Extraction and Analyses of Flavonoids and Phenolic Acids from Canadian Goldenrod and Giant Goldenrod

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Abstract: Invasive alien plant species Canadian goldenrod (Solidago canadensis L.) and giant goldenrod (Solidago gigantea Aiton) were investigated as a source of phytochemicals and yellow dyes. Flavonoids and phenolic acids were extracted from the inflorescence of Canadian goldenrod with thirteen extraction solvents ethanol, methanol, acetone, water, and mixtures of organic solvents (70%, 80%, and 90%) with water. High performance thin-layer chromatography (HPTLC) coupled to densitometry and high-performance liquid chromatography with photo-diode array detector (HPLC-PDA) were used for analyses of the obtained sample test solutions (STSs), which showed the best and comparable extraction efficiencies for 70% acetone (aq), 70% methanol (aq), and 70% ethanol (aq). HPTLC combined with image analyses in fluorescent mode resulted in different chromatographic fingerprints for Canadian goldenrod and giant goldenrod STSs (70% acetone (aq)) after development, after post-chromatographic derivatization with NP reagent and after use of PEG reagent. The developed HPLC methods enabled analyses of phenolic acids and flavonoids (aglycones and glycosylated) in STSs and hydrolyzed STSs form inflorescence of Canadian and giant goldenrod. Different contents of chlorogenic acid, rutin, hyperoside, isoquercetin, and quercetin were observed in STSs of both goldenrod species. The analyses of hydrolyzed STSs confirmed that glycosylated flavonoids in Canadian and giant goldenrod inflorescence are mainly glycosides of quercetin, kaempferol, and isorhamnetin. Additional analyses using HPTLC and HPLC coupled to tandem mass spectrometry (MS/MS; HPTLC-MS/MS and LC-MS/MS) enabled tentative identification of phenolic acids and flavonoids (10 with HPTLC-MS/MS and 15 with LC-MS/MS), from which several were identified in Canadian (4 with HPTLC-MS/MS and 8 with LC-MS/MS) and in giant (7 with HPTLC-MS/MS and 9 with LC-MS/MS) goldenrod for the first time.

Keywords: invasive alien plants; Solidago; goldenrod; flavonoids; flavonols; phenolic acids; dyes; phytochemicals; chromatography; mass spectrometry

1. Introduction

Canadian goldenrod (Solidago canadensis L.) and giant goldenrod (Solidago gigantea Aiton) are two highly variable plant species that belong to the genus Solidago and family Asteraceae [1,2]. They are herbaceous perennial plants that originate from North America where they are found from Florida to Alaska in the US as well as in Canada [3]. Both goldenrods are aggressive plants that thrive in ruderal habitats, meadows, pastures, fields, forests, on road sides, riversides, trenches, etc. [4,5]. When spreading it negatively affects the diversity of species by replacing native plants [4]. Although both goldenrod species share their invasive reputation it is easy to distinguish giant goldenrod from Canadian goldenrod due to longer rhizomes, glabrous stems, and a denser inflorescence structure [2].

Canadian goldenrod is one of the first ornamental plants that were brought to Europe from North America. Its presence in England originates from 1645 [3]. Both Canadian and giant goldenrod were popular in botanical gardens and nurseries due to attractive looks with characteristic golden flowers and easy growth [3]. Hence, they quickly spread around...
Two centuries later their presence was noted in European countries from Austria to Norway [3]. Today, Canadian and giant goldenrod are both very problematic invasive alien plant species on a global scale as it has spread over Europe, Asia, Australia, and New Zealand [3,6,7]. Both goldenrods are recognized as invasive weeds [4,7]. Based on the widespread growth of alien plant species invading ecosystems throughout Europe the European and Mediterranean Plant Protection Organization (EPPO) has listed Canadian goldenrod among the “Top 20 environmental weeds for classical biological control in Europe” [8].

However, there is another, more positive side to Canadian and giant goldenrod, which is shown through their medicinal use. For a long time European phytotherapy has used both giant and Canadian goldenrod in urological and anti-inflammatory treatments [3,9]. However, the ecological risks that both goldenrods pose on our environment are still of great concern. Due to their invasiveness both giant and Canadian goldenrod are under active plant management in Europe. Switzerland even has a sale and planting ban for giant goldenrod, which is also known to alter some characteristics of soil (pH, and concentrations of C and P) and affect soil biota [7]. It is still unclear what consequence this holds for possible reestablishment of native species in previously invaded areas, thus making this reestablishment an even bigger challenge [7]. In spite that methods of mechanical control (like mowing twice per year and soil rotation in summer) do exist [3] the issue at hand is so widespread the solution to this problem will probably require a multimethod approach. A part of the solution could also be finding positive uses for these two goldenrods, which would require further knowledge of their properties and studies of different compounds present in the plants.

Canadian goldenrod extracts prepared with hot water were used as natural yellow dyes for textile dying [10,11]. Low dye stuff content restricted the amount of dry residue produced from plant material [10]. Aboveground parts (stems, leaves, and inflorescences) of Canadian and giant goldenrod extracts prepared with 50% ethanol\(_{aq}\) and hexane showed strong antibacterial activity to Gram-positive bacteria and weaker antibacterial activity to some Gram-negative bacteria [9]. Ethanol extract was more efficient than hexane extract [9]. Essential oil from roots of Canadian goldenrod exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria, but only moderate antifungal activity against pathogenic yeast [12]. Essential oils from aboveground (stems with leaves and inflorescence) parts of Canadian goldenrod also showed antifungal activity [13].

Among secondary metabolites discovered in different parts of Canadian [12–17] and giant [16–20] goldenrod are phenolic acids [14,16,17,19], flavonoids [14,16,17,19], carotenoids [15], triterpenoids [19], terpenes [12,13,18], and diterpenes [20]. Phenolic compounds (mainly, phenolic acids and flavonoids) were investigated in Canadian goldenrod leaves [16,17], inflorescence [16,17] and herbal tea extracts [14], in giant goldenrod (Solidago gigantea Aiton) leaves [16,17] and inflorescence [16,17], as well as in European goldenrod (Solidago virgaurea L.) leaves [21,22], stalks [22] and inflorescence [22]. Analyses of phenolic acids and flavonoids were also performed in leaves and inflorescence of the interspecific hybrid between Canadian and European goldenrod known as Solidago × niederederi Khek [17].

Analyses of phenolic acids and flavonoids in the extracts prepared from inflorescence of Canadian and giant goldenrod were performed with high-performance liquid chromatography (HPLC) and thin-layer chromatography (TLC) methods. Extracts of Canadian [9,14,16,17,23] and giant [16,17] goldenrod were prepared with aqueous ethanol [14,23] and aqueous methanol [16,17]. HPLC analyses of phenolic acids and flavonoids in extracts of Canadian goldenrod [14,16,17,23] and giant goldenrod [16,17] were performed on different C18 columns (Hypersil ODS [14], Luna C18 [23], YMC-Pack ODS-A [16,17]), using isocratic [23] or gradient [14,16,17] elutions. TLC was only used for preliminary analysis of Canadian goldenrod extracts prepared from herbs. This analysis was performed on TLC silica gel plate and visual evaluation of the chromatograms was performed at 254 nm.
and in Vis after development [23]. To the best of our knowledge there is no report on high performance thin-layer chromatography (HPTLC)- tandem mass spectrometry (MS/MS) and LC-MS/MS analyses of flavonoids and phenolic acids in inflorescence of Canadian and giant goldenrod. Chromatographic fingerprinting is an important tool for the valorization of plant materials. It is a key approach in quality control of dietary supplements and detection of adulterants [24]. Chromatographic fingerprinting combined with different chemometric methods is also used to distinguish plants and plant resins from different geographical origin [25].

The aim of our study was to (i) select solvents for extraction of flavonoids from inflorescences Canadian and giant goldenrod inflorescences; (ii) compare chromatographic fingerprints for sample test solutions (STSs) of Canadian and giant goldenrod obtained by HPTLC-image analysis; and (iii) facilitate using HPLC, LC-MS/MS, HPTLC, and HPTLC-MS/MS methods for the analysis of phenolic acids, flavonoid aglycones, and glycosylated flavonoids in STSs.

2. Materials and Methods

2.1. Chemicals

All solvents used were at least of analytical grade. Ethyl acetate, tetrahydrofuran, phosphoric acid (85%), formic acid (98–100%), and sodium hydrogen carbonate were obtained from Merck (Darmstadt, Germany). Acetone, acetonitrile (HPLC and LC-MS grade), and methanol (HPLC grade) were obtained from Honeywell Riedel-de Haén (Seelze, Germany). Ethanol (absolute anhydrous; HPLC grade) was obtained from Carlo Erba (Val de Reuil, France). Polyethylene glycol (PEG) 4000 was acquired from Fluka Chemie (Buchs, Switzerland) and two-aminoethyl diphenylborinate (Natural Product Reagent, NP) was acquired from Sigma-Aldrich (Steinheim, Germany). Ultrapure water was supplied by a Milli-Q water purification system (18 MΩ−1 cm) (Millipore, Bedford, MA, USA).

Standards of chlorogenic acid (97%) and quercetin (95%) were obtained by Fluka (Buchs, Switzerland) and Sigma (St. Louis, MO, USA), respectively. Kaempferol, rutin, hyperoside, isoquercitrin, and quercitrin were all of analytical grade and obtained from Extrasynthese (Genay, France).

2.2. Preparation of Standard Solutions

Stock solutions of individual standards (1.0 mg/mL) were prepared in 70% methanol(aq). Separate working standard solutions (20 µg/mL for qualitative analyses; 2, 5, 10, 20, and 50 µg/mL for quantitative analyses) were prepared by diluting stock solutions with 70% methanol(aq). All standard solutions were stored in amber glass storage vials at −20 °C.

2.3. Plant Material

Canadian goldenrod (Solidago canadensis) and giant goldenrod (Solidago gigantea) were collected in Ljubljana (46°01'57.2" N 14°28'31.6" E—Canadian goldenrod, 46°01'24.0" N 14°30'01.2" E—giant goldenrod), Slovenia in August 2018. Plant materials were air-dried and the inflorescences were separated (as much as possible) from the leaves, which are present in small amounts in the inflorescences. Dried inflorescences were stored in the dark at room temperature until they were used for the preparation of sample test solutions.

2.4. Preparation of Sample Test Solutions (STSs)

Dried inflorescence of Canadian goldenrod and giant goldenrod were pulverized by Grindomix GM200 (Retsch, Haan, Germany). The STSs (10 mg/mL) from inflorescences of Canadian goldenrod and giant goldenrod were prepared separately by dispersing 30 mg of powdered plant material in 3 mL of the extraction solvent. Suspension was vortexed (1 min) and sonicated using ultrasonic bath (Iskra Pio, Šentjernej, Slovenia) for 30 min. The following extraction solvents were tested: methanol, 90% methanol(aq), 80% methanol(aq), 70% methanol(aq), ethanol, 90% ethanol(aq), 80% ethanol(aq), 70% ethanol(aq), acetone, 90% acetone(aq), 80% acetone(aq), 70% acetone(aq) (all % were v/v), and water.
After centrifugation for 5 min at 2000 \( \times \) g the supernatants were filtered through 0.45 \( \mu \)m polyvinylidene fluoride (PVDF) membrane filters (LLG labware, Meckenheim, Germany) into amber glass storage vials. The obtained STSs (10 mg/mL) were stored at \(-20^\circ\)C and were used undiluted for HPTLC and spectrophotometric analyses. Diluted (2 mg/mL) STSs were used for HPLC and LC-MS analyses.

For hydrolysis, 30 mg of pulverized inflorescence of Canadian or giant goldenrod was treated with 3 mL of 1.2 M HCl in 50% methanol\((aq)\) under reflux for 1 h. After hydrolysis, the extract was neutralized with NaHCO\(_3\), filtered through 0.45 \( \mu \)m PVDF membrane filter, diluted 5-fold in 70% methanol\((aq)\) and analyzed by HPLC or LC-MS instruments.

2.5. HPTLC with Densitometry and Image Analysis

The utilized method for high-performance thin-layer chromatography was adapted from ref. [26]. The analysis was performed on 20 cm \( \times \) 10 cm or 10 cm \( \times \) 10 cm silica gel 60 HPTLC plates (Art. No. 1.05641, Merck, Germany). The plates were pre-developed to the top with chloroform–methanol (1:1, \( v/v \)) and dried in an oven at 110 \( ^\circ \)C for 30 min before use. All standard solutions and sample test solutions were applied by means of Linomat 5 (Camag, Muttenz, Switzerland). The plates were developed up to 9 cm in 34 min in a saturated (10 min) twin trough developing chamber with ethyl acetate–water–formic acid (85:15:10, \( v/v/v \)) as the developing solvent. The developed plates were dried in a stream of warm air for 3 min.

For post-chromatographic derivatization the plates were heated on a TLC plate heater III (Camag) at 110 \( ^\circ \)C (3 min) and immediately dipped for 1 s in NP reagent. After drying in a stream of warm air (hair dryer) for 2 min, followed by cooling in the air for 5 min, the plates were dipped into PEG 4000 reagent and were again dried in a stream of warm air (hair dryer) for 2 min. Natural product detection reagent (NP reagent) was prepared by dissolving 1 g of NP in 200 mL of ethyl acetate [27], while PEG reagent was prepared by dissolving 10 g of PEG 400 [27] or 4000 in 200 mL of dichloromethane. Both reagents were prepared for dipping and were stored protected from light at 5 \( ^\circ \)C.

The documentation of the chromatograms was performed by DigiStore 2 documentation system in conjunction with Reprostar 3 (Camag) at 254 nm, 366 nm, and white light illumination after the development, after the post-chromatographic derivatization with NP reagent, as well as after the enhancement and the stabilization of fluorescent zones with PEG 4000 reagent. For image analyses images of the HPTLC plate captured at 366 nm were converted to a different format using WinCATS software and then converted to videodensitograms in fluorescence mode using VideoScan software (Camag). After the post-chromatographic detection the plates were also scanned at 370 nm by a slit-scanning densitometer TLC Scanner 3 (Camag, Muttenz, Switzerland) set in the absorption/reflectance mode. The wavelength 370 nm was selected based on the absorption maxima of flavonoid standards which were determined in-situ on the developed HPTLC silica gel plate in our previous study [28]. DigiStore 2 documentation system and TLC Scanner 3 were controlled by winCATS software (Version 1.4.9.2001).

2.6. HPTLC-MS/MS Analyses

HPTLC-MS/MS analyses were performed on HPTLC silica gel plates (10 cm \( \times \) 10 cm). Plates were pre-developed twice, firstly, with methanol-formic acid (10:1, \( v/v \)), and secondly, with methanol like in our previous study [29]. STSs from Canadian and giant goldenrod prepared with 70% methanol\(_{(aq)}\) were analyzed on separate plates. Each STS (100 \( \mu \)L) was applied by Linomat 5 as a 60 mm band 10 mm from the bottom of the plate. The plate was developed up to 9 cm with ethyl acetate–water–formic acid (85:15:10, \( v/v/v \)) [26] in a saturated twin-trough chamber. A TLC-MS interface (Camag) was used for on-line elution of chromatographic zones of interest into a mass spectrometer (LTQ Velos MS, Thermo Fisher Scientific, Waltham, MA, USA). A flow rate of the eluent 70% methanol\(_{(aq)}\) was 0.2 mL/min. A pre-column filter 0.5 \( \mu \)m (Idex, Health & Science, Oak Harbor, WA, USA) was mounted between TLC-MS interface and the MS to prevent con-
tamination of MS ion source. A heated electrospray ionization (HESI) probe in the negative ion mode was used for ionization of the compounds. Heater temperature was set to 200 °C, capillary temperature to 350 °C, s voltage to 2.5 kV, S-lens RF level to 69%, sheath gas flow rate to 60 a.u. (arbitrary units), and auxiliary gas flow rate to 10 a.u. [29]. MS and MS/MS spectra were acquired in the m/z range 50–1000. The fragmentation of the selected ions was performed at 35% collision energy and isolation width of 2.00 m/z. Xcalibur software (version 2.1.0, Thermo Fisher Scientific, Waltham, MA, USA) was used for evaluation of the collected data.

2.7. HPLC and LC-MS Analyses

An HPLC system (Agilent 1260 Infinity, Santa Clara, CA, USA) equipped with a photodiode-array UV–Vis detector was used for the HPLC analysis. The separations were performed on a Hypersil ODS C18 (150 mm × 4.6 mm, 5 µm i.d.) column (Thermo-Fisher Scientific, Waltham, MA, USA). The mobile phase consisted of 0.4% ortho-phosphoric acid in water (A) and 4% tetrahydrofuran in acetonitrile (B). The following gradient elution was applied at 25 °C and the flow rate of 1.0 mL min⁻¹: 12% B (0–5 min), linear gradient 12–32% B (5–30 min), 12% B (30–40 min). The injection volume was 5 µL. The acquisition wavelength was set to 360 nm. The hydrolyzed sample test solutions were analyzed using the following gradient program: 25% B (0–5 min), linear gradient 25–45% B (5–20 min) and 25% B (15–20 min).

HPLC-MS/MS analyses were performed on a LC-MS system (Dionex Ultimate 3000-LCQ Fleet system, Thermo Scientific, Waltham, MA, USA). The flow rate, the column temperature, the acquisition wavelength and the injection volume remained the same as for HPLC analysis, while ortho-phosphoric acid used in the mobile phase for HPLC analyses was replaced with formic acid. The mobile phase consisted of 0.1% formic acid in water (A) and 4% tetrahydrofuran in acetonitrile (B). The following gradient was applied: 16% B (0–5 min), linear gradient 16–40% B (5–30 min), 16% B (30–40 min). Standard solution of rutin (20 µg mL⁻¹) at the flow rate of 5 µL min⁻¹ (direct injection) was used to optimize the MS parameters. Heated electrospray ionization (HESI) probe in the negative ion mode was used for the ionization of compounds. HESI ion source conditions were as follows: transfer capillary temperature 300 °C, vaporizer temperature 350 °C, spray voltage 3.0 kV, capillary voltage -24.0 V, tube lens -125 V, sheath gas flow rate 16 a.u. and auxiliary gas flow rate 5 a.u. MS and MS/MS spectra were acquired in the m/z range 50–1000. The fragmentation of the selected ions was performed at 35% collision energy and isolation width of 2.00 m/z. The collected data were evaluated with Xcalibur software (version 2.1.0).

3. Results and Discussion

3.1. Evaluation of Extraction Solvents

As described in the literature extracts from inflorescence of Canadian [9,14,16,17,23] and giant [16,17] goldenrod were prepared with aqueous ethanol (50% [23], 70% [14], and 96% [14]) and aqueous methanol (70% [16,17]). Extraction of aboveground parts (stems, leaves, and inflorescence) of both goldenrod species was performed with 50% ethanol(aq) and hexane. For both goldenrod species the use of 50% ethanol(aq) as the extraction solvent resulted in an almost two times higher extraction yield than the use of hexane. The flavonoid content in Canadian goldenrod was 44 times higher in the case of 50% ethanol(aq) (1.76 mg/g dry matter) than hexane (0.04 mg/g dry matter). The flavonoid content in giant goldenrod was 30 times higher in the case of 50% ethanol(aq) (4.57 mg/g dry matter) than hexane (0.15 mg/g dry matter) [9]. Other comparisons of the effect of extraction solvents on extraction of phenolic compounds from Canadian or giant goldenrod are not available in the literature.

In our study extraction of flavonoids from inflorescences of Canadian goldenrod was performed using pure solvents (water, ethanol, methanol, and acetone) and mixtures of organic solvents with water (70% ethanol(aq), 80% ethanol(aq), 90% ethanol(aq), 70% methanol(aq), 80% methanol(aq), 90% methanol(aq), 70% acetone(aq), 80% acetone(aq), and
90% acetone\textsubscript{(aq)}. The sample test solutions (STSs) obtained were analyzed by HPTLC and HPLC methods.

All STSs, except STS prepared with water, were analyzed on the HPTLC silica gel plate developed with ethyl acetate–water–formic acid (85:15:10, \textit{v/v/v}). Differences were observed in the chromatograms on the HPTLC plate documented at 366 nm and at white light after development, after post-chromatographic derivatization with NP reagent and after use of PEG reagent (Figure 1). At the first glance profiles of the most intensive bands in all tracks, except in tracks 8 (STS in ethanol) and 12 (STS in acetone), look similar at both illumination conditions (Figure 1). Although at 366 nm after development, after derivatization with NP reagent and after use of PEG reagent other 10 tracks look similar, a closer look revealed differences in a number of less intensive bands (Figure 1A–C). The effect of the extraction solvents on the qualitative densitometric profiles of STSs prepared with different solvents is shown in the densitograms scanned on the developed plate at 370 nm in absorption/reflectance mode (Figure 2). As shown in densitograms (Figure 2) the peaks of STSs prepared with ethanol (track 8) and acetone (track 12) were much lower than the peaks of STSs prepared with all other extraction solvents. Comparison of the total peak areas of the densitograms of STSs prepared from Canadian goldenrod with different extraction solvents was used for the evaluation of the extraction efficiency (Figure 3). Normalized total peak areas were lower for STSs prepared with pure organic solvents (methanol, ethanol, and acetone) than for STSs prepared with mixtures of organic solvents with water (70%, 80%, and 90% methanol\textsubscript{(aq)}, 70%, 80%, and 90% ethanol\textsubscript{(aq)}, 70%, 80%, and 90% acetone\textsubscript{(aq)}) (Figure 3).

![Figure 1. HPTLC chromatograms of Canadian goldenrod sample test solutions (STSs) prepared with 70% methanol\textsubscript{(aq)} (track 1), 80% methanol\textsubscript{(aq)} (track 2), 90% methanol\textsubscript{(aq)} (track 3), methanol (track 4), 70% ethanol\textsubscript{(aq)} (track 5), 80% ethanol\textsubscript{(aq)} (track 6), 90% ethanol\textsubscript{(aq)} (track 7), ethanol (track 8), 70% acetone\textsubscript{(aq)} (track 9), 80% acetone\textsubscript{(aq)} (track 10), 90% acetone\textsubscript{(aq)} (track 11), and acetone (track 12). HPTLC silica gel plate developed with ethyl acetate–water–formic acid (85:15:10, \textit{v/v/v}) was documented at 366 nm (A–C) and at white light (D–F) before derivatization (A, D), after derivatization with NP reagent (B, E) and after use of PEG 4000 (C, F).]
Figure 2. Densitograms of Canadian goldenrod STSs scanned at 370 nm in absorption/reflectance mode on the HPTLC silica gel plate developed with ethyl acetate–water–formic acid (85:15:10, v/v/v). STSs were prepared with 70% methanol (aq) (1), 80% methanol (aq) (2), 90% methanol (aq) (3), methanol (4), 70% ethanol (aq) (5), 80% ethanol (aq) (6), 90% ethanol (aq) (7), ethanol (8), 70% acetone (aq) (9), 80% acetone (aq) (10), 90% acetone (aq) (11), and acetone (12).

Figure 3. Comparison of normalized total peak areas obtained by HPTLC and HPLC analyses of Canadian goldenrod STSs prepared with different extraction solvents. Total peak areas for HPTLC analyses were obtained from the densitograms scanned at 370 nm (Figure 2), while HPLC chromatograms were recorded at 360 nm (Figure 4).
Figure 4. HPLC chromatograms of Canadian goldenrod STSs prepared with 70% methanol\(_{aq}\) (A1), 80% methanol\(_{aq}\) (A2), 90% methanol\(_{aq}\) (A3), methanol (A4), 70% ethanol\(_{aq}\) (B1), 80% ethanol\(_{aq}\) (B2), 90% ethanol\(_{aq}\) (B3), ethanol (B4), 70% acetone\(_{aq}\) (C1), 80% acetone\(_{aq}\) (C2), 90% acetone\(_{aq}\) (C3) acetone (C4), and water\(D\) recorded at 360 nm.

Methanol gave the highest normalized total peak area among the tested pure organic solvents. The highest normalized total peak area was achieved with 70% acetone\(_{aq}\) and the lowest normalized total peak area with pure acetone (Figure 3).
All 13 STSs (including STS prepared with water) were also analyzed using the HPLC method. HPLC chromatograms of STSs recorded at 360 nm (Figure 4) showed lower peaks for STSs prepared with pure organic solvents and water than for STSs prepared with mixtures of organic solvents with water. Peak areas for the 15 separated peaks obtained for STSs prepared with 70% methanol\(_{(aq)}\) were from 1.1 up to 1.7 times higher than peak areas for STSs prepared with methanol. Peak areas for STSs prepared with 70% ethanol\(_{(aq)}\) were from 1.7 up to 4.8 times higher than peak areas for STSs prepared with ethanol. Peak areas for STSs prepared with 70% acetone\(_{(aq)}\) were from 2.6 up to 35 times higher than peak areas for STSs prepared with acetone. The highest differences in peak areas were observed when extraction solvents with acetone. Like in the case of the HPTLC analyses total peak areas of STSs were normalized and were used for the evaluation of the extraction efficiency.

Comparison of the normalized total peak areas for all 13 STSs (Figure 3) showed comparable results to those obtained by the HPTLC analyses for 12 STSs (all STSs except STS prepared with water). The addition of water (20% or 30%) to the organic solvent used for the extraction resulted in higher normalized total peak areas than were observed by extraction with pure organic solvents. The normalized total peak areas for STS prepared only with water and STS prepared only with acetone were about 90% lower than the highest normalized total peak area which was achieved for STSs prepared with 70% acetone\(_{(aq)}\) (Figure 3).

The normalized total peak areas for STS prepared only with water and STS prepared only with acetone were about 90% lower than the highest normalized total peak area which was achieved for STSs prepared with 70% acetone\(_{(aq)}\) (Figure 3). Further evaluation of the peak areas of the 15 separated peaks in the HPLC chromatograms of STSs prepared with extraction solvents 70% methanol\(_{(aq)}\) (Figure 4A1), 70% ethanol\(_{(aq)}\) (Figure 4B1), and 70% acetone\(_{(aq)}\) (Figure 4C1) revealed comparable extraction efficiencies of all three solvents for the compounds present in each of the 15 peaks in the HPLC chromatograms (Figure 5). Based on our results STSs from inflorescence of Canadian and giant goldenrod prepared with 70% methanol\(_{(aq)}\) or 70% acetone\(_{(aq)}\) were applied for further HPTLC, HPLC, HPTLC-MS/MS, and LC-MS/MS analyses.

**Figure 5.** Peak areas for each of the chromatographic peaks determined by the HPLC analyses of Canadian goldenrod STSs prepared using 70% methanol\(_{(aq)}\), 70% ethanol\(_{(aq)}\) and 70% acetone\(_{(aq)}\). Peak numbering is based on the chromatograms A1, B1, and C1 presented in Figure 4 (peak 1 at \(t_R = 4.98\) min, peak 2 at \(t_R = 14.51\) min, peak 3 at \(t_R = 14.87\) min, peak 4 at \(t_R = 15.38\) min, peak 5 at \(t_R = 15.91\) min, peak 6 at \(t_R = 16.34\) min, peak 7 at \(t_R = 17.55\) min, peak 8 at \(t_R = 17.91\) min, peak 9 at \(t_R = 18.13\) min, peak 10 at \(t_R = 18.61\) min, peak 11 at \(t_R = 19.05\) min, peak 12 at \(t_R = 19.44\) min, peak 13 at \(t_R = 20.99\) min, peak 14 at \(t_R = 22.89\) min, peak 15 at \(t_R = 23.32\) min).
3.2. HPTLC and HPTLC-Image Analyses

Data on (HP)TLC analysis of flavonoids and phenolic acids in inflorescence of Canadian and giant goldenrod are rather scarce according to the literature. TLC was only used for preliminary analyses of Canadian goldenrod extracts prepared with 70% ethanol\(_{(aq)}\) from herbs, while HPLC was used for analyses of extracts from flowers and leaves [23]. TLC silica gel plates were developed with ethyl acetate–acetic acid–water (7.5:1.5:1.5) in a saturated (24 h) chromatographic chamber. Visual evaluation of the chromatograms was performed at 254 nm and in Vis after development. The results (R\(_F\) values of chlorogenic acid, rutin, hyperoside, and quercetin) were only tabulated without showing the image of the plate [23].

In our study both HPTLC and HPLC were used for the investigation of phytochemicals in Canadian and giant goldenrod. As shown in Figure 6 for Canadian goldenrod (tracks 1 and 2) extraction solvents 70% methanol\(_{(aq)}\) (tracks 1) and 70% acetone\(_{(aq)}\) (tracks 2) gave equal qualitative profiles (fingerprints). For giant goldenrod (tracks 3 and 4) the same two solvents 70% methanol\(_{(aq)}\) (tracks 3), and 70% acetone\(_{(aq)}\) (tracks 4) resulted in equal fingerprints (Figure 6). However, differences were observed in the qualitative profiles of Canadian goldenrod (tracks 1 and 2) and giant goldenrod (tracks 3 and 4) after development, after post-chromatographic derivatization with NP reagent and after use of PEG reagent (Figure 6). At 366 nm after development intensive dark blue bands (at R\(_F\) 0.47 and 0.82) and green bands (R\(_F\) 0.94) were detected in all tracks (Figure 6A). Blue bands were more intensive for Canadian goldenrod, while green bands were more intensive for giant goldenrod (Figure 6A). Several other less intensive blue and dark green bands were also observed in all tracks. At 366 nm (Figure 6B) and at white light (Figure 6E) after post-chromatographic derivatization with NP reagent (Figure 6B,E) and after use of PEG reagent (Figure 6C,F) the sensitivity of the method increased and differences between the qualitative profiles of both plant species become more evident. After use of NP and PEG reagents blue bands (at R\(_F\) 0.47 and 0.82) turned light blue at 366 nm (Figure 6B,C). Some dark green bands become yellow-green after use of NP reagent and turned orange-yellow after use of PEG reagent (Figure 6B,C). These bands were at R\(_F\) 0.26 for both plants, at R\(_F\)s 0.61 for Canadian goldenrod (tracks 1 and 2) and at R\(_F\)s 0.44, 0.50, 0.57, and 0.64 for giant goldenrod (tracks 3 and 4) (Figure 6B,C). The intensities of the bands (light-blue and orange-yellow) at R\(_F\) 0.26, 0.47, 0.69, and 0.82 were higher for Canadian goldenrod than for giant goldenrod (Figure 6F). At white light all the bands that were yellow after development (Figure 6D) and after derivatization with NP reagent (Figure 6E) turned orange-yellow after use of PEG reagent (Figure 6F). All orange-yellow bands for giant goldenrod, except the bands at R\(_F\) 0.26, had higher intensities than the bands for Canadian goldenrod (Figure 6F). The bands at R\(_F\) 0.47 were detected in Canadian goldenrod only at white light after use of PEG reagent (Figure 6F). Bands at R\(_F\)s 0.26, 0.44, 0.57, 0.61, and 0.64 that were dark (almost black) at 254 nm after development (Figure 6G) were orange-yellow at white light after derivatization with NP and use of PEG reagent (Figure 6F).
As the images captured at 366 nm (Figure 6A–C) showed the highest number of bands, these images were used for comparison of chromatographic fingerprints of STSs of Canadian and giant goldenrod. For that purpose the images of the HPTLC plate captured at 366 nm were converted to videodensitograms in fluorescence mode. Chromatographic fingerprinting of phenolic compounds (mainly flavonoids and phenolic acids) was performed with image analysis after development, after post-chromatographic derivatization with NP reagent and after use of PEG reagent (Figure 7). The videodensitograms of STSs of Canadian (Figure 7A) and giant (Figure 7B) goldenrod showed that post-chromatographic derivatization with NP reagent drastically enhanced the sensitivity of the HPTLC method which is seen from the considerable increase in heights of the majority of the peaks. The sensitivity of the method was further improved after use of PEG reagent (Figure 7). Chromatographic fingerprints of STSs inflorescence of Canadian goldenrod (Figure 7A) and giant goldenrod (Figure 7B) are different. The most pronounced qualitative differences between the two profiles appear in the interval of R\textsubscript{F} values from 0.5 to 0.8 (Figure 7), where more peaks are present in the profile of giant goldenrod (Figure 7B). However, all major peaks (at R\textsubscript{F} 0.26, 0.47, 0.62, 0.69, and 0.82) present in both videodensitograms (Figure 7) are higher in the videodensitogram of Canadian goldenrod (Figure 7A). One of the highest peaks that appeared at R\textsubscript{F} 0.57 in the videodensitogram of giant goldenrod (Figure 7B) was not present in the videodensitogram of Canadian goldenrod (Figure 7A). It can be concluded that the HPTLC method used provides different chromatographic fingerprints for Canadian and goldenrod extracts.
Figure 7. Comparison of videodensitograms of STSs from Canadian goldenrod (A) and giant goldenrod (B) prepared with 70% acetone\(_{aq}\). Videodensitograms were obtained in fluorescence mode by image analysis of HPTLC silica gel plate (at 366 nm) after development with ethyl acetate–water–formic acid (85:15:10, \(v/v/v\)) (A: dashed green line; B: red line), after post-chromatographic derivatization with NP reagent (A: blue line; B: black line) and after use of PEG reagent (A: red line; B: blue line).

3.3. HPLC Analyses

The octadecylsilyl stationary phase was used for the separation of phenolic compounds from the extracts of Canadian and giant goldenrod in the published HPLC [16,17,23] and LC-MS [14] methods. Analyses were performed on nonhydrolyzed [14,16,17,23] and hydrolyzed [23] extracts. Isocratic [23] and gradient elution [14,16,17] were applied for analyses of phenolic acid and flavonoids in inflorescence of Canadian [16,17] and giant [14,23] goldenrod. Acetonitrile/water with addition of different acidifiers (acetic acid [14], trifluoroacetic acid [16,17], or trichloroacetic acid [23]) were used as mobile phases. Addition of tetrahydrofuran (THF) to the mobile phase improved the separation of phenolic compounds in hydrolyzed plant extracts (on Eurospher C18 column, 250 × 4.6 mm, i.d. 5 \(\mu\)m) [21].

Based on these data, the octadecylsilyl stationary phase was selected also for our study. Our method development was performed using Canadian and giant goldenrod inflorescence STSs in 70% methanol\(_{aq}\) and the following standards from the group of phenolic acids (chlorogenic acid) and groups of glycosylated flavonoids (rutin, hyperoside, isoquercetin, and quercitrin standards). The separation of glycosylated flavonoids was performed on Hypersil C18 column with mobile phase (4% THF in acetonitrile (A) and 0.4% ortho-phosphoric acid in water (B)). The mobile phase was taken from previous study on C18 column from another producer [21], where the percentage of organic modifier was 35%. Since mainly phenolic acids and glycosylated flavonoids are present in the inflorescence of goldenrods, in our method the percentage of organic modifier was lower (from 12% to 32%), as these compounds elute under relatively polar conditions. Based on the absorption spectra of standards (chlorogenic acid, rutin, hyperoside, and isoquercetin) which were recorded during the HPLC analyses the acquisition wavelength 360 nm was selected.

Our method enabled baseline separation of chlorogenic acid, rutin, hyperoside, isoquercetin, and quercitrin standards (Figure 8A). A comparison of HPLC chromatograms of Canadian and giant goldenrod STSs (prepared with 70% methanol\(_{aq}\)) as well as the chromatogram of standards revealed that except quercitrin all studied compounds (chlorogenic acid, rutin, isoquercetin, and hyperoside) were present in both plant species (Figure 8). Chromatograms show both qualitative and quantitative differences between Canadian and giant goldenrod. There were 15 peaks in the chromatogram for Canadian goldenrod (Figure 8B), while there were 14 for giant goldenrod (Figure 8C). The peak at \(t_R\) for chlorogenic acid is higher for Canadian goldenrod than for giant goldenrod. The chromatogram
of Canadian goldenrod also had a much higher peak at $t_R$ for rutin than the chromatogram of giant goldenrod. The peak at $t_R$ for hyperoside was visibly higher for giant goldenrod than Canadian. The chromatographic peaks $t_R$ for isoquercitrin were also different with higher intensity for giant goldenrod. The intensity of the peak at $t_R$ for quercitrin was bigger for giant goldenrod than Canadian. The same compounds were also reported by other authors [14,16,17].

Figure 8. HPLC chromatograms of mixture of standards (A) phenolic acid (chlorogenic acid) and glycosylated flavonoids (rutin, hyperoside, isoquercetin and quercetin)) and STSs prepared in 70% methanol$_{aq}$ from inflorescences of Canadian goldenrod (B) and giant goldenrod (C) recorded at 360 nm. Peak numbering: chlorogenic acid (1), rutin (2), hyperoside (3), isoquercitrin (4), quercitrin (5).

Quantitative analyses were performed using external standard calibration method using standards of chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin. The limits of detection (LOD) and the limits of quantification (LOQ) were calculated on the basis of signal-to-noise ratios (S/N) of 3 and 10, respectively. The limits of detection (LOD) were 30, 15, 7, 25, and 5 ng/mL for chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin, respectively. The limits of quantification (LOQ) were 100, 50, 25, 85, and 20 ng/mL mL for chlorogenic acid, rutin, hyperoside, isoquercitrin, and quercitrin, respectively. Our quantitative results for the contents of phenolic acids and flavonoids in inflorescence of Canadian and giant goldenrod (Table 1) showed both similarities and differences with available published quantitative studies [16,17,23]. For inflorescence of giant goldenrod our contents of chlorogenic acid were lower (6.51 mg/g dry mass (DM)) than in comparable studies (10.03 mg/g DM [16] and 13.72 mg/g DM [17]). The determined content of quercitrin (6.61 mg/g DM) was also lower than in other available studies (17.60 mg/g DM [16] and 18.10 mg/g DM [17]), while the contents of rutin (1.67 mg/g DM) was comparable to literature data (2.04 mg/g DM [16] and 1.42 mg/g DM [17]). On the other hand the contents of isoquercitrin (6.93 mg/g DM) was in the range presented in the literature (1.36 mg/g DM [16]–12.78 mg/g DM [17]). A similar trend was observed for the
contents of hyperoside (7.63 mg/g DM) which was midway between reported literature data (1.59 mg/g DM [17]–11.72 mg/g DM [16]).

| Compounds       | Canadian Goldenrod | Giant Goldenrod |
|-----------------|--------------------|-----------------|
| Chlorogenic acid| 9.05 ± 0.05        | 6.51 ± 0.08     |
| Rutin           | 27.62 ± 0.45       | 1.67 ± 0.03     |
| Hyperoside      | 0.80 ± 0.01        | 7.63 ± 0.04     |
| Isoquercitrin   | 6.78 ± 0.05        | 6.93 ± 0.09     |
| Quercitrin      | <LOD               | 6.61 ± 0.07     |

1 Values (mean ± SD).

The results for phytochemical contents in inflorescence of Canadian goldenrod also showed some similarities and differences with literature data. Our content of chlorogenic acid (9.05 mg/g DM) was similar to literature results (8.35 mg/g DM [16] and 9.34 mg/g DM [17]). On the other hand quercitrin was not detected in our study, although available literature data show a diverse range of quercitrin contents (2.6 mg/g DM [16], 0.23 mg/g DM [17], and 7.73 mg/g of raw herb material [23]). For rutin our content (27.62 mg/g DM) was quite higher compared to results in other studies (18.22 mg/g DM [17] and 6.87 mg/g of raw herb material [23]). On the other hand the content of isoquercitrin (6.78 mg/g DM) in our study was within the range presented in the literature (0.34 mg/g DM [17] and 3.53 mg/g of raw herb material [23]). A similar trend was observed for the contents of hyperoside (0.80 mg/g DM) which was also midway between the literature data (0.66 mg/g DM [17] and 2.32 mg/g of raw herb material [23]).

By changing the gradient program our HPLC method was adapted for analysis of hydrolyzed STSs, containing mainly less polar flavonoid aglycones. After hydrolysis new differences in the HPLC chromatograms were evident (Figure 9). The chromatograms of standards (Figure 9A) confirmed that both Canadian (Figure 9B) and giant (Figure 9C) goldenrod contained quercetin and kaempferol, while the presence of isorhamnetin was confirmed with LC-MS/MS analyses. The chromatographic profile of Canadian goldenrod showed more peaks of higher intensity with the highest peak belonging to quercetin (Figure 9B). Based on these data it can be concluded that glycosylated flavonoids in Canadian (Figure 9B) and giant (Figure 9C) goldenrod inflorescence are mainly glycosides of quercetin and kaempferol. This was observed also in another study, in which the contents of flavone aglycones expressed in mg/g of raw herb material (inflorescence of Canadian goldenrod) were 39.56, 1.71, and 0.61 for quercetin, kaempferol, and isorhamnetin, respectively [23].
Figure 9. HPLC chromatograms of mixture of standards ((A) quercetin and kaempferol) and hydrolyzed STSs prepared in 70% methanol\((aq)\) from inflorescences of Canadian goldenrod (B) and giant goldenrod (C) recorded at 360 nm. Peak numbering: quercetin (1), kaempferol (2), isorhamnetin (3*—tentatively identified by LC-MS/MS analyses).

3.4. HPTLC-MS/MS and LC-MS/MS Analyses

There is only one published study [14] dealing with HPLC-MS analyses in Canadian goldenrod inflorescence, while mass spectrometric data for giant goldenrod inflorescence are not available. MS data were obtained with a single quadrupole MS instrument [14].

In our study tentative identification of phenolic acids and glycosylated flavonoids in STSs (in 70% methanol\((aq)\) from inflorescence of Canadian goldenrod and giant goldenrod was performed using HPTLC-MS/MS (Table 2) and HPLC-MS/MS (Table 3) analyses. Chlorogenic acid and glycosylated quercetin analogues (rutin, hyperoside, and isoquercitrin) were tentatively identified in both plant species. For the first time dicaffeoylquinic acid was identified in both plant species, and quercitrin was identified in giant goldenrod. In both plants we identified isomer of rutin with parent ion at \(m/z\) 609. The MS\(^2\) spectrum of the signal at 609 \(m/z\) gave a base ion at \(m/z\) 301 with neutral loss of 308 Da, which indicated the loss of rhamnosyl-hexosyl moiety [30,31] as in rutin. Two additional glycosylated analogues of quercetin were identified in giant goldenrod for the first time. The MS\(^2\) spectrum of the signal at 595 \(m/z\) gave a base ion at \(m/z\) 301 with neutral loss of 294 Da, which indicated the loss of (pentosyl)-hexoside [31]. Therefore, the signal at \(m/z\) 595 [M−H]\(^−\) was assigned to [M−H]\(^−\) of quercetin (rhamnosyl)-hexoside. The signal at \(m/z\) 433 [M−H]\(^−\) could correspond to afzelin (kaempferol-3-O-rhamnoside), which was previously detected in Canadian goldenrod inflorescence [14]. However, the MS\(^2\) and MS\(^3\) fragmentation patterns in our study were typical for quercetin with base ion signal...
at m/z 300 in MS² spectrum and at m/z 271 in MS³ spectrum. In addition, the signal at m/z 433 gave a neutral loss 132 Da, which indicated pentosyl unit [31]. Consequently, the signal at m/z 433 was identified as quercetin pentoside. The signal at m/z 505, which was only detected in Canadian goldenrod gave a neutral loss 204 Da, which indicated acetyl-hexosyl sugar moiety [32]. The MS² and MS³ fragmentation patterns were characteristic for quercetin. Therefore, the signal at m/z 505 was identified as quercetin-(acetyl)-hexoside which was for the first time identified in Canadian goldenrod. From the group of flavonols kaempferol and isorhamnsetin glycosylated analogues were detected only in Canadian goldenrod inflorescences. Signals at m/z 593 for both kaempferol isomers gave neutral loss to 308 m/z, which indicated the loss of rhamnosyl-hexosyl residue [30,31] as in the case of rutin isomer. The MS² and MS³ fragmentation patterns were similar to the fragmentation patterns of kaempferol standard [30]. Therefore, the kaempferol isomers were identified as kaempferol-(rhamnosyl)-hexosides. The same as quercetin-(acetyl)-hexoside also the compound with mass peak at m/z 489 [M−H]− lost a sugar unit with 205 Da. Therefore, this compound was for the first time identified in Canadian goldenrod inflorescence as kaempferol-(acetyl)-hexoside. Another compound with signal at m/z 519 lost 205 Da, and had similar MS² and MS³ fragmentation patterns as isorhamnsetin standard [30]. Therefore, this compound was identified as isorhamnsetin-(acetyl)-hexoside. Compound at m/z 489 [M−H]−, which lost 308 Da ((rhamnosyl)-hexosyl sugar moiety) [30,31] and had the same MS² and MS³ fragmentation patterns as isorhamnsetin, was identified in Canadian goldenrod inflorescence as isorhamnsetin-(rhamnosyl)-hexoside.

HPTLC-MS/MS method enabled tentative identification of 2 phenolic acids and 8 glycosylated flavonoids, while HPLC-MS/MS method enabled identification of 2 phenolic acids and 13 glycosylated flavonoids including four quercetin isomers and two kaempferol isomers. Using HPTLC-MS/MS method we tentatively identified two phenolic acids and six glycosylated flavonoids in Canadian and two phenolic acids and five glycosylated flavonoids in giant goldenrod. Even more compounds were tentatively identified using HPLC-MS/MS methods, two phenolic acids and 10 glycosylated flavonoids in Canadian and two phenolic acids and 7 glycosylated flavonoids in giant goldenrod.

We were the first to identify dicaffeoylquinic acid, quercetin-(acetyl)-hexoside, quercetin-(rhamnosyl)-hexoside, isorhamnsetin-(rhamnosyl)-hexoside, isorhamnsetin-(acetyl)-hexoside, and two kaempferol-(rhamnosyl)-hexoside isomers in inflorescence of Canadian goldenrod. Chlorogenic acid, dicaffeoylquinic acid, rutin, hyperoside, isoquercitrin, quercetin pentoside, quercetin-(pentosyl)-hexoside, quercetin-(rhamnosyl)-hexoside, and quercitrin were for the first time identified in inflorescence of giant goldenrod.
| Compound                          | [M – H]$^-$ (m/z) | $^{m}MS^n$ Fragmentation                       | Rf   | Goldenrods | Ref. |
|----------------------------------|------------------|-----------------------------------------------|------|------------|------|
| Chlorogenic acid                 | 353              | MS2 [353]: 191 (100), 179 (10)                 | 0.61 | +          | +    | [33] |
|                                  |                  | MS3 [353→191]: 173 (95), 127 (100), 85 (90)    |      |            |      |      |
|                                  |                  | MS2 [433]: 301 (70), 300 (100)                 |      |            |      |      |
|                                  |                  | MS3 [433→300]: 271 (100), 255 (50)             |      |            |      |      |
| Quercetin pentoside              | 433              | MS2 [447]: 301 (100), 300 (30)                 | 0.63 | –          | +    | [30] |
|                                  |                  | MS3 [447→301]: 273 (20), 257 (20), 179 (100), 151 (80) |      |            |      |      |
| Quercitrin                       | 447              | MS2 [447]: 301 (100), 300 (30)                 | 0.66 | –          | +    | [30,34] |
|                                  |                  | MS3 [447→301]: 273 (30), 271 (30), 257 (15), 255 (30) 179 (100), 151 (80) |      |            |      |      |
| Quercitrin isomer                | 447              | MS2 [463]: 301 (100), 300 (30)                 | 0.60 | +          | –    | [34] |
|                                  |                  | MS3 [463→301]: 273 (20), 257 (25), 179 (100), 151 (80) |      |            |      |      |
| Hyperoside                       | 463              | MS2 [463]: 301 (100), 300 (30)                 | 0.56 | +          | +    | [34] |
| Kaempferol-(acetyl)-hexoside     | 489              | MS2 [489]: 284 (100), 285 (80)                 | 0.73 | +          | +    | [30] |
|                                  |                  | MS3 [489→284]: 255 (100), 227 (20)             |      |            |      |      |
| Quercetin-(acetyl)-hexoside      | 505              | MS2 [505]: 301 (100), 300 (30)                 | 0.65 | +          | –    | [32] |
|                                  |                  | MS3 [505→301]: 273 (20), 257 (20), 179 (100), 151 (80) |      |            |      |      |
|                                  |                  | MS2 [515]: 353 (100)                           |      |            |      |      |
| Dicaffeoylquinic acid            | 515              | MS2 [515→353]: 191 (50), 179 (60), 173 (100)   | 0.80 | +          | +    | [33] |
| Kaempferol-(rhamnosyl)-hexoside  | 593              | MS2 [593]: 285 (100), 284 (10)                 | 0.36 | +          | –    | [30] |
|                                  |                  | MS3 [593→285]: 267 (45), 257 (100), 229 (50), 241 (30) |      |            |      |      |
|                                  |                  | MS2 [609]: 301 (100), 300 (45)                 |      |            |      |      |
| Rutin                            | 609              | MS3 [609→301]: 273 (25), 257 (20), 179 (100), 151 (80) | 0.29 | +          | +    | [30,32] |

Table 2. Phenolic acids and glycosylated flavonoids tentatively identified (+ Detected; – Not detected) in Canadian and giant goldenrod STSs by HPTLC-MS/MS.
### Table 3. Phenolic acids and glycosylated flavonoids tentatively identified (+ Detected; – Not detected) in Canadian and giant goldenrod STSs by LC-MS/MS.

| Compound                     | Peak Number | $t_R$ (min) | $[M+H]^+$ (m/z) MS2 Fragmentation | Goldenrods                  | Ref. |
|------------------------------|-------------|-------------|-----------------------------------|----------------------------|------|
| Chlorogenic acid             | 1           | 3.2         | 353                               | +                          | [33] |
| Quercetin-(pentosyl)-hexoside| 2           | 8.9         | 595                               | –                          | [31] |
| Quercetin-(rhamnosyl)-hexoside| 3           | 9.2         | 609                               | +                          | [31,34] |
| Rutin                        | 4           | 9.5         | 609                               | +                          | [34] |
| Hyperoside                   | 5           | 10.3        | 463                               | +                          | [34] |
| Isoquercitrin                | 6           | 10.8        | 463                               | +                          | [31] |
| Kaempferol-(rhamnosyl)-hexoside| 7          | 11.2        | 593                               | +                          | [30] |
| Kaempferol-(rhamnosyl)-hexoside| 8          | 12.3        | 593                               | +                          | [30] |
| Quercetin pentoside          | 9           | 12.5        | 433                               | –                          | [30] |
| (Iso)rhamnetin-(rhamnosyl)-hexoside| 10        | 12.7        | 623                               | –                          | [32] |
| Dicaffeoylquinic acid        | 11          | 13.2        | 515                               | +                          | [33] |
| Quercitrin                   | 12          | 13.7        | 447                               | –                          | [34] |
| Quercetin-(acetyl)-hexoside  | 13          | 14.1        | 505                               | +                          | [32] |
| Kaempferol-(acetyl)-hexoside | 14          | 17.2        | 489                               | –                          | [30] |
| Isorhamnetin-(acetyl)-hexoside| 15         | 17.6        | 519                               | +                          | [32] |
4. Conclusions

From an analytical point of view, a combination of solvents with water is probably a logical choice. However, the use of solvent without the addition of water may still be worth considering, especially for larger scale extractions, since avoiding water significantly simplifies the whole process in terms of isolation of the extracted compounds. Thirteen extraction solvents (water, ethanol, methanol, acetone, ethanol\(_{(aq)}\) (70\%, 80\%, and 90\%), methanol\(_{(aq)}\) (70\%, 80\%, and 90\%), and acetone\(_{(aq)}\) (70\%, 80\%, and 90\%)) were examined in our study for extraction of flavonoids (mainly flavonols—yellow dyes) and phenolic acids form inflorescence of Canadian goldenrod. Applied solvents were chosen because of their affordability and relatively high environmental acceptability, which also makes these solvents appropriate for larger scale routine work. Although it was expected that pure organic solvents will give lower extraction yields than mixtures of organic solvent with water, pure solvents were used because they can easily be removed and recycled. HPTLC and HPLC analyses of the obtained sample test solutions showed the best and comparable extraction efficiencies for the following extraction solvents: 70\% acetone\(_{(aq)}\), 70\% methanol\(_{(aq)}\), and 70\% ethanol\(_{(aq)}\). Therefore, only 70\% methanol\(_{(aq)}\) or 70\% acetone\(_{(aq)}\) were used for preparation of STSs from inflorescence of Canadian and giant goldenrod, which were further analyzed using HPTLC, HPLC, HPTLC-MS/MS, and LC-MS/MS. HPTLC combined with image analysis in fluorescent mode resulted in different chromatographic fingerprints for Canadian and goldenrod extracts after development, after post-chromatographic derivatization with NP reagent and after use of PEG reagent. The HPLC methods developed in this study enabled analyses of chlorogenic acid as well as flavonoids in STSs and hydrolyzed STSs form inflorescence of Canadian goldenrod and giant goldenrod. Our study showed differences in the content of chlorogenic acid, rutin, hyperoside, isorquercetin, and quercetin in STSs of both goldenrod species. Our HPLC analyses of hydrolyzed STSs confirmed that glycosylated flavonoids in Canadian and giant goldenrod inflorescence are mainly glycosides of quercetin, kaempferol and isorhamnetin. Using HPTLC-MS/MS analyses we tentatively identified eight compounds in Canadian and seven in giant goldenrod. From those four in Canadian and seven in giant goldenrod were identified for the first time. Even more compounds were tentatively identified using LC-MS/MS analyses, 12 in Canadian, and 9 in giant goldenrod. From those eight in Canadian and nine in giant goldenrod were identified for the first time.

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