Studii şi Cercetări	Biologie	12	15 - 19	Universitatea din Bacău	2007

ALLIUM URSINUM L.: A POTENTIAL SOURCE FOR COMPLETING THE RECOMMENDED DAILY INTAKE OF ESSENTIAL MINERAL ELEMENTS

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Keywords: *Allium ursinum, content in mineral elements*

INTRODUCTION

Wood garlic (*Allium ursinum*) enjoys a large popularity in Romania, its leaves being consumed fresh, as salad (in spring) or dried, as a spice. Known from traditional folk medicine for its stomachic, anthelmintic, antibacterial, depurative, expectorant activities [1], several recent pharmacological investigations have documented the impressive therapeutic potential of this plant in cardio-vascular disorders. Its content in gamma-glutamyl peptides and sulfur-containing compounds is believed to be responsible for the inhibition of the angiotensin I - converting enzyme (ACE) and the interference with the nitric oxide system [2,3]. On the other hand, high levels of adenosine [4] relax smooth vascular muscles by acting upon the ATP-dependent K-channels [5], thus enhancing the blood pressure lowering effects of this plant. Additionally, cardioprotective effects were pointed out, based on the significant reduction of the incidence of ventricular fibrillation [6]. *Allium ursinum* (AU) also inhibits 5- lipoxygenase, cyclooxygenase, thrombocyte aggregation, lowers the levels of total cholesterol and increases circulating insulin [7,8]. Several authors suggest that AU has a greater therapeutic benefit compared to *Allium sativum* [3,4,8].

Although the content in organic active principles of AU has thoroughly been investigated [9], the inorganic part received less attention. However, plants are known to contain a large variety of mineral elements, according to their specific biochemical features and site of development [10]. Following the ingestion of vegetal products, human organisms are exposed to these inorganic constituents, some of them essential, some toxic to man. As AU represents a very popular vegetable and spice, it is consumed in relatively large daily amounts. Thus, the intake of inorganic compounds through AU preparations could be significant.

In this concept, the objective of the present research was to perform an extensive investigation of the inorganic constituents of AU leaves, evaluating their content in 47 elements. All samples were gathered from non-polluted sites of the wild flora, in order to assess the natural level of inorganics in the leaves.

MATERIAL AND METHODS

Plant material. Samples of wood garlic leaves (*Allium ursinum L.* subsp. *ucrainicum* Kleopow et Oxner) were gathered from 5 populations of the wild flora of the Banatian Mountains (Romania). Voucher samples were deposited in the Herbarium of the Faculty of Pharmacy, Timisoara. After natural drying, the vegetal products 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 performed through Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP- AES). Samples of 2-3 g herb were 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).

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 performed through 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). 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 based on a calibration curve established with ICP Multi Element Standard Solution XXI CertiPUR Merck. Internal standard was rhodium.

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

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

RESULTS AND DISCUSSIONS

In the present work, 47 elements have been analyzed in wood garlic leaves. The investigated elements exist in quantities of different orders of magnitude, ranging from grams/kg dry mass (potassium, calcium, magnesium) to some micrograms/kg (lithium, mercury, arsenic, lanthanides etc). The mineral content displays a large variation interval, due to high values of standard deviations. This fact is common when evaluating plants from the wild flora, as the geogenous occurrence of the investigated element, and the nature of the weathering rocks have a high impact on the content of a mineral in plants [10]. However, in our study we could not point out a correlation between the nature of the parent rock (lime; granite) and the mineral content of the vegetal product; this situation indicates that the content of inorganics depends on many site-specific variables.

Of the investigated elements, K occurs in the highest concentration (29.3 g/kg), being followed by Ca (14.6 g/kg) and Mg (2.3 g/kg); this situation is similar in most plants [11]. When assessing the content of trace elements, aluminum is best represented, with a mean concentration of 224 mg/kg, being followed by iron, sodium, manganese, zinc, boron, barium, strontium, copper and nickel (Table 1). Ultratrace elements like vanadium (995 µg/kg), iodine (910 µg/kg), chromium (369 µg/kg), neodymium (259 µg/kg), cadmium (190 µg/kg), molybdenum (242 µg/kg) and cerium (239 µg/kg) are present in even lower amounts, of hundreds of µg/kg DM. Other constituents: thallium, mercury, uranium and certain lanthanides (terbium, holmium, thulium, lutentium) could only be detected in very small amounts, of some µg/kg DM.

Table 1. The content in d-block and p-block mineral elements of *Allium ursinum* leaves

Element		Site 1 Lime	Site 2 Lime	Site 3 Granite	Site 4 Lime	Site 5 Lime	MEAN VALUE	Strd deviat.
s-Block elements (alkaline and alkaline earths)	Li (µg/kg)	56	104	198	130	248	147	76
	Na(mg/kg)	327	123	201	125	42	164	107
	K(mg/kg)	28825	30070	30532	31180	25772	29276	2140
	Rb(mg/kg)	14.4	6.7	13.0	30.8	6.4	14.3	9.9
	Cs(µg/kg)	28.3	18.9	28.9	44.0	29.1	29.8	9.0
	Be(µg/kg)	4.2	36.5	44.2	11.3	8.6	20.9	18.1
	Mg(mg/kg)	2575	2816	1610	2618	1789	2282	543
	Ca(mg/kg)	14886	13470	7463	18684	18703	14641	4631
	Sr(mg/kg)	13.2	2.3	15.1	20.6	11.1	12.5	6.7
	Ba(mg/kg)	6.5	1.9	26.7	73.0	2.8	22.2	30.2
Lanthanides	Ce(µg/kg)	134	215	230	345	271	239	77
	Pr(µg/kg)	70	30	28	192	42	72	69
	Nd(µg/kg)	252	96	113	681	152	259	244
	Sm(µg/kg)	45.4	17.5	24.5	115.2	28.0	46.1	40.0
	Eu(µg/kg)	8.0	3.5	4.2	29.7	6.1	10.3	10.9
	Gd(µg/kg)	51.8	23.7	27.7	154.4	29.6	57.4	55.3
	Tb(µg/kg)	5.7	2.7	2.6	19.4	4	6.9	7.1
	Dy(µg/kg)	28.1	14.1	16.9	103.4	21.1	36.7	37.6
	Ho(µg/kg)	4.8	2.6	2.7	21.5	4.2	7.2	8.1
	Er(µg/kg)	11.8	7.4	7.7	51.1	11.2	17.8	18.7
	Tm(µg/kg)	1.7	1.0	1.1	6.3	2.3	2.5	2.2
	Yb(µg/kg)	6.3	6.6	8.1	30.2	9.2	12.1	10.2
	Lu(µg/kg)	1.1	0.6	0.5	3.9	1.1	1.4	1.4
Actinides	Th(µg/kg)	16.3	20.0	24.7	31.6	55.6	29.6	15.6
	U(µg/kg)	1.6	5.4	14.5	8.1	6.0	7.1	4.8

Transitional elements	V(µg/kg)	543	942	1021	1190	1280	995	286
	Cr(µg/kg)	502	561	193	335	253	369	158
	Mn(mg/kg)	66	59	175	100	72	94	48
	Fe(mg/kg)	167	136	142	235	250	186	53
	Co(µg/kg)	161	210	105	98	100	135	49
	Ni(mg/kg)	4.1	5.3	3.1	2.9	3.5	3.8	1.0
	Cu(mg/kg)	12.1	11.2	12.3	9.3	8.2	10.6	1.8
	Zn(mg/kg)	30	25	36	17	17	25	8.3
	Mo(µg/kg)	118	264	455	144	231	242	133
	Ag(µg/kg)	0.8	2.4	50.4	2.2	1.2	11.4	21.8
	W(µg/kg)	1.0	16.1	15.8	6.3	6.2	9.1	6.6
	Cd(µg/kg)	228	165	175	193	189	190	24
	Hg(µg/kg)	17.5	20.2	22.6	46.0	15.4	24.3	12.4
p-Block elements	Pb(mg/kg)	4.67	2.30	0.39	2.81	3.58	2.75	1.59
	Al(mg/kg)	196	234	314	157	217	224	58
	Tl(µg/kg)	1.0	4.1	25.3	10.3	6.0	9.3	9.5
	Sn(µg/kg)	161	317	487	63	265	259	161
	Sb(µg/kg)	9.3	41.3	17.0	25.4	27.8	24.2	12.1
	B(mg/kg)	21.4	23.1	19.9	36.2	16.9	23.5	7.5
	As(µg/kg)	163	45	43	68	112	86	51
	Se(µg/kg)	147	11	1	24	60	49	59
	Te(µg/kg)	0.1	12.2	10.4	2.3	3.2	5.6	5.3
	I(mg/kg)	0.63	0.39	1.52	0.64	1.36	0.91	0.50

The presence of all the investigated elements in AU leaves shows that 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 their environment. In fact, it can be assumed that every natural element of the periodic system is present in higher plants. It is rather the sensibility of the employed analytical method and the ability to secure highly pure reagents / work environment that limit the identification of a given element in vegetal materials. Some of the mineral elements are essential to plants while others, termed bulky elements, have no known function in the plant organism [12]. Constituents of both categories are however passed on, to different degrees, during the consummation of the plants by animals or humans. From the viewpoint of their relevance to man, mineral constituents present in medicinal plants may contribute to the outcome of a treatment with a beneficial effect (through essential elements like Ca, Mg, K, Zn, Fe, I, V, Cr, Ni, Mn etc.) or with an undesired, toxic one (in case of an overload with Cd, Pb, Hg, Tl, Cs etc) [13].

In order to evaluate the impact of AU leaves consummation, the average daily intake of mineral micronutrients through this product was calculated (table 2), based on the daily normative requirements (NDR) recommended by the World Health Organization [14]. Encouraging results show that AU leaves provide significant amounts of essential minerals like Ni, V, Mn, Ca, Fe, K, enhancing the beneficial effects of wood garlic in case of their prophylactic/ therapeutic utilization, especially in cardio-vascular disorders.

Table 2. The average daily intake of mineral elements through 5 g dried leaves

Element	NR/day	Content in minerals	% to which the daily NR is covered
K (mg)	1500	146.4	9.8
Mg (mg)	200	11.4	5.7
Ca (mg)	500	73.2	14.6
Fe (µg)	6600	931.0	14.1
Zn (µg)	4600	125.0	2.7
Mn (µg)	975	468.0	48.0
Cu (µg)	750	53.2	7.1
Mo (µg)	25	1.21	4.9
Ni (µg)	32.5	18.9	58.2
I (µg)	65	4.55	7.0
Se (µg)	40	0.24	0.6
Cr (µg)	33	1.84	5.6
V (µg)	9.75	4.98	51.0
Li (µg)	97.5	0.73	0.8

NR – normative requirement (WHO, 1996) [14]

More particularly, the cardioprotective effect of AU [6] is sustained by the high Ni content of AU leaves, as researches proved that in cases of cardiac infarction the Ni content is lower in cardiac muscle compared to control persons [15]. On the other hand, vanadium enhances glucose uptake by the cells [16] and consequently has a positive impact on the nutrition of the cardiac muscle. Antioxidative micronutrients like Mn, which is the constituent / activator of several enzymes, including the mitochondrial manganese-superoxyde-dismutase, protect the cells against the oxydative damage [17] and are believed to affect the blood pressure. The Fe supplementation has a positive effect due to its role in the distribution and utilization of oxygen; through its integration in the cytochrome C, it is implicated in oxydative phosphorylations [18]. In fact, the Fe content of AU leaves is approximately threefold than that of stinging nettle [11], considered to be one of the Fe-richest plants. The content in potassium, calcium and magnesium, vital ions in the contraction and electrophysiology of the heart [19], also have a positive impact on cardiovascular health.

Unlike other medicinal plants (*Hypericum perforatum* and *Viola tricolor* - accumulating Cd and Pb; *Equisetum arvense* – taking up large amounts of As [11]), wood garlic is not prone to accumulating toxic elements. Its average content in noxious metals is below the admitted limit for medicinal plants: 0.2 mg/kg DM for Cd, 5 mg/kg DM for Pb, 100 μg/kg DM for Hg and 1mg/kg DM for As [20,21] – table 1. Other toxic elements, like U, Cs, Be, Tl, Sn are present in extremely low quantities and do not pose any threat to human health. AU leaves also contain a series of elements (lanthanides), about which the present knowledge of the medical community is yet reduced. In comparison with the lanthanide concentration of other plants [10], the leaves of AU have a comparable content that can be considered non-toxic.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. M. Grün and Dr. M. Runge from "Agrar- und Umweltanalytik Jena" for their support in the analytical determinations.

SUMMARY

The purpose of this study was to perform an extensive investigation of the inorganic components of Alii ursini folium, appreciated both as an aliment as well as a phytotherapeutical remedy. Forty-seven elements were analyzed leaves of wild-growing wood garlic. The high content in essential minerals encourages their consummation, completing the daily intake of essential mineral elements: 5 g dried leaves contribute with 58% of the normative daily intake for Ni, 51% for V, 48% for Mn, 15% for Ca, 14% of Fe, 10% for K and 7% for Cu.

CONCLUSIONS

The results of the present study encourage the consummation of wood garlic leaves. Besides the benefits owed to organic constituents, they complete the daily intake of essential inorganic elements. Amounts of as little as 5 g dried leaves contribute with 58% of the normative daily intake for Ni, 51% for V, 48% for Mn, 15% for Ca, 14% of Fe, 10% for K, 7% for Cu and I, and 6% for Mg.

The wide-spread character of this plant in Romania and the regenerable nature of its leaves enhance the potential of wood garlic to become a source for the completion of minerals with physiologic/pharmaceutical relevance.

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