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Herbal remedies of Solidago—correlation of phytochemical characteristics and antioxidative properties

P. Apáti [a,b,*], K. Szentmihályi [b], Sz.T. Kristó [a], I. Papp [a], P. Vinkler [b], É. Szoke [a], Á. Kéry [a]

[a] *Faculty of Pharmacy, Department of Pharmacognosy, Semmelweis University, Budapest Ülloi str.26, Hungary*
[b] *Hungarian Academy of Sciences, Chemical Research Centre, Institute of Chemistry, Budapest Pusztaszeri út 59-67, Hungary*

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Abstract

In this study the correlation of phytochemical characteristics and antioxidative properties of classical herbal tea extracts—*Infusum solidaginis*, *Decoctum solidaginis*, *Maceratum solidaginis*—and tinctures prepared by various concentration of ethanol (40, 70, 96% v/v) have been examined for the release of flavonoids and their antioxidant activity. Quantitative and composition determination of flavonoids were carried out by spectrophotometry, high-performance liquid chromatography and capillary electrophoresis, respectively. Hydrogen-donating ability and reducing power properties were used to define in vitro radical scavenging activity of *Solidago* extracts, but integral antioxidative capacity was determined by luminometry (Photochem®), calculating the ascorbic acid equivalents. Chlorogenic acid, quercetin-3-*O*-β-D-rutinoside, quercetin-3-*O*-β-D-galactoside, quercetin-3-*O*-β-D-glucoside, quercetin-3-*O*-β-D-rhamnoside, kaempferol-3-*O*-α-L-rhamnoside and quercetin were confirmed by retention times and UV spectra. Based on the dissolution rate, variance of flavonoid release and ascorbic acid equivalents it was concluded, that *Tinctura solidaginis* (70% v/v ethanol) and *Infusum solidaginis* are the most appropriate preparations.

Keywords: *Solidago canadensis* L.; Classical herbal extracts; Flavonoids; H-donating activity; Reducing power; Integral antioxidative capacity; Luminometry

1. Introduction

Herbal remedies are increasingly used by general public to replace or supplement conventional medicine. Canadian goldenrod (*Solidago canadensis* L., Asteraceae) has been used in European phytotherapy for 700 years for the treatment of chronic nephritis, cystitis, urolithiasis, rheumatism and as an antiphlogistic drug [1–3].

The importance of polyphenolic compounds in different in vivo free radical-mediated deleterious-degeneration of lipid reach structures, e.g. membranes, a process known to be involved in the etiology and the pathology of numerous diseases [4–6]. The use of synthetic antioxidants to prevent free radical damage is frequently accompanied by

* Corresponding author. Fax: +36-1-317-2979.
E-mail address: apatipal@drog.sote.hu (P. Apáti).

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toxic side effects. This makes interesting to investigate natural compounds with antioxidant properties [7,8].

Most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals. The flavonoids are one of the most numerous and widespread groups of natural constituents in the plant kingdom, some of them due to inhibit free radical-mediated processes. As a rule flavonoids have low toxicity which, combined with high antioxidant capacity, makes these compounds extremely useful as pharmacological agents. The capacity of flavonoids to act as antioxidants in vitro has been the subject of several studies in the past years, and important structure-activity relationships of the antioxidant activity have been established [9–11].

Medicinal plants containing phenolic substances are in the centre of interest, however the release of biologically active ingredients—responsible for antioxidant activity—from plant material is less documented.

In this study the correlation of phytochemical characteristics and antioxidative properties of classical herbal tea extracts and tinctures prepared by various concentration of ethanol have been examined for the release of flavonoids and their antioxidant activity.

2. Materials and methods

2.1. Reagents, standards and plant material

Methanolic solutions (200 μg ml^{-1}) of the following pure commercial substances were employed: chlorogenic acid, rutin, hyperoside, isoquercitrin, afzelin, quercetin from Sigma. Methanol and acetonitrile of high-performance liquid chromatography (HPLC) grade, and all other chemicals of analytical-reagent grade were from Carlo Erba and REANAL, respectively. HPLC grade water was prepared by double distillation. All solvents were filtered through 0.5 μm (Millipore) membranes and degassed in an ultrasonic bath, while Solidaginis herba extracts were passed through C-18 cartridge before use.

Solidago canadensis L. was collected as wild plant in Hungary and was identified in the Department of Pharmacognosy, Semmelweis University where herbarium specimen is also deposited [12,13].

2.2. Extraction procedure

Air-dried herb was extracted with MeOH in Soxhlet apparatus. This solution were concentrated to give a dried residue, and before investigation it was redissolved in MeOH and purified for HPLC analysis.

Decoction, infusion, maceration and different tinctures were used to make aqueous and alcoholic extracts from plant drugs. For preparation of extractions the drug and solvents were used in the ratio of 1:40.

The drug was boiled for *Decoctum solidaginis* in bidistilled water for 5 min, while it was infused with boiling water for *Infusum solidaginis*. The hot mixture was filtered immediately or after cooling for decoction and infusion, respectively. In the case of *Maceratum solidaginis* the drug had been macerated in bidistilled water at room temperature for a day and then filtered.

To obtain *Tinctura solidaginis* the drug had been steeped in diluted (40, 70, 96% v/v) ethanol for 6 days and then filtered.

2.3. HPLC conditions

HPLC separation was performed with an ABL&E Jasco system consisting of PU-980 gradient pump and RHEODYNE 7725 (20 μl) injector. The instrument was equipped with a PU-980 UV–VIS detector in combination with and IBM-PC and fitted with a Hypersil ODS (5 μm) reverse-phase C-18 column (250×4 mm^2) protected with a precolumn of the same material.

Two solvent mixtures were employed for elution:

Eluent A: AcCN
Eluent B: H_2O–CH_3COOH (40:1)

Separation was achieved at ambient temperature with a flow rate 1.0 ml min^{-1}. The gradient

begun with 14% eluent A and 86% eluent B, and was held at this concentration for the first 15 min. This was followed by a linear gradient to 35% eluent A over the next 30 min and then a sharp transition to 100% eluent A over the next 2 min. Data were collected at 360 nm. Peaks were identified by co-chromatography with authentic standards and according to UV spectra and retention times.

2.4. Antiradical activity

Hydrogen-donating ability of extracts was quantified in the presence of 1,1-diphenyl-picryl-hydrazyl stable radical (DPPH). The scavenging effect of DPPH radical was determined as described on the basis of Blois method [14], modified by Hatano et al. [15]. Used as a reagent, DPPH—which presents an intensive purple colour at 517 nm-evidently offers a convenient and accurate method for titrating the oxidizable groups of natural or synthetic antioxidants [16]. The degradation of DPPH was evaluated by comparison with a control sample, which did not contain hydrogen-donating compounds.

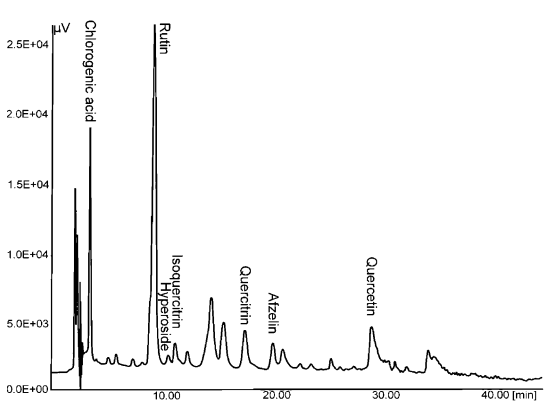
Aliquot parts of extracts (100, 50, 25 and 12.5 μg; 1 ml methanol) were added to a methanolic solution of DPPH (20 μg, 1 ml). The mixture was shaken and left to stand at room temperature for 30 min. The degree of decoloration indicates the scavenging efficiency of the added substances. The characteristic 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.

The reducing power of the samples was measured according to the spectrophotometric method of Oyazu [17]. Aliquot parts of the extracts (1 ml extract or 0.02 g freeze-dried sample in 1 ml methanol) were mixed with phosphate buffer (2.5 ml, 0.2 mol l^{-1}, pH 6.6) and potassium ferricyanide /$K_3Fe(CN)_6$/(2.5 ml, 1% v/v); the mixture was incubated at 50 °C for 20 min. A portion of trichloroacetic acid (2.5 ml, 10% v/v) was added to the mixture, which was then centrifugated at 3000 rpm, for 10 min. The upper layer of solution (2.5 ml) was mixed with bidistilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1% v/v), and the absorbance was measured at 700 nm. Increased absorbance of

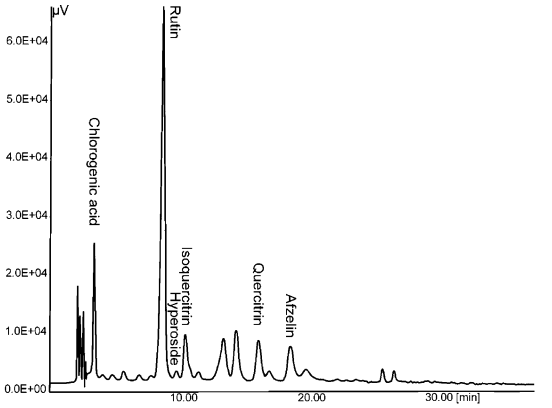
Table 1
HPLC analysis of flavonoid content and dissolution in Solidaginis herba extracts

Component	Soxhlet extract C ± S.D. (mg kg^{-1})	t_R min	Dissolution[a]						
			Tinctura sol. 40%	Tinctura sol. 70%	Tinctura sol. 96%	Infusum sol.	Decoctum sol.	Maceratum sol.
(1) Chlorogenic acid	15307.7 ± 507.4	3.46	72%	78%	42%	98%	85%	36%
(2) rutin	27297.4 ± 585.2	8.92	34%	81%	53%	81%	53%	2%
(3) Hyperoside	249.7 ± 15.1	10.21	30%	65%	58%	59%	65%	~ 0%
(4) Isoquercitrin	4012.6 ± 181.9	10.78	25%	65%	57%	59%	56%	~ 0%
(5) Quercitrin	5747.3 ± 337.2	16.45	25%	59%	44%	59%	40%	~ 0%
(6) Afzelin	6439.8 ± 192.3	18.98	12%	52%	47%	46%	31%	3%
(7) Quercetin	*t*[b]	28.80	ni	ni	ni	ni	ni	ni

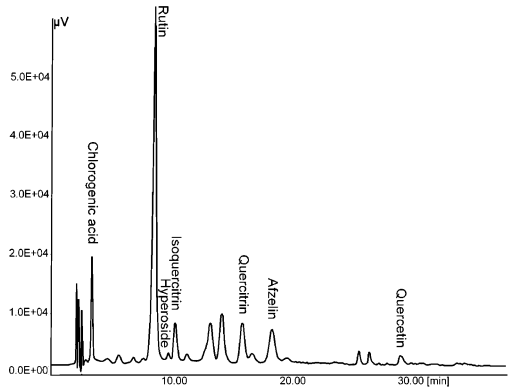
ni—not interpretable.
[a] 100% = Soxhlet extract.
[b] traces.



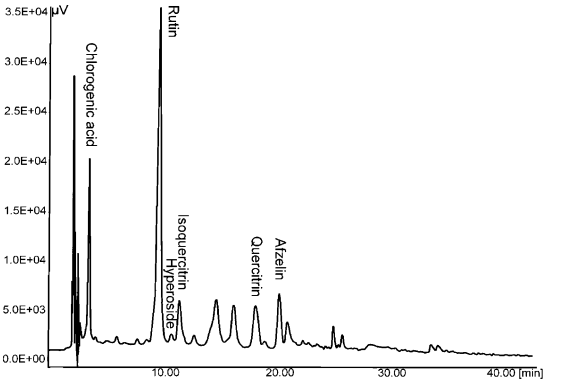
Tinctura solidaginis 40%-ethanol



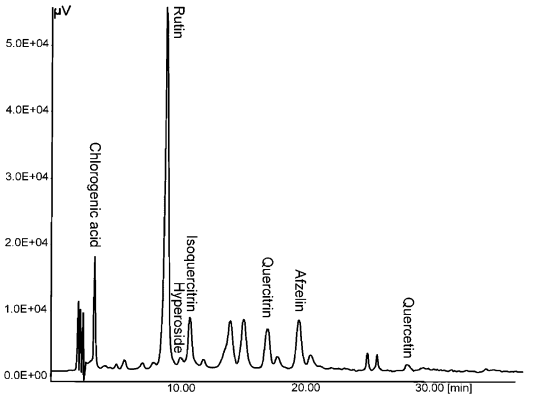
Infusum solidaginis



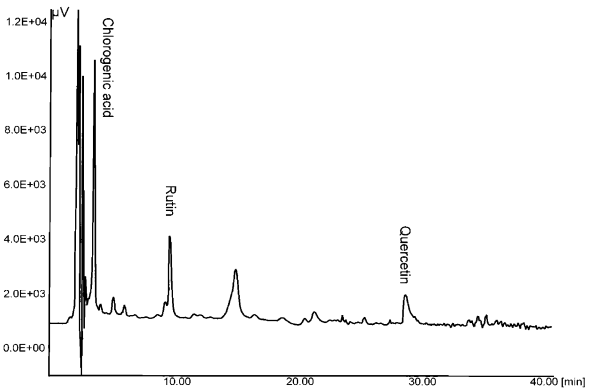
Tinctura solidaginis 70%-ethanol



Decoctum solidaginis



Tinctura solidaginis 96%-ethanol



Maceratum solidaginis

Fig. 1. HPLC analysis of solidaginis herba extracts.

Table 2
Hydrogen-donating activity of different Solidaginis herba extracts

Concentration (mg 100 ml^{-1})	Rutin		Quercetin		Ascorbic acid	
	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)
12.5	0.199±0.011	57.20	0.319±0.018	31.40	0.276±0.034	40.65
25	0.089±0.005	80.86	0.197±0.034	57.63	0.174±0.014	62.58
50	0.012±0.001	97.42	0.079±0.011	83.01	0.072±0.007	84.52
100	0±0.000	100.00	0.015±0.003	96.77	0.012±0.001	97.42
	Aqueous extracts					
	Infusum solidaginis		*Decoctum solidaginis*		*Maceratum solidaginis*	
	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)
12.5	0.346±0.022	25.59	0.358±0.021	23.01	0.407±0.012	12.47
25	0.240±0.015	48.39	0.265±0.010	43.01	0.385±0.008	17.20
50	0.106±.0009	77.20	0.131±0.008	71.83	0.356±0.011	23.44
100	0.025±0.001	94.62	0.029±0.002	93.76	0.323±0.010	30.54
	Alcoholic extracts					
	Tinctura solidaginis 40%		*Tinctura solidaginis* 70%		*Tinctura solidaginis* 96%	
	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)	Absorption (700 nm)	Inhibition (%)
12.5	0.372±0.009	20.00	0.375±0.023	19.35	0.378±0.020	18.71
25	0.321±0.013	30.97	0.301±0.019	35.27	0.346±0.011	25.59
50	0.212±0.008	54.41	0.153±0.09	67.10	0.269±0.012	42.15
100	0.153±0.008	67.10	0.036±0.002	92.26	0.190±0.021	59.14

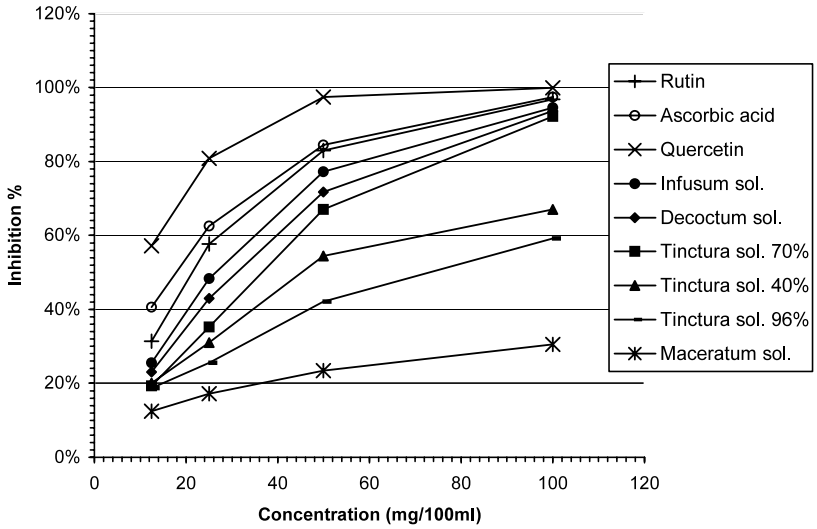


Fig. 2. Hydrogen-donating activity of different solidaginis herba extracts $I_{50\text{-Rutin}}$: <12.5 mg 100 ml^{-1}, $I_{50\text{-Ascorbic acid}}$: 16.61 mg 100 ml^{-1}, $I_{50\text{-Quercetin}}$: 21.29 mg 100 ml^{-1}, $I_{50\text{-}\textit{Infusum solidaginis}}$: 26.72 mg 100 ml^{-1}, $I_{50\text{-}\textit{Decoctum solidaginis}}$: 31.03 mg 100 ml^{-1}, $I_{50\text{-}\textit{Maceratum solidaginis}}$: >100 mg 100 ml^{-1}, $I_{50\text{-}\textit{Tinctura solidaginis 96\%}}$: 73.52 mg 100 ml^{-1}, $I_{50\text{-}\textit{Tinctura solidaginis 70\%}}$: 36.71 mg 100 ml^{-1}, $I_{50\text{-}\textit{Tinctura solidaginis 40\%}}$: 45.65 mg 100 ml^{-1}.

reaction mixture indicated elevated reducing power.

Luminometry: For the determination of the integral antioxidative capacity (AC) of water soluble substances in Solidago herbal preparations the method of photochemiluminescence (PCL) was used.

Apparatus: Photochem®.

Standard kit: ACW (Analitik Jena AG)—where the luminol plays a double role as photosensitizer and also as oxygen radical detection reagent.

Liophylized samples of *Solidago* extracts were measured in appropriate concentrations (0.1 g in 1.00 ml of water; dilutions 1:1000). Results were calculated in ascorbic acid equivalents (μmol g^{-1}).

3. Results and discussion

Three aqueous and three alcoholic extracts prepared according to the traditional methods were examined in present study. HPLC has been used to determine the phytochemical characteristics of the classical herbal tea extracts—*Infusum solidaginis*, *Decoctum solidaginis*, *Maceratum solidaginis*—and tinctures prepared by various concentration of ethanol (40, 70, 96%v/v). During separation process and quantification of phenolics in the samples chlorogenic acid, quercetin-3-*O*-β-D-rutinoside (rutin), quercetin-3-*O*-β-D-galactoside (hyperoside), quercetin-3-*O*-β-D-glucoside (isoquercitrin), quercetin-3-*O*-β-D-rhamnoside (quercitrin), kaempferol-3-*O*-α-L-rhamnoside (afzelin) and quercetin were identified by standard addition, with comparison their retention times and UV spectra. (Table 1, Fig. 1).

Our observations proved that the applied extraction technologies resulted efficient dissolution of phenolic compounds from crude drug to the extracts. Owing to relatively high hydrophilous characteristics of phenolic acids and flavonoid glycosides investigated pharmaceuticals contained large amounts of chlorogenic acid and diglycosidic rutin present in every extracts as the main components. Dissolution rates of *Tinctura solidaginis* 70%v/v-ethanol (81%) and *Infusum solidaginis* (81%) proved to be the best source of rutin, while chlorogenic acid measured at high concentrations and dissolution rates in *Tinctura solidaginis* 40%v/v-ethanol (72%), *Tinctura solidaginis* 70%v/v-ethanol (78%), *Infusum solidaginis* (98%) and *Decoctum solidaginis* (85%). Due to the fact that Solidaginis herba is a reach source of rutin, and because of high dissolution rate of it the characteristics of extracts are presumably mainly depend on the presence of this compound. Similarly to crude drug, only traces of free flavonoids were present in decoction and infusion. Presumably due to the enzyme activity, preparation procedure of extracts, and the relatively long extraction period respectively in aqueous and alcoholic extracts there were detectable amounts of the aglycon quercetin.

These polyphenols have an important role in the prevention of lipid oxidation via direct antioxidant processes and modification of several enzyme activities. The antioxidant activity of extracts were investigated by hydrogen-donating ability, reducing power and scavenging ability determined by luminometry.

The DPPH system is a stable radical-generating procedure. because it can accommodate a large number of samples in a short period, and is sensitive enough to detect active principles at low concentrations, it was used in the present study for primary screening of the antiradical activities of six different extracts obtained from Solidaginis herba. The results in Table 2. and Fig. 2. demonstrate that various preparations investigated have a significant hydrogen-donating ability which are concentration dependent in the presence of 1,1-diphenyl-2-picrylhydrazyl radical.

Hydrogen-donating ability is an index of the primary chain-breaking antioxidants. These antioxidants serve hydrogen to free radicals. This conversion leads to nonradical species and thus to inhibition of the propagation phase of lipid peroxidation [18].

Most nonenzymatic antioxidant activity is mediated by redox reactions. The antioxidant activity of extracts containing polyphenols, as ferric-reducing power, was determined using a modified ferric reducing-antioxidant power assay [19]. Reaction was followed for 20 min, and both standards and samples were dissolved in the same

Table 3
Reducing power of different Solidaginis herba extracts

Sample	ASE ml^{-1}
Rutin	0.68
Quercetin	0.51
Infusum solidaginis	0.56
Decoctum solidaginis	0.95
Maceratum solidaginis	1.25
Tinctura solidaginis 96%	1.05
Tinctura solidaginis 70%	0.78
Tinctura solidaginis 40%	0.59

solvent. Rutin and quercetin standards were included for comparison of results.

The samples exhibited strong reducing power (Table 3.) as can be seen in Fig. 3. The absorbances of the reaction mixtures containing different amounts of rutin and quercetin standards are also demonstrated in this figure. The reducing power of the samples depends on the concentrations in the reaction mixture. Secondary or preventive antioxidants can produce the rate of chain initiation in the lipid peroxidation process or can react with the temporary or end products of lipid peroxidation. This conversion leads to non-radical, non-deleterious products [18].

The reducing power was also expressed as ascorbic acid equivalent (ASE ml^{-1}). When the reducing power is 1 ASE ml^{-1}, the reducing power of 1 ml extract is equivalent to 1 μmol ascorbic acid.

Photochem® apparatus and method allowed precise as well as time and cost-effective determination of the integral AC of *Solidago* extracts [20]. Free radicals are generated in the measuring system itself by means of photosensitizer. The free radicals were detected by their reaction with a chemiluminogenic substance. Luminol played a double role as photosensitizer and also as oxygen radical detection reagent. In the presence of substances in plant extracts acting as radical traps, the intensity of the PCL was attenuated as a function of concentration. In this way, the antiradical properties of the analyzed substances could reliably be quantified. The results for *Solidago* extracts are presented in equivalent concentration units of ascorbic acid (Fig. 4 and Table 4).

4. Conclusions

The present study aimed to evaluate the possible effects of various preparation methods on the antioxidant properties of different Solidaginis herba extracts. These experiments have considered the effects of commonly used domestic methods of

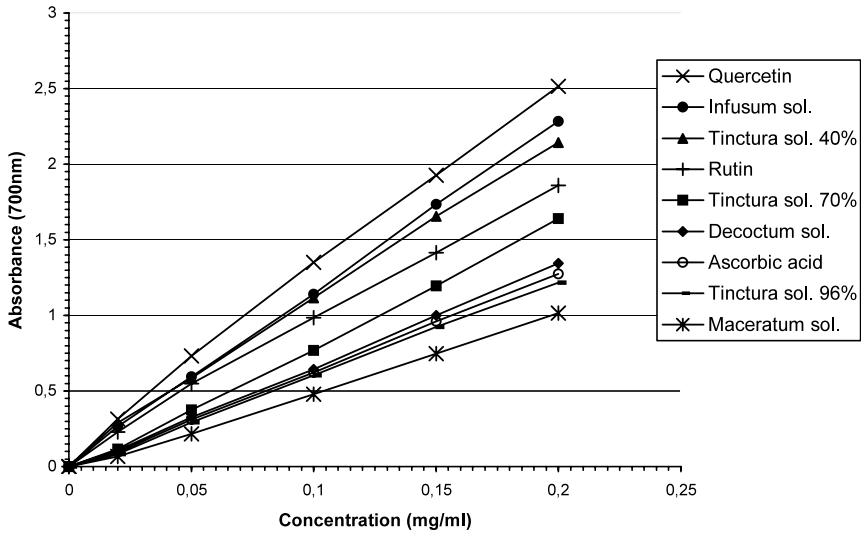


Fig. 3. Reducing power of different Solidaginis herba extracts.

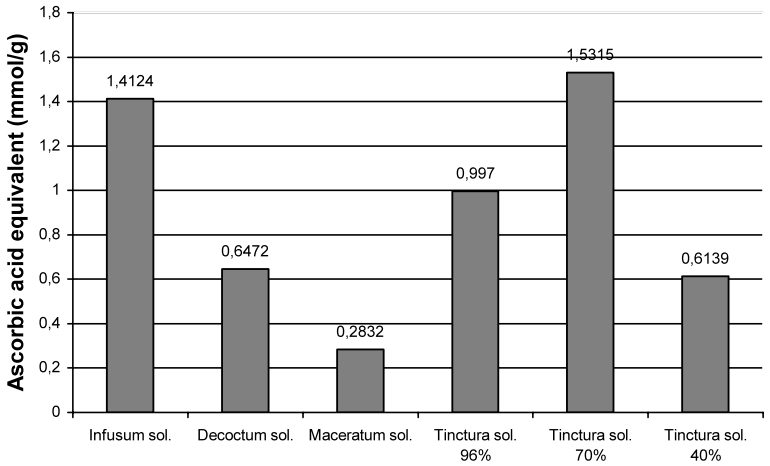


Fig. 4. Water soluble antioxidative capacity (ACW) of different Solidaginis herba extracts.

Table 4
Water soluble antioxidative capacity (ACW) of different Solidaginis herba extracts

Sample (mmol g^{-1})	Ascorbic acid equivalent ±SD	SD%
Infusum solidaginis	1.4124±0.0131	0.92
Decoctum solidaginis	0.6472±0.0012	0.18
Maceratum solidaginis	0.2832±0.0013	0.45
Tinctura solidaginis 40%	0.6139±0.0067	1.09
Tinctura solidaginis 70%	1.5315±0.0129	0.84
Tinctura solidaginis 96%	0.9973±0.0113	1.13

preparation on the in vitro antioxidant potential of extracts.

Investigated pharmaceuticals obtained from Solidaginis herba showed in our work—H-donating ability depending on the concentration. Extracts, containing higher flavonoid and chlorogenic acid levels proved to be more effective in scavenging DPPH radical, than others.

The method of PCL used for the determination of the integral AC in diverse substance mixtures of medicinal plants and their pharmaceutical products seemed to be simple, quick, sensitive, economical and convenient technique.

It is concluded that best antioxidant capacity and hence best health benefit may be derived from Solidaginis herba extract prepared by infusion technique. Further studies are required to assess the antioxidant actions of *Solidago* extracts in vivo.

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