

**RESEARCHES UPON INDIGENOUS HERBAL
PRODUCTS FOR THERAPEUTIC
VALORIFICATION IN METABOLIC DISEASES
NOTE I. *BETULAE FOLIUM* AND *RUBI IDAEI
FOLIUM*, SOURCES OF MICRO- AND
MACROELEMENTS**

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Abstract

The role of micro- and macroelements in some metabolic diseases' pathology (diabetes mellitus, dyslipidaemia) is well known. The purpose of the study was a screening of macro- and microelements found in birch (*Betulae folium*) and raspberry (*Rubi idaei folium*) leaves and the quantitative determination of those elements (calcium, potassium, magnesium, manganese, zinc, iron, copper, nickel, chromium and vanadium) implicated in diabetes and dyslipidaemia pathology, for therapeutic valorification of both herbal products as adjuvants in these metabolic disorders' treatment. Regarding this, qualitative (X-ray fluorescence = XRF) and quantitative (inductively coupled plasma atomic emission spectroscopy = ICP-AES, flame atomic absorption spectroscopy = F-AAS) methods have been used. Calcium, potassium, zinc, manganese, iron, copper and nickel were identified in both herbal products using XRF. *Betulae folium* and *Rubi idaei folium* are considerable sources of: calcium (11,600; 19,400 mg kg⁻¹), potassium (16,200; 40,300 mg kg⁻¹), magnesium (6,300; 7,200 mg kg⁻¹), manganese (3,200; 94 mg kg⁻¹), zinc (225; 48.5 mg kg⁻¹), iron (49.3; 335 mg kg⁻¹), copper (19.09; 15.05 mg kg⁻¹) (ICP-AES) and chromium (69.85; 23.28 mg kg⁻¹) (F-AAS). Nickel and vanadium were not quantified, being under the detection limit of the used methods. The presence of chromium in raspberry leaves hasn't been mentioned before.

Rezumat

Rolul micro- și macroelementelor în patologia unor afecțiuni metabolice (diabet zaharat, dislipidemii) este bine cunoscut. Scopul lucrării a constat într-un *screening* al macro- și microelementelor, din frunzele de mesteacăn (*Betulae folium*) și de zmeur (*Rubi idaei folium*), precum și în determinarea cantitativă a acestora (calciu, potasiu, magneziu, zinc, mangan, fer, cupru, nickel, crom, vanadiu), pentru valorificarea terapeutică a ambelor produse vegetale, ca adjuvante în tratamentul acestor afecțiuni. S-au utilizat metode calitative (XRF = fluorescență de raze X) și cantitative (ICP-AES = spectrometrie de emisie

atomică cu plasmă cuplată inductiv și F-AAS = spectrometrie de absorbție atomică în flacără). Prin XRF s-au identificat în ambele produse vegetale ioni de calciu, potasiu, zinc, mangan, fer, cupru, nichel. *Betulae folium* și *Rubi idaei folium* au un conținut semnificativ de: calciu (11.600; 19.400 mg kg⁻¹), potasiu (16.200; 40.300 mg kg⁻¹), magneziu (6.300; 7.200 mg kg⁻¹), mangan (3.200; 94 mg kg⁻¹), zinc (225; 48,5 mg kg⁻¹), fer (49,3; 335 mg kg⁻¹), cupru (19,09; 15,05 mg kg⁻¹) (ICP-AES) și crom (69,85; 23,28 mg kg⁻¹) (F-AAS). Conținutul de nichel și vanadiu nu a fost determinat, fiind sub limita de detecție a metodelor utilizate. Prezența cromului în frunzele de zmeur nu a fost raportată anterior.

Keywords: *Betulae folium*, *Rubi idaei folium*, microelements, macroelements

Introduction

The role of micro- and macroelements in some metabolic diseases' pathology (diabetes mellitus, dyslipidaemia) is well known. Among microelements, chromium stimulates insulin secretion through phosphorylation of its receptor IRS₁ [12] and together with niacin lowers total cholesterol and LDL levels [16]. Zinc is a cofactor for superoxide dismutase and NADPH-oxidase and mutations of zinc conveyor (ZNT₈) genes represent a major risk for developing diabetes [5]. Recent work have shown that dyslipidaemic patients have low zinc/copper ratios [8].

Research studies have shown that serum levels of manganese are low in diabetic patients with hepatic disorders [12]. The role of manganese superoxide dismutase (Mn-SOD) as a key mitochondrial enzyme with a crucial role in the antioxidant defense system, is also well known [15], since oxidative stress is responsible for vascular complications of diabetes mellitus [3].

Vanadium and vanadium compounds have been reported to mimic insulin action in liver, skeletal muscles and adipose tissues by increasing glucose uptake, stimulating expression of insulin-sensitive glucose transporter protein (GLUT-4), stimulating glycogen synthesis and lipogenesis; vanadium is also able to regenerate β-cells in the pancreas tissue of diabetic rats [4, 14]. Iron, copper and nickel, which are responsible for lipid peroxidation, have elevated serum concentrations in diabetic patients [12].

Among macroelements, calcium mediates phosphorylation of insulinic receptor IRS₁ and low magnesium levels were found in diabetic patients with severe retinopathy and microalbuminuria [21]; magnesium level is also interrelated with HLD cholesterol [17]. It has been demonstrated that potassium levels of diabetic patients are low in muscles and red blood cells [18].

The purpose of the study was a screening of macro- and microelements, found in birch and raspberry leaves, as well as the quantitative determination of those elements implicated in diabetes and dyslipidaemia pathology (calcium, potassium, magnesium, manganese, zinc, iron, copper, nickel, chromium and vanadium), for therapeutic valorification of both herbal products, as adjuvants in metabolic disorders' treatment.

According to scientific literature, raspberry leaves are a source of: calcium, potassium, manganese, magnesium, copper, zinc [10], vanadium ($0.138\text{--}1.958\text{ mg kg}^{-1}$) [1] and birch leaves contain: calcium ($3,200\text{--}16,200\text{ mg kg}^{-1}$), potassium ($4,600\text{--}16,800\text{ mg kg}^{-1}$), magnesium ($1,050\text{--}4,030\text{ mg kg}^{-1}$), manganese ($130\text{--}9,743\text{ mg kg}^{-1}$) [9], chromium (0.39 mg kg^{-1}), zinc ($13.6\text{--}275\text{ mg kg}^{-1}$) [11], iron (492 mg kg^{-1}) [19], nickel, copper [13] and vanadium ($0.072\text{--}0.183\text{ mg kg}^{-1}$) [1]. Both herbal products are also known for their high polyphenols' content [2, 20].

Materials and Methods

For all determinations, *Betulae folium* (leaves of *Betula pendula* Roth.) and *Rubi idaei folium* (leaves of *Rubus idaeus* L.) harvested in May 2010, from Romania, Morărești village, Argeș District and Jilava village, Ilfov District have been used. Both herbal products were dried in shadow and stored in laboratory conditions.

For determination of macro- and microelements, qualitative (X-ray fluorescence = XRF) and quantitative (inductively coupled plasma atomic emission spectroscopy = ICP-AES and flame atomic absorption spectroscopy = F-AAS) methods have been used.

XRF determinations were performed using a PW4025 MiniPal 2 (PANalytical, Netherlands) EDXRF spectrometer. The method offered a screening (qualitative results) of macro- and microelements, being able to identify elements from Na to U ($11 < Z < 90$) and it didn't require a special sample preparation like ICP-AES and F-AAS methods. Determinations were carried out in helium atmosphere, for 300 seconds, without any filter, the voltage was 20 and the current intensity was automatic. Characteristic X-rays (with a specific energy for each element) were sent to a detector with a resolution of 150 eV at 5,89 keV (Mn-K α line). The signal produced by the detector was processed through a multichannel analyser (MCA), in order to produce a digital spectrum. The detection limit of the method was 100 ppm [7].

ICP-AES was used for the quantitative analysis of calcium, potassium, zinc, manganese, iron, copper and nickel. Measurements were performed using a Varian 110 spectrometer (Varian, USA). The generator

had 60 MHz, the argon flow was 12L/min, a V-Groove nebulizer and a Czerny-Turner monochromator with 1800 lines/mm have been used. The spray chamber was resistant to fluorhydric acid. The detection limits of device range (ppm) and the wavelengths (nm) for the analysed elements were: 0.33; $\lambda=769.896$ (potassium), 0.36; $\lambda=422.673$ (calcium), 0.48; $\lambda=285.13$ (magnesium), 0.03; $\lambda=257.61$ (manganese), 0.42; $\lambda=213.856$ (zinc), 0.02; $\lambda=259.94$ (iron), 0.35; $\lambda=213.604$ (nickel), 0.01, $\lambda=324.754$ (copper). Calibration curves for Ca, K, Mg, Mn, Zn, Fe, Cu, Ni were for 1-10 ppm range with a good linearity ($R^2 > 0.9998$, $n = 5$). Results were expressed in mg kg^{-1} dried herbal product [6].

Preparation of samples: 0.4177 g raspberry leaves and 0.5485 g birch leaves were mineralized in Berhof microwave digester (for 2 hours), by a mixture of concentrated nitric acid (Sigma-Aldrich) and hydrogen peroxide (Chemopar) in a ratio of 20:5 (v/v) and diluted with water in a 25 mL volumetric flask [6].

F-AAS was used for determination of vanadium and chromium content. Measurements were performed with Varian SpectrAA-220 spectrometer (Varian, USA), equipped with deuterium background corrector. Metals hollow-cathode lamps (Varian) were employed as radiation source. The flame type, its temperature, the lamp current, the slit widths and the wavelengths used were: air/acetylene, 2100-2400°C, 7mA, 0.2nm, $\lambda=357.9$ nm (chromium) and nitrous oxide/ acetylene, 2600-2900°C, 20 mA, 0.2nm, $\lambda=318.5$ nm (vanadium). A Czerny-Turner monochromator with 1200 lines/mm has been used. The detection limit of the method was 0.06 ppm (chromium) and 1 ppm (vanadium). Calibration curve for chromium was in 2-10 ppm range ($R^2 = 0.9998$, $n = 3$) and for vanadium 2-5 ppm ($R^2 = 0.9980$, $n = 3$). Results were expressed as mg kg^{-1} dried herbal product [22].

Preparation of samples: 15 g herbal products were calcinated with sulphuric acid at $650^\circ\text{C} \pm 5^\circ\text{C}$, for 3-4 hours (Microterm L 1103 Caloris oven). The ash (0.5 g birch leaves and 1g raspberry leaves) was mineralized for 2-3 hours with 5 mL nitric acid : clorhidric acid = 1:1 ratio (v/v) (Merck) and diluted to 100 mL with ultrapure water (Merck) in a volumetric flask [22].

Results and Discussion

Using XRF, calcium, potassium, zinc, manganese, iron, copper and nickel were identified in both herbal products. Errors have occurred for the qualitative determination of magnesium, due to its small atomic number ($Z=12$). Vanadium and chromium were not identified, as they were proven to be under the detection limit of the method (table I). By analysing XRF

spectra (figures 1, 2), one can note the presence of other elements: sodium, phosphorus, sulphur, aluminium and chlorine in both leaves and titanium only in *Betulae folium*, with no clinical significance in diabetes and dyslipidaemia pathology.

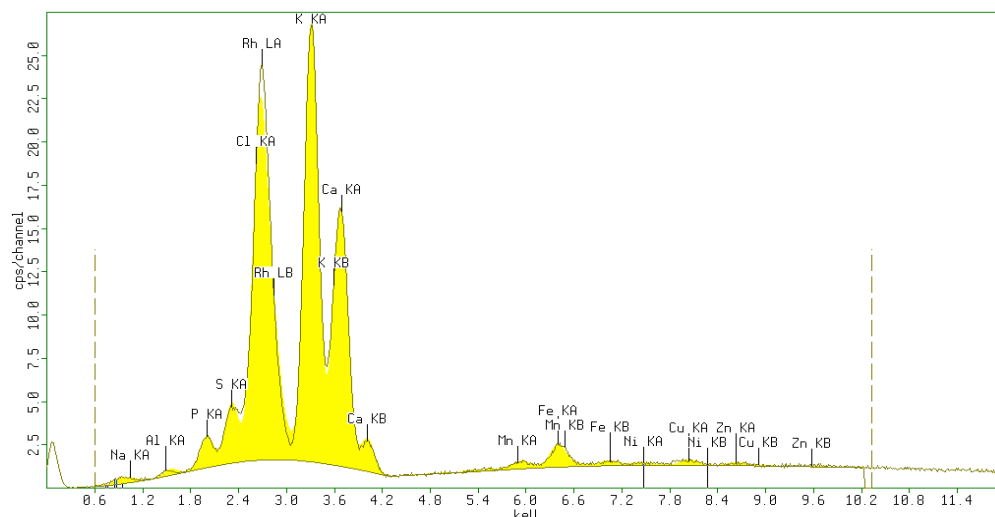


Figure 1
XRF spectrum of *Rubi idaei folium*

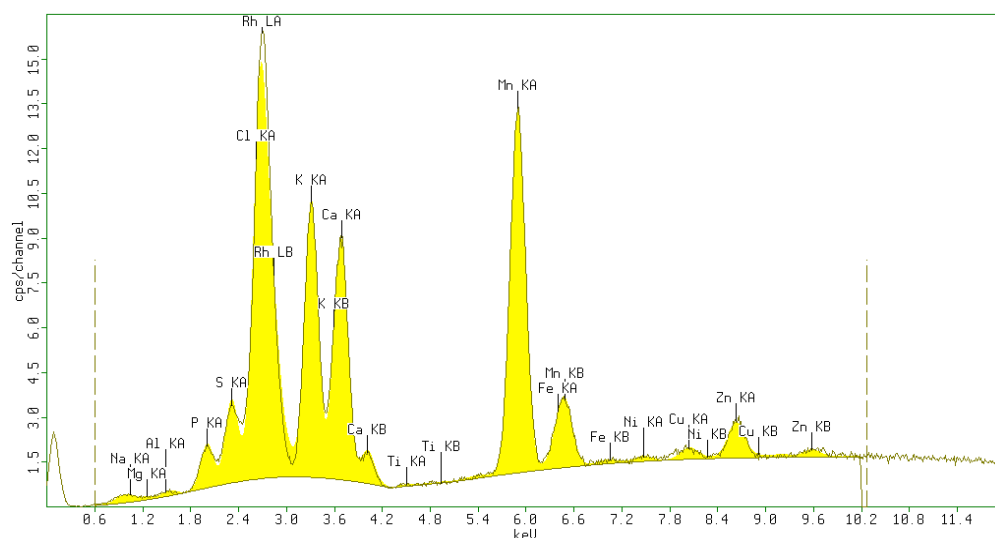


Figure 2
XRF spectrum of *Betulae folium*

ICP-AES and F-AAS quantitative results are shown in table II.

Table I
XRF qualitative results

Element (%)	<i>Betulae folium</i>	<i>Rubi idaei folium</i>
calcium	18.9	29.7
potassium	22.4	42.9
magnesium	NA	NA
zinc	1.2	0.1
manganese	14.9	0.26
iron	0.2	1
copper	0.43	0.24
nickel	0.74	0.09
chromium	SLD	SLD
vanadium	SLD	SLD

„NA” – not applicable; „SLD” - under the detection limit

Table II
ICP-AES and F-AAS quantitative results

Element (mg kg ⁻¹)	<i>Betulae folium</i>	<i>Rubi idaei folium</i>	Method
calcium	11,600	19,400	ICP-AES
potassium	16,200	40,300	
magnesium	6,300	7,200	
zinc	225	48.5	
manganese	3,200	94	
iron	49.3	335	
copper	19.9	15.05	
nickel	SLD	SLD	F-AAS
chromium	69.85	23.28	
vanadium	SLD	SLD	

„SLD” - under the detection limit

ICP-AES method showed that calcium, potassium and magnesium content is higher for raspberry leaves, but manganese and zinc quantities are smaller compared to birch leaves (correlation with XRF qualitative results). For both herbal products, the amount of iron and copper (responsible for lipid peroxidation) is low compared to the rest of elements, with beneficial effects.

The content of magnesium, manganese and chromium in *Betulae folium* is higher compared to scientific literature [9, 11]. High quantities of manganese are associated with an acid pH of the soil [9], whereas high content of calcium and low content of nickel, copper and iron are associated with low pollution extent, in correlation with the place of harvest [13].

The presence of chromium and nickel in *Rubi idaei folium* has not been mentioned before, nor the amounts of the other analysed elements [1, 8]. Opposite to scientific literature [1], the content of vanadium was not determined, being under the detection limit for both herbal products.

Conclusions

Raspberry and birch leaves represent a considerable source of macro- and microelements. The originality of our work consists in qualitative analysis of macro- (calcium, potassium) and microelements (zinc, manganese, iron, copper, nickel) from both herbal products using XRF analysis and the quantitative determination of these elements and magnesium in *Rubi idaei folium* using ICP-AES. The presence of chromium in raspberry leaves and its content (determined by F-AAS) have not been mentioned before.

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