

Analytical, Nutritional and Clinical Methods Section

# Comparative evaluation of *Helichrysi flos* herbal extracts as dietary sources of plant polyphenols, and macro- and microelements

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## Abstract

The inflorescence of *Helichrysum arenarium* (L.) Moench (*Helichrysi flos* syn. *Stoechados flos*) has long been known in herbal medicine in Europe for its choleric, diuretic, antiinflammatory and detoxifying activities. Organic bioactive compounds and the inorganic element content of *Helichrysi flos* drugs of different origin (from cultivation or from the commercial network) and their water extracts were examined. The polyphenol and flavonoid content in the drug (61.4–92.3 and 9.4–12.7 g kg<sup>-1</sup>, respectively) and tea samples (1200–1730 and 47–71 mg l<sup>-1</sup>, respectively) were determined by a spectrophotometric method. The concentration of 23 macro- and microelements was measured in crude drugs and their water extracts by inductively coupled plasma–atomic emission spectrometry (ICP–AES). The cultivated drug sample from 1999 (Hungary) contained aluminum (353 mg kg<sup>-1</sup>), chromium (6 mg kg<sup>-1</sup>), copper (19 mg kg<sup>-1</sup>), manganese (349 mg kg<sup>-1</sup>) and phosphorus (2907 mg kg<sup>-1</sup>) in highest level, while the concentration of barium (19 mg kg<sup>-1</sup>), calcium (7575 mg kg<sup>-1</sup>), iron (159 mg kg<sup>-1</sup>), and zinc (59 mg kg<sup>-1</sup>) was highest in a commercial sample from 1998 (Poland). © 2002 Published by Elsevier Science Ltd.

**Keywords:** *Helichrysi flos*; Polyphenol; Flavonoid; Macroelement; Microelement

## 1. Introduction

Several epidemiological studies verify that the consumption of fresh fruits, vegetables and beverages of plant origin (such as herbal teas) can play a preventive role in free radical mediated disorders (e.g. cancer, heart disease, stroke), which is due to a variety of constituents, including vitamins, minerals, fiber and numerous phytochemicals, including polyphenols (Eastwood, 1999; Hertog et al., 1995).

*Helichrysum arenarium* (L.) Moench (everlasting, immortelle—Asteraceae) is a native perennial herb in Europe from the Netherlands southwards to Germany and Bulgaria. The inflorescence of *H. arenarium* (*Helichrysi flos* syn. *Stoechados flos*) has long been used in folk medicine for its choleric, diuretic, anti-inflammatory and detoxifying properties; and applied in the form of infusion for cystitis, arthritis, rheumatism, for stimulating gastric secretion and for the treatment

of gallbladder disorders (Szadowska, 1962; Skakun & Stepanov, 1988; Krivenko, Potebnia, & Loiko, 1989). The active ingredients of *Helichrysi flos* are mainly phenolics, such as flavonoids, phtalides (Vrkoc, Ubik, & Sedmera, 1973), coumarins (Derkach, Komissarenko, & Chernobai, 1986),  $\alpha$ -pyron derivatives (Vrkoc, Dolejs, Sedmera, Vasickova, & Sorm, 1971), phenolic acids (Dombrowicz, Swiatek, & Kopycki, 1994) further more, terpenoids, essential oils, volatile- and fatty acids (Czinner, Lemberkovics, Bihátsi-Karsai, Vitányi, & Lelik, 2000). The beneficial effects of the drug are attributed mainly to the presence of flavonoids, and may be related to their antioxidant activity; but they are also influenced by other organic (phenolics: coumarins, phenolic acids etc.) and inorganic (macro- and microelements: Cu, Fe, Mg, Mn, Zn etc.) ingredients (Litvinenko, Popova, Popova, & Bubenchikova, 1992).

In the course of our phytochemical study on phenolic compounds of *Helichrysi flos* we identified eight flavonoids, namely kaempferol, kaempferol-3-*O*-glucoside, apigenin, apigenin-7-*O*-glucoside, naringenin, naringenin-5-*O*-glucoside, naringenin-4'-*O*-glucoside, quercetin-3-*O*-glucoside (isoquercitrin), the chalcone derivative

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isosalipurposide (2',4,4',6'-tetrahydroxychalcone-6'-O-glucoside) and chlorogenic acid in methanolic extract by HPLC–DAD–MS. The water extract of the drug also contained the above phenolic compounds, except aglycones (kaempferol, apigenin and naringenin), which are rather apolar, and therefore insoluble in water (Czinner et al., in press).

Our earlier study provides evidence of the fact that lyophilized water extracts of *Helichrysi flos* (with standardized polyphenol and flavonoid contents) exhibit interesting antioxidant and antilipoperoxidant properties expressed either by their capacity to scavenge reactive oxygen species or diminish enzymatically induced lipid peroxidation (Czinner et al., 1999, 2000).

A large number of laboratory studies indicate that reactive oxygen species, especially lipid peroxidation products play a causative role in aging and several degenerative diseases associated with it, such as heart diseases, atherogenesis (Witzum, 1994), neurodegenerative diseases (Beal, 1995; Jenner, 1994) and cancer (Cerruti, 1994; Flagg, Coates, & Greenberg, 1995). Generation of free radicals is an integral part of normal metabolism. However, if the production of oxygen reactive species is inadequately controlled, they may damage biological macromolecules, altering their properties, and thus, the structure and function of cells (Potterat, 1997).

Under normal physiological conditions, humans have evolved antioxidant systems for protection against free radicals. These systems include some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous). The first include enzymatic (Se-glutathione peroxidase, catalase, superoxide dismutase) and nonenzymatic (glutathione, histidine-peptides, the iron-binding proteins transferrin and ferritin, reduced CoQ<sub>10</sub>) defenses (Pietta, 2000). Macro- and microelements, e.g. Fe, Cu, Mn, Co, Se etc. are critical components for a number of these processes, and a deficiency of any of these essential elements may impair the function of the overall antioxidant system (Zidenberg-Cherr & Keen, 1991).

Well established antioxidants derived from the diet are vitamins C, E, A, and carotenoids, etc. which have been studied intensively. Besides these antioxidant vitamins, other substances might account for at least part of the health benefits associated with plant food consumption. Over the past decade evidence has been accumulated showing that plant polyphenols, such as flavonoids, phenolic acids, coumarins, tannins, and lignans, constitute an important class of defense antioxidants (Potterat, 1997). These compounds are almost ubiquitous in plant foods (vegetables, cereals, legumes, fruits, nuts, etc.) and beverages (wine, cider, beer, tea, herbal teas, cocoa, etc.) (Gorinstein, Caspi, Zemser, & Trakhtenberg, 2000; Lugasi, 2000; Zhao, Li, He, Cheng, & Xin, 1989).

The dietary intake of the nutrients and non-nutrients mentioned above may modify the activity of the antioxidant defense system, and thus effect the degree of protection provided to the cell or tissue against oxidative reactions. Thus, dietary deficiency of one of these defense components may impair the ability of the organism to defend itself against excessive free radical damage and associated pathologies (Zidenberg & Keen, 1991).

The aim of this study was, to determine the content of organic and inorganic ingredients in *Helichrysi flos* herbal drugs and extracts, and estimate their real value as dietary sources of plant polyphenols, flavonoids, macro-nutrient elements (Ca, K, Mg, Na, P, S), micronutrient elements (Co, Cr, Cu, Fe, Mn, Mo, V, Zn), and some other microelements (Al, As, B, Ba, Cd, Hg, Li, Pb, Ti).

## 2. Materials and methods

### 2.1. Chemicals

All chemicals and reagents were analytically clean, and obtained from Reanal Finomvegszergyár Rt. (Budapest, Hungary).

### 2.2. Plant material

Six different inflorescence drugs of *Helichrysum arenarium* (L.) Moench (*Helichrysi flos* syn. *Stoechados flos*, Asteraceae) and its water extracts (teas) were used for the examinations. Sample Nos. 1, 2, 3 and 4 were purchased from the commercial network of several countries (Kawon-Hurt S.C., Poland, 1998 and 1999; Caesar and Loretz GmbH, Germany, 1999; Herbária, Hungary, 1999), samples 5 and 6 were cultivated in Hungary (Soroksár, 1999 and 2000) by ourselves (Table 1).

### 2.3. Preparation of tea

The procedure adopted was as follows: 200 ml boiling water was poured onto 5 g of the preparation, covered and left to infuse for 30 min, then filtered, the moisture squeezed out, and the volume made up to 200 ml.

Table 1  
Origin of *Helichrysi flos* drug samples

Sample No.	Originating from	Year
1	Commercial (Kawon-Hurt S.C., Poland)	1998
2	Commercial (Kawon-Hurt S.C., Poland)	1999
3	Commercial (Caesar and Loretz GmbH, Germany)	1999
4	Commercial (Herbária, Hungary)	1999
5	Cultivated (Soroksár, Hungary)	1999
6	Cultivated (Soroksár, Hungary)	2000

## 2.4. Methods for the determination of organic ingredients

The polyphenol content of the drugs and teas was measured at 760 nm by spectrophotometry (Ph.Hg.VII., 1986), using pyrogallol as reference standard (modified method of AOAC, Official Methods of Analysis, 1990). This method is based on the formation of blue reaction products by redox reaction with the Folin reagent (phosphotungstic acid solution). The absorbance of coloured solution was proportional to polyphenol concentration.

Flavonoid content in the samples was determined by spectrometry according to the German Pharmacopoeia (DAB 10, 1996) method, measuring the flavonoids in  $AlCl_3$ -complex form of purified ethyl acetate phase obtained after acid hydrolysis. Glycosides and aglycones were determined together in aglycone form. The flavonoid content was calculated in hyperoside.

The moisture and ash content of the crude drugs and the crude material content of teas were measured using the Hungarian Pharmacopoeia (Ph.Hg.VII., 1986).

## 2.5. Determination of element content

Concentration of the elements of samples of different origin were determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES). 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 8000–10,000 K. Optical system is composed of a Czerny–Turner vacuum monochromator and two photoelectron multipliers. The quantity of the following 23 elements was examined in three parallel measurements: Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Li, Mg, Mn, Mo, Na, P, Pb, S, Ti, V, Zn. The detection limit was equal to the values given by Thermo Jarrell Ash. Standard solutions (prepared from Merck ICP standards) were in the same matrix as in the samples.

Sampling: 0.5 g of each drug sample was digested with 10 ml of  $HNO_3$  (67%) in MARSx microwave instrument. After digestion the samples were diluted to 25 ml with bi-distilled water in a volumetric flask. In the case of tea, after evaporation, sampling was carried out in the same way.

## 2.6. Statistical analysis

Mean values and standard deviation (S.D.) were calculated from the results. One way analysis of variance (ANOVA) was applied for comparison of the mean values.  $P$ -value < 0.05 was regarded as significant.

## 3. Results

The amount of total polyphenols and flavonoids in crude drug samples as well as moisture and ash content are shown in Fig. 1. Highest polyphenol content (92.3 g

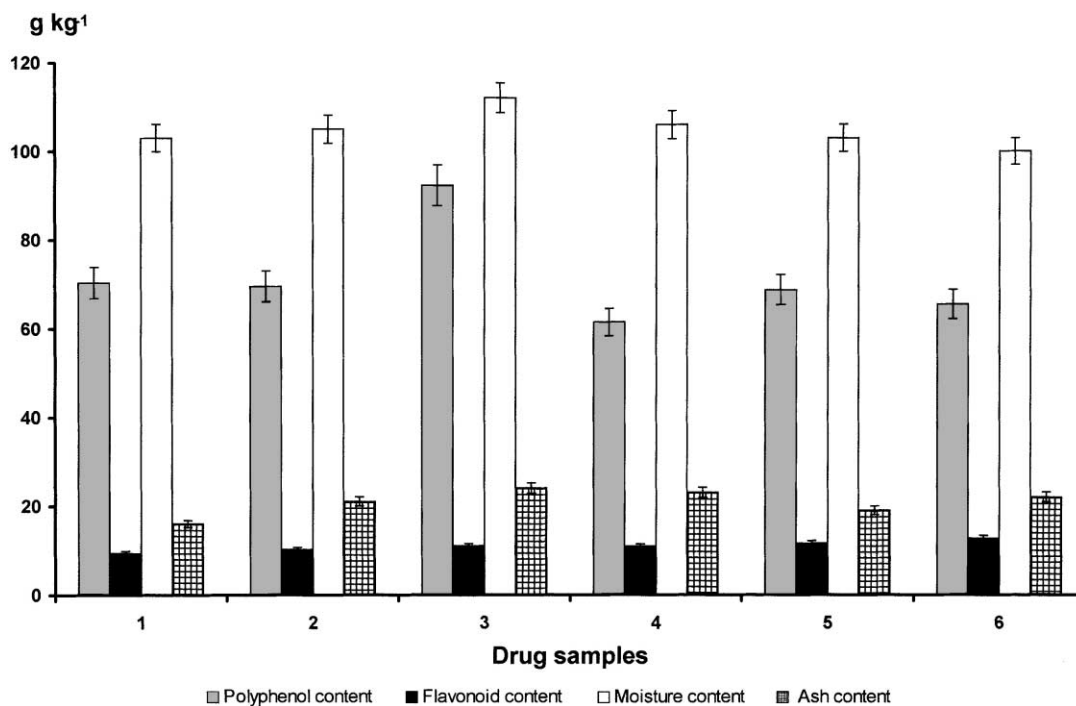


Fig. 1. Total polyphenol, flavonoid, moisture and ash content ( $g\ kg^{-1}$ ) of *Helichrysis flos* drug samples and  $\pm$  standard deviation. ■ Polyphenol content; ■ flavanoid content; □ moisture content; ▨ ash content.

Table 2

Total polyphenol, flavonoid and crude material content of *Helichrysi flos* teas,  $\pm$  standard deviation and the dissolution (%) of polyphenols and flavonoids (in parentheses)

Tea samples	Polyphenol content (mg l <sup>-1</sup> )	Flavonoid content (mg l <sup>-1</sup> )	Water soluble materials (g l <sup>-1</sup> )
1	1520 $\pm$ 120 (86.5)	47 $\pm$ 2.5 (20.2)	4.0 $\pm$ 0.12
2	1460 $\pm$ 90 (84)	49 $\pm$ 2.6 (19.2)	4.5 $\pm$ 0.21
3	1730 $\pm$ 140 (75)	67 $\pm$ 3.0 (24.4)	6.7 $\pm$ 0.35
4	1200 $\pm$ 80 (78.1)	71 $\pm$ 4.0 (26)	5.5 $\pm$ 0.28
5	1370 $\pm$ 100 (79.8)	59 $\pm$ 2.3 (20.4)	5.3 $\pm$ 0.20
6	1280 $\pm$ 80 (78.2)	62 $\pm$ 3.8 (19.7)	4.7 $\pm$ 0.22

kg<sup>-1</sup>) was measured in the German commercial drug sample (No. 3). No significant difference in the polyphenol concentration of other drugs could be observed (61.4–70.3 g kg<sup>-1</sup>). Cultivated drugs (5 and 6) proved to contain the largest amounts of flavonoids (11.6, and 12.7 g kg<sup>-1</sup>, respectively), which were significantly higher ( $P < 0.05$ , and  $P < 0.01$ , respectively) than in commercial drugs (range 9.4–11.0 g kg<sup>-1</sup>). The mean values of moisture (100–112 g kg<sup>-1</sup>) and ash content (19–24 g kg<sup>-1</sup>) determinations did not show significant difference between the drug samples.

The most frequently applied method of herbal drug consumption is tea making. Total polyphenol and flavonoid content, further amount of water soluble materials of 6 *Helichrysi flos* herbal tea samples (obtained from drugs Nos. 1–6), and the amount of polyphenols

and flavonoids dissolved in tea are also summarized in Table 2. In accordance with expectations, the highest polyphenol concentration (1730 mg l<sup>-1</sup>) was determined in tea sample No. 3, since drug sample No. 3 contained polyphenols in highest amount. Tea sample No. 4, obtained from the Hungarian commercial drug, proved to contain the highest amount of flavonoids (71 mg l<sup>-1</sup>), but the difference between teas was not significant, even in the case of water soluble materials (ranging between 4.0 and 6.7 g l<sup>-1</sup>).

The mean values (%) of polyphenol and flavonoid dissolution into teas from plant drugs are shown in Fig. 2. Extremely high dissolution (80.27%) was found for polyphenols, while average flavonoid dissolution was only 21.65%.

Element concentrations in *Helichrysi flos* drug and teas are depicted in Tables 3 and 4. One way analysis of variance was used for the statistical comparison of samples.

Macronutrient elements are needed in relatively large amounts in the human diet such as calcium, phosphorus, sodium, potassium, magnesium, and sulphur. The amount of potassium (15612–17063 mg kg<sup>-1</sup>), magnesium (1316–1661 mg kg<sup>-1</sup>), phosphorus (2486–2907 mg kg<sup>-1</sup>) and sulphur (1893–2212 mg kg<sup>-1</sup>) was found to be similar ( $P > 0.1$ ) in all drug samples. Significant difference ( $P < 0.05$ ) was found in the calcium and sodium concentrations of drugs. Content of Ca ranged from 4242 to 7575 mg kg<sup>-1</sup>; minimum and maximum values of Na were 15.9 and 73.5 mg kg<sup>-1</sup>.

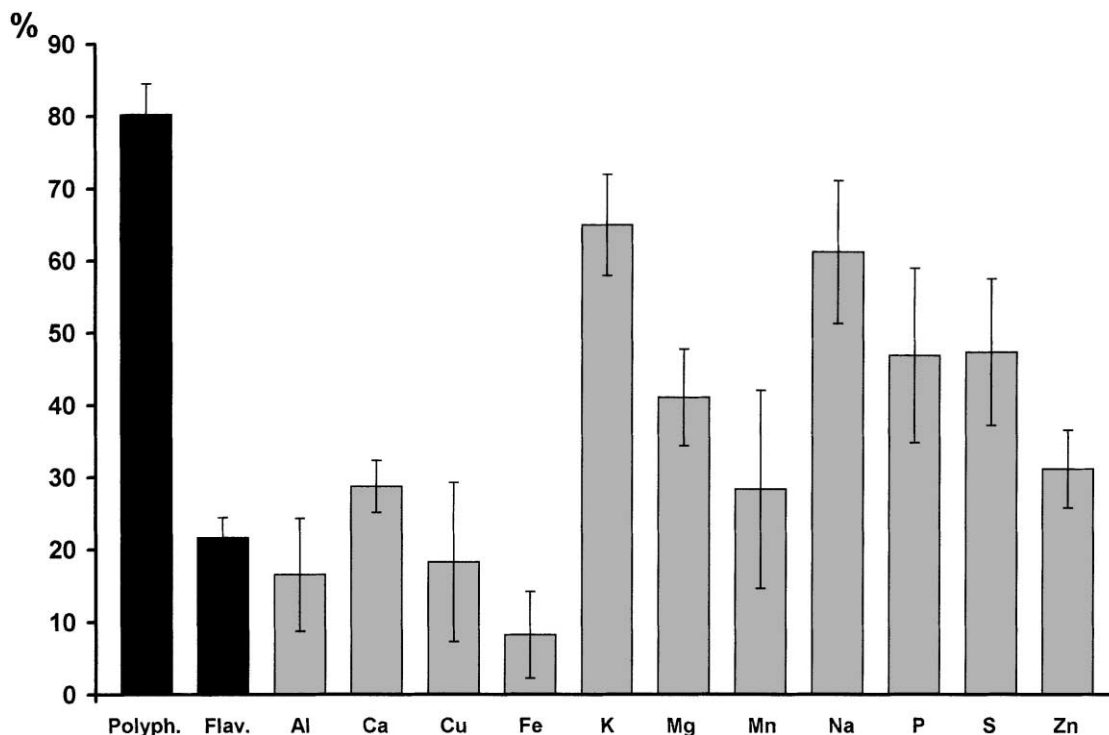


Fig. 2. Average dissolution (%) of polyphenols, flavonoids and mineral elements from *Helichrysi flos* drugs into herbal teas and  $\pm$  standard deviation.

Table 3  
Element concentrations of *Helichrysi flos* drugs and  $\pm$  standard deviation (mg kg<sup>-1</sup>)

Elements	Element concentration (mg kg <sup>-1</sup> )					
	Sample No.					
	1	2	3	4	5	6
Al	242.6±22.6	223.0±11.6	160.9±55.7	232.3±18.5	352.7±70.6	134.0±6.1
As	<1.0	<1.0	<1.0	<1.0	<1.0	<1.0
B	<2.5	<2.5	<2.5	<2.5	<2.5	<2.5
Ba	19.2±5.1	10.4±1.4	6.4±2.6	11.4±2.2	11.4±1.0	8.1±2.0
Ca	7575±303	6416±662	6993±775	7149±668	4242±67	5309±46
Cd	<1.26	<1.26	<1.26	<1.26	<1.26	<1.26
Co	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Cr	2.8±0.2	2.6±0.4	2.9±0.1	3.4±0.3	6.0±0.8	3.1±0.5
Cu	22.1±1.7	17.4±4.1	8.8±0.9	14.6±1.8	19.2±1.1	11.7±2.8
Fe	159.4±8.0	116.7±12.1	88.0±13.0	156.5±47.7	48.0±1.6	31.1±9.7
Hg	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
K	16524±285	17063±611	16047±480	16471±810	15612±234	15942±1587
Li	<0.15	<0.15	<0.15	<0.15	<0.15	<0.15
Mg	1463±46	1590±344	1377±151	1426±82	1316±6	1661±62
Mn	171.3±25.9	155.1±32.8	155.7±23.6	166.3±8.7	349.4±29.0	129.4±6.5
Mo	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Na	72.6±6.8	49.1±4.0	57.9±7.4	73.5±5.1	15.9±1.2	46.3±7.6
P	2486±143	2782±277	2795±204	2578±258	2907±103	2830±198
Pb	<1.26	<1.26	<1.26	<1.26	<1.26	<1.26
S	2062±82	2212±249	2113±233	1959±185	1893±11	2171±143
Ti	2.5±0.4	1.3±0.7	1.3±0.4	2.8±0.3	2.6±0.2	1.6±0.4
V	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
Zn	59.4±12.2	56.2±9.4	37±5.2	58.1±29.7	40.5±0.2	28.0±1.2

Table 4  
Element concentrations of herbal teas obtained from *Helichrysi flos* drugs and  $\pm$  standard deviation (mg l<sup>-1</sup>)

Elements	Element concentration (mg l <sup>-1</sup> )					
	Sample No.					
	1	2	3	4	5	6
Al	0.94±0.05	0.45±0.10	0.82±0.06	0.85±0.11	0.27±0.01	0.36±0.01
As	<0.025	<0.025	<0.025	<0.025	<0.025	<0.025
B	<0.06	<0.06	<0.06	<0.06	<0.06	<0.06
Ba	0.060±0.005	<0.005	0.025±0.001	0.05±0.003	<0.005	<0.005
Ca	51.5±2.5	41.1±3.6	48.6±8.1	45.5±5.3	33.6±0.7	41.2±6.7
Cd	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
Co	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cr	<0.013	<0.013	<0.013	<0.013	<0.013	<0.013
Cu	0.120±0.003	0.070±0.020	0.081±0.001	0.075±0.004	0.005±0.003	0.045±0.010
Fe	0.140±0.001	0.095±0.008	0.155±0.007	0.194±0.008	0.065±0.020	0.200±0.013
Hg	<0.003	<0.003	<0.003	<0.003	<0.003	<0.003
K	291.5±25.7	265.5±4.9	270.0±6.0	289.0±18.1	203.2±3.8	264.0±0.2
Li	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Mg	16.0±0.8	16.2±0.01	16.5±2.1	16.3±0.4	9.7±0.1	16.1±2.8
Mn	1.31±0.04	1.64±0.26	0.95±0.14	1.03±0.26	0.47±0.05	1.35±0.30
Mo	<0.0003	<0.0003	<0.0003	<0.0003	<0.0003	<0.0003
Na	1.12±0.10	0.69±0.08	0.84±0.48	0.91±0.08	0.32±0.01	0.79±0.19
P	32.7±1.9	35.8±0.8	35.2±0.8	35.7±1.3	20.7±0.4	29.6±2.1
Pb	<0.006	<0.006	<0.006	<0.006	<0.006	<0.006
S	26.8±0.3	28.8±0.8	26.8±4.8	22.7±0.2	18.1±0.3	24.1±0.9
Ti	<0.0063	<0.0063	<0.0063	<0.0063	<0.0063	<0.0063
V	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025	<0.0025
Zn	0.47±0.02	0.42±0.06	0.37±0.16	0.35±0.04	0.23±0.01	0.27±0.08

The commercial drug sample from Poland (No. 2) contained potassium ( $17063 \text{ mg kg}^{-1}$ ) and sulphur ( $2212 \text{ mg kg}^{-1}$ ) in highest level, while the concentration of calcium ( $4242 \text{ mg kg}^{-1}$ ), potassium ( $15612 \text{ mg kg}^{-1}$ ), magnesium ( $1316 \text{ mg kg}^{-1}$ ), sodium ( $15.9 \text{ mg kg}^{-1}$ ) and sulphur ( $1893 \text{ mg kg}^{-1}$ ) was the lowest in the cultivated drug sample from 1999 (No. 5).

Significant difference ( $P < 0.05$ ) was found in the macronutrient element concentrations of herbal teas. The highest amount of calcium ( $51.46 \text{ mg l}^{-1}$ ), potassium ( $291.5 \text{ mg l}^{-1}$ ) and sodium ( $1.12 \text{ mg l}^{-1}$ ) was found in tea sample No. 1 (commercial drug from Poland, 1998). The highest magnesium level ( $16.5 \text{ mg l}^{-1}$ ) was determined in tea sample No. 3. Tea sample No. 5, obtained from the Hungarian cultivated drug (1999), contained all macronutrient elements in lowest concentration.

The average dissolution of macronutrient elements (Fig. 2) from plant drugs varies as follows: highest dissolution was observed for potassium (65%), somewhat lower for sodium (61.1%), phosphorus (46.9%), sulphur (47.3%) and magnesium (41%), lowest dissolution was found for calcium (28.7%).

Micronutrient elements, such as iron, zinc, manganese, copper, molybdenum, cobalt, chromium and vanadium are present in extremely small amounts in the human body, but, as with the other essential nutrients, we could not live without them. The concentrations of cobalt, molybdenum and vanadium were below the detection limit in all drug and tea samples. Chromium was detectable in drug samples, but in low quantity (range  $2.6\text{--}6 \text{ g kg}^{-1}$ ), while no concentration higher than the detection limit was measured in Cr in herbal teas.

Significant difference ( $P < 0.05$ ) was found in the micronutrient element concentrations of drugs and teas. The cultivated drug sample from 1999 (No. 5) contained chromium ( $6.0 \text{ mg kg}^{-1}$ ), copper ( $19.2 \text{ mg kg}^{-1}$ ) and manganese ( $349.4 \text{ mg kg}^{-1}$ ) in highest level, while the concentration of iron ( $159 \text{ mg kg}^{-1}$ ), and zinc ( $59.4 \text{ mg kg}^{-1}$ ) was highest in commercial sample No. 1. Cultivated *Helichrysi flos* drugs (5 and 6) proved to contain all micronutrient elements in significantly different ( $P < 0.05$ ) quantities, and the concentration of iron was the lowest in these samples ( $48$  and  $31 \text{ mg kg}^{-1}$ , respectively).

The highest amount of copper ( $0.12 \text{ mg l}^{-1}$ ) and zinc ( $0.47 \text{ mg l}^{-1}$ ) was found in tea sample No. 1 (commercial drug from Poland, 1998); while tea sample No. 5 contained all micronutrient elements in lowest concentration. The best source of iron ( $0.194 \text{ mg l}^{-1}$ ) proved to be tea No. 4, and the highest magnesium level ( $16.5 \text{ mg l}^{-1}$ ) was determined in tea sample No. 3.

The mean values (%) of micronutrient element dissolution into teas from plant drugs (Fig. 2) are relatively lower than macronutrient elements: 31.1% for zinc,

28.3% for manganese, 18.3% for copper, and lowest dissolution was observed in the case of iron (8.2%).

In addition to macro- and micronutrient concentrations, we analysed some other microelements (Al, As, B, Ba, Cd, Hg, Li, Pb, Ti) in *Helichrysi flos* herbal drugs and teas, which can be found in the human body, and for which essentiality has not yet been established, or for which no metabolic role has been elucidated. No concentrations higher than the detection limit were determined in elements arsenic, boron, cadmium, mercury, lithium, and lead. Titanium was detectable in drug samples, but in low quantity (range  $1.3\text{--}2.8 \text{ g kg}^{-1}$ ), while Ti concentration was below the detection limit in all herbal teas.

Content of aluminum in drug samples ranged from 134 to  $352.7 \text{ mg kg}^{-1}$ ; the average Al dissolution into teas from herbal drugs was 16.5% (Fig. 2). Minimum and maximum values of barium were 6.4 and  $19.2 \text{ mg kg}^{-1}$  in drugs, but Ba was detectable only in three tea samples (Nos. 1, 2 and 4) in very low quantity (ranging between 0.025 and  $0.06 \text{ mg l}^{-1}$ ).

#### 4. Discussion

Free radicals are now known to participate in the pathogenesis of a growing number of disorders including the two major causes of death: cancer and atherosclerosis (Cerruti, 1994; Witzum, 1994). Reactive oxygen species are also reported to be involved in asthma, inflammation, arthritis, neurodegeneration, Parkinson's disease, mongolism and perhaps dementia (Adams & Odunze, 1991; Perry et al., 2000). Aging may also be the sum of deleterious free radical reactions, which occur continuously throughout cells and tissues (Ashok & Ali, 1999).

Promising epidemiological findings (Van de Vijver, Kardinaal, Grobbee, Princen, & Van Poppel, 1997) have indicated that a diet adequate to, or supplemented with, antioxidants (vitamins and trace elements) may play an important role in protection against disorders caused by oxidant damage. Both epidemiological and animal studies have suggested that microchemicals (e.g. polyphenols, flavonoids etc.) present in several herbs and plants with antioxidant properties are useful agents for the prevention of pathologies in which free radicals are involved (Lahiri-Chatterjee, Katiyar, Mohan, & Agarwal, 1999).

Polyphenolic compounds are ubiquitous in all plant organs and are, therefore, an integral part of the diet. Recent interest in food phenolics has greatly increased, owing to their antioxidant capacity and possible beneficial implications in human health. There is a large body of literature on the polyphenolic composition and content of plant foods and beverages. The polyphenolic content of fruit juices is usually in the range of 2–500

mg l<sup>-1</sup>, although juices from certain orange varieties have much higher values (up to 7000 mg l<sup>-1</sup>) owing to their extremely high hesperidin content. A cup of black tea (200 ml) contains 150–200 mg, a cup of coffee (150 ml) 200–550 mg, while a glass (200 ml) of red wine contains 200–800 mg polyphenols (Bravo, 1998; Hertog, Hollman, & van der Putte, 1993). The polyphenolic content of *Helichrysi flos* herbal teas, according to our findings, ranges from 1200 to 1730 mg l<sup>-1</sup>, therefore one cup of the tea (240–346 mg/200 ml) is almost equal to a cup of coffee or a glass of red wine regarding their polyphenol quantity. Moreover, these herbal teas do not contain caffeine and alcohol, which can be useful in the diet of children or for women during pregnancy.

Flavonoids are plant phytochemicals that cannot be synthesized by humans. Apart from their physiological role in plants, flavonoids are important components of the human diet, although they are generally considered as non-nutrients. Flavonoids are increasingly recognized as playing potentially important roles in health, including but not limited to their role as antioxidants. Flavonoid dietary intake can range between 50 and 800 mg/day, depending on the consumption of vegetables and fruits, and specific beverages, such as red wine, tea and unfiltered beer (Pietta, 2000). Thus, variation in the consumption of these beverages is mainly responsible for the overall flavonoid intake in different national diets. Different medicinal plants and related phytomedicines are another significant source of flavonoids. According to Hertog et al. (Hertog et al., 1993; Hertog, Hollman, & Katan, 1992) plant sources with medium flavonoid level (< 50 mg kg<sup>-1</sup> or < 50 mg l<sup>-1</sup>) are e.g. apples, grapes, red wine, tea, tomato juice; with high flavonoid level (> 50 mg kg<sup>-1</sup> or > 50 mg l<sup>-1</sup>) onions, kale, broccoli, endive and celery. *Helichrysi flos* drugs contain flavonoids in extremely high amount (9.4–12.7 g kg<sup>-1</sup>) compared to the plant foods mentioned above, while herbal teas obtained from crude drugs are high level sources of flavonoids (47–71 mg l<sup>-1</sup>).

The biologically damaging effects of reactive oxygen species are controlled in vivo by a wide spectrum of antioxidant defense mechanisms. The antioxidant defense system comprises a number of interconnecting, and overlapping, components which include both enzymatic and nonenzymatic factors. Essential elements, such as Fe, Cu, Mn, Co, Zn, are critical components of the body's antioxidant defense system, thus, dietary deficiency of one of these components may impair the ability of the organism to defend itself against free radical damage and associated disorders.

Most plants which possess medicinal properties contain some macro- and microelements in therapeutic concentrations (Parmar, Gupta, Jha, Varma, & Lohar, 1993), furthermore they are capable to accumulate some elements in relatively high (toxic) amount (Fytianos, Katsianis, Triantafyllou, & Zachariadis, 2001; Yang,

Wong, Yang, & Zhou, 2001). In the case of low daily intakes, there is a risk of developing element-deficiency, but in excess (high dietary intakes) it can be harmful, that is, both deficiency and overload have severe consequences (Nordberg, Sandstrom, Becking, & Goyer, 2001). Therefore, it is important to know the level of macro- and microelements in medicinal plants, and estimate their role as sources of these components in the human diet.

From a nutritional aspect, a good source of element contains at least 10% of the US Recommended Dietary Allowances (1989) in the selected serving amount. According to the above, *Helichrysi flos* teas are good sources of manganese (2–5 mg/day/adult) and potassium (RDA 2000 mg/day/adult). Calcium, magnesium and phosphorus are present approximately in 5% of RDA recommendations (800 mg/day/adult, 280–350 mg/day/adult, 800 mg/day/adult, respectively), contents of sodium, iron and zinc are ~1–2% of RDA recommended daily dose (500, 10–15 and 12–15 mg/day/adult, respectively) in 1 l of herbal teas. Some microelements, such as arsenic, cadmium, lead and mercury, are regarded as harmful to the human body, therefore from a therapeutic aspect it is beneficial that neither *Helichrysi flos* herbal drugs, nor their teas contain these elements in concentrations higher than the detection limit. In conclusion, these teas do not contain enough macro- and microelements to cover our daily needs, nevertheless they may be important as supplementary food.

Our results were compared with element content of other herbal drugs belonging to the Asteraceae family, with antioxidant activity published in the literature (György, Antus, Blázovics, & Földiák, 1992; Hagymási et al., 1999; Safayhi, Sabierai, Sailer, & Ammon, 1994) such as inflorescence of chamomile (*Chamomillae anthodium*, *Matricaria recutita* L., cultivated Degumil type), chicory herb (*Cichorii herba*, *Cichorium intybus* L.) and mille thistle fruit (*Cardui mariae fructus*, *Silybum marianum* L. Gaertner). It has been established, that all examined *Helichrysi flos* drugs (Table 3) contained manganese in significantly higher concentration than the other medicinal herbs mentioned above (30.92, 20.7–127 and 29.95 mg kg<sup>-1</sup>, respectively). The amount of chromium, copper, iron and zinc was found to be in similar range in all drugs (2.05, 6.91, 179.5 and 40.1 mg kg<sup>-1</sup>, respectively for chamomile; below the detection limit–4.57, 8.56–12.7, 88–351 and 19.3–80.6 mg kg<sup>-1</sup>, respectively for chicory herb; and 5.32, 18.51, 112 and 51.97 mg kg<sup>-1</sup>, respectively for mille thistle fruit). The calcium, magnesium and sodium content was higher in other drugs (10764, 2847 and 951.3 mg kg<sup>-1</sup>, respectively for chamomile; 10397–18077, 1999–4050 and 31.3–818 mg kg<sup>-1</sup>, respectively for chicory herb; and 8664, 4351 and 220 mg kg<sup>-1</sup>, respectively for mille thistle fruit) than in our *Helichrysi flos* samples examined (Table 3) (Máday, Szentmihályi, Then, & Szőke, 2000; Szentmihályi et al., 1996, 1998).

In the case of nutritional deficiencies or malnutrition there is an increased risk of occurrence of several disorders or illnesses such as cancer, atherosclerosis, cardiovascular diseases etc. The protection against oxidative damage and related diseases is best served by the variety of antioxidant substances found in fruit and vegetables. Therefore, nutritional modulation is one of the possible methods for the prevention of pathologies in which free radical oxidation plays a fundamental role. Consequently, *Helichrysi flos* herbal teas containing high levels of antioxidant polyphenols, especially flavonoids, in addition to mineral elements, may have important applications in the prevention of highly prevalent human diseases associated with oxidative damage. For a full understanding of the actual significance of these natural plant extracts, it is necessary to investigate (in vitro and in vivo) not only their bio-availability but also their mechanisms of action and their possible synergism with other constituents either in the diet or within the human body.

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