

Comparative study of the morphological and phytochemical characterization of Romanian *Solidago* species

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Abstract: In the present study, three indigenous species of *Solidago* genus (*Solidago gigantea*, *Solidago virgaurea* and *Solidago canadensis*) have been analyzed for the assessment of polyphenolic, phenyl propane derivates and essential oil contents. In addition, a comparative morphological study was also described. The leaves and the flowers of the three *Solidago* species were studied by scanning electron microscopy (SEM). The qualitative and quantitative characterizations of the main polyphenolic compounds from the hydrolyzed extracts were carried out by using high performance liquid chromatography with UV detection (HPLC-UV), high performance liquid chromatography coupled with mass spectrometry (HPLC-MS) and gas chromatography coupled with mass spectrometry (GC-MS) for the essential oil determination. The dominant flavonoidic aglycone found for all three species was quercetol with its highest concentration registered in *Solidago canadensis*. Four components, α -pinene, mircene, bornyl acetate and germacrene D, were detected in all the analyzed samples of essential oils. According to the comparative morphological analysis, morphoanatomical differences were observed for the tryhomes, stomata and flowers of the studied Romanian *Solidago* species.

Keywords: *Solidago virgaurea* L., *Solidago gigantea* Ait., *Solidago canadensis* L., polyphenols and essential oil, scanning electron microscopy.

INTRODUCTION

Solidago gigantea Ait., *Solidago canadensis* L. and *Solidago virgaurea* L. belong to the genus *Solidago* (goldenrod) within the family of the *Asteraceae*, each of them having several varieties. *Solidago gigantea* and *Solidago canadensis* are originary from North America, they were first introduced to Europe as an ornamental plant in the 18th century, then following a widely spread. *Solidago virgaurea* is the only one native to Europe (Weber, 1997). *Solidago* species are successful worldwide invaders. The mechanism of their presence in particular areas is not clear. It is controlled by two factors: Competitiveness or dispersion ability (Szymura and Szymura, 2013).

The medicinal material *Herba Solidaginis* including *S. virgaurea*, *S. gigantea* and *S. canadensis* presents several therapeutic effects such as: antibacterial, antimycotic, antiinflammatory, analgesic, anticancerogenic, sedative and hypotensive reported so far. The pharmacological use of *Herba Solidaginis* included the diseases treatment of the urinary tract, nephrolithiasis and prostate conditions (Thiem and Golinska, 2002). *Solidaginis virgaureae herba* is included in the European Scientific Cooperation for Phytotherapy monographs and according to the French Pharmacopoeia goldenrod herb should be obtained from *S. virgaurea*.

The principal compounds identified in *Solidago* species are: terpenoids, saponins, flavonoids, phenolic glycosides, coumarins and essential oil (Weber and Jacobs, 2005). *S. gigantea* exhibits moderate spasmolytic, diuretic properties and antimycotic activity. The sesquiterpene hydrocarbon profile of the *Solidago* species containing germacrene D, germacrene A, α -humulene and β -caryophyllene was examined by GC-MS (Prosser *et al.*, 2002).

Many phytochemical studies of *Solidago* species collected from various countries all over the world such as: Poland, Turkey, Italy, Hungary, Russia and India were conducted. The constituents of the essential oil of *Solidago* species harvested from Poland were investigated by GC, GC-MS, NMR spectroscopy identifying 60, 90 and 85 components respectively (Kalemba, 1998; Kalemba *et al.*, 2001; Kalemba and Thiem, 2004; Demir *et al.*, 2009; Bertoli *et al.*, 1999; Mishra *et al.*, 2010; Tkachev *et al.*, 2006; Apati *et al.*, 2003).

To the best of our knowledge, there are no reports so far on the complete chemical composition and morphological description of the *Solidago* species from Romania. Only few preliminary studies concerning the phytochemical and pharmacological characterization of *Solidago* sp. were reported (Racz *et al.*, 1980; Tămaș *et al.*, 1983; Dobjanschi *et al.*, 2012; Dobjanschi, 2008).

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Due to this reason, the aim of the present work was to study the morphological features of the flowers and leaves and also to identify and quantify the phenolic content and the essential oil composition of aerial parts of *Solidago* species (*S. canadensis*, *S. virgaurea* and *S. gigantea*) harvested from Romania (fig. 1A-C). In addition, we wanted to make a distinguishable comparison of these three Romanian goldenrods species from phytochemical and morphoanatomical point of view. The morphological studies and the quantitative determinations of the chemical compounds were achieved by using chromatographic methods (HPLC-UV, HPLC-MS and GC-MS) and scanning electron microscopy (SEM).

MATERIALS AND METHODS

Plant material

For the morphological studies and phytochemical analysis, the plant material (*Solidago* herba) from the three *Solidago* species were collected from Cluj county (a province from Transylvania with hills having a continental climate) during the blooming period, being identified by prof. Tămaş from Faculty of Pharmacy, Cluj-Napoca, Romania.

Sample preparation for SEM analysis

The plant materials were preserved in 96% ethanol-acetic acid mixture (3:1) and they were kept at 4°C (Andrei *et al.*, 1987). Then the leaves and the flowers were dried and covered with metal under vacuum and after that they were studied by using a scanning electron microscope (JEOL-JSM 5510 LV from the Faculty of Biology and Geology, University Babes-Bolyai Cluj-Napoca) (Tarnavschii *et al.*, 1981, Dobjanschi and Barbu, 2004).

Extraction procedure for HPLC analysis

The vegetal material was dried at room temperature and grinded to fine powder (*herba-pulvis*).

For the phenolic content determination, 1.25g of vegetal powder were extracted with 25ml of 50° ethanol on a water bath, at 60°C, and then filtered. The samples were then hydrolyzed: 1 part of extract was diluted with 1 part of 2N HCl for 40 min on a water bath, at 80°C. The essential oil was extracted by the method mentioned in Romanian Pharmacopoeia, X Edition: 25g of vegetal material were distilled with 500 ml water during 3 hours.

Chemicals

The 18 polyphenolic standard compounds: caftaric acid, gentisic acid, caffeic acid, chlorogenic acid, p-coumaric acid, ferulic acid, sinapic acid, hyperoside, isoquercitrin, rutozid, myricetol, fisetin, quercitrin, quercetol, patuletin, luteolin, kaempferol and apigenin were purchased from Sigma (Germany). HPLC grade methanol, acetic acid and hydrochloric acid were purchased from Merk (Germany).

Apparatus and chromatographic conditions

The quantitative and qualitative determination of polyphenols was achieved by using HPLC-UV and

HPLC-MS, meanwhile for essential oil analysis was used GC-MS.

HPLC analysis

The experiments were carried out by using an Agilent 1100 HPLC Series system (Agilent, Santa Clara, CA, USA) equipped with G1322A degasser, HP 1100 Series binary pump, UV HP 1100 detector and coupled with an Agilent Ion Trap 1100 VL mass spectrometer. A reverse-phase analytical column Zorbax SB-C18 (100× 3.0mm i.d, 3.5µm particle) was employed working at 48°C. The UV detector was set at 330 nm until 17 min, then at 370 nm. The MS system used an electrospray ion source in negative mode.

The mobile phase was a binary gradient: methanol and acetic acid 0.1% (v/v). The elution started with a linear gradient of 5% methanol and ended at 42% methanol for 35 minutes; then for the next 3 minutes 42% methanol and till 45min 5% methanol (Mocanu *et al.*, 2015; Hanganu *et al.*, 2016). The flow rate was set on 1mL/min and the injected volume was 5µL.

The MS signal was used only for qualitative analysis while the UV detection was used for the quantification of the previously identified compounds. MS spectra obtained from a standard solution of polyphenols and integrated in a mass spectra library allowed the positive identification of the compounds from the samples compared to spectra from library based on spectral match.

The external standard method was used for quantitative determinations with calibration curves in the range 0.5-50 µg/mL for each substance ($R^2 > 0.999$, five points plot) (Conea *et al.*, 2014; Hanganu *et al.*, 2016). The limits of detection were between 18-92ng/ml, meanwhile the accuracy was in the range 94.13-105.3% (Hanganu *et al.*, 2016).

The four polyphenols which could not be quantified in the mentioned chromatographic conditions due overlapping (caftaric acid with gentisic acid and caffeic acid with chlorogenic acid) were selectively identified in MS detection for a qualitative analysis.

GC-MS analysis

The GC-MS experiments were performed by using a Hewlett-Pakard 5890II GC with a SM-5 column (300 × 0.25mm i.d) coupled with an MSD 5972 mass spectrometer. The injection volume was set on 0.2-0.5µL. The identification of the compounds was achieved by comparing the obtained spectra from the samples with those from the soft database (Chem Information, Library Winey 275), meanwhile the quantitative determination was done by calculating the area under the curve.

STATISTICAL ANALYSIS

The analytical results were presented as mean of three measurements and as standard deviation (SD). The statistical analysis was carried out by using Excel software and ANOVA (Tukey's Multiple Comparison Test and t test unpaired test).

RESULTS

SEM analysis

According to the morphological studies of *S. virgaurea*, *S. gigantea* and *S. canadensis*, their folium and flos were studied more thoroughly. The trichomes, stomata and flowers of these three Romanian *Solidago* species were compared. The results are illustrated in the figs. 2-4.

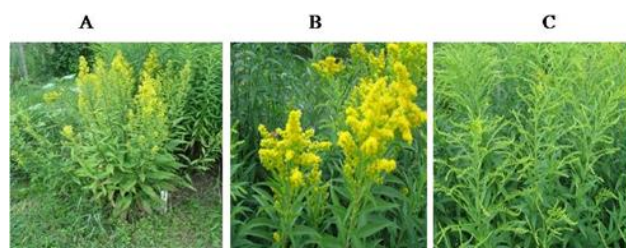


Fig. 1: A) *Solidago virgaurea* L.; B) *Solidago gigantea* Ait.; C) *Solidago canadensis* L.

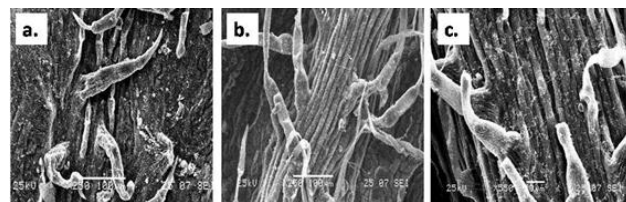


Fig. 2: SEM images of trichomes on abaxial epidermis of *S. virgaurea* (a), *S. gigantea* (b) and *S. canadensis* (c)

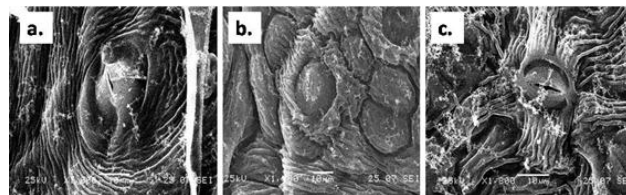


Fig. 3: SEM images of stomata on abaxial epidermis of *S. virgaurea* (a), *S. gigantea* (b) and *S. canadensis* (c)

Polyphenols analysis

The quantitative determination of the polyphenols was performed by using the external standard method. The HPLC chromatograms of the 18 polyphenolic compounds were the same as presented by Conea et al. (Conea et al., 2014). The retention time values and the parameters of the calibration line equation for the polyphenolic standards obtained by HPLC-UV were the same as demonstrated by Conea et al. (2014). For the two pairs of phenolic acids with incomplete separation (caftaric acid-gentisic acid,

caffeic acid-chlorogenic acid) only a qualitative analysis was achieved by UV detection.

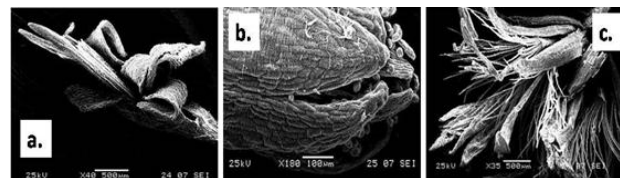


Fig. 4: SEM images of flowers of *S. virgaurea* (a), *S. gigantea* (b) and *S. canadensis* (c).

Due to the presence of at least one phenolic function in the molecule of the polyphenolic compounds (and one carboxyl group for polyphenolic acids), they can be transformed into negative ions (M-H) and thus they can be analyzed by negative ionization, as indicated by Conea et al. (2014).

The HPLC-UV and HPLC-MS chromatograms of the hydrolyzed samples from the three *Solidago* species are shown in fig. 5.

The chromatographic profiles of the three *Solidago* species revealing some differences in their chemical compositions are presented in table 1. It can be observed that quercetol was found in the highest concentration in all three analyzed samples.

Essential oil analysis

The GC-MS chromatograms revealed that the essential oil of *S. virgaurea*, *S. gigantea* and *S. canadensis* contained 27, 30 and 12 respectively compounds. The composition of the essential oil for the three samples is detailed in table 2.

DISCUSSION

SEM analysis

According to the SEM analysis, there are some distinguishable differences between the three Romanian *Solidago* species. The abaxial epidermis of the leaf of *S. virgaurea* presented non-glandular, long and unicellular trihomes (200µm) with spiked edges at the leaf blade and globular edges at the midrib. The leaf blade had smaller and unicellular trihomes than those near the midrib (fig. 2a). The trihomes of *S. gigantea* had only spiked edges (fig. 2b). According to Weber E. and Jacobs G (Weber and Jacobs, 2005) leaf blades are usually glabrous above and below, but may occasionally be pubescent on the leaf underside. The abaxial epidermis of *S. canadensis* leaf also presented numerous non-glandular, long and unicellular trihomes (140µm) with globular edges. On the adaxial epidermis of this species, there were both non-glandular and glandular trichomes (fig. 2c).

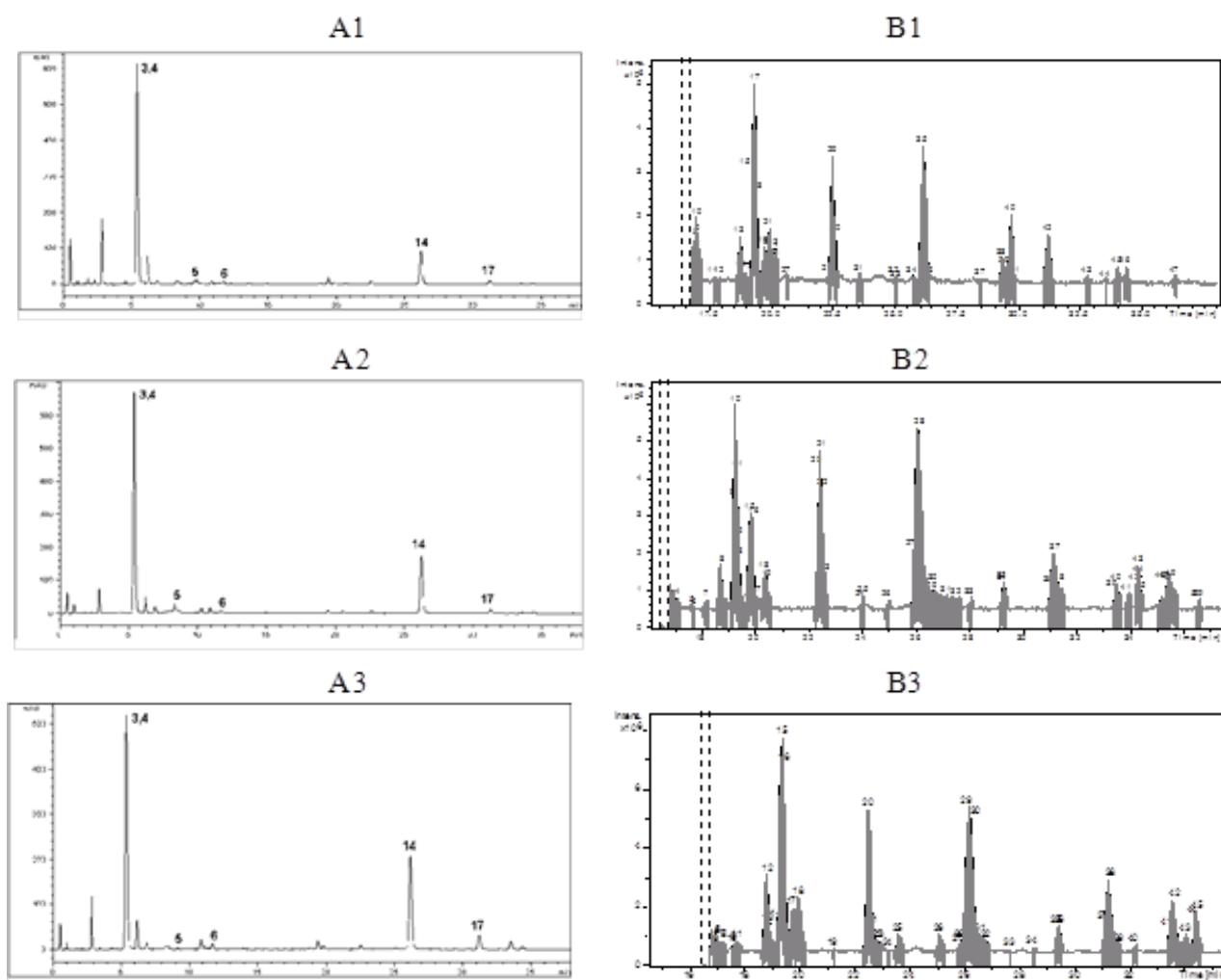


Fig. 5: HPLC-UV (A1, A2, A3) and HPLC-MS chromatograms (B1, B2, B3) of the hydrolyzed extracts from: (A) *S. virgaurea* (B) *S. gigantea* and (C) *S. canadensis*

The mesomorphic stoma of *S. virgaurea* and *S. gigantea* had smaller stomatal opening ($<10\mu\text{m}$) than *S. canadensis* (fig. 3a, 3b). The stomatal opening of *S. canadensis* was bigger than the others ($>10\mu\text{m}$) (fig. 3c). According to both our studies and to Birute K. (Birute and Jolita, 2016), *Solidago* species were amphistomatic as stomata were found on both sides of the leaf surfaces, with the abaxial epidermis having higher stomatal density than the adaxial epidermis. Even though, some leaves of *S. canadensis* were hypostomatic similarly to earlier reported data. The observed stomata were anisocytic type at all three studied species. Our data were in concordance with Szymura and Wolski who described anisocytic type in the epidermis of *S. canadensis*, *S. gigantea* and *S. virgaurea* and anomocytic type in *S. altissima* and *S. graminifolia* (Szymura and Wolski, 2011). However, stomatal characteristics are not highly reliable for taxonomic differentiation of species as some previous studies suggested that stomatal development, their size and density are affected by different environmental factors

such as light intensity, amount of atmospheric CO_2 , moisture, temperature and internal factors such as genome size (Birute and Jolita, 2016).

S. canadensis presented more stigmatic papillae than the tubular flower of *S. virgaurea*. The abundant and cellulosic pappus of flowers have hairs with branched edge (fig. 4c). According to Szymura and Szymura (Szymura and Szymura, 2013), plant height, inflorescence length and width of *Solidago* species are correlated with the amount of available soil nutrients. The European *S. gigantea* and *S. canadensis* samples tend to produce smaller shoots and inflorescences, but more rhizomes with more buds than the American samples. The reason of this observation is also the difference of soil nutrients (Gusewell *et al.* 2006). In addition, according to Szymura *et al.*, the inflorescences of *S. gigantea* were smaller than the less frequent species (*S. altissima*, *S. canadensis* and *Euthamia graminifolia*) from south-western Poland (Szymura *et al.*, 2015).

Table 1: Polyphenolic compounds content found in *Solidago* species (hydrolyzed extract, µg/ml)

Compound	No.	UV identified	MS identified	<i>S. virgaurea</i>	<i>S. gigantea</i>	<i>S. canadensis</i>
Caftaric acid	1	NO	YES	-	-	-
Gentisic acid	2	NO	YES	-	-	-
Caffeic acid	3	NO	YES	-	-	-
Chlorogenic acid	4	NO	YES	-	-	-
p-coumaric acid	5	YES	YES	1.536	1.422	1.296
Ferulic acid	6	YES	YES	3.151	2.938	6.240
Quercetol	14	YES	YES	64.730	124.560	144.955
Luteolin	16	YES	YES	0.773	-	-
Kaempferol	17	YES	YES	9.954	10.250	25.389
Apigenin	18	YES	YES	1.122	1.021	-

Values are the mean $\pm$ SD ($n = 3$, SD between ± 0.09 and ± 1.85).

Table 2: Essential oil compounds from the three *Solidago* species

R_t	Compound	<i>S. virgaurea</i> (%)	<i>S. gigantea</i> (%)	<i>S. canadensis</i> (%)
5.57	α -pinene	13.27	4.19	37.8
6.64	sabinene	-	1.11	-
6.68	β -pinene	1.76	-	2.48
7.29	mircene	46.3	1.45	8.74
7.66	α -felandren	12.57	1.03	-
8.027	p-cimene	9.27	2.38	-
8.33	limonene	-	-	24.98
8.98	β -ocimene	2.16	-	-
10.45	α -terpinene	0.69	-	-
12.79	trans-verbenol	-	0.92	-
18.68	Bornyl acetate	0.8	6.04	0.8
22.58	α -copaene	0.67	-	-
23.29	β -elemene	-	1.45	2.26
23.29	β -cubebene	4.08	-	-
24.01	α -gurjunen	-	5.22	-
26.67	γ -gurjunen	-	2.19	-
27.11	Germacone D	1.56	7.5	14.08
27.7	isolede	-	0.93	3.93
27.71	α -amorfen	0.76	-	-
28.85	Delta-cadinen	0.72	0.21	-
30.64	palustrol	-	1.71	-
31.16	spathulenol	1.74	6.44	-
31.68	leden	-	1.47	-
32.09	Azulen-4-ol	-	3.75	-
34.17	Cicloizolongifol-5-ol	-	3.1	-
35.45	3,4-difluoro-4—metoxi phenil	-	2.62	-
38.21	aristolon	-	36.73	-

Values are the mean $\pm$ SD ($n = 3$, SD between ± 0.22 and ± 2.75).

The pappus frequency of *S. virgaurea* was more numerous than the pappus of *S. gigantea*, but this contained more cells and it was more branched and divergent than the pappus of the other two *Solidago* species (fig. 4a, 4b). These observations are in accordance with the results reported by Weber E. and Jacobs G (Weber and Jacobs, 2005). According to Takahashi and Matsuki, *S. virgaurea* presented smaller number of large flower heads and thicker stem diameter at higher altitudes (2000-2400 m a.s.l.) than at lower ones (1600-1900 m a.s.l.). This aspect

suggests that this plant adapts to high altitude conditions (cool temperature, short growing season and strong wind) by changing its vegetative and reproductive traits (Takahashi and Matsuki 2016).

S. gigantea can be easily distinguished from the closely related *S. canadensis* by its longer rhizomes, brownish white pappus, glabrous stems and the denser inflorescence architecture (Weber and Jacobs, 2005). In addition, Semple and Uesugi also mentioned that the middle and

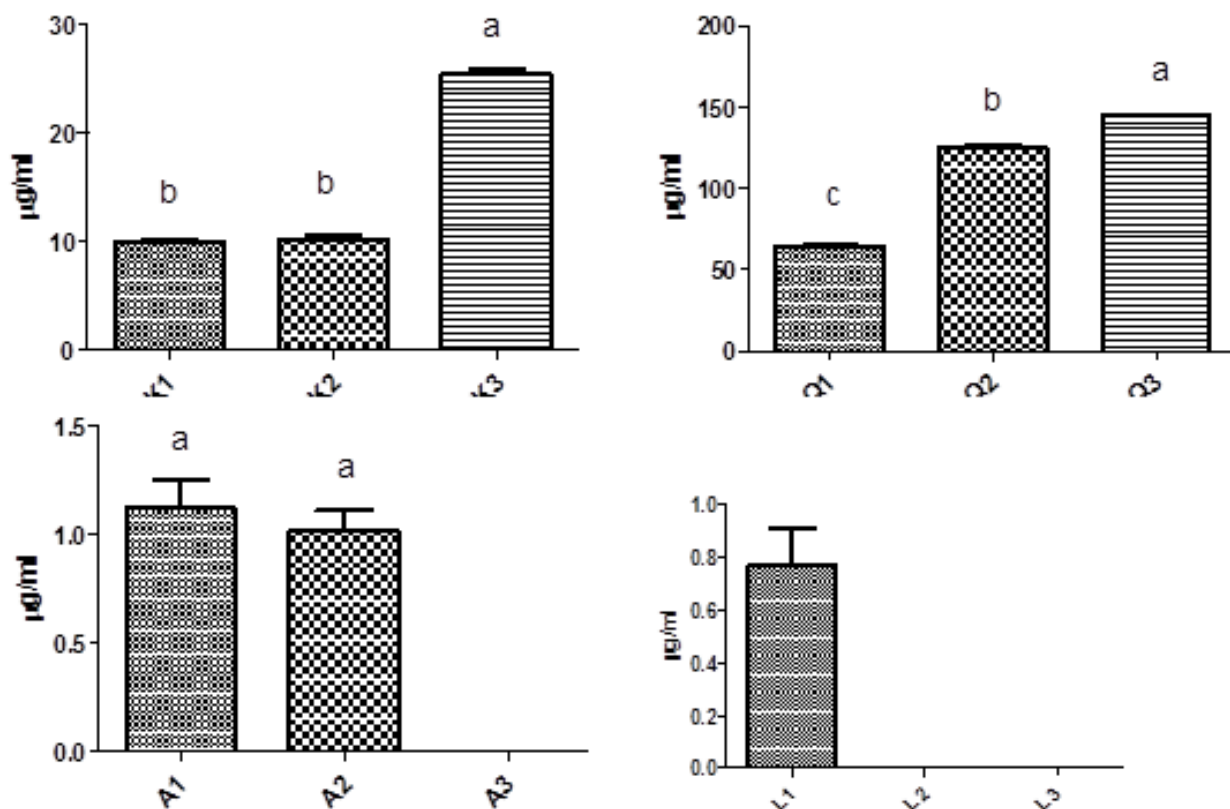


Fig. 6: The content of flavonoid aglycones in the three *Solidago* species from the hydrolyzed extracts; mean $\pm$ SD ($p < 0.05$, One way ANOVA, Tukey's Multiple Comparison test, t test – unpaired test) K = kaempferol, Q = quercetol, A = apigenin, L = luteolin, 1 = *S. virgaurea*, 2 = *S. gigantea*, 3 = *S. canadensis*

upper stem leaf margins were serrate and the inflorescence usually was nearly as wide as tall at *S. canadensis* and *S. gigantea* (Semple and Uesugi 2017).

The pollen grains of these three studied species are tricolporate which is characteristic to *Asteraceae* family. The earlier published features described higher pollen viability at *S. canadensis*, *S. gigantea* and *S. virgaurea* plants than the other *Solidago* species (Birute and Jolita, 2016).

HPLC analysis

The HPLC-UV and HPLC-MS methods that were employed allowed the identification and quantification of the polyphenolic content of the three *Solidago* species.

In the hydrolyzed extract of *S. virgaurea*, four flavonoid aglycones were identified and quantified, namely quercetol (64.73 $\mu\text{g/ml}$), kaempferol (9.95 $\mu\text{g/ml}$), apigenin (1.12 $\mu\text{g/ml}$) and luteolin (0.77 $\mu\text{g/ml}$). Analyzing the phenyl propane derivatives, it appeared that chlorogenic and caffeic acids were the main compounds, ferulic and p-coumaric acids presented lower levels (3.151 $\mu\text{g/ml}$ and 1.536 $\mu\text{g/ml}$), meanwhile caftaric and gentisic acids were confirmed only by MS spectra (fig. 5 and table 1).

In the case of the samples from *S. gigantea*, quercetol was the major aglycone (124.53 $\mu\text{g/ml}$) followed by kaempferol (10.25 $\mu\text{g/ml}$) and apigenin (1.02 $\mu\text{g/ml}$). The ferulic and p-coumaric acids were detected in lower concentration in comparison with *S. virgaurea* (2.93 $\mu\text{g/ml}$ and 1.42 $\mu\text{g/ml}$ respectively), meanwhile the other phenyl propane compounds presented the same tendency as for *S. virgaurea* and *S. canadensis* (fig. 5 and table 1).

Regarding the composition of *S. canadensis* extract, it can be observed that two flavonoids presented the most increased concentration from all three *Solidago* species: quercetol 144.95 $\mu\text{g/ml}$ and kaempferol 25.38 $\mu\text{g/ml}$. Ferulic acid was also found in the highest amount (6.24 $\mu\text{g/ml}$), contrary to the p-coumaric acid concentration (1.296 $\mu\text{g/ml}$) (fig. 5 and table 1).

Luteolin was detected only in *S. virgaurea*, meanwhile apigenin was absent only from *S. canadensis*. Quercetol was found as dominant compound in all three species, the best source being *S. canadensis* extract (fig. 6).

The statistical analysis of kaempferol and quercetol contents indicated significant correlation ($p < 0.0001$, ***). The concentrations of kaempferol in *S. virgaurea* and in *S. gigantea* were not statistically significant ($p = 0.2364$).

calculated by using t test-unpaired test). The same it was indicated in the case of apigenin concentrations in *S. virgaurea* and in *S. gigantea* ($p=0.3349$, t test – unpaired test) (fig. 6).

Flavonoids (polyphenols) are among the most studied bioactive compounds from plants due to their significant antioxidant potential and antimicrobial activity (Apati *et al.*, 2003; Apati *et al.*, 2006; Demir *et al.*, 2009; Hanganu *et al.*, 2016; Jurca *et al.*, 2016; Mocanu *et al.*, 2015; Patay *et al.*, 2016; Thiem *et al.*, 2002; Vicas *et al.*, 2015). *S. virgaurea* harvested from Poland presented three flavonoids, hyperoside, rutin and astragalín, in a concentration range from 600 to 1850 mg/100 g, and two polyphenolic acids, rosmarinic and chlorogenic, in a concentration of 440-1200mg/100 g (Roslon *et al.*, 2014). The content of polyphenols and tannins from two *Solidago* species from Ukraine (one native and one invasive) were analyzed pointing out the enemy release hypothesis (Omelchuk *et al.*, 2013). It was demonstrated that the ripeness stage, tissue type and extraction method can influence the composition of the *S. canadensis* extracts (contents of total phenolic, tannins and flavonoids) and therefore the antioxidant activities also (Denga *et al.*, 2015). Sutovska *et al.*, revealed that the *S. canadensis* complex contained carbohydrates (43 wt%), protein (27 wt%), phenolics (12 wt%), uronic acids (10 wt%) and inorganic compounds (8 wt%), exhibiting an antitussive activity (Sutovska *et al.*, 2013).

Concerning the essential oil composition of the three analyzed species, it can be observed that there are four common compounds, but in different concentration for each plant: α -pinene (the major compound for *S. canadensis* 37.8%), mircene (the highest amount in *S. virgaurea* 46.3%), bornyl acetate (the dominant compound in *S. gigantea* 6.04%) and germacrene D (*S. canadensis* 14.7%).

In contrast, some compounds were identified only in certain species: sabinene (*S. gigantea* 1.11%), limonene (*S. canadensis* 24.98%), β -ocimene (*S. virgaurea* 2.16%), trans-verbenol (*S. gigantea* 0.92%), α -gurjunene (*S. gigantea* 5.22%), γ -gurjunene (*S. gigantea* 2.19%), palustrol (*S. gigantea* 1.75%), aristolon (*S. gigantea* 36.73%). The major compounds found in the essential oil of *S. virgaurea* were: mircene (46.3%), α -pinene (13.27%) and α -felandren (12.57%). In the case of *S. gigantea*, the highest quantified concentrations were: aristolon (36.73%), germacrene D (7.5%) and spathenol (6.44%), meanwhile for *S. canadensis* the main detected compounds were: α -pinene (37.8%), limonene (24.98%) and mircene (8.74%) (table 2).

In the Polish *S. virgaurea* samples, the main constituents of all the essential oils were found α -pinene, myrcene, β -pinene and germacrene-D, meanwhile for *S. gigantea*, α -pinene, myrcene, p-cymene, bornyl acetate, α - and γ -

gurjunene, germacrene D, ledol, eudesma-4(15),7-dien-1 β -ol and cyclocolorenone were the main constituents (Kalemba *et al.* 2001). The three dominated essential oils in *S. canadensis* harvested in Poland were α -pinene, limonene and germacrene D (Kalemba and Thiem, 2004). The hexane and alcoholic extracts of three *Solidago* species analyzed by Kolodziej presented 39 and 49 compounds for *S. virgaurea*, 62 and 46 substances for *S. canadensis* and 73 and 45 compounds for *S. gigantea*. The ethanolic extracts exhibited antimicrobial activity (especially against gram positive bacteria), meanwhile the hexane extracts presented an antimutagenic activity (Kolodziej *et al.*, 2011). In the essential oil of *S. canadensis* from China, 53 compounds were detected among which the most important were: β -cubebene (26.9 %), α -pinene (13.8%), D-limonene (12.2%), β -pinene (9.3%) and bornyl acetate (3.2%). This essential oil presented cytotoxic activity (Huang *et al.*, 2012). Meanwhile, the essential oil obtained from *S. canadensis* from India contained as major components germacrene D (64.06%), limonene (4.23%) and bornyl acetate (3.37%); and exhibited analgesic activity and antimicrobial effect mainly against gram positive bacteria (Mishra *et al.*, 2011).

CONCLUSIONS

Morphological and phytochemical investigations were carried out by using plant materials obtained from three Romanian species of *Solidago* genus: *Solidago virgaurea*, *Solidago gigantea* and *Solidago canadensis*. The morphoanatomical analysis was performed by using SEM method which offered us detailed characterization of plant samples of the studied species. HPLC method assisted by UV-Vis and mass spectrometry detection was employed for the identification and quantification of a wide range of polyphenolic compounds and GC method for the determination of the essential oil composition. The dominant flavonoidic aglycone was quercetol and *S. canadensis* was found to be the richest specie in quercetol. Concerning the essential oil composition, the three species of *Solidago* presented qualitative and quantitative differences, but also some similarities. The flavonoidic and the essential oil contents of the three *Solidago* species represent a valuable natural source of antioxidants and the assessment of their therapeutic potential will be a following to this work. The identified morphoanatomical differences, the polyphenolic compounds and the essential oil composition completed the available scientific data about these species and they can be considered as chemotaxonomic and taxonomic markers for the identification and differentiation of these selected species.

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REFERENCES

- Andrei M, Anghel I, Popescu I and Stoica E (1975). *Lucrări practice de botanică*, Editura Didactica și Pedagogică, București, Romania, p.188.
- Apati P, Szentmihályi K, Kristo ST, Papp I, Vinkler P, Szoke E and Kery A (2003). Herbal remedies of *Solidago*-correlation of phytochemical characteristics and antioxidative properties. *J. Pharmaceut. Biomed.*, **32**(4-5): 1045-1053.
- Apati P, Houghton PJ, Kite G, Steventon GB and Kery A (2006). *In vitro* effect of flavonoids from *Solidago canadensis* extract on glutathione S-transferase. *JPP*, **58**(2): 251-256.
- Bertoli A, Cioni PL, Flamini G, Morelli I, Spinelli G and Tomei PE (1999). Constituents of the Essential Oil of *Solidago litoralis*, an Endemic Plant from Northern Tuscany (Italy). *J. Essent. Oil Res.*, **11**(2): 215-216.
- Birute K and Jolita R (2016). Morphological and Anatomical Characterization of *Solidago* × *niederederi* and Other Sympatric *Solidago* Species. *Weed Science*, **64**(1): 61-70.
- Conea S, Vostinaru O and Vlase L (2014). LC-MS analysis and anti-inflammatory activity of a tincture from *Eryngium planum* in a rat model of acute inflammation. *Dig. J. Nanomater. Bios.*, **9**(3): 1039-1045.
- Demir H, Açık L, Bali EB, Koç LY and Kaynak G (2009). Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. *Afri. J. Biotechnol.*, **8**(2): 274-279.
- Denga Y, Zhaob Y, Padilla-Zakourc O and Yang G (2015). Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. *Ind Crops Prod.*, **74**: 803-809.
- Dobjanschi L, Antonescu A, Zdrinca M, Muresan M, Vicas L, Micle O and Vicas S (2012). HPLC-MS analysis of flavonoids obtained from *Solidago* sp. (Asteraceae). *Analele Universității din Oradea, Fascicula Protecția Mediului*, **XIX**: 75-79.
- Dobjanschi L and Barbu L (2004). Studii morfoanatomice asupra unor specii indigene de *Solidago*. *Revista de Medicină și Farmacie Târgu-Mureș*, **50**: 344.
- Dobjanschi L (2008). Implicațiile biologice și biochimice ale saponinelor triterpenice izolate din speciile genului *Solidago* și *Anagallis Arvensis*. Editura Universității din Oradea, Oradea, ISBN 978-973-759-487-7, p.59.
- Gusewell S, Jakobs G and Weber E (2006). Native and introduced populations of *Solidago gigantea* differ in shoot production but not in leaf traits or litter decomposition. *Funct Ecol.*, **20**: 575-584.
- Hanganu D, Olah NK, Benedec D, Mocan A, Crisan G, Vlase L, Popica I and Oniga I (2016). Comparative polyphenolic content and antioxidant activities of *Genista tinctoria* L. and *Genistella sagittalis* (L.) Gams (Fabaceae). *Pak. J. Pharm. Sci.*, **29**(1): 301-307.
- Huang B, Lei Y, Qin L and Liu J (2012). Chemical Composition and Cytotoxic Activities of the Essential Oil from the Inflorescences of *Solidago canadensis* L., an Invasive Weed in Southeastern China. *Jeobp*, **15**(4): 667-671.
- Jurca T, Vicas L, Toth I, Braun M, Marian E, Teusdea A, Vicas S and Muresan M (2016). Mineral elements profile, bioactive compounds and antioxidant capacity of wild blueberry and of pharmaceutical preparations from blueberry (*Vaccinium myrtillus*). *Farmacia*, **64**(4): 581-587.
- Kalemba D, Marschall H and Bradesi P (2001). Constituents of the essential oil of *Solidago gigantea* Ait. (giant goldenrod). *Flavour Fragr. J.*, **16**(1): 19-26.
- Kalemba D and Thiem B (2004). Constituents of the essential oils of four micropropagated *Solidago* species. *Flavour Fragr. J.*, **19**(1): 40-43.
- Kołodziej B, Kowalski R and Bogdan Kędzia B (2011). Antibacterial and antimutagenic activity of extracts aboveground parts of three *Solidago* species: *Solidago virgaurea* L., *Solidago canadensis* L. and *Solidago gigantea* Ait. *J. Med. Plants Res.*, **5**(31): 6770-6779.
- Mishra D, Joshi S, Bisht G and Pilkhwal S (2010). Chemical composition and antimicrobial activity of *Solidago canadensis* Linn. root essential oil. *J. Basic Clin. Pharm.*, **1**(3): 187-190.
- Mishra D, Joshib S, Sahc SP and Bisht G (2011). Chemical composition, analgesic and antimicrobial activity of *Solidago Canadensis* essential oil from India. *J. Pharm. Res.*, **4**(1): 63-66.
- Mocanu A, Vlase L, Raita O, Hanganu D, Păltinean R, Dezsı Ş, Gheldiu AM, Oprean R and Crișan G (2015). Comparative studies on antioxidant activity and polyphenolic content of *Lycium barbarum* L. and *Lycium chinense* Mill. leaves. *Pak. J. Pharm. Sci.*, **28**(4): 1511-1515.
- Omelchuk O, Heitman A and Prots B (2013). The content of phenolics and tannins in native and invasive *Solidago* species. *Mod. Phytomorphol.*, **4**: 91-92.
- Patay EB, Nemeth T, Nemeth S, Filep R, Vlase L and Papp N (2016). Histological and phytochemical studies of *Coffea benghalensis* B. Heyne ex Schult., compared with *Coffea arabica* L. *Farmacia*, **64**(1): 125-130.
- Prosser I, Phillips AL, Gittings S, Lewis MJ, Hooper AM, Pickett JA and Beale MH (2002). (+)-(10R)-Germacrene A synthase from goldenrod, *Solidago canadensis*; cDNA isolation, bacterial expression and functional analysis. *Phytochemistry*, **60**(7): 691-702.
- Racz G, Racz-Kotilla E and Jozsa J (1980). Hipotensive activity- a possible pharmacotaxonomic character of *Solidago* L. *Herba. Hungarica.*, **19**(1): 15-17.

- Roslon W, Osinska E, Mazur K and Anna Geszprych A (2014). Chemical characteristics of European goldenrod (*Solidago virgaurea* L. subsp. *virgaurea*) from natural sites in central and eastern Poland. *Acta Sci. Pol., Hortorum Cultus.*, **13**(1): 55-65.
- Semple JS and Uesugi A (2017). *Solidago altissima* var. *pluricephla* (Asteraceae: Astereae) in Australia, Tonga and Hawaii. *Phytoneuron*, **40**: 1-16.
- Sutovska M, Capek P, Kocmalova M, Franova S, Pawlaczyk I and Gancarz R (2013). Characterization and biological activity of *Solidago canadensis* complex. *Int. J. Biol. Macromolec.*, **52**: 192-197.
- Szymura M and Szymura TH (2013). Soil preferences and morphological diversity of goldenrods (*Solidago* L.) from south-western Poland. *Acta. Soc. Bot. Pol.*, **82**(2): 107-115.
- Szymura M and Wolski K (2011). Leaf epidermis traits as tools to identify *Solidago* L. taxa in Poland. *Acta Biol Cracov. Bot.*, **53**(1): 38-46.
- Szymura M, Szymura TH and Kreitschitz A (2015). Morphological and cytological diversity of goldenrods (*Solidago* L. and *Euthamia Nutt.*) from south-western Poland. *Biodiv. Res. Conserv*, **38**: 41-49.
- Takahashi K and Matsuki S (2016). Morphological variations of the *Solidago virgaurea* L. complex along an elevation gradient on Mt Norikura, central Japan. *Plant Species Biol.*, **32**(3): 238-246.
- Tarnavski I.T, Șerbănescu-Jitariu G, Mitroiu Rădulescu N and Rădulescu D (1981). *Practicum de morfologie și anatomie vegetală, ediția a 2-a*, București, Romania, p.424.
- Tămaș M, Hodișan V, Kory M and Gugu P (1983). Cercetări fitochimice și farmacologice asupra unor plante indigene utilizate în afecțiuni urogenitale. *Herba Romanica*, **4**: 67-73.
- Thiem B and Goslinska O (2002). Antimicrobial activity of *Solidago virgaurea* L. from *in vitro* cultures. *Fitoterapia.*, **73**(6): 514-516.
- Tkachev AV, Korolyuk EA and Letchamo W (2006). Volatile oil-bearing Flora of Siberia VIII: Essential oil composition and antimicrobial activity of wild *Solidago virgaurea* L. from the Russian Altai. *J. Essent. Oil Res.*, **18**(1): 46-50.
- Vicaș L, Teușdea A, Vicaș S, Marian E, Jurca T, Mureșan M and Gligor F (2015). Assessment of antioxidant capacity of some extracts for further use in therapy. *Farmacia.*, **63**(2): 267-274.
- Weber E (1997). Morphological variation of the introduced perennial *Solidago canadensis* L. sensu lato (Asteraceae) in Europe. *Bot. J. Linn. Soc.*, **123**(3): 197-210.
- Weber E and Jakobs G (2005). Biological flora of central Europe: *Solidago gigantea* Aiton. *Flora*, **200**(2): 109-118.