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Macro- and micro-nutrients and their bioavailability in polish herbal medicaments

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Abstract

Several polish herbal medicaments were studied to determine trace elements and their bioavailable forms by the use of the ICP-AES method. The contents of Al, B, Ba, Bi, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Ni, P, Pb, Se, Si, Ti, V and Zn were determined. The total concentrations were measured in samples mineralised with concentrated nitric acid and hydrogen peroxide in a microwave system. The one-step extraction was applied, using, as extractants: deionised water, diluted hydrochloric acid and buffer solution containing pepsin. Efficiencies of the leaching versus time were investigated. The extraction efficiencies were analysed, taking into account bioavailability of elements under conditions simulating digestion processes in the alimentary system. The contents of the examined elements in prescribed amounts of medication, were compared to the nutritional requirements and daily permissible dose. The results are considered in terms of the utility of the natural herbal medicaments as a source of minerals indispensable for proper functioning of the human organism. Some points of merit (precision of measurements, accuracy by Standard Reference Material analysis) are also considered.

Keywords: Herbal medicinal products; Solid–liquid extraction; Trace elements; Macro- and micro-nutrients; ICP-AES

1. Introduction

The trace elements, together with other essential nutrients, are necessary for growth, normal physiological functioning, and maintaining of life; they must be supplied by food, since the body cannot synthesis them. The exact classification of trace versus macro minerals is not clear cut, but traces are often considered as minerals required by the body in amounts less than 100 mg daily. While some of them are vitally important for health, the roles of others are unclear. Recommended intakes have been set for some trace elements and their deficiency can lead to disease, but a lack of others does not cause any recognised problems. To decide whether a micro-nutrient is ''essential'' or not, several criteria are used, such as the presence of the nutrient in healthy tissue, if it appears in the fetus and newborns and if the body maintains homeostatic control over its uptake in the bloodstream or tissue and its excretion. The following are considered essential micro-nutrients: cobalt, copper, chromium, fluorine, iron, iodine, manganese, molybdenum, selenium and zinc. On the other hand, nickel, tin, vanadium, silicon and boron have recently been found to be important micro-nutrients, whereas aluminium, arsenic, barium, bismuth, bromine, cadmium, germanium, gold, lead, lithium, mercury, rubidium, silver, strontium, titanium and zirconium are all found in plant and animal tissue, yet their importance is still being determined. The nutritionally essential values and toxicity values have also been examined and widely discussed (Goldhaber, 2003; The Columbia Encyclopedia, 2001–2004). Reference Doses set by The US Environmental Protection Agency, Acceptable Daily Intakes calculated

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by The World Health Organisation, Dietary Reference Intakes established by The US Food and Nutrition Board of the Institute of Medicine and Reference Daily Intakes examined by The US Food and Drug Administration enable evaluation of foods as a micro-nutrient sources.

Based on the World Health Organization definitions (WHO, 1991), *Herbal medicines* are plant-derived materials and preparations with therapeutic or other human health benefits, which contain either raw or processed ingredients from one or more plants, inorganic materials or of animal origin. Herbal medicines have been used for hundreds of years and recognised as a valuable and readily available resource for health in nations all over the world. In recent years, herbal medicine has been increasingly used by the general public on a self-selection basis, to either replace or complement conventional medicine therapies. A World Health Organization survey indicated that about 70–80% of the world population, especially in developing countries, rely on non-conventional medicine, mainly from herbal sources in their primary healthcare (Akerele, 1993). Botanical product consumption is expected to grow exponentially. The distinct need to study herbal medicines scientifically is obvious. With significant advances in preclinical and clinical research, there are now several paths to achieve a knowledge base on the composition (chemical features) and pharmacological properties of specific herbs and medicines.

Quality control monitoring ensures safety, efficacy and quality of herbal medicines and their preparations. Therefore, quality control consists of a regular check of the quality of herbal medicines, and is performed according to the specifications of the product which detail the requirements for identity, purity and content of characterising compounds (Benthin, Danz, & Hamburger, 1999; Guo et al., 2001). Also, contents of undesirable components, such as herbicides, pesticides, heavy metals, alfatoxins, micro-organisms, mycotoxins, insects and undeclared herbal constituents, should be verified in medicinal plant raw materials and herbal remedies. Trace elements have both a curative and a preventive role in combating diseases. It is very important to know the level of macro- and micro-elements in medicinal plants and herbal medicaments and to estimate their role as sources of these components in the human diet because, at elevated levels, these metals can also be dangerous and toxic. These precautions are indispensable when larger amounts of the products are consumed, i.e. when recommended dosages are followed and long-term therapy is undertaken.

Many studies have been performed on active herbal plant constituents and their activity (for example, Chen et al., 2003; De Mastro, Ruta, Mincione, & Poiana, 2004; Dragland, Senoo, Wake, Holte, & Blomhoff, 2003; Lee, Jung, Kim, & Chung, 2004; Mannila, Lang, Wai, Cui, & Ang, 2003; Oreopoulou, 2003). Several attempts have been made to determine of the macro- and micro-nutrient contents of herbal, medicinal and aromatic plants from many countries all over the world. For example medicinal, aromatic and spice plants growing in Argentina (Scarpa, 2004), Austria (Chizzola, Michitsch, & Franz, 2003), Egypt (Abou-Arab, Kawther, El Tantawy, Badeaa, & Khayria, 1999), Hungary and Germany (Lemberkovics, Czinner, Szentmihályi, Balázs, & Szöke, 2002), India (Kumar, Nair, Reddy, & Garg, 2005; Naidu, Denschalg, Mauerhofer, Porte, & Balaj, 1999; Rajurkar & Damame, 1997), Nigeria (Ajasa, Bello, Ibrahim, Ogunwande, & Olawore, 2004), Poland (Lemberkovics et al., 2002; Łozak, Sołtyk, Ostapczuk, & Fijałek, 2002; Solecki, Reszka, & Chibowski, 2003), Serbia (Ražić, Onjia, & Potkonjak, 2003), Spain (Garcia, Cabrera, Lorenzo, & López, 2000; López, Cabrera, Lorenzo, & López, 2000) and the USA (Kumar et al., 2005) were monitored. As far as herbal remedy chemical composition is concerned, especially for traditional Indian and Chinese remedies (see for example, Ernst, 2002; Li & Deng, 2003; Wang, Duo, Chang, & Yang, 1996; Yang, Guangyu, & Lin, 2004), the data for European medicines are still missing. It is also very important to estimate mineral bioavailability. The amount of HCl-extractable minerals indicates their availability from foods. Extractable minerals are those which are soluble in diluted HCl, the acid found in human stomach (Duhan, Khetarpaul, & Bishnoi, 2004). Digestion, using simulated gastric and intestinal fluid, also provides useful information on mineral bioavailability (Elless, Blaylock, Huang, & Gussman, 2000). In the case of herbs, fractionation of the macro- and micro-nutrients was performed only by quantitative determination of the elements in plant materials and plant infusions (see Fernández, Pablos, Matrin, & González, 2002; Łozak et al., 2002). As far as we know, no such studies on herbal remedies have been undertaken. Also, to the best of our knowledge, enzymatic extraction has not been applied to the herbal medicaments. Enzymatic hydrolysis procedures were employed for the biological samples, e.g., for human hair and mussel (Bermejo- Barrera, Fernández-Nocelo, Moreda-Piñeiro, & Bermejo-Barrera, 1999), selenium enriched onion, garlic and yeast (Ponce de Leon, Sutton, Caruso, & Uden, 2000), bovine milk (Silva, Lopes, Nóbrega, Souza, & Nogueira, 2001), selenised mushroom (Dernovics, Stefánka, & Fodor, 2002) and Indian mustard (*Brasicca juncea*) (Elless et al., 2000).

The objectives of this study were to determine the total contents of the macro- and micro-elements in commercial herbal drugs, to estimate amounts of the micronutrients provided by herbal medicine consumption and to verify essential-nutrient intake by the extraction investigation under conditions simulating food digestion in the stomach.

2. Materials and methods

2.1. Samples

Several commercial herbal drugs, from traditional medicine, were purchased from a drug store in Wroclaw. Detailed description of analysed herbal medicinal products, composed of a single herb or of a herb collection, is presented in Table 1. Composition and medical application of examined remedies is given to allow a comparison with similar herbal medicaments used in other countries for non-conventional therapies.

All medicaments from the packages (tablets and granules produced in 2002) were ground into a fine powder and stored in plastic bags at room temperature prior to use.

The Standard Reference Material (INCT-MPH-2: Mixed Polish Herbs) was used for verification of measurement accuracy.

2.2. Reagents, glassware and plastics

All reagents used in this study were of analytical grade. For sample digestion, concentrated HNO_3 (Merck KGaA, Darmstadt, Germany) and 30% (m/v) H_2O_2 (POCh S.A., Gliwice, Poland) were applied. Extractants were prepared using fuming 37% hydrochloric acid (Merck KGaA, Germany) pepsin from hog stomach, 766 U/mg (Fluka, GmbH, Switzerland) and sodium chloride (POCh S.A., Gliwice, Poland).Aqueous standard solutions were prepared by dilution of the ICP multi-element standard (Merck KGaA, Darmstadt, Germany) solution. All dissolutions and dilutions were performed with deionised water obtained from EASY-pure™ (Barnstead, Thermolyne Corporation, USA).

Glassware and plastic bottles were cleaned with 10% nitric acid in an ultrasonic bath and rinsed several times with deionised water.

2.3. Mineralisation method

About 500 mg of powdered herbal material were accurately weighed into a Teflon digestion vessel. Next, 6 ml of concentrated nitric acid and 1 ml of hydrogen peroxide were added. Decomposition of samples was carried out in a microwave digestion system (Milestone, MLS-1200, MEGA). A six-step programme, with maximum power 650 W, was simultaneously applied to the six samples. After cooling, the sample solutions were quantitatively transferred into 25 ml volumetric flasks and made up to the mark. Before analysis, all the solutions were filtered through hard filter paper.

Table 1
Herbal medicinal products analysed here, their composition and medicinal use

Confection producer	Composition	Medicinal properties
Alax *tablets* Herbapol, Poznań	*Aloe vera*, *Glycyrrhiza glbra* L., *Rhamnus frangula* L., *Atropa belladonna*	Constipation, purgative
Alliofil *tablets* Herbapol, Poznań	*Allium* spp., *Urtica dioica* L.	Cold, immunity improvement, acceleration of cells regeneration, atheromatosis, diabets
Cholegran *granule* Herbapol Pruszków	*Marrubium vulgare*, *Leonurus cardiaca* L., *Hypericum perforatum* L., *Matricaria chamomilla* L., *Rheum emodi*, *Rhamnus frangula* L., *Mentha piperita* L. (Huds.), *Levisticum officinale Koch*	Hepathopathy, cholecystopathy, cholepathy, dyspepsia, habitual constipation
Gastrogran *granule* Herbapol Pruszków	*Ricinus communis*, *Trigonella foenum-graecum* L., *Menyanthes trifoliate* L., *Glycyrrhiza glbra* L., *Hypericum perforatum* L., *Salvia officinalis* L., *Levisticum officinale Koch*	Chronic peptic ulcer disease, gastritis, duodenitis, alimentary tract disorder
Normogran *granule* Herbapol, Pruszków	*Fucus vesiculosus* L., *Althaea officinalis* L., *Glycyrrhiza glbra* L., *Hypericum perforatum* L., *Achillea millefolium* L., *Rhamus frangula* L., *Mentha piperita* L. (Huds.)	Food poisoning, constipation
Digestive tablets *tablets* Laborfarm	*Silibum marianum* L., *Taraxacum officinale* L., *Carum carvi* L., *Mentha piperita* L. (Huds.), *Rhamus frangula* L.	Dispepsia, habitual constipation, meteorism, alimentary obesity, gastric secretion disorder
Tonic tablets *tablets* Laborfarm	*Crataegus oxyacantha* L., *Leonurus cardiaca* L., *Melilotus officinalis* (L.) Pallas	Cardiovascular system disorder, cardioneurosis, agioneurosis, general weakness, exhaustion
Sedative tablets *tablets* Laborfarm	*Melissa officinalis* L., *Leonurus cardiaca* L., *Valeriana officianalis* L., *Humulus lupulus* L., *Mentha piperita* L. (Huds.), *Lavandula officinalis Chaix et Kitt*	Nervous excitability, vegetative neurosis, tension, anxiety, motor agitation, menopause
Urogran *granule* Herbapol Pruszków	*Solidago virgaurea* L., *Equisetum arvense* L., *Betula pendula* L., *Cichorium intybus* L., *Levisticum officinale* L., *Acorus calamus* L.	Urinary tract inflammation, oligouria, metabolic disease

2.4. Extraction method

A conventional one-step extraction was carried out at 37 °C, in a water-bath shaker (elpan – Laboratory Instruments, type 357). Extractants used were deionised water, diluted hydrochloric acid (0.1 M) and a solution of composition similar to gastric juice, containing sodium chloride, hydrochloric acid and pepsin (1.6 g pepsin, 0.3 g of NaCl and 3.5 ml of 37% HCl made up to the 500 ml with deionised water).

15.0 ml of extractant solution were added to 0.3 g of dry herbal material in a glass test-tube. The closed tube was shaken for 1 h at 37 °C on a mechanical shaker at a speed 200 r/min. The supernatant was seperated from the solid residue by centrifugation for 0.5 h at 12,000 r/min (High Speed Brushless Centrifuge – MPW 350). All investigated extracts were stored in clean polyethylene bottles at 4 °C before analysis.

For evaluation of the influence of the leaching time, samples with the solution containing pepsin were also agitated for 16 h.

Extraction efficiency was calculated as a ratio of metal concentration in the extractant to element concentration in the samples obtained after complete decomposition in the microwave system.

With each set of digested and leached samples, a blank sample was passed through the entire procedure (under the same extraction or digestion experimental conditions), analysed and then used for correction of analytical signals.

2.5. Measurement method

The element concentrations (Al, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, P, Pb, Si, Sr, Ti, V and Zn) in digests and extracts were measured by atomic emission spectrometry with inductively coupled argon plasma as the excitation source. A Jobin-Yvon 38S spectrometer was equipped with a cross-flow nebuliser and Scott-type spray chamber for digest measurements and V-groove nebuliser and cyclonic chamber for extracted samples. The operating parameters and analytical line wavelengths used are in Table 2.

3. Results and discussion

Total concentrations of the elements determined in analysed herbal medicaments are shown as a arithmetic means and standard deviations in Table 3. Low contents of most of the elements (Al, B, Ca, Cr, Cu, P, Si and Zn) were observed in Alax. In Aliofil, the lowest concentrations of Ba, Fe, Mn, Sr and Ti were found. Both preparations contained Co, Ni and V below the detection limit. None (except Sedative and Digestive Tablets) of the examined medicaments contained detectable amount

Table 2
Instrumental and operating conditions for ICP-AES

Discharge parameters	
Forward power	1000 W[a]/1200 W[b]
Frequency	40.68 MHz
Plasma gas flow rate	13 l min^{-1}[a]/14 l min^{-1}[b]
Sheath gas flow rate	0.2 l min^{-1}
Nebuliser gas flow rate	0.3 l min^{-1}
Sample uptake	1.5 ml min^{-1}[a]/2.0 ml min^{-1}[b]
Monochromator	
	1 m Czerny-Turner
	Type: HR 1000
Gratings	4320 and 2400 grooves mm^{-1}
Slit width (entrance/exit)	20 μm/50 μm
Photomultiplier	R 955
Plasma observation zone	Radial, 12 mm above load coil

Analytical lines (wavelengths in nm)			
Al	396.152	Mn	259.373
B	249.773	Mo	202.030
Ba	233.527	Ni	221.647
Ca	317.933	P	214.914
Cd	226.502	Pb	220.353
Co	228.616	Si	251.611
Cr	267.716	Sr	407.771
Cu	324.754	Ti	334.941
Fe	259.940	V	292.402
Mg	285.213	Zn	202.548

[a] Applied for digested samples.
[b] Applied for extracted samples.

Table 3
Contents of the macro- and micro-elements in the analysed medicaments (μg/g)

	Alax $n=5$	Alliofil $n=5$	Cholegran $n=5$	Gastrogran $n=5$	Normogran $n=5$	Digestive tablets $n=5$	Tonic tablets $n=5$	Sedative tablets $n=5$	Urogran $n=5$
Al	98.1 ± 5.9	306 ± 18	240 ± 6	197 ± 12	156 ± 9	259 ± 12	263 ± 9	695 ± 23	112 ± 5
B	5.44 ± 0.30	6.18 ± 0.72	13.1 ± 0.4	11.2 ± 0.6	16.6 ± 0.4	20.4 ± 0.6	21.0 ± 0.5	19.6 ± 0.2	13.8 ± 1.0
Ba	10.7 ± 0.1	4.82 ± 0.79	23.9 ± 1.3	8.0 ± 0.3	13.2 ± 1.1	38.2 ± 1.3	38.3 ± 1.3	22.5 ± 0.3	11.2 ± 0.8
Ca	3740 ± 180	5840 ± 600	7700 ± 240	5870 ± 400	5930 ± 280	9180 ± 330	11320 ± 350	7710 ± 150	4600 ± 230
Cd	0.42 ± 0.02	0.38 ± 0.03	0.16 ± 0.04	0.09 ± 0.05	0.24 ± 0.08	0.13 ± 0.03	0.23 ± 0.04	0.18 ± 0.02	0.16 ± 0.05
Co	[a]	0.13 ± 0.06	0.17 ± 0.05	0.13 ± 0.06	0.13 ± 0.04	[a]	0.28 ± 0.09	0.33 ± 0.01	0.16 ± 0.09
Cr	0.30 ± 0.01	0.30 ± 0.10	1.46 ± 0.10	1.07 ± 0.15	0.74 ± 0.15	15.0 ± 1.1	20.6 ± 1.3	63.1 ± 2.6	0.83 ± 0.12
Cu	3.30 ± 0.19	9.04 ± 0.61	5.29 ± 0.55	5.63 ± 0.50	5.41 ± 0.89	10.1 ± 1.4	11.5 ± 0.7	28.4 ± 0.9	4.21 ± 0.95
Fe	148 ± 10	91.8 ± 6.0	349 ± 7	230 ± 8	204 ± 19	475 ± 36	429 ± 29	1032 ± 35	177 ± 5
Mg	5170 ± 70	1670 ± 30	1730 ± 30	1660 ± 30	2110 ± 50	2320 ± 50	2040 ± 40	2120 ± 20	1130 ± 50
Mn	44.8 ± 2.2	16.9 ± 1.2	112 ± 2	52.9 ± 2.4	71.1 ± 1.6	117 ± 3	82.6 ± 2.0	98.7 ± 1.5	136 ± 7
Ni	1.06 ± 0.19	0.56 ± 0.19	1.69 ± 0.08	1.53 ± 0.29	1.37 ± 0.22	9.15 ± 0.49	10.9 ± 0.4	32.3 ± 0.5	1.21 ± 0.09
P	622 ± 15	5220 ± 140	3670 ± 60	3490 ± 130	3070 ± 40	6950 ± 150	6530 ± 240	8070 ± 180	3270 ± 80
Pb	[a]	0.27 ± 0.89	[a]	[a]	[a]	1.34 ± 0.38	2.39 ± 1.39	2.14 ± 0.84	[a]
Si	32.4 ± 3.7	66.7 ± 4.8	76.7 ± 3.5	59.2 ± 2.8	51.3 ± 3.2	51.9 ± 10.8	38.9 ± 2.8	55.2 ± 3.5	1580 ± 50
Sr	34.8 ± 1.3	14.4 ± 0.3	28.3 ± 1.5	20.2 ± 1.0	74.2 ± 3.6	32.7 ± 1.8	24.1 ± 0.8	22.1 ± 1.1	14.5 ± 0.3
Ti	1.66 ± 0.06	0.72 ± 0.07	3.43 ± 0.35	2.25 ± 0.03	6.30 ± 0.15	5.85 ± 0.24	7.61 ± 0.03	16.4 ± 0.5	3.32 ± 0.17
V	[a]	[a]	1.44 ± 0.41	[a]	[a]	1.57 ± 0.41	2.64 ± 0.41	2.52 ± 0.57	0.45 ± 0.27
Zn	16.3 ± 1.7	18.6 ± 0.9	19.4 ± 1.4	24.0 ± 2.0	22.2 ± 2.2	22.7 ± 1.2	37.2 ± 3.3	36.6 ± 0.3	34.3 ± 2.1

n is the number of independent determinations.
[a] Concentration below detection limit.

of Mo or Pb. The highest concentrations of all examined elements were observed in Tonic Tablets, Sedative Tablets and Digestive Tablets. Therefore, those three herbal products were selected for extraction experiments.

Comparing the dose recommended by the medicine producer and assessed content of the essential minerals, it is possible to estimate the nutritive value of the examined herbal medicinal products. The first column of Table 4. presents results calculated for the remedies

Table 4
Dietary value of the analysed herbal medicaments measured here (calculated on the prescribed amount) compared to recommended values

Element	Daily intake for this study min.–max. (μg/day) (taking recommended dosage)	Reference data for adults acquired from literature			
		Daily dietary intake (μg/day) (Alberti-Fidanza et al., 2003; Biego et al., 1998; Iyengar 1998; Jorhem et al., 1998)	Recommended Dietary Allowances/Adequate Daily Dietary Intake (μg/day) (www.nap.edu, 1997, 2001; WHO, 1996)	Tolerable Upper Intake Level (mg/day) (www.nap.edu, 1997, 2001)	Maximum level of daily intake without detriment to health (mg) (Edited by Seńczuk, 1999)
Al	174–2650	2000–45,000	70,000[a]		36.4
B	9.67–153	300–20,000		11–20	0.01–0.02
Ba	11.1–124	600–750			16
Ca	6650–78,500	582–1340 (mg)	1000–1200 (mg)	2500	
Cd	0.08–2.26	23–120	70[a]		0.018–0.20
Co	0.10–1.68	7–50			0.3
Cr	0.70–14.3	20–320	20–35		0.06
Cu	5.82–75.2	1000–4800	900	10	3.2
Fe	212–3080	8100–22,700	*8–18* (mg)	45	15
Mg	2300–22100	163–433 (mg)	*320–420* (mg)	350	500
Mn	39.0–1190	1700–5900	1.8–2.3 (mg)	11	5
Ni	1.30–67.8	50–799		1.0	0.45
Pb	0–4.91	34–440	250[a]		0.3
Sr	32.4–685	858–1900			2
Ti	1.65–58.2	800			0.3
V	0.57–7.47	10–100		1.8	2.5
Zn	14.8–320	6800–22,500	*8–11* (mg)	40	17

Recommended Dietary Allowances, are set to meet the needs of almost all (97–98%) individuals in a group.
Adequate Daily Dietary Intake, is believed to cover the needs of all individuals in a group, but lack of data prevents being able to specify, with confidence, the percentage of individuals covered by this intake.
Tolerable Upper Intake Level, the maximum level of daily nutrient intake that is likely to pose no risk of adverse effect.
[a] Calculated from Provisional Tolerable Weekly Intake (WHO, 1996).

analysed here according to the prescription of those medicines (minimum and maximum amounts). Element concentration ranges, mentioned above, are estimated for data collected in Table 3. In Table 4 reference values for the micro-nutrient intake are also shown. As can be concluded, by comparing submitted data, namely Recommended Dietary Allowances, Adequate Daily Dietary Intake, Tolerable Upper Intake Level and maximum level of daily intake without detriment to health, concentrations of all of them were within safety baseline levels for human consumption. These results indicate that analysed herbal products are not negligible as a source of dietary micro-nutrients. It is clear that examined herbal drugs should be considered as trace elements supplements. This is especially important when taking into account accumulation of toxic elements and their effects on the human organism. Comparing results found here and the average daily intakes of various elements presented in the literature (Alberti-Fidanza, Burini, Perriello, & Fidanza, 2003; Biego, Joyeux, Hartemann, & Debry, 1998; Iyengar, Wolf, Tanner, & Morris, 2000; Jorhem, Becker, & Slorach, 1998), is possible to conclude that, for most of the examined nutrients, daily intake by the patients taking these popular herbal medicaments ranges from 0.5% to 10%. For Al (in Gastrogran), Ba (Cholegran, Gastrogran, Normogran and Urogran), Cr (in Digestive and Sedative Tablets), Fe (Cholegran, Gastrogran and Normogran), Mn (Cholegran, Gastrogran, Normogran and Urogran), Ni (in Sedative Tablets), Sr (Cholegran, Gastrogran and Normogran) and V (Cholegran and Sedative Tablets), maximal daily intake is above 10% and could even reach 50% (for Sr in Normogran). Analysing daily micro-nutrient consumption of the commercial herbal medicaments examined here, Alax and Aliofil cover bodily needs the least – below 5% of all element requirements is satisfied by the daily dose. In the case of Tonic Tablets, only Ba and Cr demand is met by over 5% (6 and 9, respectively). The highest micro-nutrient intake, is observed via Cholegran, Gastrogran and Normogran taking. Over 10% of the normal daily intake of Ba, Fe, Mn, Sr and V for Cholegran, Al, Ba, Fe, Mn, and Sr for Gastrogran and Ba, Fe, Mn and Sr for Normogran is met by taking the prescribed medicine dosage.

Analysis of Standard Reference Material (INCT-MPH-2: Mixed Polish Herbs) was performed for validation of the applied analytical procedures. Concentrations of elements were measured in samples mineralised by mixture of nitric acid and hydrogen peroxide (as is commonly used) in a closed system with the aid of microwave energy. Results of these analyses are shown in Table 5.

For most of the examined elements, good agreement between obtained data and certified values was observed. For Ba, Ca, Cd, Mg, Mn, Ni, Sr, V and Zn the differences in concentrations (measured and certified) were found to be with in the limits of standard deviation uncertainties. Only for Al, Co, Cr, Cu and Pb were significant differences between values noticed. For Fe, Mo, P and Ti, the standard reference material producer gave information values. Obtained values, except for Fe concentration, differed considerably from those given in attestation. Similar effects were observed during the Norway spruce needles (CRM 101-Norway spruce needles collected in Europe) analysis (Leśniewicz, Żyrnicki, & Schroder, 2003). Meaningful differences between Al, Ca, Cr, Fe and Mg concentrations and certified values were observed. Application of hydrofluoric and nitric acids to the microwave digestion led to Al, Ca, Cu, Mg, Pb, Sr and Ti concentrations increasing with simultaneous precision deterioration.

Table 5
Verification of digestion procedure and measurement accuracy – Standard Reference Material analysis (INCT-MPH-2: mixed polish herbs)

Element	Concentration (μg/g)	
	Certified value	Experimental data
Ba	32.5 ± 2.5	30.6 ± 01.0
Ca	10,800 ± 700	10,900 ± 400
Cd	0.199 ± 0.015	0.15 ± 0.06
Co	0.210 ± 0.025	0.12 ± 0.07
Cr	1.69 ± 0.13	0.99 ± 0.06
Cu	7.77 ± 0.53	9.98 ± 0.80
Fe	460[a]	455 ± 4
Mg	2920 ± 180	2800 ± 40
Mn	191 ± 12	181 ± 3
Ni	1.57 ± 0.16	1.66 ± 0.38
Pb	2.16 ± 0.23	1.62 ± 0.80
Sr	37.6 ± 2.7	34.4 ± 2.0
V	0.952 ± 0.163	0.85 ± 0.16
Zn	33.5 ± 2.1	34.8 ± 1.2

[a] Information value.

Precision of the measurements was investigated by analysis of five sub-samples, done in two runs, and was found to be below 5% for the digests and 15% for the extracts. In the case of elements extracted in very low quantities, e.g. Al, Cr, Ni, Si, the RSD values exceeded 30%.

Extraction experiments were performed to study trace element bioavailability by assessment of leaching effectiveness under various conditions, including those simulating digestion in the alimentary system.

Extraction efficiency for selected elements is shown in Fig. 1. In the figures, initials TT, ST and DT were used instead of full names of examined herbal medicines (Tonic Tablets, Sedative Tablets and Digestive Tablets). Based on results obtained for parallel samples, it was found that extraction efficiency depended on extractant solution, element and sample. Generally, significant differences in extraction effectiveness were found for various elements. Those differences indicated possibility of applications used here for extractants in the fractionation analysis of herbal supplements. For elements such

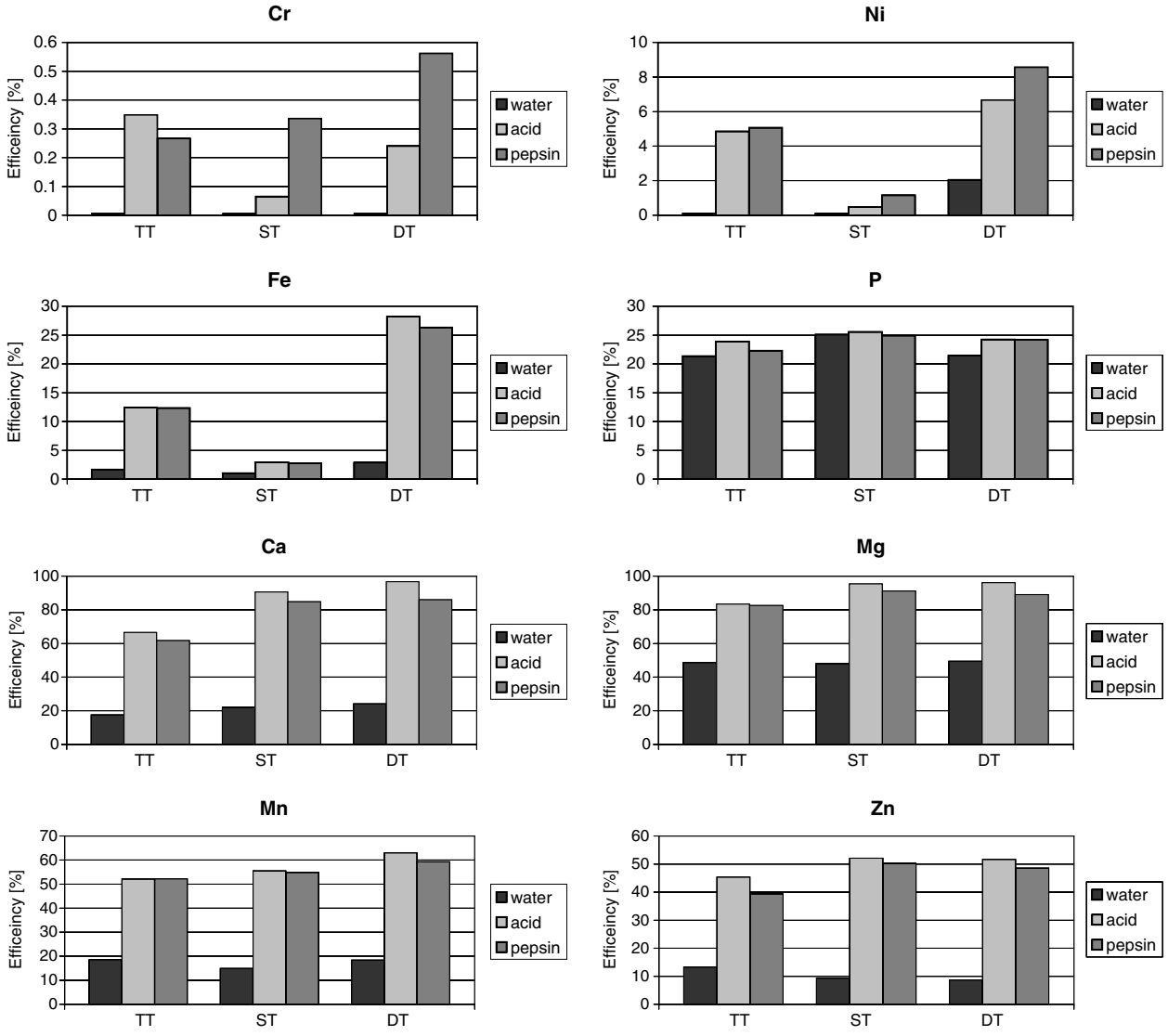

Fig. 1. Different extractant leaching efficiencies for selected elements.

as B, Ba, Ca, Mg, Mn, Sr and Zn, the highest leaching efficiency was achieved (at least 50% for the acid and pepsin solution). Very small concentrations of Al, Cr, Ni and Si were determined in all extractants. As can be seen in Fig. 1, water, an extractant removing the water-soluble metal fraction, leached the least amounts of all examined elements from all analysed medicaments. Only in the case of B, Ca, Mg, Mn and P was almost 20% of the total element content is present in extractable (i.e. water-soluble) form. Dilute hydrochloric acid, an agent liberating acid-soluble metal and the solution containing pepsin, for digesting proteins, extracted comparable, within standard deviation range, element amounts. As mentioned in the Introduction, leaching using those solutions leads to determination of the bioavailable fraction of the elements. In our investigation, the highest efficiency (over 40% for all samples) was observed for Ba, Ca, Mg, Mn, Sr and Zn. Almost quantitative leaching (i.e. effectiveness of over 80%) was achieved for Ca and Mg. For those two elements, extraction with dilute acid or with acidic solution containing proteolytic enzyme, could replace the mineralisation procedure using concentrated acids. No differences in properties of analysed materials were noticed.

According to the literature data, the entire digestion process takes up to 16 h (Harper, Rodwell, & Mayes, 1983); therefore to evaluate the influence of the extraction time on leaching effectiveness, samples with the solution containing pepsin were shaken for 1 and 16 h. Comparison of both processes efficiencies for selected elements is shown in Fig. 2. Our previous studies (Leśniewicz & Żyrnicki, 2003a, 2003b) showed that significant increases of element concentrations determined in extracts with shaking time elongation were possible only for Al and

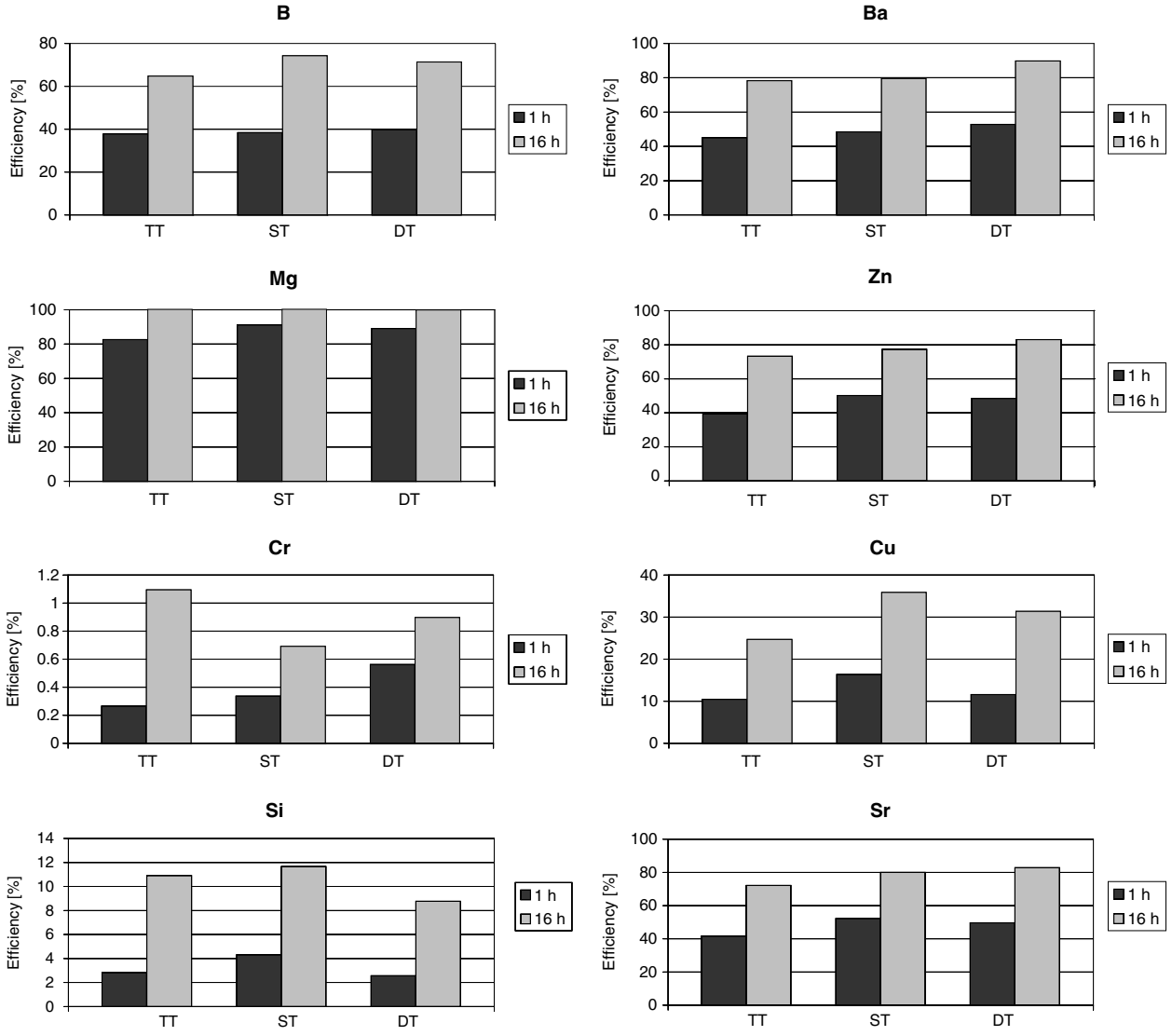

Fig. 2. Leaching time influence on extraction efficiency.

Fe. The present results, unequivocally, indicate a considerable rise of the enzymatic extraction efficiency with time. The minimum differences, observed for Al, Cr, Ni, and Si, amount to 3–10%. The average effectiveness increase is close to 30% and was achieved for B, Ba, Cu, Fe, Mn, P, Sr and Zn. Maximal efficiency increase was achieved for Ca, Cd, Co, Mg and Pb and it ranged from 50% to over 100%. It indicates full bioavailability of all above specified elements. Comparing examined medicaments, it was found that element leaching effectiveness increases were similar. Taking into account data presented in Table 4 (i.e. element daily intake) and nutrient bioavailability estimated by extraction efficiency, it is clear that the investigated herbal remedies are meaningful sources of major and trace elements in the human diet. Commercial herbal drug consumption cannot be ignored because the element toxicity risk assessment is based rather on habitual than on incidental intake.

4. Conclusions

For the first time, the total concentrations of macro- and micro-elements were measured in polish herbal medicaments. Significant numbers of herbal medicines, of various medical properties being used usually in long-term therapy, were examined. Element bioavailabilities were assessed using water, dilute hydrochloric acid and synthetic gastric juice as extractants. Nutritive values of the examined herbal medicinal products were evaluated. Results presented here clearly show that the examined herbal remedies play a meaningful role in

human nutrition as micro-nutrients sources. Their recommended daily dose consumption is not harmful.

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