MACRO, TRACE AND ULTRATRACE ELEMENTS IN THE HERB AND AQUEOUS EXTRACT OF SAINT JOHN'S WORT (HYPERICUM PERFORATUM L.)

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REZUMAT

Introducere: Prezentul studiu își propune investigarea componentelor anorganice ale Hyperici herba, precum și evaluarea ratei de extracție prin decocție. **Material și metode:** Probe vegetale de la 7 populații de sunătoare, dezvoltate pe soluri cu origine geologică diferită, au fost culese din flora spontană a Munților Banatului; în fiecare probă au fost analizate 46 de elemente prin plasmă cuplată inductiv cu spectroscopia de emisie atomică (ICP-AES) și plasmă cuplată inductiv cu spectrometria de masă (ICP-MS). **Rezultate:** Potasiul este prezent în cea mai mare cantitate (9,5 ± 1,9 g/kg greutate uscată - GU), fiind urmat de calciu (5,9 ± 2,0mg/kg GU), magneziu (2,0 ± 0,4 g/kg GU), mangan (145 ± 63 mg/kg GU), fier (80 ± 43,1 mg/kg GU) și zinc (51 ± 16,5 mg/kg GU). Conținutul elementelor toxice din probe variază, fiind situat sub limita admisă pentru plantele medicinale în cazul mercurului, plumbului și arsenului, dar depășind-o în cazul cadmiului (725 ± 481 mg/kg GU). **Concluzii:** Având în vedere conținutul relativ redus al acestei specii în elemente esențiale omului, și cantitatea mică în care Hyperici herba este recomandată în scop terapeutic, impactul său asupra aportului zilnic de elemente anorganice este redus (situat sub 5% din necesarului normativ). Deși culese din locuri nepoluate, probele analizate posedă un conținut crescut de Cd, demonstrând capacitatea sunătoarei de a acumula acest element. Diferențele privind conținutul elementelor minerale al plantelor culese din locuri diferite sunt mari, mai ales în cazul ultramicroelementelor, unde variații cu două ordine de mărime sunt frecvente.

Cuvinte cheie: Hypericum perforatum, macroelemente, microelemente, ultramicroelemente, rată de extracție, decocție

ABSTRACT

Introduction: The present study aims to investigate the inorganic components of Hyperici herba, and to assess their extraction ratio through decoction. **Material and methods:** Samples of seven populations of Saint John's wort growing on soils of different geologic origin were collected from the wild flora of the Banatian Mountains. In each sample 46 elements were analyzed by Inductively Coupled Plasma - Atomic Emission Spectroscopy (ICP-AES) and Inductively Coupled Plasma - Mass Spectrometry (ICP-MS). **Results:** Potassium is present in the largest quantities (9.5 ± 1.9 g/kg dry weight - DW), being followed by calcium (5.9 ± 2.0 mg/kg DW), magnesium (2.0 ± 0.4 g/kg DW), manganese (145 ± 63 mg/kg DW), iron (80 ± 43.1 mg/kg DW) and zinc (51 ± 16.5 mg/kg DW). The content in toxic elements varies, being below the admitted limit for medicinal plants in the case of mercury, lead and arsenic, but exceeding it in the case of cadmium (725 ± 481 mg/kg DW). **Conclusions:** Given this species' relatively low content in elements essential to humans, and the small quantity in which Hyperici herba is recommended for therapeutic purposes, its impact on the daily intake of inorganic essential elements is reduced (being below 5% of the normative requirement). Though originating from non-polluted sites, the investigated samples display a high Cd level, showing that Hypericum perforatum is prone to accumulating this element. The differences in the mineral content of samples gathered from different sites are high, especially in the case of ultratrace elements, where variations of two orders of magnitude are common.

Key Words: Hypericum perforatum, macro elementes, trace elements, ultratrace elelents, extraction ratio, decoction

INTRODUCTION

The aerial part of Saint John's wort (*Hypericum perforatum L.*) enjoys a high appreciation in today's phytotherapy, due to its clinically documented anti-inflammatory, cicatrizing, choleretic, antibacterial and antidepressant actions.[1,2] Its content in organic active principles (naphtodianthrones, volatile oils, flavonoids, polyphenolic acids and other compounds) has thoroughly been investigated, unlike the inorganic part, mainly researched in what toxic elements are concerned.[3] However, plants are known to contain a large variety of mineral elements, according to their specific biochemical features and site of development.[4] Following the ingestion of vegetal products, human organisms are exposed to these inorganic constituents, some of them essential, some toxic to man.

From the viewpoint of pharmacognosy, mineral constituents of medicinal plants may contribute to the outcome of a treatment with a beneficial effect (through essential elements like Ca, Mg, Zn, Fe, I, V,

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Received for publication: Jul. 20, 2005. Revised: Dec. 2, 2005.

Cr, Ni, Mn etc.) or with an undesired noxious one (in case of an overload with Cd, Pb, Hg, Tl, Cs etc).[5]

In this concept, the main objective of the present research was to perform an extensive investigation of the inorganic constituents of *Hyperici herba*, evaluating its content in 46 elements. In order to research the factors influencing the composition of the inorganic part, various vegetal samples were collected from the wild flora of the Banatian Mountains, originating from soils with differing geologic substrates: limestone, granite and phyllite, as the nature of the parent rock is known to have a major influence on the mineral content of the plants.[4] All samples were gathered from non-polluted sites, to evaluate only the natural level of inorganics in the herbs.

Subsequently, the degree to which the mineral elements pass from the biologic matrix into solution after decoction was also assessed. These measurements aimed at conferring biologic relevance to the chemical data regarding the composition of the dried plant, as soluble components are readily absorbed by human organisms.[6]

MATERIAL AND METHODS

Plant material

Seven samples of flowering aerial parts of *Hypericum perforatum L.* were gathered from non-polluted sites in the Banatian Mountains. The samples originated from soils with different geologic origin: limestone (4 samples), granite (2 samples) and phyllite (1 sample). After natural drying, the herbs were pulverized with ceramic instruments, avoiding the contact with metals.

Analysis of mineral elements

1. The assessment of the content in Al, B, Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, Pb and Zn was done by using Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-AES). Samples of 2-3 g herb were weighed in porcelain cups and heated to 105˚C in order to establish the dry mass. Matrix destruction was done after heating at a temperature of 550˚C for 6 hours, until the plant material was transformed into white ash. The ash was dissolved after boiling in hydrochloric acid 10%, followed by the quantitative transfer of the content in a test-tube. The acid solution was made up with bidistilled water to 25 ml, filtrated and measured by ICP-AES without further dilution.

The apparatus employed was IRIS Interprid II ICP-AES (Thermo Electron, Dreieich, Germany). The parameters of the measurement were as follows: nebulizer - Meinhard/K-type; nebulization chamber - glass, Scott chamber; torch-quartz; power 1150 W; frequency 27.12 MHz; nebulizing pressure - 2.6 bar; observation height 9.5 mm; pumping rate 1.8 l/min; number of measurements - 3; line positions (reading wavelengths): Al 396.1; B 208.9; Ca 318.1; Cr 267.7; Cu 324.7; Fe 240.4; K 766.4; Mg 279.0; Mn 293.9; Mo 202.0; Na 589.5; Ni 231.6; Pb 220.3; Zn 206.2.

2. The investigation of the content in Li, Be, V, Co, As, Se, Ag, Cd, In, Sn, Sb, Te, I, Cs, Tl, lanthanides and thorium was done by inductively coupled plasma - mass spectrometry (ICP-MS). Weighed samples of 0.3-0.4 g dried plant material were placed in Teflon crucibles and 4 ml of nitric acid (subboiled), 0.25 ml hydrochloric acid and 1 ml hydrogen peroxide were added. Mineralization was performed in a closed system with the use of microwave energy. The digestion solutions were transferred into volumetric flasks and made-up to 15 ml with water (nanopure); 1 ml of each solution was diluted 1:10 and analyzed.

The apparatus used for this research was ThermoElemental X Series ICP-MS (Thermo Electron, Dreiech, Germany, 2004). The determination parameters were set as follows: excitation power of plasma: 1360 W; flow rate for plasma gas: 13 l/min; for nebulization gas: 0,9 l/min; for auxiliary gas 0,9 l/min; extraction voltage: -173 V; pole bias: -2 V; hexapole bias: -2V; lens 1: 0,3 V; lens 2: -20,4 V; lens 3: -127,1 V; torch position: horizontal - 54 V, vertical - 577 V; amount of double charged ions CeO/Ce < 2% and Ba2+/Ba < 4%; measurement time 10 s in threefold repetitions; sampling depth 130.

As a first step, a semi-quantitative analysis was performed, allowing the estimation of the concentration ranges in the digestion solutions of the plant materials. The quantitative determinations were carried out by a calibration curve using ICP Multi Element Standard Solution XXI CertiPUR Merck, diluted to obtain optimal measurement range. Internal standard was rhodium (except for the measurement of iodine, where tellurium was used).

3. Mercury was assessed in the microwave-digested solutions by atomic fluorescence (apparatus Mercur, Analytik Jena AG, Germany, 2004).

Preparation of the aqueous extract

To 2,000 g dried herb 50 ml bidistilled water were added and heated to boiling; the temperature of 100˚C was maintained for 15 minutes. After cooling and filtration, 5 ml extract were introduced in a teflon crucible, and treated with 3 ml nitric acid subboiled and 0.250 ml hydrochloric acid suprapur. The solution was microwave-digested and analyzed by ICP-AES, ICP-MS and atomic fluorescence.

Accuracy of the data has been verified in case of ICP-OES and -MS by a parallel analysis of two certified reference materials: Peach Leaves 1547 and Oriental Tobacco Leaves CTA-OTL-1.

Statistic analysis was performed using Windows 2000 Excel, using the functions for the calculation of average values, standard deviation, t-test and Pearson's coefficient (r). The threshold for statistic significance was considered to be 0.05.

RESULTS

Following the analysis of the mineral content of Saint John's wort herb, 46 elements have been assessed. These exist in quantities of different orders of magnitude, ranging from grams/kg dry weight (DW) (potassium, calcium, magnesium) to some micrograms/kg DW (lithium, mercury, arsenic, lanthanides etc). Potassium is present in the largest quantities (with a mean of 9.5 ± 1.9 g/kg DW), being followed by calcium (5.9 ± 2.0 mg/kg DW) and magnesium (2.0 ± 0.4 g/kg DW). (Table 1) Of these three macro elements, potassium shows the largest variations in function of the site of harvesting, but no statistic correlation with the geologic substrate could be pointed out ($r < 0.8$).

When assessing the content in trace elements, manganese is best represented, with a mean content of 145 ± 63 mg/kg DW, being followed by iron, zinc, sodium, boron, aluminum, copper, nickel, cobalt and molybdenum. (Table 2) In case of manganese and iron, the site-specific variations are the largest; the boron and copper content are the most stable.

The ultratrace elements show a high variation in function of the site of harvesting, of up to two orders of magnitude (for vanadium, selenium, indium). Iodine, cadmium, tin and vanadium are present in the highest quantities (hundreds of µg/kg DW), while thallium, mercury, uranium and lanthanides like terbium, holmium, thulium and lutentium could be detected in only very small amounts, of some µg/kg DW. (Table 3)

A weak correlation ($0.80 < r < 0.88$) between the geologic substrate and the content in ultratrace elements could be pointed out only in case of elements like cadmium, chromium and selenium (where plants grown on limestone are richer in the considered element); arsenic and cesium (where plants grown on phyllite have a higher content in the given mineral) and lead - highest in plants grown on phyllite and granite substrates.

The extraction ratio of mineral elements from the vegetal matrix by decoction shows a large interval of variation, being high for the macro elements, arsenic,

Table 1. The content in mineral macroelements (mg/kg dry weight) of some samples of Hyperici herba and their extraction ratio through decoction.

Element	Site 1 lime	Site 2 lime	Site 3 lime	Site 4 lime	Site 5 *phyllite*	Site 6 granite	Site 7 granite	Mean content	Std. deviation	Extract. ratio (%)
Potassium	9297	10252	5677	8764	11247	11045	10316	9514	1909	68
Calcium	8112	3848	8309	3860	6694	6488	3844	5879	2011	64
Magnesium	1971	1919	2073	1226	2836	2227	1783	2005	485	65

Table 2. The content in trace mineral elements (mg/kg dry weight) of some samples of Hyperici herba and their extraction ratio through decoction.

Element	Site 1 lime	Site 2 lime	Site 3 lime	Site 4 lime	Site 5 *phyllite*	Site 6 granite	Site 7 granite	Mean content	Std. deviation	Extract. ratio (%)
Manganese	109	177	60	87	196	149	236	145	62.99	9.4
Iron	47	51	113	34	98	153	61	80	43.10	5.1
Zinc	37	57	44	45	84	54	36	51	16.53	25.9
Sodium	55	48	57	22	32	52	14	40	17.25	2.0
Boron	38	37	34	31	32	30	21	32	5.64	43.4
Aluminum	13	13	46	21	47	37	30	30	14.44	2.7
Copper	10.7	9.8	8.3	11.3	9.6	9.3	9.2	9.7	0.99	15.1
Nickel	4.1	4.5	3.3	6.2	9.7	6.3	3.6	5.4	2.24	34.9
Cobalt	0.28	0.31	0.28	0.18	0.91	0.18	0.07	0.32	0.27	32.4
Molybdenum	0.18	0.05	0.11	0.07	0.13	0.20	0.34	0.15	0.10	6.7

Table 3. The content in ultratrace mineral elements (μg/kg dry weight) of some samples of Hyperici herba and their extraction ratio through decoction.

Element	Site 1 lime	Site 2 lime	Site 3 lime	Site 4 lime	*Site 5* phyllite	Site 6 granite	Site 7 granite	Mean content	Strd deviation	Extract. ratio (%)
Iodine	920	558	2093	360	1450	725	1035	1020	589	21.8
Cadmium	551	1047	1550	945	825	320	125	752	481	34.5
Tin	149	127	139	2432	1268	355	662	766	855	8.5
Vanadium	423	237	284	239	2060	92	1470	686	762	35.9
Lead	320	190	223	379	521	334	812	397	212	9.1
Arsenic	366	39	51	40	875	21	41	205	320	88.2
Cerium	446	135	251	32	183	81	219	192	135	8.3
Chromium	161	240	401	173	64	42	188	181	119	4.3
Neodym	431	58	218	31	91	42	140	144	142	3.4
Lithium	181	5	158	133	53	108	109	107	61	34.3
Cesium	52	17	56	12	191	135	19	69	69	88.4
Praseodym	124	18	64	8	26	13	39	42	41	2.8
Selenium	177	19.1	23.9	12.4	3.1	4.0	18.2	36.8	62.3	45.0
Gadolinium	78	23	62	10	19	12	28	33	26	4.5
Indium	67.7	0.2	0.5	119	6.4	0.2	3.2	28.2	47.0	*
Samarium	72	11	35	6	16	7	25	25	23.3	4.6
Thorium	40.1	8.2	12.6	2.5	44.7	9.5	24.0	20.2	16.5	2.1
Dysprosium	47.5	12.4	31.8	7.1	12.1	5.6	18.2	19.2	15.2	4.7
Antimony	26.2	8.6	13.1	17.7	14.7	19.3	22.3	17.4	5.9	1.1
Tungsten	1.8	3.0	15.8	12.2	36.1	15.5	7.1	13.1	11.6	31.6
Beryllium	1.6	12.4	5.1	1.9	10.2	23.2	31.4	12.3	11.3	*
Tellurium	16.6	6.0	8.3	10.4	27	10.1	0.2	11.2	8.5	*
Silver	12.3	10.2	5.5	4.8	2.3	34.0	4.0	10.4	11.0	*
Erbium	23.0	6.2	16.8	4.1	5.2	2.6	9.2	9.6	7.5	*
Thallium	18.8	3.2	7.5	3.4	13.5	4.0	11.1	8.8	6.0	27.5
Mercury	8.3	4.7	2.2	5.0	24.2	8.6	1.5	7.8	7.7	9.9
Ytterbium	16.5	6.4	10.3	4.2	4.6	1.5	7.1	7.2	4.9	*
Uranium	7.5	2.5	4.1	1.3	16.4	2.9	6.4	5.9	5.1	73.1
Europium	12.2	2.1	6.7	2.1	6.2	0.6	6.2	5.2	4.0	*
Terbium	9.0	2.8	6.0	1.9	3.1	1.2	4.3	4.0	2.7	*
Holmium	8.5	2.9	6.4	1.7	2.2	1.3	4.1	3.9	2.7	*
Thulium	2.8	1.9	1.6	1.5	1.0	0.5	1.1	1.5	0.7	*
Lutentium	2.1	0.2	1.0	1.0	1.2	0.9	1.0	1.1	0.6	*

* The extraction ratio of the elements in question could not be established due to their very low concentration in the herbal decoction, situated below the detection limit of the apparatus.

cesium and uranium (where it surpasses 60%), median (20-50%) in case of zinc, nickel, boron, cobalt, iodine, cadmium, vanadium, lithium, selenium, tungsten, thallium, and low (below 10%) in case of the other researched elements (like manganese, iron, sodium, aluminum, molybdenum, the lanthanides).

DISCUSSIONS

Plants are able to take up various elements from their environment, according to both their physiological requirement, as well as to the presence of the given element in the environment. The existence of over 40 chemical elements has reliably been proven in higher plants, and this number is increasing along with the improvement of the available analytical methods.[7] All of the 46 mineral elements investigated in the present study proved to exist in the herb and most of them could be detected in the aqueous extract. For some elements of the ultratrace domain however (beryllium, silver, indium, some lanthanides), the detection limit of the apparatus did not allow their analysis in teas.

The herb of Saint John's wort contains at highest amounts potassium, calcium and magnesium, elements essential both to higher plants and humans.[8-10] In food plants, like cereals, potatoe, cauliflower and carrots, the concentration of these elements is lower than in *Hyperici herba*.[10-12] However, when compared to other vegetal medicinal products (*Cichorii herba*, T*araxaci herba*, *Virgaureae herba* or *Violae tricoloris herba*), the K, Ca and Mg content of *Hyperici herba* is on the average 30-50% less.[13] Even so, due to the high extraction ratio of the three macro elements through decoction, teas prepared of Saint John's wort could have a certain contribution to the completion of the human daily requirement in these elements. (Table 1)

The group of trace elements in plants, slightly differing from the trace elements in man, comprises Mn, Fe, Zn, Na, B, Al, Cu, Ni, Co, Mo.[7] They are either essential to vegetal species or possess a mere stimulating effect on the growth of plants, without being essential. There exist differences of up to one order of magnitude between the uptake of a given trace elements by Saint John's wort (Table 2), however these differences could not be attributed to the type of geologic substrate, being due to the specific local conditions existing on the site where the samples were gathered. The levels of trace elements in the analyzed samples are within the range of concentration found in other plants, showing a balanced uptake of these elements by *Hypericum perforatum*.[4]

The manganese content of *Hyperici herba* is relatively high in comparison with the average Mn concentration of herbaceous species that commonly make up the pastures (54 mg Mn/kg), or compared to vegetables 5-34 mg/kg and fruits <10 mg/kg.[14,15] *Hyperici herba* contains even higher amounts of Mn than its average content in medicinal plants (102 mg/kg).[13] In contrast, with a content of 80 ± 43 mg Fe/kg, the herb of *Hypericum perforatum* is poorer in iron than most medicinal plants (143 mg/kg), although richer than vegetables (3-6 mg/kg) and fruits (2-4 mg/kg).[13,16]

With a zinc content of 51 ± 16mg/kg, *Hyperici herba* surpasses the average of Zn in medicinal plants (40 mg/kg), but its concentration lies within the interval typical to non-polluted sites (15-100 mg/kg).[13,17] The Zn content of the herb is, when compared to the normative zinc requirement for humans (4.6 mg/day), very low.[18] A similar situation also applies for copper: *Hyperici herba* contains 9.7+0.9mg Cu/kg, the requirement being 0.75 mg/day.[18,19]

Given the rather low content of trace elements of *Hypericum perforatum*, and the low quantity in which it is consumed, the impact upon the daily intake of essential elements is reduced, being below 5% of the normative requirement established by the WHO.[18]

The herb of Saint John's wort also contains a high number of elements with no known function in the vegetal organism, included in the category of ultratrace (bulky) elements. (Table 3) The content of these elements shows very strong variations, which are not dependent on the type of geologic substrate, but rather on the particular site of harvesting.

The content in ultratrace elements of the plants gathered from site 1 (lime) differs significantly ($p < 0.05$) from the content of plants collected from site 2 (also lime), but the average content of ultratrace elements in the plants gathered from limestone shows no significant difference from the average content in plants harvested from granite weathering soils ($p > 0.05$).

Elements like iodine (1020 ± 589 µg/kg), selenium (36.8 ± 62.3 µg/kg) and vanadium (686 ± 762µg/kg), essential for man, are present in a low quantity, when compared to human normative requirement: 65 µg I/day, 40 µg Se/day and 9.75 µg V/day; as such, Saint John's wort could not be proposed as a mineral supplementation factor like other medicinal herbs (*Viola tricolor, Taraxacum officinale, Allium ursinum*).[13,18,20-22]

In what the content of toxic elements is concerned, *Hyperici herba* possesses with an average of 725 ± 481 µg/kg DW a very high cadmium concentration, exceeding the amount of 200 µg/kg DW allowed by regulations for medicinal plants.[23] This fact is mainly due to the particular capacity of Saint John's wort to accumulate cadmium, a feature also observed by other authors.[24,25] A second reason for the high cadmium level is the elevated geogenous occurrence of the element in the region where the plants were collected.[26]

The calcophilic character of the considered element accounts for its significantly higher Cd content in the plants grown on limestone. Although the cadmium content of the researched herb is relatively high, a direct threat to human health following the consummation of pulverized herb or aqueous extract for therapeutic purposes is not to be expected, since the tolerable daily intake for Cd is considered by the WHO to be 65 µg, contained in 40g dried plant material, when considering the Cd-richest samples (thus exceeding by ten times the average recommended dose of 4g Hyperici herba/day).[18] The lead, mercury and arsenic contents are below the required threshold limits (5 mg/kg DW for Pb, 100 µg/kg DW for Hg and 1mg/kg DW for As).[23,27]

CONCLUSIONS

The herb of Saint John's wort contains a large variety of mineral elements, ranging from concentrations of the order of grams/kg DW (K, Ca, Mg) to the order of micrograms/kg DW. The differences in the mineral content of samples gathered from different sites are high, especially in the case of ultratrace elements, where variations with two orders of magnitude are common. In case of macro- and trace elements, the content tends to be more stable. The type of the geologic substrate influences the mineral content in the case of only few elements, like Cd, Pb, As, Cr, Cs, Se, Te.

The extraction ratio of mineral elements through decoction shows a large interval of variation, being high for the macro elements, arsenic, cesium and uranium (where it surpasses 60%), median (20-50%) in case of zinc, nickel, boron, cobalt, iodine, cadmium, vanadium, lithium, selenium, tungsten, thallium, and low (below 10%) in case of the other researched elements (like manganese, iron, sodium, aluminum, molybdenum, lanthanides).

Given this species' low content in elements essential to man, and the low quantity in which *Hyperici herba* is recommended for therapeutic purposes, its impact upon the daily intake of inorganic essential elements is reduced, being below 5% of the normative requirement.

Though originating from non-polluted sites, the samples display a high Cd level, showing that *Hypericum perforatum* is prone to accumulating this element. Six of the seven samples surpass the threshold value of 200 µg Cd/kg imposed upon medicinal plants; however when considering intakes of 4g herb/day, and the extraction ratio of Cd through decoction (35%), the Cd content of the plant is not dangerous to human health. The content in other potentially harmful elements (lead, mercury, arsenic) is far below the required threshold limits.

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