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Metalic Trace Elements in Medicinal Plants from Macedonia

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Abstract: Medicinal plants are a source of biologically important elements, which may play a part in the observed therapeutic uses of these plants. It is therefore of major interest to establish the levels of some elements in common herbal plants because, at elevated levels, these elements can also be dangerous and toxic. It was therefore imperative to explore the present status of local plants *Urtica dioica L. Taraxacum officinale*, *Robinia pseudoacacia* and *Matricaria recutita*, in terms of selected heavy metals and macronutrients (Zn, Cu, Fe, Cr, Ca, K, Li, Mg and Na). All the elements were accumulated to greater or lower extents by all 4 plant species studied. Elemental studies of the plants showed that they contained large amounts of nutrients and were rich in Mg, Ca and K.

Key words: Macroelements % Metals % Medicinal plants % Macedonia

INTRODUCTION

A number of minerals essential to human nutrition are accumulated in different parts of plants. They accumulate minerals essential for growth from the environment including metals such as Cd, Co and Ag, which are of unknown direct benefit to the plant [1]. Recently, plant species have been identified that contain nutrients displaying new beneficial medicinal or therapeutic properties [2]. The past decade has seen a significant increase in the use of herbal medicine due to their minimal side effects, availability and acceptability to the majority of the population, so medicinal plants play an important and vital role in traditional medicine and are widely consumed as home remedies. Environment, pollution, atmosphere, soil, harvesting and handling are some of the factors, which play an important role in contamination of medicinal plants by metals and microbial growth. Trace elements have curative and preventive role in combating diseases. It is therefore of major interest to establish the levels of some metallic elements in common used plants because, at elevated levels, these metals can also be dangerous and toxic [3]. Thus the analytical control of metals in plants, especially medicinal plants, is part of quality control, which should establish their purity, safety and efficacy, as a World Health Organization (WHO) recommended in a number of resolutions [4]. Furthermore, the European Commission has established the lead, cadmium and mercury limits in food supplements that have been in force since March 2001 [5].

Several attempts have been made at determination of the metal contents of medicinal and aromatic plants from all over the world [6-11], but reports are scanty with respect to plants growing in R. Macedonia, beside papers on metal content in Thymus species from all over the country [12-15]. It was therefore imperative to explore the present status of local plants *Urtica dioica L.* (*Urticaceae*), *Taraxacum officinale* (*Asteraceae*), *Robinia pseudoacacia* (*Fabaceae*) and *Matricaria recutita* (*Asteraceae*), in terms of selected heavy metals and macronutrients (Zn, Cu, Fe, Cr, Ca, K, Li, Mg, Na). These herbs are commonly used by local people to prepare infusions and use them for phytotherapeutical purposes.

MATERIALS AND METHODS

Sample Collection and Preparation: Samples from all four investigated plant species were taken from Pla...kovica Mountain, located in the eastern part of R. Macedonia which highest point is peak Lisec (1754 m) (Figure 1). The plants were identified at the Department of Pharmacognosy, Faculty of Pharmacy, Skopje, R. Macedonia, where specimens of all the plants are deposited in the herbarium. All samples were collected

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Fig. 1: Pla…kovica Mountain as sampling area in R. Macedonia

Table 1: Characteristics (synonyms, plant part used, medicinal properties) of plants studied

Plant	Synonyms	Plant part used	Medicinal properties
Robinia pseudoacacia	false acacia; black locust;	Flowers	Antispasmodic, antiviral, aromatic, cholagogue, diuretic, emetic, purgative, emollient, febrifuge, tonic;
Taraxacum officinale	dandelion; blowball;	Leaves	Aperients, cholagogue, depurative, diuretic, hepatic, laxatice, stomachic, tonic;
Urtica dioica	stinging nettle;	Leaves	Antiasthmatic, astringent, diuretic, galactogogue, haemostatic, hypoglycaemic, tonic;
Matricaria recutita	German chamomile; camomile	Flowers	Anti-inflammatory, antiseptic, antispasmodic, carminative, cholagogue, diaphoretic, homeopathy, nervine, stomachic;

Table 2: Instrumentation and operating conditions for ICP-AES system (Varian, 715ES)

RF Generator					
Operating frequency	40.68 MHz free-running,				
Power output of RF generator	air-cooled RF generator.				
Power output stability	700-1700 W in 50 W increments				
	Better than 0.1%				
Introduction Area					
Sample Nebulizer	V- groove				
Spray Chamber	Double-pass cyclone				
Peristaltic pump	0-50 rpm				
Plasma configuration	Radially viewed				
Spectrometer					
Optical Arrangement	Echelle optical design				
Polychromator	400 mm focal length				
Echelle grating	94.74 lines/mm				
Polychromator purge	0.5 L min^{-1}				
Megapixel CCD detector	1.12 million pixels				
Wavelength coverage	177 nm to 785 nm				
Conditions for program					
RFG Power	1.0 kW	Pump speed	25 rpm		
Plasma Ar flow rate	15 L min^{-1}	Stabilization time	30 s		
Auxiliary Ar flow rate	1.5 L min^{-1}	Rinse time	30 s		
Nebulizer Ar flow rate	0.75 L min^{-1}	Sample delay	30 s		
Background correction	Fitted	Number of replicates	3		
Element	*Wavelength*	*Element*	*Wavelength*	*Element*	*Wavelength*
Zn	213.857 nm	Fe	238.204 nm	Li	670.783 nm
Cu	324.754 nm	Cr	267.716 nm	Mg	279.553 nm
Ca	370.602 nm	K	769.897 nm	Na	589.592 nm

during May 2009. The identities, as well as the medicinal properties of the plant samples under investigation are shown in table 1. About 200 g (fresh weight) of each plant investigated, were collected. All plant samples were air dried, milled in a micro-hammer (without metal parts in it) and stored in clean paper bags.

Analytical Techniques: For standards and samples preparation demineralized water and high purity reagents (Tracepur and *p.a.*) were used. Standards of selected metals were set by dilution of stock-standards which were prepared using analytical grade salts of metals (Merck multielement standard 1000 mg/L) with HNO_3.

From fine crushed tissues of each item approximately 0.5 g were weighed and placed into PTFE vessels and 5 ml HNO_3 (69% Merck, Tracepur) and 2 ml H_2O_2 (30%, m/V; Merck) were added, mixture was left at room temperature for 1 h and then placed in the rotor of microwave oven (MARS CEM XP 1500) and mineralized with two step procedure at 180 ºC. Digests were filtered on the filter paper (Munkteil), quantitatively transferred in 25 ml calibrated flasks, diluted with demineralized water and analyzed by ICP-AES (Varian, Model 715-ES, USA) for selected metals. The instrumental parameters for the determination of the investigated elements are given in table 2.

RESULTS AND DISCUSSION

A total of nine elements (Zn, Cu, Fe, Cr, Ca, K, Li, Mg and Na) was determined in the powdered medicinal plant samples by ICP-AES. Figure 2 shows the mean content ± SD of various elements in investigated plants. All samples were analyzed three times. Each data represent means ± SD of three samples. All results were calculated on a dry weight basis (mg kgG^{1} dw).

From the study, it was revealed that all the metals were accumulated to greater or lower extents by all 4 plant species studied. Elemental studies of the plants showed that they contained large amounts of nutrients and were rich in Mg, Ca and K (Figure 2b).

Zn content varied from 10.20±0.16 to 35.34±0.18 mg kgG^{1}. *T. officinale* had the lowest Zn content and *M. recutita* the highest. The contents of Zn were comparable in *U. dioica* (with lowest) and *R. pseudoacacia* (with highest), too.

Cu contents varied from 5.50±0.13 to 9.74±0.09 mg kgG^{1}. The lowest content is determined in *T. officinale* and the highest in *M. recutita*. It can be noted that for both this elements, considered as micronutrients, *T. officinale* and *M. recutita* showed the lowest and the highest value in this study. Although Cu is an essential micronutrient for plant growth, it can be more toxic than non-essential Pb when extraneous Cu is present in soil environments [16]. Shaw *et al.* [17] reported 4-15 mg kgG^{1} for normal copper contents in plants. Zn is not considered to be highly phytotoxic, but high contents of zinc in plants may cause the loss of leaves production, whereas low contents may cause deformations of leaves. A plant foliar content of 100 mg kgG^{1} Zn has been quoted by various authors [18], as a critical indicator of whether the environment is polluted with Zn. The WHO limits for these metals have not yet been established.

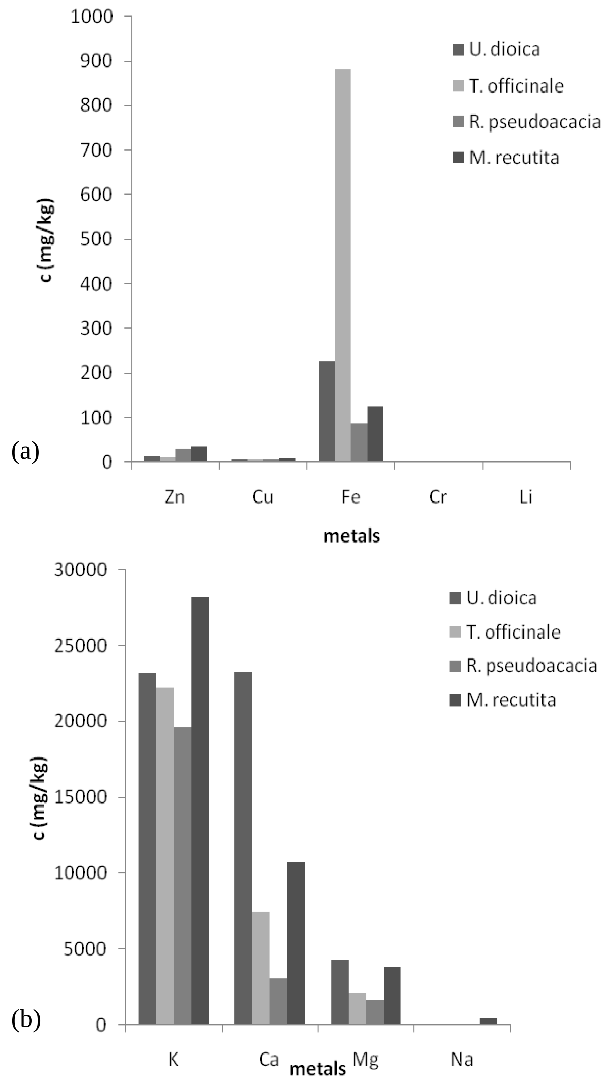

Fig. 2: Metal content (mg kgG^{1}) in investigated medicinal plants: (*Urtica dioica L. Taraxacum officinale*, *Robinia pseudoacacia* and *Matricaria recutita*) a) trace elements; b) macroelements

After comparison of the metal limits in the studied plants, it was found that all species studied accumulated Cu beyond these ranges and Zn contents of all the samples were also found to be within the range.

R. pseudoacacia had the lowest Fe content while *T. officinale* had the highest. The tolerable upper limit of iron for human is 45 mg per day [19].

Chromium contents ranged from 0.227±0.03 mg kgG^{1} (*R. pseudoacacia*) to 1.88±0.07 mg kgG^{1} (*T. officinale*). Jabeen *et al.* [20] in their study determined Cr levels in plants between 1.2 and 29.49 mg kgG^{1} which is high above obtained values for Cr in this study.

Chronic exposure to Cr in humans may result with liver, kidney and lung damage [21]. However, for medicinal plants the WHO limits are not yet been established for Cr. Although in medicinal plants, permissible limits for Cr set by Canada, were 2 mg kg^{-1} in raw medicinal plant material and 0.02 mg/day in finished herbal products [22].

The Ca contents varied from 3057.94±1.71 mg kg^{-1} (*R. pseudoacacia*) to 23279.56±7.59 mg kg^{-1} (*U. dioica*).

M. recutita had the highest K content (28173.24±1.66 mg kg^{-1}) in this study, whereas *R. pseudoacacia* had the lowest (19624.51±0.72 mg kg^{-1}). All the studied plants show high K contents and our results coincided with previous studies on medicinal herbs [23, 20].

Sodium content is in range from 23.11±0.29 (*T. officinale*) to 457.59±1.02 (*M. recutita*). Similar results for Na in a wide range in different plants were obtained from other authors, as levels from 44 to 614 mg kg^{-1}, for example [8].

Li content showed big differences in investigated plants, from 0.0063±0.0006 mg kg^{-1} for *R. pseudoacacia* to 0.5048±0.03 mg kg^{-1} for *M. recutita*. Although Li is not known to be an essential plant nutrient, there is some evidence that Li can affect plant growth and development. The highest mean Li content is found in plants of the Rosaceae family, about 2.9 mg kg^{-1}[24]. The U.S. Environmental Protection Agency (EPA) in 1985 estimated the daily Li intake of a 70 kg adult to range from 650 to 3100 µg [25].

Finally, *R. pseudoacacia* had the lowest Mg content (1648.67±0.31 mg kg^{-1}) and *U. dioica* had the highest (4296.66±1.07 mg kg^{-1}). The results indicated that the studied plants showed a high content of Mg and this is agreeable with the previous findings [8, 20, 26]. *R. pseudoacacia* showed the lowest metal content, in 67% for investigated elements, from all plants investigated in this study.

The results above indicated that the plants contain large amounts of nutrients and are rich in Fe, Mg, Ca and K. The abundance of Mg, Ca and K, in the result of this analysis, was in agreement with previous findings that these three elements represent the most abundant metal constituents in plants [11, 23].

Though much is known about the functional role of a number of elements, the best foreseeable benefit for human health, by mineral nutrition, lies in obtaining the correct amount of supplementation in the right form at the right time. The medicinal values of some plant species used in homeopathic system may be due to the presence of Ca, Cr, Cu, Fe, Mg, K and Zn [27]. In contrast with macronutrients, which are consumed in large amounts (hundreds of grams daily), micronutrients, such as magnesium, zinc and chromium, are ingested in very small amounts (micrograms and milligrams per day). The importance of these micronutrients is revealed by the diversity of metabolic processes they help to regulate. Magnesium, a ubiquitous element that plays a fundamental role in many cellular reactions, is involved in >300 enzymatic reactions in which food is catabolized and new chemical products are formed [28]. In addition, some zinc-containing enzymes, such as carbonic anhydrase and lactate dehydrogenase, are involved in intermediary metabolism. Another zinc-containing enzyme, superoxide dismutase, protects against free radical damage [29]. The element chromium is the subject of growing interest in the public and scientific communities. Mammals need trivalent chromium to maintain balanced glucose metabolism and thus chromium may facilitate insulin action [30, 31]. Cr, Mg and Zn have important roles in the metabolism of cholesterol as well heart diseases, also. The presence of Cr and Mg in plants may be correlated with therapeutic properties against diabetic and cardiovascular diseases [32]. Deficiency or axcess of Cu, Mn, Zn, Cr, Ca, Mg and K may cause a number of disorders [33, 34]. High contents of Ca are important, because of its role in human and studied plants show satisfactory level of Ca accumulation. The biochemical mechanisms of action of lithium appear to be multifactorial and are intercorrelated with the functions of several enzymes, hormones and vitamins, as well as with growth and transforming factors. The available experimental evidence now appears to be sufficient to accept lithium as essential element; a provisional RDA for a 70 kg adult of 1000 µg/day is suggested [35]. Persons who seek to optimize the benefit of nutrition and physical activity on health and function may resort to using mineral supplements.

Conclusions: In view of above facts, the medicinal plants studied are a source of biologically important elements, which may play a part in the observed therapeutic uses of these plants. Medicinal plants from R. Macedonia are rich with Fe, Mg, Ca and K and it is expected that plants with high contents of the above-mentioned macro and micronutrients, might play an important role in maintenance of human health. Also, all of the detected values for metallic elements in plants studied here are below the WHO permissible levels and may not constitute a health hazard for consumers.

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