present study can be explained by genotypic differences, as the plants grew under close conditions. Sexual reproduction helps maintain a high level of genetic and phenotypic diversity in goldenrods. Our previous study showed a high morphological diversity in *Solidago* spp. both between populations and between individual genets [3]. Similarly, high variability in volatiles and morphological characters was observed in wild *Mentha longifolia* L. accessions growing in the same field [70]. According to Zhao et al. [71], high genetic variations are characteristic within invasive and native areas of *S. canadensis* populations. Considerations suggested that the study of local populations makes it possible to identify intraspecific diversity that potentially reflects local genetic changes rather than the controversial dependence of terpenes synthesis under changing environmental conditions. The outstanding diversity in the goldenrods studied allows the selection of accessions in terms of the desired composition of EO volatiles. Phytochemical profiling of plant raw materials is an informative tool to learn about their potential for further development of new natural products.

4. Material and Methods

4.1. Plant Material

Plant material of four *Solidago* spp. in the flowering phase was collected from six different sites in Vilnius district, Lithuania, in August 2018. Eighteen accessions of *S. canadensis*, seven of *S. gigantea*, nine of *S.* × *niederederi*, and three accessions of *S. virgaurea* were collected at least one kilometre apart from each other in abandoned dry grasslands and disturbed farmlands (Table 3). The vegetation of the collection sites was characterized as semi-ruderal dry grassland dominated by plant communities of *Agropyretea intermedii-repentis* and *Artemisietea vulgaris*. The EOs in habitats were sand or sandy loam, with low to moderate humus content (1.8–2.7%), rich in phosphorus (126–260 P_2O_5 mg kg^{-1}) and potassium (146–205 K_2O mg kg^{-1}), pH_{KCl} varied from 5.8 to 7.1.

Table 3. Collection sites data on *Solidago gigantea* (SG), *S. canadensis* (SC), *S.* × *niederederi* (SN), and *S. virgaurea* (SV).

Collection Site	Altitude, m	Latitude N	Longitude E	Number of Accessions			
				SG	SC	SN	SV
Pavilnys, Vilnius distr.	215	54°40′35″	25°23′01″	3	3	1	–
Didieji Pupojai, Vilnius	206	54°42′38″	25°23′29″	1	3	3	2
Rokantiškės, Vilnius	203	54°40′03″	25°22′58″	3	2	1	–
Raudondvaris, Vilnius distr.	149	54°52′32″	25°31′08″	–	4	–	–
Dvariškės, Vilnius distr.	149	54°49′17″	25°16′23″	–	3	3	1
Karklinė, Vilnius distr.	164	54°54′26″	25°33′40″	–	3	1	–

The harvested plant material consisted of shoots of a single genet derived from a single seed. Individual genets were identified by phenological and morphological characteristics and by rhizome connections. The accessions of the same species were collected at least five meters apart from each other if more than one accession was collected from the same site. The plant material was dissected into inflorescences and leaves and dried separately at 25 °C. The botanical identification of species was based on morphological diagnostic characters such as the shape and size of inflorescences and ray flowers, stem color, and stem hairiness by Birutė Karpavičienė and Jolita Radušienė [3]. The specimens of evaluated

Solidago spp. were deposited in the Herbarium of the Institute of Botany of Nature Research Centre (BILAS), Vilnius, Lithuania.

4.2. Isolation of Essential Oils

The plant material from 30 g of air-dried leaves and inflorescences was hydrodistilled separately for three hours using a Clevenger type apparatus. Each sample of yellowish EO was dried over anhydrous sodium sulphate and stored in a sealed vial at 4 °C until analysis. A sample preparation for chemical analysis included 1.0 µL of EO added into 1.0 mL of *n*-hexane following previous studies [23,33]. The essential oil content was calculated as relative percentages per 100 g of dry plant material.

4.3. Analysis of Essential Oils

The EOs analysis was performed using the GCMS-QP 2010 Ultra system equipped with a Shimazu autoinjector AOC-5000 (Shimadzu, Europa GmbH). A capillary column RXi-5MS (30 m × 0.25 mm i.d. × 0.25 film thickness µm) (Restek, Bellefonte, PA, USA) was used. The sample injection volume was 1 µL, a split ratio was 1:60 (*v*:*v*) and the split injector temperature was 260 °C. Helium was used as carrier gas with flow rate of 1.22 mL min^{-1}. The initial column temperature was 50 °C, held for 5 min and raised to 200 °C at the rate of 2 °C min^{-1}, then raised from 200 to 315 °C at the rate of 15 °C min^{-1} and held for 5 min. The detector ion source and interface temperatures were 200 °C and 280 °C, respectively. Mass spectra were acquired at an ionization voltage of 70 eV, a scan rate of 2500 *m*/*z* within the range of 29–500 *m*/*z* and a scan time of 0.2 s. The chromatographic analysis was run in triplicate.

4.4. Identification and Quantification of Components

The linear retention indices (LRI) of compounds were calculated using a homologous series of *n*-alkanes C8–C26 (Sigma-Aldrich, UK, purity >99.2%) injected at the beginning of the analysis and comparing the retention times of the eluted peaks with those of the alkanes [72]. Chromatographic data were analyzed using GC–MS solution software (Shimadzu, Europa GmbH). The EO constituents were identified by comparing the unique mass spectral fragmentation patterns of each peak with the mass spectral computer library database and those presented as standards in the NIST 14, FFNSC, WR10, and WR10R libraries, as well as comparing the obtained LRI with presented in NIST 14 datasets and reference [73] corresponding to the conditions for dimethylsilicone stationary phase with 5% phenyl groups. The relative percentages of analytes as the mean of the three runs were calculated from their peak areas in the chromatographic profiles without the use of correction factors corresponding to the conditions for the stationary phase of dimethylsilicone with 5% phenyl groups.

The repeatability and intermediate precision of analysis, expressed as relative standard deviation (RSD), was evaluated by performing the retention time and peak area values of five analytes in the same *S. canadensis* EO extract for intra- and inter- daily tests (Table 4).

Table 4. Repeatability and intermediate precision on the relative retention time (t_R,) and peak area (A) of the five analytes in *S. canadensis* EOs expressed as relative standard deviation (RSD, %).

Analytes	Repeatability (Run-to-Run)				Intermediate Precision (Day-to-Day)			
	t_R, min	RSD, %	A, %	RSD, %	t_R, min	RSD, %	A, %	RSD, %
α-pinene	11.85	0.23	24.57	0.45	11.92	0.82	24.83	1.51
limonene	15.24	0.29	9.07	0.33	15.31	0.73	9.17	1.42
trans-verbenol	23.20	0.22	10.36	0.41	23.36	0.92	10.54	2.21
bornyl acetate	32.70	0.32	10.21	0.43	32.77	0.54	10.26	0.98
β-cubebene	44.59	0.26	2.82	0.38	44.74	0.60	2.84	1.02

Repeatability was determined in five consecutive injections of EO in the same day. The RSD for the retention time ranged from 0.22 to 0.32% and for relative peak area from 0.33 to 0.45%. Intermediate precision was assessed by five injections over two different days, with RSD values ranging from 0.54 to 0.92% for retention time and 0.98 to 2.21% for peak areas. The accuracy of the quantification was satisfactory, as RSD value within the 3% range is generally considered as acceptable.

4.5. Data Analysis

Multivariate statistical analysis was performed using software package Statistica 10.0 (StatSoft Inc.). The Kruskal–Wallis ANOVA was used to determine the differences between species. Significant differences were specified by two-tailed test at $p \leq 0.05$. Principal component analysis (PCA) was used to identify the similarities and differences between the EOs analyzed using statistically independent variables. The PCA was based on 24 inflorescence and 16-leaf standardized variables that different significantly between species and that represented constituents detected in 30% or more of EO samples in at least one species. Leaf and inflorescence data sets were pooled and used in PCA, resulting in more convincing results than separate leaf and inflorescence PCAs.

5. Conclusions

The frequency and percentage of distribution in the volatile constituents of *Solidago* spp. varied depending on the species, accessions, and plant parts. The principal compounds common to all *Solidago* spp. inflorescence and leaf EOs were α- and β-pinene, limonene, bornyl acetate, germacrene D, spathulenol, and caryophyllene oxide. *Solidago* spp. differed significantly in some of the distinctive terpenes that can be considered as compounds with high potential for chemophenetic and taxonomic studies of the genus. A comparison of volatile profiles for *Solidago* spp. confirmed the interspecific origin of *S.* × *niederederi* between *S. canadensis* and *S. virgaurea* with a higher metabolic contribution of alien species than native ones. The findings provide the bioprospecting of *Solidago* spp. as a source for specified composition of volatiles. The vast resources of invasive goldenrods are of great interest as a convenient and readily acceptable, underutilized source of natural bioactive compounds that can be used for different applications.

The combination of fingerprint and multivariate data analysis demonstrated a simplified assessment of the quality of wild plant materials. Correct species identification is essential for the development of raw material quality control protocols for the targeted collection and assessment of raw materials from wild populations. The screening of a relatively large number of plant accessions from mixed populations of different species allows for a more reasonable comparison of their volatile profiles and enables prediction of the most likely quality of raw materials harvested from the wild.

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Title: Antioxidant profiles of leaves and inflorescences of native, invasive, and hybrid Solidago species

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Antioxidant profiles of leaves and inflorescences of native, invasive and hybrid *Solidago* species

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ABSTRACT

Solidago species is a rich source of phenolic compounds with multi-pharmacological properties, particularly for the complementary treatment of various inflammatory diseases of the urinary tract. Phenolic acids and flavonoids are main compounds in the phytochemical profiles in *Solidago* spp. The aim of this study was to determine antioxidant profiles of leaves and inflorescences of four species as *S. gigantea*, *S. canadensis*, *S. virgaurea*, *S.* ×*niederederi* from the wild populations in Lithuania. HPLC-ABTS assay coupled with UPLC-QTOF-MS was applied for the elucidation of antioxidant active compounds and determination of activity markers. Antioxidant profiles significantly vary within the species and plant part investigated. Chlorogenic acid and 3,5-di-caffeoylquinic acid were identified as the radical scavenging markers in leaves and inflorescences of *Solidago* species. The leaves of *S. gigantea* and *S. virgaurea* distinguished from other species by the highest amounts of radical scavengers. *Solidago* species is a promising source of fast acting high-capacity antioxidants.

1. Introduction

The genus *Solidago* L. (Asteraceae Bercht. & J.Presl) is comprised of over 100 species, most of which originated from North America, eight species occur in Mexico, four in South America, and six to ten species are native to Europe and Asia (Beck et al., 2014; Semple and Cook, 2006). Raw materials of goldenrods have a long usage history for the complementary treatment of various inflammatory diseases of the urinary tract and degenerative locomotor diseases (European Medicines Agency, 2008). Scientific studies have shown the cytotoxic, anti-inflammatory, antimicrobial, antioxidant, analgesic, and anti-adipogenic effects of plant extracts (Abdel Motaal et al., 2016; Anzlovar and Dolenc Koce, 2014; Hwang et al., 2013, 2017; Paun et al., 2016). In Europe, the most commonly used and the most studied species is naturally growing goldenrod *Solidago virgaurea* L. (European Medicines Agency, 2008). Due to the extensive distribution of invasive species *S. gigantea* Aiton and *S. canadensis* L. together with a natural hybrid *S.* ×*niederederi* Kekh between *S. canadensis* and *S. virgaurea* (Karpavičienė and Radušienė, 2016; Pliszko and Pliszko, 2015; Szymura et al., 2016) more scientific studies are performed to justify them as medicinal plants and to elucidate the profiles of biologically active compounds (Elshafie et al., 2019; Woźniak et al., 2018). On the other hand, *S. canadensis* and *S. gigantea* are included in European Pharmacopoeia that specifies their quality with certain amounts of flavonoids (European Pharmacopoeia, 2016). The allelopathic activity of *Solidago* species interferes with the physiological processes of plant and determines the superiority of these species in the ecosystems (Balezentiene, 2015). Invasive goldenrods create a negative impact on other plant species and hamper the diversity of pollinators, and disturb other ecosystem elements. The spread of invasive populations must be under strict control with the development of their eradication programs (Szymura et al., 2016). On the other hand, raw materials of invasive *Solidago* spp. is considered as a promising, economically reasonable source of biologically active phenolic compounds (Radušiene et al., 2015). An intensive collection of

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goldenrods could be one of the eradication measures that meet the requirements of Regulation EU No. 1143/2014. Eradication of these species results in waste material being bio-economically turned into value-added products ensuring the utilization of no-waste-technologies (Zihare and Blumberga, 2017).

One of the main groups of biologically active compounds in goldenrods is phenolic compounds which express the pharmacological effects and possess antioxidant activity (Demir et al., 2009; Marksa at al., 2016). The profile of phenolic compounds in goldenrods varies significantly in qualitative and quantitative composition and strongly depend on the species, plant part, ontogenetic development and environmental conditions (Apati et al., 2002; Choi et al., 2004; Demir et al., 2009; Deng et al., 2015; Hwang et al., 2013, 2017; Radušiene et al., 2018). The phenolic and antioxidant profiles may differ because the latter elucidates the compounds with expressed activity and their markers do not always correspond to the phytochemical markers (Raudonis et al., 2009). Therefore, it is essential to apply combined detection systems to detect even the minor components in the phytochemical profile as they could possess highly expressed antioxidant activity. HPLC post-column assays are widely used for the coupled determination of phytochemical and antioxidant profiles (Marksa at al., 2016; Nalewajko-Sieliwoniuk et al., 2017; Raudone et al., 2016, 2015; Raudonis et al., 2009). Post-column assays enable the determination of the most active compounds and can be used for the screening and selecting plant material rich in specific antioxidants of interest (Shi et al., 2009). Applied assays and identified markers may be used to control the distribution and stability of antioxidants, to measure antioxidant activity after all processing has undergone. The quantitative expression in standard Trolox equivalents enables the quality control of extracts in functional ingredients and their preparations (Raudonis et al., 2012). Pharmaceutical and food industries have an increased demand for natural antioxidants because they are more effective than synthetic antioxidants, which possess also the carcinogenic effects (Augustyniak et al., 2010).

The aim of the present study was to identify and quantify antioxidant profiles of leaves and inflorescences of four *Solidago* species (*S. gigantea*, *S. canadensis*, *S. virgaurea*, *S. ×niederederi*) from wild populations in Lithuania and to predict the importance of these plant raw materials for possible use as the source of antioxidants. To the best of our knowledge, this is the first comprehensive report on the individual antioxidant active compounds of certain *Solidago* species.

2. Materials and methods

2.1. Plant materials

Sample collection of *Solidago* species was performed from wild mixed populations in Vilnius district, Lithuania (Table S1). The sample of the plant material consisted of the three tops of two to three shoots of the same clonal plant which considered as a group of individuals (ramets) vegetatively originated from the same genet. Plants were dissected into inflorescences and leaves and dried at 25 °C. The botanical identification of taxa was performed following to the morphological description of Central European *Solidago* species (Wagenitz, 1979) and according to the morphological diagnostic characters defined in the previous study (Karpavičienė et al., 2016). The herbarium vouchers of the specimen were deposited at the Herbarium of the Nature Research Centre, Vilnius, Lithuania (BILAS) with the appropriate collection number.

2.2. Chemicals and solvents

HPLC grade standard materials were used: chlorogenic acid (5-*O*-caffeoylquinic acid) (purity ≥ 95.3 %), rutin trihydrate (purity ≥ 97.1 %) and isoquercetin (purity ≥ 94.1 %), were purchased from HWI ANALYTIK GmbH (Ruelzheim, Germany), quercitrin (purity ≥ 98.0 %) and hyperoside (purity ≥ 98.0 %) were obtained from Roth GmbH (Karlsruhe, Germany. Neochlorogenic acid (3-*O*-caffeoylquinic acid, purity ≥ 95.0 %), 4-*O*-caffeoylquinic acid (purity ≥ 98.0 %), 3,5-dicaffeoylquinic acid (purity ≥ 95.0 %) and 3,4-dicaffeoylquinic acid (purity ≥ 90.0 %) were purchased from Sigma–Aldrich GmbH (Steinheim, Germany). The acetonitrile and methanol were of HPLC grade and supplied by Roth GmbH (Karlsruhe, Germany); the ethanol was produced by Spiritus Vilnensis (Vilnius, Lithuania). 2,2′ -azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, purity ≥ 98 %), potassium persulphate (purity ≥ 99 %)were purchased from Sigma (St. Louis, MO, USA), Trolox (purity ≥ 98 %) and TFA (purity ≥ 99 %) were received from Fluka Chemika (Buchs, Switzerland). Ultrapure water was purified with a Millipore water cleaning system (Bedford, MA, USA).

2.3. Sample preparation

All the samples of air-dried plant material were mechanically ground up to the homogenous powder. Approximately 0.1 g (precise weight) of each sample was extracted in 10 ml of a methanol and water mixture (70:30, v/v) by ultra-sonication at 25 °C for 50 min. The extracts were filtered through 0.22 mm nylon syringe filters (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) and stored at 4 °C until analysis.

2.4. HPLC-PDA conditions and HPLC post-column assay

HPLC-PDA and HPLC-ABTS were performed according to Marksa et al. (2016). A Waters Alliance 2695 (Waters, Milford, USA) separation module system was equipped with Waters 2487 UV/VIS and Waters 996 PDA diode-array detector (DAD). The separation of the compounds was carried out using 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). After the PDA detection, the mobile phase with the analytes was mixed in a reaction coil with the ABTS solution, which was prepared following the methods described by Raudonis et al. (2009, 2012). The data were analyzed using the Empower Software chromatographic manager system (Waters Corporation, Milford, USA). The ABTS post-column chromatograms were registered at 650 nm using Waters 2487 UV/VIS detector (Waters Corporation). Calibration curves were established from a standard antioxidant Trolox. The antioxidant activity was expressed as µmol Trolox equivalent (TE) for 1 g of dry mass (DM) of plant material using the following formula:

$$TE = c \times V/m \ (\mu mol/g)$$

where c is the concentration of Trolox established from the calibration curve, µM; V is the volume of plant material extract, L; m is the weight (precise), g.

2.5. UPLC-QTOF-MS conditions

The analysis was performed according to Kraujaliene et al., 2017, using Waters Aquity UPLC system with PDA detector (Waters, Milford, Mass., U.S.A.) and compounds were analyzed by MAXIS 4 G QTOF mass spectrometer (BRUKER DALTONIK GmbH, Bremen, Germany) equipped with an ESI ionization source (Kraujaliene et al., 2017). Waters Aquity HSS T3 column was used. Eluent A – 2 % acetic acid, and B – acetonitrile. The gradient was formed as follows: 0–1.2 min 80 % A, 1–8 min 88 % to 70 % A, 8–9 min 70 % to 90 % A, 9–10 min 90 % A.

2.6. Statistical analysis

Trolox equivalent antioxidant activity of phenolic compounds was expressed as mean ± standard error (SE). An ANOVA and posthoc Tukey HSD multiple comparison test was completed to identify significant differences at $p \leq 0.05$. According to the radical scavenging

Table 1
UPLC-QTOF-MS (negative ionization mode) data of phenolic compounds for *Solidago* species inflorescences and leaves.

Compounds	RT[a] HPLC	RT UPLC	Formula $[M-H]^-$	$[M-H]^-$	UV max
Neochlorogenic acid	8.19	1.55	C13H15O9	353.09	326
Chlorogenic acid	11.00	1.96	C16H17O9	353.09	325
4-*O*-Caffeoylquinic acid	13.74	2.44	C16 H17O9	353.00	326
Coumaroylquinic acid	14.71	2.47	C16 H17O8	337.00	309
Caffeoylshikimic acid	16.11	2.64	C17H19O9	367.10	324
Feruloylquinic acid	16.23	2.97	C17H19O9	367.00	324
Quercetin pentoside hexoside	23.02	3.45	C26H27O16	595.13	255, 355
Coumaroylquinic acid	21.63	3.15	C16H17O8	337.00	309.00
Quercetin hexoside hexoside	23.39	3.50	C27H29O16	609.00	255, 354
Rutin	23.90	3.55	C27H29O16	609.15	253, 353
Hyperoside	24.66	3.72	C21H19O12	463.00	253, 354
Isoquercitrin	25.39	3.85	C21H19O12	463.00	253, 355
Kaempferol deoxyhexose hexoside	26.85	4.20	C27H29O15	593.00	263, 347
Quercetin pentoside	28.09	4.53	C20H17O11	433.08	253, 353
3,5-dicaffeoylquinic acid	29.57	4.73	C25H23O12	515.12	215,6, 239,4sh, 326,6
Quercitrin	30.18	4.87	C21H19O11	447.09	253, 353
Quercetin 3-*O*-(6"-acetyl-hexoside)	32.00	5.10	C23H22O13	505.40	253, 353
Kaempferol 3-*O*-arabinoside	32.75	5.14	C20H17O10	417.00	263,2, 344,7
3,4-dicaffeoylquinic acid	33.89	5.51	C25H23O12	515.12	326.60
Quercetin caffeoyl hexoside	35.00	5.81	C35H33O19	757.16	250, 332
Kaempferol 3-*O*-rhamnoside	35.27	5.99	C21H19O10	431.00	264, 347
Quercetin	44.13	7.17	C15H9O7	301.23	252, 370

activities of identified compounds, the tested samples were compared by the method of cluster analysis using squared Euclidean distances. Principal component analysis (PCA) was performed taking into account factors with eigenvalues higher than 1. The data was processed using Microsoft Office Excel 2010 (Microsoft, JAV) and SPSS 20 software packages.

3. Results

3.1. Identification of phenolic compounds and their antioxidant activities

UPLC-QTOF-MS was applied to identify the phenolic compounds. The results are presented in Table 1, which includes all identified compounds with their mass after ionization, the molecular ion formulas that have been compared with literary data (Willems et al., 2016; Woźniak et al., 2018). Negative ionization mode was applied, and twenty-three compounds of different phenolic origin were identified in the tested *Solidago* spp. samples. Fourteen compounds were elucidated with significant antioxidant activity. The antioxidant profiles of leaves and inflorescences of *Solidago* species constituted mainly of caffeoylquinic acid and quercetin derivatives (Fig. 1). The greatest radical scavenging activities ($p \leq 0.05$) were determined for the individual components of the caffeoylquinic acid derivatives complex. Three monocaffeoylquinic acids gave the *m/z* ion of 353, and their identities, namely neochlorogenic, chlorogenic and 4-*O*-caffeoylquinic acid, were confirmed by matching their retention time with reference compounds. Dicaffeoylquinic acid derivatives gave the *m/z* of 515 and their identities were confirmed by reference compounds. The detected caffeoylquinic acid derivatives were determined in leaves and inflorescences of all investigated species. 3,5-Dicaffeoylquinic acid was the predominant radical scavenger in samples of *S. virgaurea*, *S. canadensis* and *S.* ×*niedereder*i (Table 2). It comprised about 43 and 54 % of total radical scavenging activity in leaf and inflorescence samples, respectively. The predominant radical scavenger in *S. gigantea* was chlorogenic acid, accounting for 32 and 66 % of total inflorescence and leaf activity, respectively. Neochlorogenic acid was detected in all tested goldenrod samples with the lowest activity of *S. gigantea* inflorescences and leaves, 0.33 ± 0.16 and 0.51 ± 0.12 µmol/g of TE, DM, respectively. Significant radical scavenging activity for the caffeoylshikimic acid was determined only in the inflorescence and leaf samples of *S. gigantea*. 4-*O*-Caffeoylquinic acid was detected as a radical scavenger in leaf samples of all *Solidago* species tested, but not in inflorescences. The greatest radical scavenging activity of 3,4-dicaffeoylquinic acid was expressed in the inflorescences and leaves of *S.* ×*niedereder*i and *S. virgaurea*, 16.45 ± 1.00 and 12.27 ± 0.84, µmol TE/g DM, respectively (Table 1). Chlorogenic acid can be considered as a marker of radical scavenging activity in *S. gigantea* whereas 3,5-dicaffeoylquinic acid – in *S. virgaurea*, *S. canadensis* and *S.* ×*niedereder*i. Trolox equivalent radical scavenging activities of the tested *Solidago* species were significantly greater in the leaf samples compared to the inflorescences ($p \leq 0.05$).

The flavonoid complex was predominated by quercetin derivatives and showed a great variation within the species and plant parts. Quercetin pentoside-hexoside *m/z* 595, quercetin hexoside-hexoside *m/z* 609, quercetin 3-*O*-(6"-acetyl-hexoside) *m/z* 505, quercetin caffeoyl-hexoside derivative *m/z* 757 correspond to the following molecular ion formula: C26H27O16, C27H 29O16, C23H22O13 and C35H33O19, respectively (Table 1). The presence and structures of rutin, hyperoside, isoquercitrin, quercitrin, and quercetin were ascertained by matching them with the reference compounds. The radical scavenging activities of quercetin derivatives were significantly lower compared with caffeoylquinic acids. Quercetin pentoside and hyperoside were predominant flavonol-derived radical scavengers in *S. gigantea* inflorescences and leaves, wheres rutin had the greatest value up to 11 % of total antioxidant activity in *S. virgaurea*, *S. canadensis* and *S.* ×*niedereder*i samples. The other identified compounds, namely feruloylquinic and coumaroylquinic acids, kaempferol rutinoside, kaempferol arabinoside, kaempferol 3-*O*-rhamnoside, had no significant antioxidant activity. On the other hand, phytochemical profiles of corresponding compounds highly differed in *Solidago* species.

3.2. Hierarchical cluster analysis of radical scavenging compounds

The hierarchical analysis was applied separately to both leaves and inflorescences, based on the mean quantities of Trolox antioxidant equivalents of phenolic compounds as clustering variables. The clustering of the inflorescences resulted in grouping samples into two main clusters (Fig. 2). The first cluster grouped all samples of *S. gigantea* inflorescences. The corresponding samples were differed from the others by the high antioxidant activity of chlorogenic acid and other antioxidants in the following decreasing order: 3,5-dicaffeoylquinic acid > quercetin pentoside > hyperoside > quercitrin and >

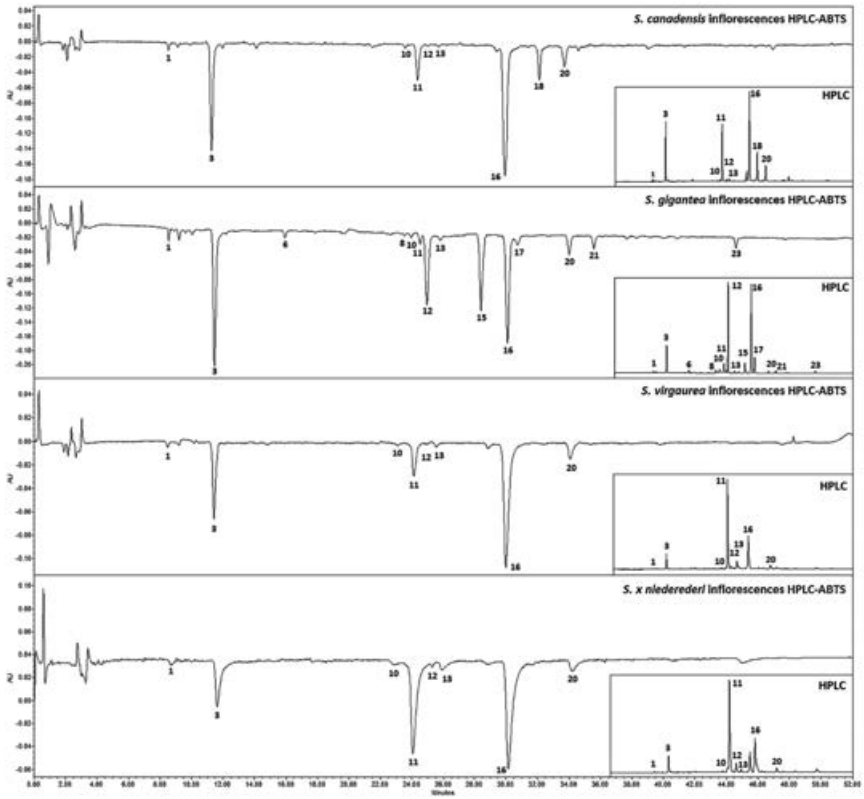

Fig. 1. HPLC and HPLC-ABTS chromatograms of the *Solidago* species inflorescences. (1) – neochlorogenic acid, (3)– chlorogenic acid, (6) – caffeoyl shikimic acid, (8) – quercetin pentoside hexoside, (10) – quercetin hexoside hexoside, (11) – –rutin, (12) – hyperoside, (13) – isoquercitrin, (15) – quercetin pentoside, (16) – 3,5-dicaffeoylquinic acid, (17) – quercitrin, (18) – quercetin 3-*O*-(6"-acetyl-hexoside), (20) – 3,4-dicaffeoylquinic acid, (21) – quercetin caffeoyl hexoside, (23) – quercetin.

isoquercitrin. Chlorogenic and 3,5-dicaffeoylquinic acids can be considered as the principal radical scavenging markers of *S. gigantea* inflorescences followed by quercetin pentoside (24.50 ± 3.82 μmol TE/g DM) from flavonol complex (Fig. 4). The samples of *S. canadensis*, *S. virgaurea* and *S.* ×*niedererderi* formed the second cluster. The cluster was characterized by the highest antioxidant activity of 3,5-dicaffeoylquinic acid and other phenolics in the following decreasing order: chlorogenic acid > rutin > 3,4-dicaffeoylquinic acid > hyperoside > and isoquercitrin. Rutin was prevailing radical scavenging marker from flavonol complex.

The clustering of *Solidago* spp. leaves divided all samples into two clusters (Fig. 3). *S. gigantea* leaves formed the first cluster. Similar to inflorescences, chlorogenic acid was the predominant compound, followed by other decreasing phenolics, such as 3,5-dicaffeoylquinic acid > quercetin pentoside > quercitrin > and 3,4-dicaffeoylquinic acid. Meanwhile, leaves of *S. canadensis*, *S.* ×*niedererderi*, and *S. virgaurea* were subdivided into well-defined sub-clusters combined into a second cluster, except for one sample of *S. virgaurea*, which showed similarity with leaves of *S. canadensis*. The remote position of the corresponding sample may be explained by low antioxidant activity of 3,5-dicaffeoylquinic acid and high of chlorogenic acid, which revealed similarity with *S. canadensis* leaves. The samples forming the second cluster were characterized by the highest antioxidant activity of 3,5-dicaffeoylquinic acid. Radical scavenging activity of other compounds can be presented by the following decreasing order: chlorogenic acid > rutin > and 3,4-dicaffeoylquinic acid (Fig. 5). Overall, caffeoylquinic acids, namely chlorogenic and 3,5-dicaffeoylquinic acids, were presented as major markers of antioxidant activity in extracted groups of inflorescence and leaf samples (Figs. 5 and 6).

Consequently, the hierarchical cluster analysis revealed the similarity between *S. virgaurea*, *S. candensis* and *S.* ×*niedererderi* in terms of antioxidant activity of phenolic compounds. Meanwhile, the results showed that the antioxidant activity of *S. gigantea* was highly different from that of other *Solidago* species.

3.3. PCA analysis of phenolic antioxidants

A principal component analysis (PCA) was performed to detect similarities and differences between the analyzed samples according to statistically independent variables, which indicate the antioxidant

Table 2
Antioxidant activities of inflorescence and leaf compounds of *Solidago* species expressed in Trolox equivalent values (μmol TE/g DM) using HPLC-ABTS post-column assay.

Compounds	ISG[3]	ISV	ISC	ISN	LSG	LSV	LSC	LSN
Neochlorogenic acid	0.33 ± 0.16c[4]	1.73 ± 0.16a	0.99 ± 0.09b	2.17 ± 0.22a	0.51 ± 0.12c	3.01 ± 0.29b	2.63 ± 0.38b	5.60 ± 0.51a
Chlorogenic acid	52.42 ± 4.31a	23.64 ± 1.94b	49.58 ± 4.64a	40.17 ± 2.31a	122.44 ± 12.33a	51.06 ± 3.36b	64.03 ± 6.60b	68.70 ± 5.17b
4-*O*-caffeoylquinic acid	nd	ns	ns	nd	3.65 ± 0.50a	1.53 ± 0.30b	1.95 ± 0.24b	nd
Coumaroylquinic acid	ns	ns	nd	ns	nd	ns	ns	ns
Caffeoylshikimic acid	1.41 ± 0.45	ns	ns	ns	1.56 ± 0.45	ns	ns	ns
Feruloylquinic acid	ns	ns	ns	ns	ns	ns	ns	ns
Quercetin pentoside hexoside	1.15 ± 0.19	nd	ns	nd	0.89 ± 0.17a	nd	0.38 ± 0.25ab	nd
Coumaroylquinic acid	0	nd	nd	nd	nd	nd	ns	nd
Quercetin hexoside hexoside	0.55 ± 0.12b	0.79 ± 0.17b	1.64 ± 0.56b	5.07 ± 0.78a	nd	nd	10.62 ± 0.37a	0.46 ± 0.34a
Rutin	1.69 ± 0.17c	9.92 ± 1.31bc	19.91 ± 3.26a	18.32 ± 1.90ab	0.46 ± 0.21b	19.81 ± 2.26a	19.73 ± 3.42a	22.26 ± 3.33a
Hyperoside	16.18 ± 1.85a	2.12 ± 0.29bc	0.06 ± 0.06c	3.32 ± 0.55b	2.88 ± 0.49a	ns	ns	1.31 ± 0.36b
Isoquercitrin	6.09 ± 0.63a	3.44 ± 0.35b	2.58 ± 0.59b	3.41 ± 0.78b	3.07 ± 0.56a	1.15 ± 0.33b	1.07 ± 0.28b	0.37 ± 0.35b
Kaempferol deoxyhexose hexoside	nd	nd	ns	ns	ns	nd	ns	ns
Quercetin pentoside	24.51 ± 3.83	nd	nd	nd	11.57 ± 2.54	nd	nd	nd
3,5-dicaffeoylquinic acid	31.42 ± 2.74b	81.51 ± 4.10a	94.65 ± 3.54a	90.69 ± 13a	24.89 ± 3.25d	103.63 ± 4.74a	50.36 ± 3.87c	72.47 ± 6.10b
Quercitrin	6.80 ± 1.73	nd	nd	nd	10.38 ± 2.84	nd	nd	nd
Quercetin 3-*O*-(6"-acetyl-hexoside)	nd	nd	7.40 ± 2.55	ns	nd	nd	8.32 ± 2.23	ns
Kaempferol 3-*O*-arabinoside	0	nd	nd	nd	nd	nd	nd	nd
3,4-dicaffeoylquinic acid	5.03 ± 0.73c	12.27 ± 0.84b	10.46 ± 1.15b	16.45 ± 1.00a	2.83 ± 0.71c	28.41 ± 2.91a	4.43 ± 1.20c	15.55 ± 1.54b
Quercetin caffeoyl hexoside	4.14 ± 0.35a	nd	nd	nd	ns	nd	nd	nd
Kaempferol 3-*O*-rhamnoside	ns	ns	ns	ns	ns	ns	ns	ns
Quercetin	4.84 ± 1.11	ns	ns	ns	nd	nd	nd	ns

Abbreviations: [1] nd not detected; ; nsnot significant; 2RT retention time.
[3]ISG – inflorescences of *S. gigantea*; ISV – inflorescences of *S. virgaurea*; ISC – inflorescences of *S. canadensis*; ISN – inflorescences of *S. ×niederederi*; LSG – leaves of *S. gigantea*; LSV – leaves of *S. virgaurea*; LSC – leaves of *S. canadensis*; LSN – leaves of *S. × niederederi*.
[4]Values (means ± SE) marked by different letters along rows are significantly different ($p \leq 0.05$) according to posthoc Tukey HSD multiple comparison test, which was completed for inflorescences and leaves separately.

activity of phenolic compounds. Table S2 summarizes the PCA results based on the correlation matrix with PC1, PC2 and PC3, which explain 75.52 and 69.92 % of the total variance in the data sets of inflorescences and leaves, respectively. The score plot models for inflorescence (PCA1) and leaf (PCA2) samples have shown relatively good separation between the four *Solidago* species (Fig. 6).

In the PCA1 model, PC1 described 53.35 % of the total variance of data and highly correlated with positive loadings of hyperoside, quercitrin, isoquercitrin, quercetin, quercetin pentoside, quercetin pentoside hexoside, quercetin caffeoyl hexoside, and caffeoyl shikimic acid as well as with negative loading of 3,5-dicaffeoylquinic acid. The PC2 accounted for 11.78 % of the total variance and characterized by the loadings of neochlorogenic acid, 3,4-dicaffeoylquinic acid, quercetin hexoside hexoside, and quercetin 3-*O*-(6"-acetyl-hexoside) variables. Meanwhile, the PC3 described 10.39 % of the total observed variability and was associated with loadings of chlorogenic acid and rutin. The PCA1 score plots of inflorescence samples showed their arrangement into two distinct groups (Fig. 6). The first group on the right-hand positive side plots covered all the inflorescences of *S. gigantea* and was in distance from all others. The clustering of samples along the positive PC1 can be explained by the highest values of hyperoside, quercetin, quercitrin, quercetin caffeoyl hexoside, and isoquercitrin in this group. Conversely, rutin scoring low in PC1 and 3,5-dicaffeoylquinic acid with high negative loading in PC1 exposed the weak antioxidant activity in the inflorescences of *S. gigantea*. The second group of samples on the left-hand plot grouped together all samples of *S. canadensis*, *S. virgaurea* and *S. ×niederederi* indicating similarity in their antioxidant activity. Inflorescences of *S. virgaurea* and *S. ×niederederi* situated close to the PC1 and PC2 zero point, which means that the radical scavenging activity of phenolic compounds was found in the range from the lowest to the mean values. On the other hand, inflorescences of *S. ×niederederi* and S. *canadensis* demonstrated close position in PC1 vs. PC3 space scoring high in PC3 that suggested high antioxidant activity of chlorogenic acid and rutin in the corresponding samples. Meanwhile, samples of *S. virgaurea* scattering on negative PC1 vs. PC3 space exposed the weakest antioxidant activity of chlorogenic acid and rutin.

In the PCA2 model, PC1 described 47 % of the total variance in the data set of leaves and was characterized by positive loadings of quercitrin, hyperoside, isoquercitrin, rutin, quercetin pentoside, chlorogenic acid, and caffeoyl shikimic acid. Meanwhile, the PC2 explains 13.43 % of the total variance and associated with positive loading of quercetin 3-*O*-(6"-acetyl-hexoside) and with negative loadings of the 3,5- and 3,4-dicaffeoylquinic acids antioxidant activities. The PC3 explained 9.56 % of the total variance of data and was characterized by positive loadings of quercetin pentoside hexoside and negative loading of radical scavenging activity of neochlorogenic acid and quercetin hexoside hexoside. The score plots of leaf samples showed their arrangement into two distinct groups similar to inflorescences (Fig. 6). The first group on the right-hand positive side plot covered all the samples of *S. gigantea*, which were in distance from all others and clustered along PC1. Significant antioxidant activities of chlorogenic acid, quercetin pentoside, quercitrin, caffeoyl shikimic acid, hyperoside, and isoquercitrin scoring high in PC1 were found in leaves of *S. gigantea*. Consequently, quercetin pentoside hexoside scoring high in PC3 was associated with antioxidant activity of this compound in leaf samples. The second group on the left-hand plots grouped together all samples of *S. canadensis*, *S. virgaurea* and *S. ×niederederi*. On the other hand, most samples of *S. canadensis* were closely clustered along positive PC2 and PC3 and were differed from other species in expressed antioxidant activity of quercetin 3-*O*-(6"-acetyl-hexoside) and quercetin hexoside hexoside. Leaves of *S. virgaurea* located on the negative side of PC2 associated with the strongest antioxidant activities of 3,5- and 3,4-dicaffeoylquinic acids in the corresponding samples. Meanwhile, quercetin pentoside hexoside and quercetin hexoside hexoside scoring high in PC3 did not expose antioxidant activity in samples of *S. virgaurea* located near zero point of PC3. Leaves of *S. ×niederederi* situated near the zero point of PC2 indicated mean values of radical scavenging activity of 3,5- and 3,4-dicaffeoylquinic acids.

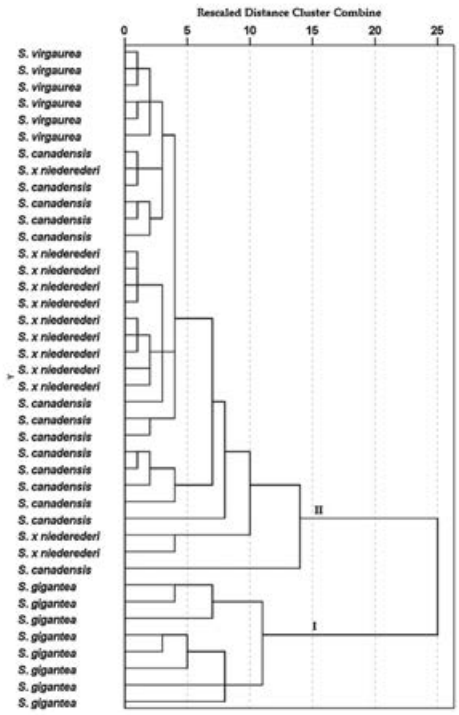

Fig. 2. The dendrogram of hierarchical cluster analysis of *Solidago* species inflorescence samples based on the phenolic radical scavenging compounds expressed as Trolox equivalents for 1 g of dry matter (DM) of plant material (μmol/ TEg DM).

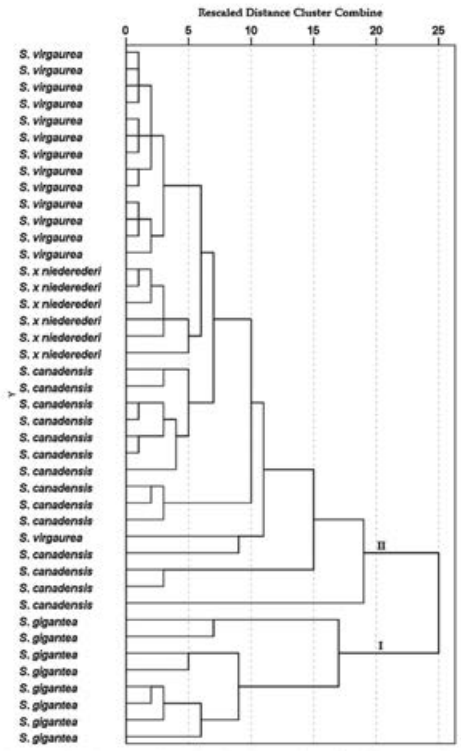

Fig. 3. The dendrogram of hierarchical cluster analysis of *Solidago* species leaf samples based on the phenolic radical scavenging compounds expressed as Trolox equivalents for 1 g of dry matter (DM)of phenolic antioxidants (μmol/ TEg DM).

PCA was present as a graphical visualization of differences between *Solidago* species on the basis of radical scavenging activity of phenolic compounds. Consequently, *S. gigantea* inflorescence and leaf samples formed a separate cluster indicating different antioxidant activity of phenolics from other *Solidago* species. On the other hand, *S. canadensis*, S. *virgaurea* and *S. ×niedererderi* revealed similarity in the antioxidant activity of their raw materials.

4. Discussion

The antioxidant activity is one of the most important features of phenolic compounds. As a result, the increased accumulation of phenolics indicates the protection against intense ROS formation in plants under the exposure to biotic and abiotic stressors (Kasote et al., 2015). High capacity antioxidants could possess notable health effects and could be used as activity markers for the standardization of plant origin extracts (Rutkowska et al., 2019). Goldenrods are a promising and an abundant source of phenolic compounds; however, data on the peculiarities of their antioxidant profiles are still lacking. Flavonoids, especially quercetin and kaempferol derivatives, are predominant compounds in phenolic profiles and are used in quality determination and standardization of goldenrod extracts (European Medicines Agency, 2008; European Pharmacopoeia, 2016; Fedotova and Konovalov, 2018). *Solidago* spp. flavonoids are considered as a group of special importance compounds because of their ability to increase urinary flow by inhibiting neutral endopeptidase (Melzig, 2004). Quercetin derivatives due to their structure peculiarities are regarded as potent antioxidants (Raudone et al., 2019). In this regard, *S. gigantea* was distinguished from other *Solidago* species by radical scavenging capacities of hyperoside, quercetin pentoside and quercitrin. Meanwhile, rutin was the predominant antioxidant active flavonol in the other three *Solidago* species. The findings are in agreement with previous studies which confirmed the presence of rutin as the predominant flavonol in extracts of *S. virgaurea* and *S. canadensis* and the absence of this compound in *S. gigantea* (Anzlovar and Dolenc Koce, 2014; Apati et al., 2002; Paun et al., 2016; Woźniak et al., 2018). In the case of pure phenolic compounds, rutin and hyperoside were found to have the same radical scavenging capacities, which were greater compared with quercetin pentoside and quercitrin (Raudone et al., 2019). Hyperoside could be considered as an important marker in assessing the phytochemical quality of the raw materials due to its contribution to the total flavonoid content, the importance of which is referred in the European Pharmacopoeia (European Pharmacopoeia, 2016). Our results partly supported by this consequence, since hyperoside accounted for up to 11 % of the total radical scavenging capacity in *S. gigantea* leaves, while in other species the impact of hyperoside on total antioxidant activity was

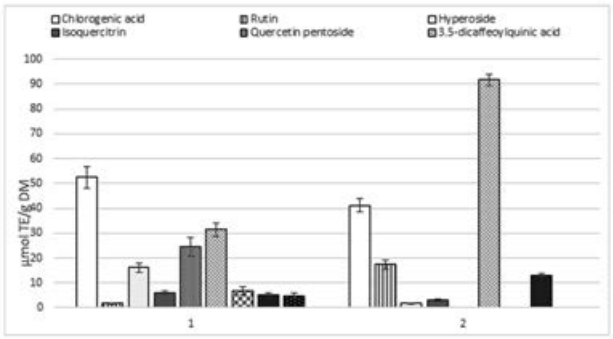

Fig. 4. Mean values of antioxidant activities of principal radical scavengers (µmol TE/g DM) of *Solidago* species inflorescence groups extracted using hierarchical cluster analysis. 1 – cluster composed of *S. gigantea*; 2 – cluster composed of *S. virgaurea*, *S. canadensis* and *S.* ×*niedereder*i.

less than 2 %. Therefore, hyperoside could be considered as a marker of antioxidant activity only for *S. gigantea*. On the other hand, Woźniak et al. (2018) detected that hyperoside was absent in the root and above-ground samples of *S. canadensis* and *S. gigantea* (Woźniak et al., 2018). Furthermore, the antioxidant profiles of leaves and inflorescences of *S. gigantea* contain a more diverse complex of quercetin derivatives with significantly greater radical scavenging capacities compared to other *Solidago* species.

Using UPLC-MS, three kaempferol derivatives such as kaempferol deoxyhexose hexoside, kaempferol 3-*O*-arabinoside, and kaempferol 3-*O*-rhamnoside, were identified in the leaf and inflorescence samples of *Solidago* spp., however, their radical scavenging activities in the antioxidant profiles were not significant. According to Murugesu et al. (2017), kaempferol provided notable antioxidant activity using different detection systems. However, the current study did not reveal any significant antioxidant capacity of kaempferol derivatives, which can be explained by the limited exposure of the corresponding compounds with the reference oxidant during continuous separation process in post-column detection. The negligible radical scavenging activity of kaempferol derivatives can be justified by the lack of easily oxidizable hydroxyl groups in the catechol group compared with quercetin derivatives (Murugesu et al., 2017). Contrary to the present results, some previous sources indicate the accumulation of methylated flavonols (isorhamnetin derivatives) in goldenrod raw materials (European Medicines Agency, 2008; Woźniak et al., 2018). On the other hand, Apati et al. (2002); Choi et al. (2004); Kraujaliene et al. (2017) and, Radušiene et al. (2015, 2018) did not report isorhamnetin derivatives in *Solidago* spp. Consequently, the flavonoid composition is highly variable and can be considered as an indication of the phytochemical heterogeneity of the genus *Solidago* (Woźniak et al., 2018).

Quinic acid esters with cinnamic acids such as caffeoylquinic, coumaroylquinic and feruloylquinic, are widely distributed in *Asteraceae* family plants with different accumulation patterns depending on the species (Gimenes et al., 2018). However, the amounts of coumaroyl and feruloylquinic acids did not expose any significant response in the antioxidant profiles of tested *Solidago* spp. Similarly, in previous post-column studies, there was no detected radical scavenging activity of the corresponding compounds (Raudone et al., 2016, 2015). Caffeoylquinic acids are regarded as potential antioxidants with neuroprotective and cardioprotective activities as well as anti-inflammatory effects and can be beneficial in the treatment of metabolic disorders. (Abdel Motaal et al., 2016; Spínola and Castilho, 2017; Upadhyay and Mohan Rao, 2013). In our study 3,5- and 3,4- dicaffeoylquinic acids had greater Trolox equivalent antioxidant values compared to monocafeoylquinic

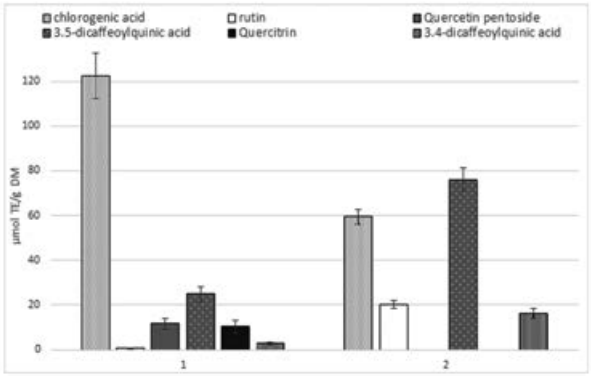

Fig. 5. Mean values of antioxidant activities of principal radical scavengers (µmol TE/g DM) of *Solidago* species leaf groups extracted using hierarchical cluster analysis. 1 – cluster composed of *S. gigantea*; 2 – cluster composed of *S. virgaurea*, *S. canadensis* and *S.* ×*niedereder*i.

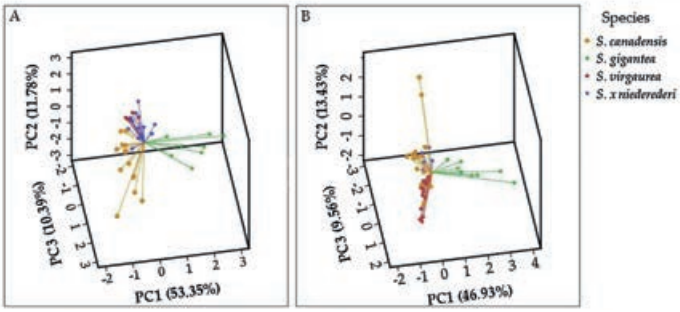

Fig. 6. PCA score (B) plots of different *Solidago* species inflorescence (A) and leaf (B) samples.

acids. This is in agreement with Li et al. (2018) and Xu et al. (2012) findings, which reported that two substitutional caffeoyl groups significantly increased the antioxidant activity of corresponding compounds. On the other hand, caffeoylquinic acids have been identified as major phenolic markers in the species of the *Asteraceae* family (Fraisse et al., 2011; Gimenes et al., 2018; Jaiswal et al., 2011). However, *Solidago* species have a specific pattern of accumulation of these constituents. Qualitative patterns of phenolic profiles of various structural origins are used in the phenetic chemistry of the studied taxon and recently have been described as chemophenetic studies (Zidorn, 2019).

The multivariate analysis of the antioxidant activity of the phenolic compounds of *Solidago* species leaves and inflorescences have revealed the principal radical scavengers. Phenolic profile of *S. gigantea* differed with predominant antioxidant activity of chlorogenic acid, 3,5-dicaffeoylquinic acid and quercetin pentoside. Accordingly, inflorescences and leaves of *S. canadensis*, *S.* × *niederederi* and *S. virgaurea* had priority in antioxidant activity of 3,5-dicaffeoylquinic acid, chlorogenic aid and rutin against *S. gigantea*. Consequently, these compounds can be considered as antioxidant activity markers in phytochemical profiles of the corresponding species. The results are consistent with previous studies which reported that *Solidago* species exposed the same profile of phenolic constituents with significant quantitative differences (Radušiene et al., 2018). Generally, the total antioxidant capacity of the detected compounds of invasive *Solidago* species was significantly higher than that of native species. The increased accumulation of flavonoids in invasive species represents their potential defense against plant herbivores and pathogens and, consequently, a higher mechanism of adaptation to the new environment (Kundu and Vadassery, 2019; Wang et al., 2012).

Phenolic compounds depending on the structural peculiarities and initial reaction with oxidant rate have different antioxidant capacities, which strongly correlate with the amount of active compounds in the raw material (Raudone et al., 2019). Antioxidant profiles obtained by post-column assay specifically include only the compounds with significant activities and fast initial reactions. Therefore, this method is appropriate for the detection of fast acting high-capacity antioxidants and identification of antioxidant activity markers. In this respect, antioxidant activity markers can be useful in ensuring the quality and effectiveness of plant materials with potentially beneficial health effects that could be incorporated in nutraceuticals, cosmeceuticals, and pharmaceuticals.

Declaration of Competing Interest

The authors declare no conflict of interest.

CRediT authorship contribution statement

Mindaugas Marksa: Investigation, Data curation. **Kristina Zymone:** Formal analysis, Data curation. **Liudas Ivanauskas:** Methodology, Resources, Supervision. **Jolita Radušienė:** Conceptualization, Resources, Writing - review & editing. **Audrius Pukalskas:** Methodology, Investigation. **Lina Raudone:** Conceptualization, Data curation, Writing - original draft, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.indcrop.2020.112123.

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SUPPLEMENTARY MATERIAL

Antioxidant profiles of leaves and inflorescences of native, invasive and hybrid *Solidago* species

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Table S1. *Collection data of the investigated populations of Solidago species*

Species	Sample no.	Collection site	Altitude, m	Latitude N	Longitude E	Habitat
SG	33101	Naujoji Vilnia, Vilnius distr.	160	54° 40'30"	25° 23'10"	Abandoned field
SG	33102	Naujoji Vilnia, Vilnius distr.	160	54° 40'32"	25° 23'05"	Abandoned field
SG	33103	Naujoji Vilnia, Vilnius distr.	160	54° 40'34"	25° 23'01"	Abandoned field
SG	33204	Rokantiskes, Vilnius distr.	200	54° 42'38"	25° 23'29"	Abandoned field
SG	33305	Rokantiskes, Vilnius distr.	200	54° 42'36"	25° 23'25"	Dry meadow
SG	33406	Rokantiskes, Vilnius distr.	200	54° 42'38"	25° 23'23"	Dry meadow
SG	33307	Suderve, Vilnius distr.	120	54° 40'13"	25° 22'48"	Abandoned field
SG	33308	Suderve, Vilnius distr.	120	54° 40'10"	25° 22'45"	Abandoned field
SV	31201	Naujoji Vilnia, Vilnius distr.	160	54° 40'35"	25° 23'01"	Abandoned field
SV	32204	Rokantiskes, Vilnius distr.	200	54° 42'35"	25° 23'24"	Dry meadow
SV	33107	Suderve, Vilnius distr.	210	54° 40'03"	25° 22'58"	Abandoned field
SV	36211	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SV	38113	Karkline, Vilnius distr.	120	54° 54'26"	25° 33'40"	Abandoned field
SV	38214	Karkline, Vilnius distr.	120	54° 54'20"	25° 33'13"	Abandoned field
SC	31201	Naujoji Vilnia, Vilnius distr.	160	54° 40'15"	25° 23'10"	Abandoned field
SC	31302	Naujoji Vilnia, Vilnius distr	160	54° 40'10"	25° 23'15"	Abandoned field
SC	32103	Rokantiskes, Vilnius distr.	200	54° 42'38"	25° 23'29"	Dry meadow
SC	32204	Rokantiskes, Vilnius distr.	200	54° 42'34"	25° 23'20"	Dry meadow
SC	32305	Rokantiskes, Vilnius distr.	200	54° 42'30"	25° 23'22"	Dry meadow
SC	33206	Suderve, Vilnius distr.	120	54° 40'03"	25° 22'58"	Abandoned field
SC	33407	Suderve, Vilnius distr.	120	54° 40'13"	25° 22'48"	Abandoned field
SC	33508	Suderve, Vilnius distr.	120	54° 40'13"	25° 22'48"	Abandoned field

Table S1. *Continued*

Species	Sample no.	Collection site	Altitude, m	Latitude N	Longitude E	Habitat
SC	36109	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SC	36210	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SC	36311	Raguvele, Vilnius distr.	130	54° 52'20"	25° 30'50"	Abandoned field
SC	38112	Karkline, Vilnius distr.	120	54° 54'25"	25° 33'11"	Roadside
SC	38213	Karkline, Vilnius distr.	120	54° 54'23"	25° 33'10"	Roadside
SN	31201	Naujoji Vilnia, Vilnius distr	160	54° 40'10"	25° 23'15"	Abandoned field
SN	31303	Naujoji Vilnia, Vilnius distr.	160	54° 40'15"	25° 22'10"	Abandoned field
SN	32104	Rokantiskes, Vilnius distr.	200	54° 42'36"	25° 23'25"	Dry meadow
SN	32205	Rokantiskes, Vilnius distr.	200	54° 42'36"	25° 23'25"	Dry meadow
SN	32306	Rokantiskes, Vilnius distr.	200	54° 42'36"	25° 23'25"	Dry meadow
SN	33107	Suderve, Vilnius distr.	120	54° 40'10"	25° 22'45"	Abandoned field
SN	33208	Suderve, Vilnius distr.	120	54° 40'10"	25° 22'45"	Abandoned field
SN	33509	Suderve, Vilnius distr.	120	54° 40'10"	25° 22'45"	Abandoned field
SN	36110	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SN	36211	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SN	36312	Raguvele, Vilnius distr.	130	54° 52'27"	25° 30'52"	Abandoned field
SN	38213	Karkline, Vilnius distr.	120	54° 54'20"	25° 33'12"	Roadside
SN	38314	Karkline, Vilnius distr.	120	54° 54'23"	25° 33'10"	Roadside

SG – *Solidago gigantea*; SV – *S. virgaurea*; SC – *S. canadensis*, SN – *S.* × *niederederi*.

The coordinates of the collecting sites were indicated using GPS versus Google Earth mode. The altitude of the growing sites was indicated using the Lithuanian elevation map: https://mapslt.maps.arcgis.com/apps/webappviewer/index.html?id=d29c96b53bc740e38fb79f8532753544

Table S2. *Correlation coefficients with the first (PC1), second (PC2) and third (PC3) principal components based on antioxidant activity of phenolic compounds in inflorescences (PCA1) and leaves (PCA2) of Solidago species*

Variables	PCA1			PCA2		
	PC1	**PC2**	**PC3**	**PC1**	**PC2**	**PC3**
Neochlorogenic acid	–0.345	0.789	–0.212	–0.316	–0.238	–0.760
Chlorogenic acid	0.196	–0.310	0.782	0.770	0.185	0.364
4-*O*-caffeoylquinic acid	n[1]	n	n	0.547	0.300	0.431
Caffeoylshikimic acid	0.624	–0.343	0.003	0.746	0.102	0.199
Quercetin pentoside hexoside	0.932	–0.204	0.071	0.444	0.328	0.461
Quercetin hexoside hexoside	–0.156	0.702	0.158	0.025	0.332	–0.747
Rutin	–0.563	0.226	0.693	–0.655	–0.286	0.249
Hyperoside	0.974	–0.006	0.073	0.919	–0.018	0.057
Isoquercitrin	0.614	0.195	0.555	0.614	0.081	0.360
Quercetin pentoside	0.943	–0.195	0.069	0.927	0.065	0.226
3,5-dicaffeoylquinic acid	–0.830	0.357	0.202	–0.529	–0.724	0.022
Quercitrin	0.896	–0.115	0.096	0.887	0.056	0.205
Quercetin 3-*O*-(6"-acetyl-hexoside)	–0.383	–0.461	0.200	–0.349	0.725	0.023
3,4-dicaffeoylquinic acid	–0.432	0.723	–0.042	–0.300	–0.837	–0.056
Quercetin caffeoyl hexoside	0.901	–0.294	0.011	n	n	n
Quercetin	0.781	–0.272	–0.005	n	n	n

Abbreviations: [1]n – variable was not included to the PCA.