Comprehensive Evaluation of Different Solidaginis Herba Extracts

P. Apáti*, T.Sz. Kristó, É. Szőke and Á. Kéry
Department of Pharmacognosy, Semmelweis University
H-1085 Budapest, Üllői út 26.Hungary

K. Szentmihályi and P. Vinkler
*Chemical Research Center, Institute of Chemistry, Hungarian Academy of Sciences, H-1025 Budapest, Pusztaszeri út 59-67. Hungary

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Abstract

Canadian golden rod (*Solidago* x *canadensis L.*, Asteraceae) has been used in European phytotheraphy for 700 years as a urological and antiphlogistical remedy. Dissolution rates of quercetin glycosides and organic acids have been studied, as well as mineral elements of Solidaginis herba into different tinctures and aqueous extracts. Determination of the flavonoids in Solidaginis herba (16.75 mg/g) and of flavonoid release in extracts (14.9-72.9 %) was carried out by spectrophotometric analysis. To study the flavonoid composition of the crude drug, an HPLC technique was applied. The concentrations of 16 mineral elements (Al, B, Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Ti, Zn) in the samples were measured by inductively coupled plasma atomic emission spectrometry (ICP-OES). Connection has been found between some ion concentrations and the presence and quantity of flavonoids.

INTRODUCTION

Applications of medicinal plants and phytopharmaceuticals in disorders of the urinary tract have been used successfully in therapy, as well as synthetic drugs. Advantages of their use are due to the mild and safe action on the salt-water and acid-base equilibrium (Bales et al., 1999).

Canadian golden rod (*Solidago canadensis* L., Asteraceae) has been used in European phytotheraphy for 700 years as an urological and antiphlogistical remedy (Bader, 1999). Flavonoids, especially quercetin and derivatives, inhibit the enzyme neutral endopeptidase, which is responsible for the interaction of the atrial natriuretic peptide and thus regulates the formation of urine via the excretion of sodium ions. This can be interpreted as the basis of enhanced urinary flow therapy (Budzianowski et al., 1990; Melzig and Major, 2000; Schiller et al., 1989; Hänsel et al., 1994). Besides controlling quality of Solidaginis herba, chemical standardisation and bioavailability of active flavonoid ingredients in the resulting herbal remedies have to be guaranteed.

Therapeutical relevance, presumably related to the combined effect of organic and inorganic compounds, like flavonoids and metal ions, has received great attention in recent years (Szentmihályi et al., 1998). Thus we have investigated the dissolution rate of quercetin glycosides and organic acids connected to the mineral element content of Solidaginis herba into different tinctures and aqueous extracts. Due to these studies it should be possible to evaluate the specific release of active principles from vegetable drug to the respective formulation.

MATERIALS AND METHODS

Plant material was collected before the full flowering state on abandoned farmlands near to Budapest (Hungary) and identified as *Solidago canadensis* L. (Asteraceae) in the Department of Pharmacognosy, Semmelweis University, where a herbarium specimen is also deposited. Aerial parts were used for extractions as Solidaginis herba, according to the Hungarian Standard.

Preparation of samples - 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 minutes, 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 v/v%, 70 v/v%, 96 v/v%) ethanol for six days and then filtered.

Flavonoid content determination - Total flavonoid content of the dried Solidaginis herba and extracts were determined by spectrophotometry according to the instructions of 10th German Pharmacopoea, after acidic hydrolysis (HCl 25 v/v%) and complexation with $AlCl_3$ (2 v/v %) from the purified ethyl acetate phase. Flavonoid content was calculated as hyperoside ($A^{1\%}_{1cm} = 500$).

HPLC conditions - The phenolic compounds were analysed by high-performance liquid-chromatography. Reversed phase columns are the most appropriate for the separation of polyphenols by HPLC (Dondi et al., 1988). Chromatography was performed using an Able Jasco HPLC system consisting of a JASCO PU-980 gradient pump, JASCO PU-980 UV-VIS detector in combination with RHEODYNE 7725 (20 μL) injector and IBM-PC. Separation was achived on a Hypersil ODS (5μm) reverse-phase C-18 (250 x 4 mm) column at ambient temperature with a flow rate 1.0 mL/min. The solvent system consisted of a step gradient starting with 14 % acetonitrile (eluent A) and 86 % diluted acetic acid 2.5 v/v% (eluent B) increasing to 35 % eluent A 65 % eluent B over 40 minutes. Data were collected at 360 nm. Peaks were identified by co-chromatography with authentic standards and according to UV spectra and retention times.

ICP-OES conditions - The element contents of samples were determined by inductively coupled plasma optical emission spectrometer (ICP-OES). Type of instrument: Atom Scan 25 (Thermo Jarrell Ash), a sequential plasma emission spectrometer with generator (2 kW, 27.12 MHz) exciting argon plasma to 8000-10000 °K. The optical system is composed of a Czerny-Turner vacuum monochromator and two photoelectron multipliers. The detection limit was equal to the values given by Thermo Jarrell Ash. Standard solutions (prepared from Merck ICP standards) were in the matrix of the samples.

The samples (0.5 g drug or 20 mL evaporated extract) were digested with HNO_3 (10 mL). After digestion these were diluted to 25 mL, and analysed for 16 elements (Al, B, Ba, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, P, S, Ti, Zn) in three parallel measurements.

RESULTS AND DISCUSSION

Extraction of organic and inorganic constituents is effected by numerous factors. Because of various technological applications, measured element and flavonoid levels were highly different in the samples (Meier, 1991; Schneider-Leukel and Franz, 1994).

Quality of tinctures (Fig. 1) and aqueous extracts (Fig. 2) was mainly affected by the solvent characteristics, temperature and duration of extraction respectively, when other parameters were unaltered.

Dissolution mainly depends on solvent and molecular characteristics, temperature and duration of extraction. Quality of tinctures - investigated in our study - is in connection with solvent characteristics, while aqueous extracts' content changed with temperature and duration of extraction – keeping the other parameters constant.

Different preparations obtained from *Solidago canadensis* were evaluated: classical herbal tea extracts, Infusum solidaginis, Decoctum solidaginis, Maceratum solidaginis and tinctures prepared by using various concentrations of ethanol - 40 v/v %, 70 v/v %, 96 v/v %. The quantitative determination of the flavonoids in Solidaginis herba and of flavonoid release in extracts was carried out by spectrophotometric analysis, as required by the German Pharmacopoea ed.10. To study the flavonoid composition and dissolution rates of the main compounds, an HPLC technique was used.

Chlorogenic acid, caffeic acid, quercetin-3-O-beta-D-rutinoside (rutin), quercetin-

3-O-beta-D-galactoside (hyperoside), quercetin-3-O-beta-D-glucoside (isoquercitrin) and quercetin were confirmed by retention times and their UV spectra (Table 1.). Reversed-phase high-performance liquid chromatographic separation of polyphenols on octadecyl sorbent Hypersil was performed, using acetonitrile:acetic acid 2.5 v/v% mobile phase in gradient elution.

Only traces of quercetin were present in Solidaginis herba and decoction/infusion, presumably due to the enzyme inhibiting effect of methanol, and the shortness of the extraction period, respectively. According to our study, the dissolution of flavonoids and organic acids from alcoholic extracts depends mainly on the solvent polarity, while aqueous preparations resulted in differences in flavonoids due to the temperature and duration of extraction. Based on the dissolution ratio and variation of flavonoid release we can conclude that Tinctura solidaginis (70%-ethanol) and Infusum solidaginis are the most appropriate preparations as far as the availability of active flavonoids is concerned.

Samples were analysed for 16 elements by inductively coupled plasma optical emission spectrometer (ICP-OES) after digestion (ccHNO_3) (Table 2).

Concentrations of elements in alcoholic extracts were relatively low, but the highest amount of copper (0.17 mg/L), sodium (2.01 mg/L) and zinc (0.35 mg/L) was found in tinctures. Other investigated elements were present in higher concentrations in the aqueous extracts.

Relatively high amount of mineral elements can be found in Maceratum solidaginis, but in almost every case, higher temperature proved to be the determinant factor, beside the extraction time, in micro elements content. In the case of Decoctum solidaginis, the short-term contact (5 minutes) with boiling water caused lower levels, both in organic and inorganic components.

According to our study, the dissolution of flavonoids and organic acids from alcoholic extracts depends mainly on solvent polarity, while differences in aqueous preparations resulted from differences in temperature and duration of extraction. A connection has been found between some ion concentrations (B, Cu, K, Mg, P, S, Zn) and flavonoid presence. Although water and aqueous tinctures proved to be the most useful medium for element dissolution, in some cases high amounts of mineral elements coincided with the highest flavonoid content, but the connection between extraction of these elements and flavonoid presence requires further investigations.

Considering the diuretic application of *Solidago* extracts, we can conclude that Tinctura solidaginis (ethanol, 70 v/v%) and Infusum solidaginis are the best sources of both flavonoid glycosides and mineral elements.

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Tables

Table 1. HPLC analysis of Solidaginis herba and dissolution of phenolic components into various extracts

	Flavonoid	Retention time [min]	Tinctura sol. 40%	Tinctura sol. 70%	Tinctura sol. 96%	Infusum sol.	Decoctum sol.	Maceratum sol.
a	chlorogenic acid	3.46	72%	78%	42%	98%	85%	36%
	caffeic acid	5.78	<DL	<DL	<DL	<DL	<DL	<DL
b	rutin	8.92	34%	81%	53%	81%	53%	2%
c	hyperoside	10.21	30%	65%	58%	59%	65%	~0%
d	isoquercitrin	10.78	25%	65%	57%	59%	56%	~0%
e	f.g. 1	14.74	24%	64%	47%	60%	37%	1%
f	f.g. 2	16.45	25%	59%	44%	59%	40%	~0%
g	f.g. 3	18.98	12%	52%	47%	46%	31%	3%
	quercetin	28.80	nd	nd	nd	nd	nd	nd

f.g. = unidentified flavonoid glycoside

nd = not defined

<DL = below determination limit

Table 2. Dissolution of mineral elements from Solidaginis herba into various extracts obtained by different technologies

Elements	Tinctura sol.40%	Tinctura sol.70%	Tinctura sol. 96%	Maceratum sol.	Infusum sol.	Decoctum sol.
Al	7,9%	3,6%	1,0%	13,3%	13,4%	8,3%
B	32,4%	43,0%	12,4%	36,6%	53,2%	33,5%
Ba	32,3%	18,7%	7,3%	23,8%	28,3%	55,9%
Ca	16,4%	6,0%	1,5%	24,2%	29,7%	28,3%
Co	11,5%	<d.l.	<d.l.	60,7%	51,7%	68,6%
Cr	10,8%	10,1%	4,4%	34,8%	25,0%	57,9%
Cu	71,0%	72,9%	12,8%	51,0%	66,4%	67,0%
Fe	4,9%	2,2%	2,5%	9,8%	4,5%	4,0%
K	53,9%	65,9%	17,9%	70,5%	66,9%	66,5%
Mg	26,4%	48,0%	4,7%	48,0%	51,0%	50,5%
Mn	15,2%	4,2%	<d.l.	28,9%	30,3%	37,4%
Na	65,2%	33,9%	32,0%	26,9%	30,8%	60,7%
P	28,6%	32,1%	5,1%	71,0%	55,0%	54,0%
S	24,9%	30,9%	5,8%	51,8%	45,8%	49,4%
Ti	1,9%	1,8%	0,6%	11,5%	3,8%	3,7%
Zn	41,3%	57,2%	9,2%	25,9%	29,0%	28,8%

<d.l. = below detection limit

Figures

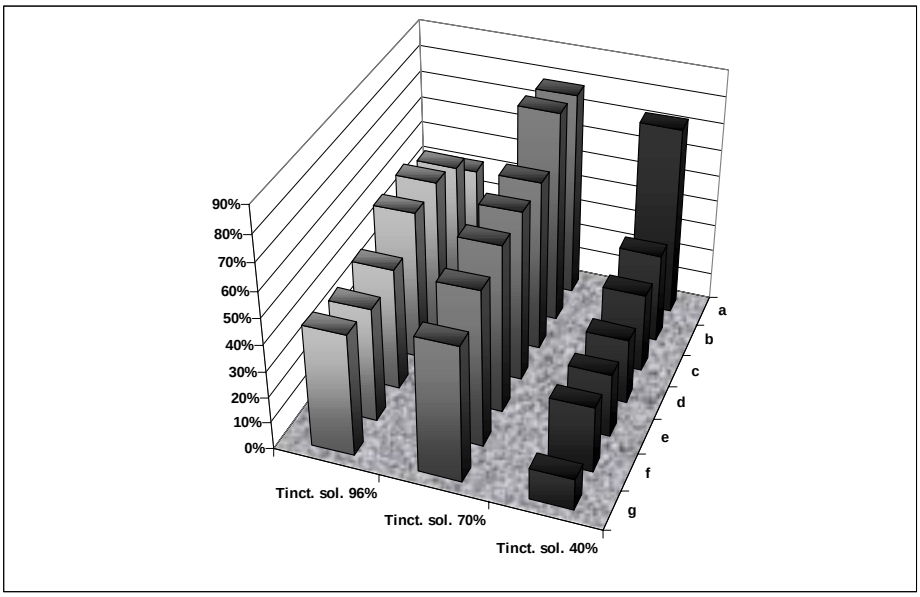

Fig. 1. Dissolution of flavonoids into various extracts of Solidaginis herba
1. Tinctures (a-chlorogenic acid, b-rutin, c-hyperoside, d-isoquercitrin, e-,f-,g-unidentified flavonoid glycosides)

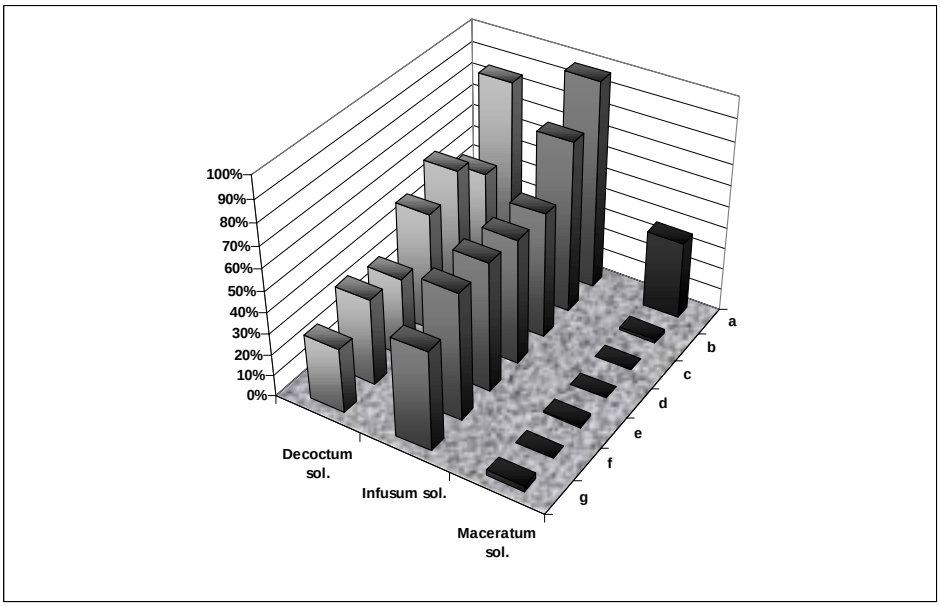

Fig. 2. Dissolution of flavonoids into various extracts of Solidaginis herba
2. Aqueous extracts (a-chlorogenic acid, b-rutin, c-hyperoside, d-isoquercitrin, e-,f-,g-unidentified flavonoid glycosides)