

ORIGINAL PAPER

COMPARATIVE PHYTOCHEMICAL RESEARCH OF *SOLIDAGO* GENUS: *S. GRAMINIFOLIA*. NOTE I. FLAVONOIDS

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Abstract: *Solidago graminifolia* L. Salisb. is one of the latest species appeared in Romania. Due to the interest for the *Solidago* species as medicinal plants, we researched its phytochemical composition in comparison with the other three species present in Romania: *Solidago virgaurea* L., *Solidago canadensis* L., *Solidago gigantea* Aiton. Starting from the chemotaxonomic value of flavonoids, and their valuable biological properties, we wanted to analyze these substances from *S. graminifolia* compared to other *Solidago* species in Romanian flora. The studied species have a high content of flavonoids (3.44-5.21%). The flavonoid substances identified in the indigenous species of *Solidago* have a high chemotaxonomic value, each species having a characteristic chromatographic profile, therefore their analysis is useful in the case of adulterations. The qualitative analysis of flavonoids was performed by TLC and HPLC-MS, meanwhile the quantitative determination was achieved by spectrophotometric method and individual fractions separated by HPLC-MS.

Keywords: *Solidago virgaurea* L., *Solidago canadensis* L., *Solidago gigantea* Aiton, *Solidago graminifolia* L. Salisb., flavonoids.

1. Introduction

In Romania Flora, vol. IX (1964), there are mentioned only three *Solidago* species (Asteraceae family), one from the spontaneous flora (*S. virgaurea* L., Golden rod) and two adventitious species, initially cultivated as ornamental plants, originating from North America, then escaped as subsponaneous: *S. canadensis* L. and *S. gigantea* Aiton. (Early Golden rod) (**Fig. 1**). Flora Europaea (Tutin et

al., 2010) presents five species of *Solidago*, four of which are found in the Romania flora. Concerning *S. altissima* L., it is not recognized as a distinct species but only as a variety of *S. canadensis* (*S. canadensis* var. *scabra* Torrey & A. Gray) and therefore Sârbu et al. (2013) mentioned it in the Observation section.

In 1975, Negrean (Sârbu et al., 2011) reported for the first time in the Flora of

Romania the fourth species of *Solidago*, *S. graminifolia* (L.) Salisb. (syn. *Euthamia graminifolia* (L.) Nutt.) in the area of Maramureş county, a species originating also from North America which was introduced in Europe as an ornamental plant, then become adventitious, but with a lower colonization rate than the other two adventitious species mentioned before. *S. graminifolia* can be distinguished by the marginal flowers of the flower-heads with short ligule (0.8-1 mm), the linear lanceolate leaves which are scabrous (rough) on the edges, and numerous flower-heads arranged in corymbose panicles (Tutin et al., 2010; Sârbu et al., 2013).

In 2012, a second area in the country was reported for *S. graminifolia* in Cluj county (place Ciucea, leg. Tămaş M.) (**Fig. 2**).

The interest for the *Solidago* species as medicinal plants (Ciulei et al., 1993; Grigorescu et al., 2001) led us to carry out phytochemical research studies also on this last species appeared in the Romanian flora in comparison with the other three species. Starting from the chemotaxonomic value of flavonoids (Tămaş, 1986), and their valuable biological properties, we wanted to analyze these substances from *S. graminifolia* compared to other *Solidago* species in

Romanian flora. In addition to the flavonoid substances from the *Solidago* species, we have also studied triterpenoid saponins (Tămaş and Roşca, 1988), phenolic compounds (Dobjanschi et al., 2005) and volatile oils (Dobjanschi, 2006). A botanical and chemical complex study was conducted on these species by Dobjanschi (2006) and a pharmacological study by Voştinaru (2007). There have also been performed researches focused on the diuretic action (Tămaş and Toader, 1989), anti-inflammatory action (Pîrvu et al., 2000) and hypotensive action (Rác-Kotilla et al., 1977). In the European Pharmacopoeia 9.0 (9.4-2018) it is mentioned in the monograph of *Solidaginis virgaureae herba* a flavonoid content of 0.5-1.5 % expressed in hyperoside, and for highlighting the substitutions with *S. canadensis* and *S. gigantea*, it is mentioned thin layer chromatography for flavonoids determination where should not be present the orange fluorescence band characteristic for quercitroside (Ph.Eu.9.0). In Ph.Eu.9.0 is also official the monograph *Solidaginis herba*, which presents the blooming aerial parts of *S. gigantea* and *S. canadensis* for which a minimum content of 2.5 % of flavonoids expressed in the hyperoside is envisaged.



Fig. 2. *Solidago graminifolia* L. Salisb. (original photo made in Ciucea, Cluj county, 2016)

Among the flavonoid substances in the *Solidago* species, there have been mentioned glycosides of quercetol and kaempferol, including rutoside, hyperoside, quercitroside, isoquercitroside, astragaloside, isorhamnetin, nicotiflorina (Bisset and Wichtl, 1994), and the pharmacological properties include diuretic and saluretic action, anti-inflammatory, kidney stone lysis, spasmolytic and disinfectant of urinary tracts (Weiss and Fintelmann, 2000).

In our country, *Virgaureae herba* is used in phytotherapy and is included in the Plafar Nomenclature (1990). It is also official in the European Pharmacopoeia 6.0 (2007), and the *Solidago virgaurea* extracts are present in the composition of some pharmaceutical products such as: Rhoival (pulv.), Prostaforton (tablets), Uralyt (tablets, caps.), Cystinol (sol.), Nieron (caps.), and others (Rote Liste, 1988). There are few phytochemical and pharmacological data recorded on *S. graminifolia*. Thus, Kalemba et al. (1994) analyzed the extracted volatile oil and identified 80 components, the main ones being β -felandren (23%), sabinen (18%) and β -pinen (10%). Derda et al. (2008) tested some plant extracts, including *S. graminifolia* for its amoebicide activity.

2. Materials and Methods

2.1. Materials

The plant material (herba) was harvested at the beginning of August 2016 during flowering from Ciucea (Cluj county) from a ruderal area on the bank of a tributary river (Surduca) of Crişul Repede river (Cluj County), dried in the shade and then ground to a fine powder (IV sieve, Romanian Pharmacopoeia 10th Edition-FR X). A voucher specimen (no.143.3.1.1) of *S. graminifolia* was deposited in the Pharmaceutical Botany Discipline, Faculty of Pharmacy, University of Medicine and Pharmacy Iuliu Haţieganu Cluj-Napoca.

2.2. Extraction method

A 2 % extract in methyl alcohol was prepared for the chemical analysis. Thus, to 1 g of vegetable powder is added 50 ml of methyl alcohol and the mixture is kept in boiling in the water bath for 30 minutes in a reflux condensed flask. After cooling, it is filtered and then methyl alcohol is added in a 50 ml volumetric flask.

2.3. The quantitative determination of total flavonoids

It was performed according to the spectrophotometric technique indicated by FR X for *Cynarae folium* monograph and the expression of rutoside content (g %) by using a calibration curve for this substance.

2.4. The qualitative analysis of flavonoids

It was performed by thin layer chromatography (TLC) using silica gel G plates (Merck) with a layer of 0.25 mm, mobile phase consisting in formic acid-water-ethyl acetate (6:9:90), reference substances (Karl Roth GmbH Karlsruhe, Germany) such as: rutoside, hyperoside, quercitroside, isoquercitroside, chlorogenic acid and caffeic acid, 0.1 % solutions in methyl alcohol, revealed with NEU-PEG reagents under UV light 365 nm (Jork et al., 1990).

2.5. High performance liquid chromatography (HPLC)

It was employed for both qualitative and quantitative analysis for the flavonoid fractions which were separated and identified by the technique indicated by Fodorea and Vlase (2005). The separated fractions were identified by using reference substances (Karl Roth GmbH Karlsruhe, Germany) and confirmed by mass spectrum assays (MS). An HPLC apparatus coupled with an HP 1100 mass spectrometer with binary pump series, HP 1100

autosempler, HP 1100 thermostat, HP 1100 UV detector, and an Agilent Ion Trap 1100 VL mass spectrometer were used.

3. Results and discussions

3.1. The quantitative determination of total flavonoids

The total flavonoid content determined by spectrophotometric method and expressed as rutoside (g %) was 4.06 % for *S. virgaurea*, 5.21 % for *S. canadensis*, 5.20 % for *S. gigantea* and 3.44 % for *S. graminifolia*. It results that the analyzed *Solidago* species are rich in flavonoid substances, the higher content of total flavonoids in two species, more than 5%, can be correlated with their higher proportion of flowers (inflorescences) compared to the lower number of flowers in the herba product of *S. graminifolia* and *S. virgaurea* species.

3.2. The qualitative analysis of flavonoids

From the TLC analysis (**Fig. 3**) it results that the richest species in flavonoid fractions are *S. gigantea* and *S. canadensis* in which 4-5 fractions with yellow fluorescence, one with green fluorescence and four with blue

fluorescence were highlighted. It can also be noticed that each *Solidago* species has a characteristic chromatographic profile. Thus, *S. graminifolia* is the only species that does not contain rutoside (Rf 0.18, **Fig. 3-1**), being present in all the other three analyzed species. *S. virgaurea* does not have quercitroside (Rf 0.65, **Fig. 3-2**), *S. canadensis* is the only one containing high concentration of isoquercitroside (Rf 0.55). Hyperoside (Rf 0.47, **Fig. 3-3**), caffeic acid (Rf 0.85) and chlorogenic acid (Rf 0.40, **Fig. 3-4**) are common substances for all analyzed species.

Therefore, the TLC analysis for flavonoids of *Solidago* species can be used for their chemical characterization and as identification criteria for substitutions between species even when referring to their extracts. Thus, for *S. graminifolia* it is characteristic the presence of hyperoside and quercitroside and the absence of rutoside, and for *S. virgaurea* the presence of rutoside and hyperoside, but also the absence of quercitroside, meanwhile for *S. canadensis*, rutoside, hyperoside and quercitroside are present, and isoquercitroside occurs in significant quantities only in *S. gigantea*.

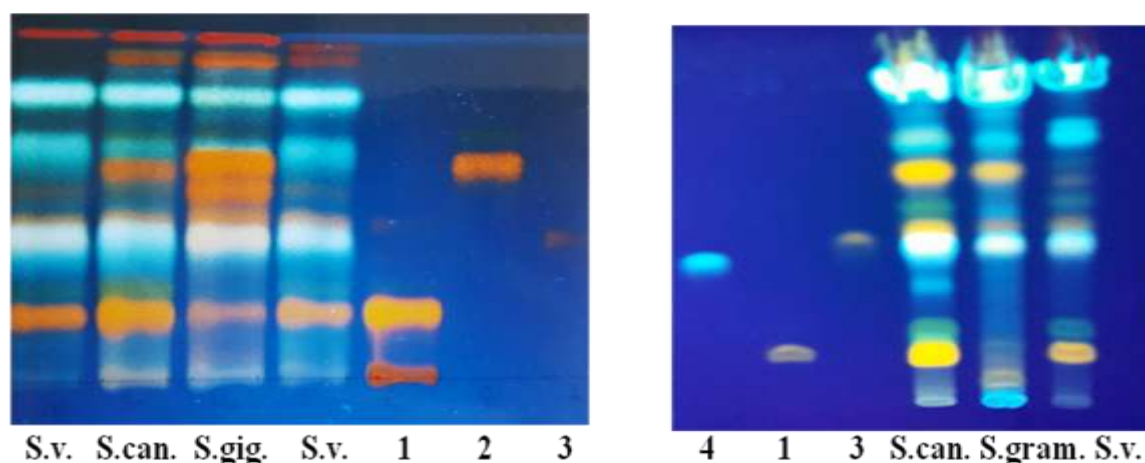


Fig. 3. TLC chromatograms of *Solidago* species (S.v. = *S. virgaurea*; S.can. = *S. canadensis*; S.gig. = *S. gigantea*; S.gram. = *S. graminifolia*; 1 = rutoside; 2 = quercitroside; 3 = hyperoside; 4 = chlorogenic acid)

3.3. High performance liquid chromatography

From the HPLC-MS analysis obtained for *S. graminifolia* and compared to those obtained for the other species (Figures 4-7), it can be noticed that both the content of the separated flavonoid fractions and their number is lower for this species (Tables 1-4). Thus, rutoside is absent only in *S. graminifolia*, being present in all three other analyzed species, the highest rutoside content being present in *S. canadensis*. Another flavonoid fraction, quercitroside, is absent in *S. virgaurea*, but it is present in the other species which is a differentiation and evidence of the substitutions for *S. virgaurea*.

Hyperoside is present in all analyzed species but in variable amounts, as well as isoquercitroside, the latter being present in the highest concentration in *S. gigantea*, and the free aglycons quercetol and kaempferol are present only in traces. In addition to the HPLC-MS quantification of each fraction separated by this technique, the sensitivity of this method is higher, so that some fractions that could not be detected by TLC appeared in HPLC-MS chromatograms, of course, in small amounts.

By the presence of quercitroside in *S. graminifolia*, this species appears to be closer to *S. canadensis* and *S. gigantea*.

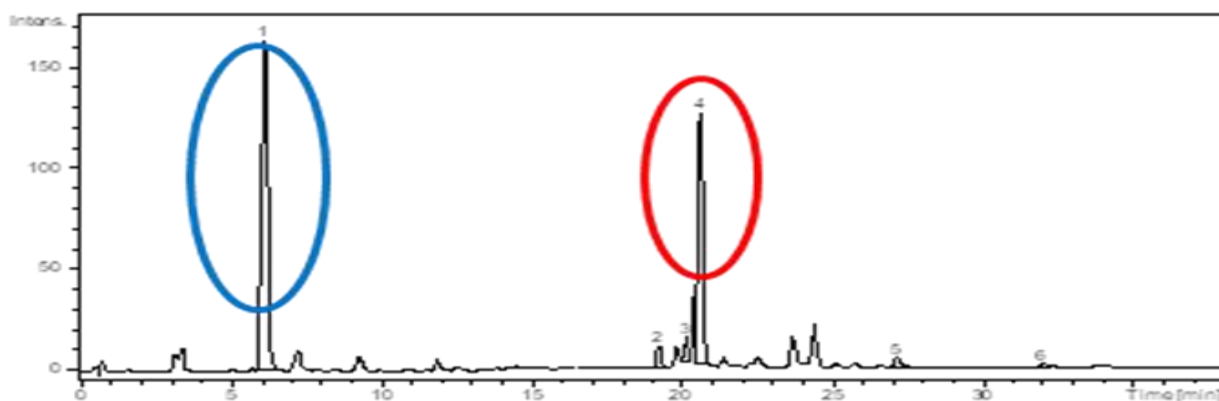


Fig. 4. HPLC chromatogram obtained for *Solidago virgaurea* extract

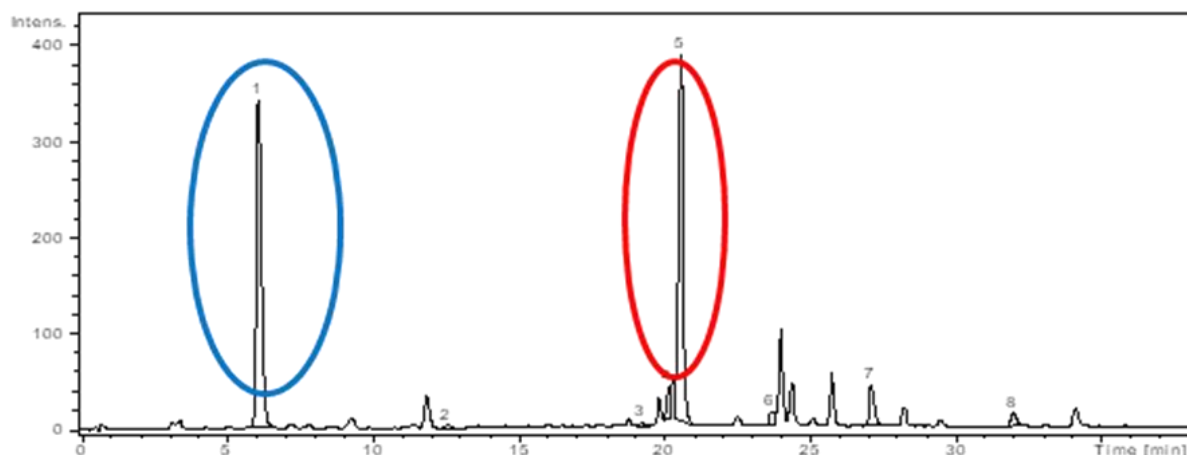


Fig. 5. HPLC chromatogram obtained for *Solidago canadensis* extract

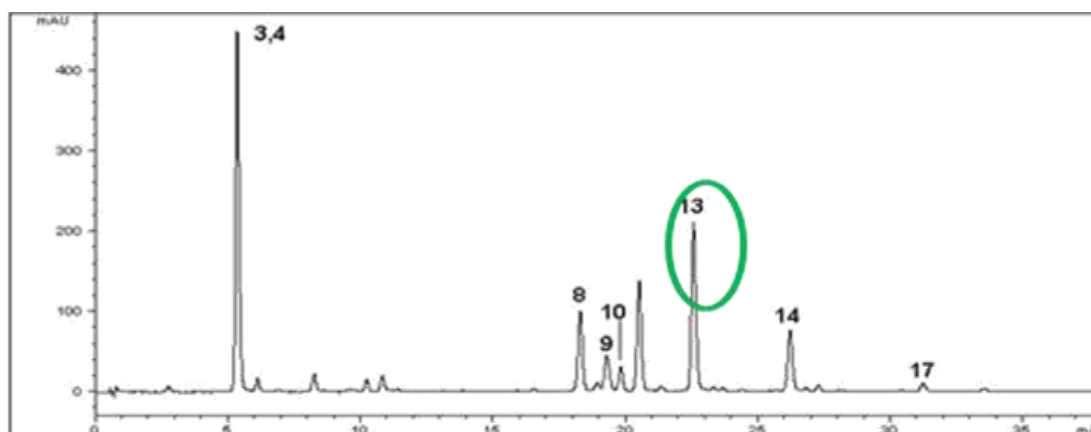


Fig. 6. HPLC chromatogram obtained for *Solidago gigantea* extract

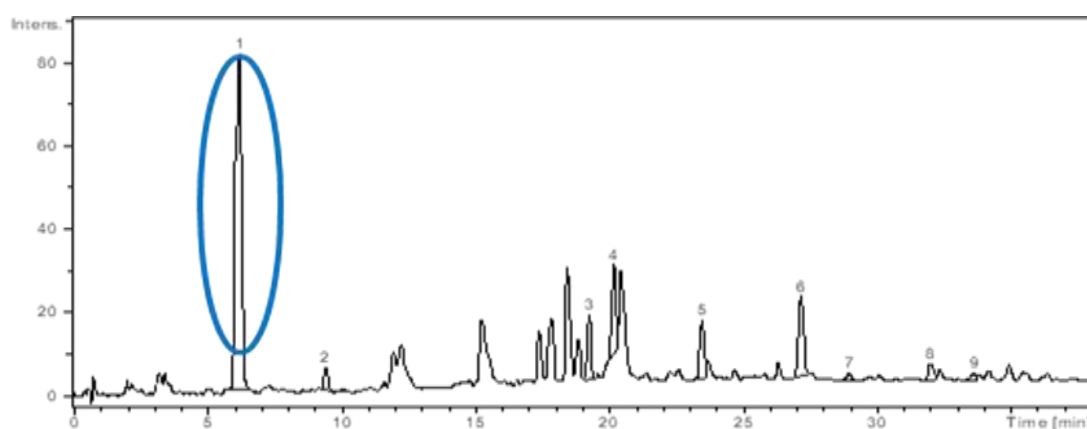


Fig. 7. HPLC chromatogram obtained for *Solidago graminifolia* extract

Table 1. HPLC-MS results obtained from *Solidago virgaurea* extract

Number on chromatogram	Compound	UV identification	MS qualitative identification	Quantity (µg/pharmaceutical product)
2	Hyperoside	YES	YES	11.288
3	Isoquercitrin	YES	YES	16.994
4	Rutoside	YES	YES	200.521
	Quercitrin	NO	YES	-
5	Quercetol	YES	YES	3.587
6	Kaempferol	YES	YES	1.212

Table 2. HPLC-MS results obtained from *Solidago canadensis* extract

Number on chromatogram	Compound	UV Identification	MS qualitative identification	Quantity (µg/pharmaceutical product)
3	Hyperoside	YES	YES	5.379
4	Isoquercitrin	YES	YES	47.507
5	Rutoside	YES	YES	694.113
6	Quercitrin	YES	YES	40.374
7	Quercetol	YES	YES	32.106
8	Kaempferol	YES	YES	12.554

Table 3. HPLC-MS results obtained from *Solidago gigantea* extract

Number on chromatogram	Compound	UV identification	MS qualitative identification	Quantity (µg/pharmaceutical product)
8	Hyperoside	YES	YES	120.000
9	Isoquercitrin	YES	YES	82.490
10	Rutoside	YES	YES	45.540
13	Quercitrin	YES	YES	450.560
17	Kaempferol	YES	YES	8.520

Table 4. HPLC-MS results obtained from *Solidago graminifolia* extract

Number on chromatogram	Compound	UV identification	MS qualitative identification	Quantity (µg/pharmaceutical product)
3	Hyperoside	YES	YES	17.507
4	Isoquercitrin	YES	YES	33.946
	Rutoside	NO	YES	-
5	Quercitrin	YES	YES	31.587
6	Quercetol	YES	YES	14.323
8	Kaempferol	YES	YES	4.130
9	Apigenin	YES	YES	2.442

Conclusions

For the first time, a qualitative and quantitative study of flavonoid substances from a new *Solidago* species, recently reported in Romania flora, *S. graminifolia*, was carried out, compared to the other three *Solidago* species from Romania flora.

In *S. graminifolia* species, both the number of flavonoid fractions identified by TLC and HPLC methods, and the content of these substances are lower compared to the other three analyzed species. Through its chromatographic profile, *S. graminifolia* appears closer to *S. canadensis* and *S. gigantea*.

From the qualitative analysis of the four *Solidago* species, we found that rutoside is absent in the case of *S. graminifolia* and quercitroside is absent in *S. virgaurea*.

The qualitative analyzes of the flavonoid substances from *Solidago* species are useful for identifying the possible substitutions between species, the chromatographic profile being a characteristic for each species.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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