Antioxidant constituents in *Solidago canadensis* L. and its traditional phytopharmaceuticals

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1. Introduction, aim of the study

Medicinal herbs have played a major role in the development of modern medicine and continue to be widely used in their original form. During the latter part of this century the practice of herbalism has become mainstream throughout the world. This is due in part to the recognition of the value of traditional medical systems and the identification of medicinal plants from indigenous pharmacopeias that have been shown to have significant healing power, either in their natural state or as the source of new pharmaceuticals.

Free radicals play an important role in development of different disorders of human body (inflammatory-immune injury, carcinogenesis, hepatic toxicity, atherosclerosis and lower-urinary tract disorders). Each antioxidant play its role in isolation from the others, but there is a dynamic interplay among the defence systems, with the various antioxidants cycles acting to prevent cell damage and disease. Combinations of antioxidants work better than separate antioxidants alone. It was thought that increased metabolism responsible for continous oxidative stress present in gastrointestinal tract, accordingly needed to focus attention on traditional medicinal herbs, applied in the prevention and therapy of gastrointestinal problems and disorders of the urinary tract. The antioxidant role of a wide spectrum of natural products has been established, thus it is advisable to investigate antioxidant activity as well as content and composition of natural phenolics in a series of medicinal plants with phytotherapeutical significance.

Medicinal plants and phytopharmaceuticals have been used successfully in the therapy of the urinary tract with parallel administration of synthetic drugs. Representatives of Solidago species have been used in European phytotherapy for centuries as a component of ulroteological and antiphlogistical remedies. The sale of herbal medicines has increased considerably over the last 10 years in the industrialised countries, mainly due to the globalising market and continuously increasing demand for healthier life. This growing trend to use herbal medicines to treat a wide range of problems has supported among others the release of a number of phytopharmaceuticals containing Solidago species – mostly common goldenrod (Solidago virgaurea L.) in European countries, especially in Germany - as active ingredient. The efficacy of these herbal remedies has been proved in numerous studies and clinical trials, wherethrough public attention turned to the whole Solidago genus. Owing to the increasing interest, other Solidago species nowadays form an integral part of European phytopharmaceuticals with parallel administration of synthetic drugs. Representatives of Solidago species must be taken into account as a constituent of new phytopharmaceuticals.

Taking the traditional application form of Solidaginis herba into consideration, phytochemical and biological investigation of active ingredients both can contribute to ensure the relative safety of self medication and provide new scientific information for the further development of modern herbal medicines. Since high quality for herbal products may be assured if the starting materials are defined rigorously and in great detail, the Member States of European Union refer to these definitions in granting marketing authorizations. In this field, the European Pharmacopoeia - in special cases the National Pharmacopoeia of any Member State - provides a collection of official, high standard monographs on herbal drugs, facilitating the harmonisation of their quality (65/65/EEC, 75/319/EEC).

The quality control of collected plant material and determination of total flavonoid content of the dried Solidaginis herba were performed according to the instructions of the X. German Pharmacopoeia. Owing to the similar appearance of Centre European Solidago species other chromatographic and spectroscopic methods have been used in addition. We have applied thin layer chromatography (TLC), high performance liquid chromatography (HPLC), liquid chromatography connected to mass spectrometric detection (LC/MS-APCI and LC/MS-ESI) and inductively coupled plasma emission spectrometry (ICP-OES) technologies to evaluate the exact phenoloid and mineral element composition of Solidaginis herba. Applied methods have enabled more specific chemotaxonomic classification for Solidaginis herba drug.

The aim of our work was to study the dissolution behaviour of some organic ingredients and micronutrients in various commonly applied plant extracts (Infusum-, Decoctum-, Maceratum solidaginis, and Tinctura solidaginis 40-, 70-, 96% ethanol). Comparison of liquid chromatography (HPLC) and capillary electrophoresis (CE) was performed regarding the efficacy in the analysis of phenoloid composition of studied extracts. As a result of reproducibility, repeatability, recovery and sensitivity tests the more sensitive HPLC method have been chosen to follow the dissolution of characteristic active ingredients to traditionally applied phytopharmaceuticals.

Solidaginis herba contains substantial quantities of agents that have a considerable role in prevention. Regarding to long-term efficacy, tolerability and effectiveness of this medical treatment validation of traditional use and careful quality control is needed. Biological activity of the drug and its phytopharmaceuticals has been confirmed in a combined test method comparing to the antioxidant activity of silibinin, with the aim of confirm the efficacy of the drug observed during the traditional application, thereby strengthen the hypothesis that these active ingredients are effective in the prevention of patbiochemical processes generated by oxidative stress. To estimate the contribution of polyphenolic compounds of extracts to the non-enzymatic antioxidant defence system of human body H-donor activity-, reducing power-, chelating activity- and total scavenger capacity tests were performed, while thiobarbituric acid (TBA) assay was applied to determine liposome lipidperoxidation inhibiting properties of samples. Hydrogen donating ability of extracts was quantified in the presence of 1,1-diphenyl-picrylhydradyl stable radical (DPPH), and for determination of scavenger capacity of water-soluble substances in Solidago herbal preparations the method of photochemiluminescence was used. Glutathione S-transfense (GST) inducing activity of Solidaginis herba extracts was assayed using human hepatoma cell line (HepG2) for the contribution to the enzymatic antioxidative defence system, to get information simultaneously about lipid peroxidation inhibiting and chemopreventive activity of extracts.
2. MATERIALS AND METHODS

2.1. Plant material

Plant material was collected before full flowering on abandoned farmlands in the vicinity of Göd-Hungary (1998. August, sample “A”) and near to Mátészalka-Hungary (2001. June, sample “B”) and identified as Solidago canadensis L. (Asteraceae) in the Department of Pharmacognosy, Semmelweis University, where a herbarium specimen has also been deposited. Aerial parts were used for the extractions as Solidaginis herba according to the Hungarian Standard (MSZ 12341-86).

2.2. Preparation of samples

Air-dried herb was extracted with MeOH in Soxhlet apparatus according to the recommendations of VII. Hungarian Pharmacopoeia (PhHgVII.). Owing to the mostly available data about traditional application of Solidaginis herba decoction, infusion, maceration (Decoctum solidaginis, Infusum solidaginis, Maceratum solidaginis) and different tinctures (Tinctura solidaginis 40-, 70-, 96% ethanol) were used to make aqueous and alcoholic extracts from plant drugs. For preparation of extractions the drug (V.) and solvents were used in the ratio of 1:40. After freeze-drying of aqueous pharmaceuticals (LABOR-MIM, 60ºC, sample -20ºC → 30ºC, t = 13 hours, p = 12-13Pa) and evaporating of tinctures (BÜCHI rotary evaporator) dry extracts have been achived.

2.3. Phytochemical analysis

2.3.1. Flavonoid content

- Total flavonoid content of the dried Solidaginis herba and extracts were determined by spectrophotometry according to the instructions of the X. German Pharmacopoea, after acidic hydrolysis with HCl and complexation with AlCl3. Glycosides and aglycones were jointly determined in aglycon form, and flavonoid content was calculated as hyperoside.

2.3.2. Chromatographic methods (qualitative and quantitative analysis of phenoloid content)

- Thin layer chromatography - Kieselgel 60F254 (Merck, 0,2 mm), 100x200 mm. Eluent: Flavonol aglycons - chloroform-aceton-formic acid (75:16.5:8.5), flavonol glycosides – ethyl acetate-formic acid-acetic acid-water (100:11:11:24).

- RP-HPLC (ABL&E JASCO HPLC, column: Hypersil ODS (C-18, 250 x 4,6 mm, 5µm, eluent: acetonitril/water+2,5%acetic acid).

- CE-CZE (Hewlett Packard, capillary: standard, fused silica, 50 µm, 70 cm, 25 kV, 25ºC, buffer: 25mM sodium tetraborate, pH 9.5, 20% MeOH).

- LC/MS-APCI (Thermo-Finnigan-Surveyor, column: Merck LiChrosphere 5µm, 250 x 4 mm, eluent: water/methanol/methanol+5%acetic acid, range (full): m/z 125-1200)

- LC/MS-ESI (1100 HPLC Hewlet Packard, column: Hypersil ODS 5µm, 250 x 4,6 mm, water/acetonitril:methanol-1:1/water+5%acetic acid, range (full): m/z 150-1000).

2.3.3. Mineral element content

- ICP-OES (Thermo Jarrell Ash, spectrometer: Atom Scan 25, generator: 2 kW, 27,12 MHz, determined elements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Hg, Mg, Mn, Na, P, Pb, S, Ti, Zn)

2.4. In vitro antioxidant assays

- DPPH assay – spray reagent: ethanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH•) stabil free radical

- Hydrogen donating ability - in the presence of 1,1-diphenyl-2-picrylhydrazyl stable radical (DPPH•), scavenging effect of DPPH radical was determined as described on the basis of Blois method, modified by Hatano and co-workers

- Reducing power – spectrophotometric method of Oyazu, according to Fe3+ → Fe2+ conversion reaction (700 nm).

- Chelating activity – spectrophotometric detection of the bithromomic shift produced by chelation of copper to the flavonoid structure in hexamine buffer, in the presence of tetramethyl murexide as an indicator (480 nm → 530 nm) according to method of Hatano.

- Integral antioxidative capacity, Photochem® – chemiluminometric detection of emitted monochromatic light (luminol → amino-phthalic acid) during the scavenging reaction of superoxide, originated from optical excitation.

- Total scavenger capacity, Lumat® - chemiluminometric method of Blázovics with Lumat LB 9051 luminometer in the H2O2/•OH-luminol-microperoxidase system. Unstable free radicals (‘OH) originating from H2O2 via the Fenton reaction result in dissolving of luminol to amino-phthalic acid, when monochromatic light is emitted.
Liposome lipid peroxidation assay – the genesis of \( *OH \) due to the prooxidant activity of ascorbic acid in the presence of transition metals (Fe\(^3\)) - induces damage to liposomes. The extent of damage and hence the antioxidant efficacy of any compound can be monitored and quantified with the aid of thiobarbituric acid.

Induction of glutation S-transferase\(_2\) (GST) – simple in vitro method of Habig was optimised for assaying the GST activity, and thus the enzyme inducing effect of plant extracts using human hepatoma cell line HepG2. 1-chloro-2,4-dinitrobenzene (CDNB), and reduced glutathione (GSH) were used as a relatively non-specific substrate for GST activity.

3. NOVEL SCIENTIFIC RESULTS

3.1. Phytochemical analysis of Solidaginis herba extracts

3.1.1. Quality control of collected Solidaginis herba drug

Standardisation of canadian goldenrod was achieved according the recommendations of X. German Pharmacopoeia, on principles to work out a rapid, simple and cost-effective test method for the quality control of the drug. Botanical identification was performed with respect to the morphological similarities of Centre European Solidago species. The stem and leaf pubescence were investigated, as a diagnostic feature to distinguish the most similar taxa from each other. Alongside the leaves 2-3 celled, hooked hairs, and multi-cellular, attenuated so-called “kneeing” hairs on the surface of the leaves were identified as a characteristic mark of Solidago Canadensis L. The quality control was performed according the requirements of X. German Pharmacopoeia (DAB 10). Investigation for physical and chemical impurities, weight-loss during exsecation, ash- and sand-content of the drug, qualitative (TLC) and quantitative (flavonoid content) analysis of active ingredients were accomplished. Quality of Solidaginis herba drug corresponded to the requirements of the DAB 10. Collected plant material was identified as Solidago Canadensis L. Its purity and flavonoid content were in acceptable range („A”=35,42 mg g\(^-1\) drug (3,542%) és „B”= 51,09 mg g\(^-1\) drug (5,109%), therefore appropriate for further phytochemical and biological analysis.

3.1.2. LC/MS analysis of Solidaginis herba

We have applied HPLC, LC/MS-APCI and LC/MS-ESI technologies to evaluate the exact phenoloid composition of Solidaginis herba. Quercetin-3-O-β-glucoside (isoquercitrin), quercetin-3-O-β-galactoside (hyperoside), quercetin-3-O-β-rhamnoside (quercitrin), quercetin-3-O-β-rutinoside (rutin), kaempferol-3-O-β-rhamnoside (afzelin), kaempferol-3-O-β-rutinoside (nicotiflorin), caffeoil-quinic acid (chlorogenic acid) were identified in sample „A”, while the presence of quercetin, quercetin-3-O-β-glucoside (isoquercitrin), quercetin-3-/6”-O-acetyl-/β-glucopiranoside, quercetin-3-O-β-rutinoside (rutin), kaempferol, kaempferol-3-O-β-glucoside (astragalin), kaempferol-3-/6”-O-acetyl-/β-glucopiranoside, isorhamnetin, isorhamnetin-3-/6”-O-acetyl-/β-glucopiranoside, isorhamnetin-3-O-β-rutinoside (narcissin), caffeoil-quinic acid (chlorogenic acid), caffeoil-shikimic acid-glucoside (dattelic acid-glucoside) were confirmed in sample „B”. According the occurrence of acetyl-glycosides and the diversity of sugar component of flavonoid glycosides Solidaginis herba samples chemotaxonomically were classified into different varieties. Sample „A” was identified as Solidago canadensis L. var. canadensis, while sample „B” has proved to be the part of Solidago canadensis L. var. scabra.

Due to the same flavonoid aglycons and the large amounts of flavonol glycosides occurring in each drug, phytochemical characteristics of investigated samples proved to be very similar.

3.1.3. Comparison of HPLC and CE techniques in the analysis of Solidaginis herba

Regarding the efficacy of phytootechnological processes and to accomplish continuous quality control, rapid and sensitive analytical methods are needed. For this purpose comparison of liquid chromatography (HPLC) and capillary electrophoresis (CE) was performed. Both chromatographic and capillary electrophoresis methods were investigated for the convenience and reliability for the routine analysis of characteristic polyphenolic compounds of Solidaginis herba (quercetin, quercetin glycosides and chlorogenic acid). Both HPLC and CE methods were found to be reliable and compatible. The reproducibility of within-day assay using both methods was generally ≥90%, and 75% in the instance of between-days. Using HPLC, the retention times of all analytes were reproducible. The relative standard deviations (RSDs) of <3% for within-day assay and <5% for between-days analysis. For the Ce method, the RSDs of migration time for within-day and between-days analysis were <1% and <2% respectively. The analysis time of CE was three-times faster, however it is ten-times less sensitive than HPLC, which has detection limits of 0,5 µg/ml.

According the UV spectra of phenoloid compounds chromatographic method was appropriate to confirm the presence of ten flavonoids, and two caffeic acid derivatives in the samples, while applying CE technique only nine flavonoids, but three hydroxycinnamates were detectable in Solidaginis herba. Therefore CE method proved to be more sensitive for the analysis of hydroxycinnamates, and HPLC method showed more efficacy in the analysis of flavonoid compounds.
3.1.4. Analysis of mineral element content

Methanolic and commonly applied aqueous and alcoholic extracts of Solidaginis herba were analysed for Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Hg, Mg, Mn, Na, P, Pb, S, Ti, Zn content. The concentrations of the minerals examined were determined by inductively coupled plasma emission spectrometry (ICP-OES).

According the results achieved we can conclude that mineral element content of Solidaginis herba is similar to the average, and nutritive value of extracts is adequate in the instance of most of the elements. In our experiment no toxic elements (As, Cd, Hg, Pb) were measured in higher concentration than the detection limit.

Solidaginis herba proved to be good source of potassium (16781±19,104 µg g\(^{-1}\)), magnesium (1961,9±1,025 µg g\(^{-1}\)), iron (9,21±0,0025 µg g\(^{-1}\)) and zinc (24,04±0,4049 µg g\(^{-1}\)). Accurate selection of the pharmaceutical technology required for curing various health problems might result in high amounts of mineral element intake.

Regarding the phytotherapeutical application of Solidaginis herba and its extracts distribution of mineral elements was found to be favourable. Occurrence of antioxidant elements (Mg, Mn, Zn) in relatively high amount and high potassium-sodium ratio (K/Na=137), with negligible quantity of toxic element together can contribute to increase of therapeutic quality of the drug.

3.2. In vitro antioxidant activity tests

Biological activity of Solidaginis herba drug has been investigated in a combined test method, with the aim of confirm the contribution of its characteristic phenoloid compounds, possessing antioxidant activity according the scientific findings of last century.

In the current experiment dose-dependent in vitro antioxidant- and free radical scavenging activity of methanolic extract obtained from Solidaginis herba was observed. Investigated samples were acted as primary (chain-breaking) and secondary (preventive) antioxidants, and additionally membrane protecting activity of Solidaginis herba extracts has been proved.

Our observations proved that the crude methanolic extract of Solidaginis herba investigated in DPPH assay have a significant hydrogen-donating ability which is concentration dependent in the presence of 1,1-diphenyl-2-picrylhydrazyl radical.

Hydrogen donating activity was expressed as I\(_{50}\), that is the amount of the sample that results in a 50% decrease of colour intensity of DPPH at 517 nm. Activity of the investigated extract at 0,1 mg/ml concentration (1744 nmolASE/mg) was comparable to the results obtained applying silibinin at 0,001 M concentration (436 nmolASE/mg), as a positive control with well documented antiradical and antioxidant activity.

For the characterisation of the scavenging effect of the plant extract on hydroxyl radical and the total scavenging capacity in \(\text{H}_2\text{O}_2/\cdot \text{OH}\)-microperoxidase/luminol system chemiluminescence technique was applied. Methanolic extract of Solidaginis herba drug was capable of scavenging hydroxyl radical in a concentration dependent manner. The activity was comparable to the effect of silibinin (I\(_{50}=56 \text{ mg l}^{-1}\)).

The lipid peroxidation assay on standardised liposome system underlined the results of the previous experiments. In vitro membrane protecting activity of methanolic extract at 0,01 mM concentration was comparable to the activity of authenticated antioxidant propyl gallate. Solidaginis herba has inhibited liposome lipid peroxidation in dose dependent manner (I\(_{50}=27,152 \text{ mg l}^{-1}\)), initiated previously by simultaneous presence of ascorbic acid and the transition metal Fe\(^{3+}\) in low concentration.

Glutathione S-transferase (GST) inducing activity of Solidaginis herba extracts was assayed using human hepatoma cell line (HepG2) for the contribution to the enzymatic antioxidative defence system of human body.

Different fractions obtained from methanolic extract of Solidaginis herba were also investigated for in vitro GST inducing activity. The results were compared to the effect of characteristic flavonoid compounds (quercetin, quercitrin and rutin representing the model structures of flavonol-aglycon, -monoglucoside and -diglucoside) of the drug, when \(\beta\)-naphthoflavone was used as positive control with well documented phase II metabolising enzyme inducing activity.

Application of methanolic extract of Solidaginis herba in the dosing solution a slight dose-dependent inhibition of the enzyme was observed. Hep G2 cells were exposed to selected fractions of Solidaginis herba obtained column chromatography, in order to investigate whether the flavonoids were responsible for the effect of the crude extract.

As it was expected the flavonoid (mainly flavonol glycosides) reach fraction, caused a slight induction on the enzyme, while dose dependent enzyme-inhibition was observed applying the fraction, containing mainly caffeic acid derivatives, but the toxic effect of the saponins present in large amount in the samples has encumbered the evaluation of the results.

Application of pure flavonoid standards caused a dose-dependent change in the activity of GST without any critical influence on cell-viability. The flavonoid glycosides exerted significant inducing effect in HepG2 cells, while definitive inhibition was observed in case of the aglycon (rutin: 51,36%, quercitrin: 24,55%, quercetin: 30,15%).

Current results demonstrate that glutathione S-transferase inducing ability of quercetin derivatives depends on the sugar moiety connected to the molecule, although inhibiting activity of the aglycon was observed. Regarding the results obtained in the assay, contribution of flavonoid glycosides to the in vitro GST inducing activity of Solidaginis herba, and therefore the possibility for contribution to enzymatic antioxidative defence system of human body can be concluded.
3.3. Dissolution of active ingredients from Solidaginis herba to traditionally applied phytopharmaceuticals obtained by different technologies

High-performance liquid chromatography (HPLC) has been used to determine the phytochemical characteristics of the classical herbal tea extracts. Our observations proved that the applied extraction technologies resulted efficient dissolution of phenolic compounds from crude drug to the extracts. The dissolution of flavonoids and organic acids to alcoholic extracts depends mainly on the solvent polarity, while aqueous preparations resulted differences in flavonoids due to the temperature and duration of extraction.

Tinctura solidaginis 70 v/v%-ethanol (79.2%) and Infusum solidaginis (63.7%) proved to be the best source of flavonoids. Varying the extraction technologies, additionally dissolution of hydroxycinnamates was observed in considerable amounts. Highest level of chlorogenic acid was found in aqueous extracts.

Rutin was the major flavonoid compound in both crude drug and extracts. Tinctura solidaginis 70 v/v%-ethanol (572.5 mg L⁻¹) and Infusum solidaginis (559.18 mg L⁻¹) proved to be the best source of this compound, while dissolution of chlorogenic acid

The highest level of this compound was found in Tinctura solidaginis 70 v/v%-ethanol (572.5 mg L⁻¹), but increased release was observed into Infusum solidaginis (559.18 mg L⁻¹) compared to other extracts, while highest amount of chlorogenic acid was measured in aqueous preparations (Infusum solidaginis – 357.5 mg L⁻¹) corresponding to our expectations.

Regarding the molecular weight of polyphenolic compounds, molar concentrations of model phenoloids were calculated in studied extracts. Molar concentrations of the main hydroxycinnamate chlorogenic acid and of the main flavonoid compound rutin was comparable to each other in all cases, moreover in the instance of Decoctum solidaginis and Maceratum solidaginis the level of chlorogenic acid proved to be higher.

<table>
<thead>
<tr>
<th>Solidaginis herba</th>
<th>Tinctura solidaginis 40%-ethanol</th>
<th>[µM]</th>
<th>Tinctura solidaginis 70%-ethanol</th>
<th>[µM]</th>
<th>Tinctura solidaginis 96%-ethanol</th>
<th>[µM]</th>
<th>Infusum solidaginis</th>
<th>[µM]</th>
<th>Decoctum solidaginis</th>
<th>[µM]</th>
<th>Maceratum solidaginis</th>
<th>[µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorogenic acid</td>
<td>45.12</td>
<td>786.7</td>
<td>785.6</td>
<td>461.9</td>
<td>1009.8</td>
<td>905.3</td>
<td>421.4</td>
<td></td>
<td></td>
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<tr>
<td>Rutin</td>
<td>44.75</td>
<td>415.9</td>
<td>941.9</td>
<td>646.8</td>
<td>916.5</td>
<td>605.9</td>
<td>18.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isoquercitrin</td>
<td>8.63</td>
<td>76.5</td>
<td>134.8</td>
<td>136.1</td>
<td>167.9</td>
<td>111.6</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Quercetin</td>
<td>12.79</td>
<td>108.4</td>
<td>187.9</td>
<td>186.9</td>
<td>244.7</td>
<td>163.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quercitrin</td>
<td>-</td>
<td>112.5</td>
<td>30.1</td>
<td>3.4</td>
<td>-</td>
<td>-</td>
<td>31.4</td>
<td></td>
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</table>

Dissolution of mineral elements into various extracts obtained by different phytotechnologies was followed by inductively coupled plasma optical emission spectrometry (ICP-OES).

According the high mineral element content of extracts prepared by aqueous technologies, the importance of solvent’s polarity have been concluded. Tinctura solidaginis (96%/v/-ethanol) proved to be the worst source of mineral elements, none of them were measured in prominent concentration. The highest amount of copper (0.166 mg l⁻¹) and zinc (0.353 mg l⁻¹) was found in Tinctura solidaginis (70%v/v-ethanol), while Tinctura solidaginis (40%/v/-ethanol) contained more sodium (1.968 mg l⁻¹) than other extracts. The highest quantity of aluminium (0.888 mg l⁻¹), boron (0.493 mg l⁻¹), calcium (45.48 mg l⁻¹), magnesium (25.34 mg l⁻¹) was found in Infusum solidaginis, while Decoctum solidaginis contained more barium (0.0485 mg l⁻¹), cobalt (0.0077 mg l⁻¹), chromium (0.0281 mg l⁻¹) and manganese (0.431 mg l⁻¹) than other extracts. Maceratum solidaginis was the best source for the remaining minerals.

Nutritive value of the samples studied was characterised by comparing nutrient concentrations of different extracts and U.S. Recommended Dietary Allowances (RDA, 1989). Aqueous extracts proved to be very good sources of chromium (RDA 50-200 µg/day/adult), potassium (RDA 2000-3500 mg/day/adult) and manganese (RDA 2-5 mg/day/adult). In the case of calcium (RDA 800 mg/day/adult), copper (RDA 1.5-3 mg/day/adult), magnesium (RDA 350 mg/day/adult) and phosphorus (RDA 800 mg/day/adult) recommended consumption of herbal teas can ensure the daily needs. Although the concentration of the other elements measured in aqueous extracts were overmatched, even such small amounts of iron (RDA 10 mg/day/adult) and zinc (RDA 15 mg/day/adult) may ensure the supply of the recommended doses (1-2%).

Tinctura solidaginis (70%/v/-ethanol) containing highest amount of flavonoids accompanied with considerable amounts of antioxidant elements possessing good capabilities to form complexes. This observation arises the hypothesis of the possibilities of influencing the dissolution behavior of mineral elements by polyphenolic compound during simultaneous dissolution.

The potassium-sodium ratio was calculated according to the measured element concentrations. The highest ratio was observed in Maceratum solidaginis (360) and Infusum solidaginis (299), while a high value of Tinctura solidaginis (70%-ethanol) (267) combined with highest flavonoid content could be observed.

3.4. Antioxidant activity of traditional phytopharmaceuticals of Solidaginis herba obtained by different technologies

Correlation of phytochemical characteristics and antioxidative properties of phytopharmaceuticals have been examined for the release of phenolic compounds and their in vitro antioxidant activity, using the previously developed test method.

Dose-dependent in vitro antioxidant- and free radical scavenging activity of traditional phytopharmaceuticals obtained from Solidaginis herba was observed. Investigated samples were acted
as primary (chain-breaking) and secondary (preventive) antioxidants, and additionally membrane protecting activity of extracts has been proved. H-donor activity and reducing power of the extracts mainly correlated with chlorogenic acid content, arising the possibility of higher activity of hydroxycinnamates than flavonoid compounds. According to our expectations chelating activity of the extract was in tight correlation with the flavonoid content of the samples, due to the role of binding sites for trace metals on B, and C rings of flavonol glycosides in formation of chelat-complexes.

Table 2. Correlation between antioxidant properties and the content of main phenoloid compounds of investigated traditional phytopharmaceuticals of Solidaginis herba

<table>
<thead>
<tr>
<th>Antioxidant Activity</th>
<th>Rutin Content</th>
<th>Chlorogenic Acid Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reducing power (ASE)</td>
<td>r = 0.5117</td>
<td>r = 0.7606</td>
</tr>
<tr>
<td>H-donating activity (μmol/g)</td>
<td>r = 0.7911</td>
<td>r = 0.9540</td>
</tr>
<tr>
<td>Chelating activity (μmol/g)</td>
<td>r = 0.9445</td>
<td>r = 0.5894</td>
</tr>
<tr>
<td>Antioxidant capacity (μmol/g)</td>
<td>r = 0.9842</td>
<td>r = 0.4881</td>
</tr>
<tr>
<td>Total scavenger capacity (RLU)</td>
<td>r = 0.8528</td>
<td>r = 0.8223</td>
</tr>
<tr>
<td>Inhibition of lipid peroxidation (%)</td>
<td>r = 0.8651</td>
<td>r = 0.8603</td>
</tr>
</tbody>
</table>

Superoxide scavenging activity were correlated with rutin content of the samples. Hydroxyl radical scavenging- and membrane protecting activity were mainly influenced by both flavonol glycoside and hydroxycinnamates of the extracts.

For the determination of the scavenging capacity of Solidago herbal preparations against superoxide and hydroxyl radical the method of photochemiluminescence was used. According the results obtained in this experiment scavenging activity against hydroxyl radical generated in H₂O₂•OH-microperoxidase/luminol system correlated similarly both with the rutin and chlorogenic acid content of the samples, possibly due to the high reactivity of the radical, while flavonoid compounds proved to be more effective in scavenging superoxide than hydroxycinnamates, as we determined in instrument Photochem®.

Liposome lipidperoxidation inhibiting property of Solidaginis herba extracts determined in TBA assay similarly to scavenger capacity was in correlation with both the flavonoid and chlorogenic acid content.

Summarising the results of antioxidant activity tests, H-donor activity and reducing power of the extracts mainly correlated with chlorogenic acid content, while chelating- and superoxide scavenging activity were correlated with rutin content of the samples. Hydroxyl radical scavenging- and membrane protecting activity were mainly influenced by both flavonol glycoside and hydroxycinnamates of the extracts.

4. ACKNOWLEDGEMENT

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5. PUBLICATIONS

5.1. In the topics of thesis


5.2. Partially in the topics of thesis


6. PRESENTATIONS, POSTERS

6.1. Citable abstracts


6.2. Abstracts


