ELEMENTAL COMPOSITION OF THREE BULGARIAN MEDICINAL PLANTS STUDIED BY MEANS OF INSTRUMENTAL NEUTRON ACTIAVATION ANALYSIS (INAA)

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ABSTRACT

The elemental composition of three Bulgarian Medicinal plants (**Valeriana offcialis, Rodiola Rosea, Salvia officialis**) was studied by means of instrumental neutron activation analysis. The main objective of the study is to determine the presence of two important groups of elements in respect to the human physiology-**Essential elements** (**Minerals**) and **Toxic elements**. INAA presents a very good opportunity for conducting such a research, because of its low detection limits and high precision. The presence of 34 elements was established in the studied plants, among them 13 essential and 4 toxic. Some plants showed high concentrations of essential elements compared to other analytical works, which was related to their medicinal properties. The concentrations of the toxic elements were compared with regulatory organs giving the maximal permissible values for toxic elements in foods and beverages.

INTRODUCTION

Medicinal plants have been known to human civilization for thousands of years. In different regions of the world, national groups have established their own primitive medicinal systems-Ayurveda in India, Shamanism in South America, Traditional Chinese medicine in China, Traditional medical practices of the Balkans and others. In most of those systems, a substantial part is taken by medicinal plants. Very early in their development, civilizations discovered the beneficial effects of plants oh human health. This is due to their natural abundance and great variety. Off course those practices are not forgotten, and many of the plants are used in modern days, especially those that have been proven to be effective. According to the World health organization (WHO), between 60 – 90% of the human population have used at least ones medicinal plants, while in some parts of the world plants are the main source of medical aid [1]. In that regard understanding the elemental content of medicinal plants is absolutely obligatory. This is the focus of the present work -to investigate the elemental content of three Bulgarian medicinal plants. As mentioned before we will try to determine the presence of two important groups of elements – **Essential elements** (**Minerals**) and **Toxic elements**.

Minerals are essential for the normal functioning of the body and cannot be produced by it [2]; therefor they should be supplied through a proper diet. Mineral poor diet might lead to serious illnesses such as anemia, hearth problems, depression and others. If a plant has a

high concentration of minerals it might be the reason for its curative properties (restoration of the mineral balance or other metabolic processes).

Toxic elements on the other hand are harmful for the human organism [3]. If the plants are contaminated and then consumed, toxic elements might be ingested and induce damage in the organism of the consumer. There are various documents prescribing the maximal permissible content of toxic elements in foods and drinks. We examined the concentrations of toxic elements in the studied plants in order to evaluate the dangers of using those plants as teas and other remedies.

The method used in the study is Instrumental neutron activation analysis (INAA). It is based on the transformation of the stable nuclei of the sample into unstable radioactive isotope (by bombardment with neutrons) and consequently measuring the induced gamma radiation. The method has very good characteristics for the context of the study, and in general is one of the leading analytical techniques in regard to its abilities [4]. Many of the elements that present interest for us are of the so called micro or trace elements, which indicate that they are usually present in minute quantities (micrograms per kilogram or even less). This makes INAA an excellent choice due to its low limits of detection and high precision (up to ppb for different elements). Another great advantage of the method is the lack of a need for chemical preparation of the samples, which minimizes the chance for contamination. Finally INAA as used at the REGATA facility at JINR, allows the detection of 53 elements, among which are most of the essential and toxic elements(Na, Mg, Al, S, Cl, K, Ca, Sc, Tl, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Zr, Nb, Mo, Ag, Cd, In, Sn, Sb, I, Ba, Cs, La, Ce, Nd, Sm, Eu, Gd, Tb, Dy, Tm, Yb, Lu, Hf, Ta, W, Ir, Pt, Au, Hg, Th, U).

MATERIALS AND METHODS

The studied plants are widely used in traditional Bulgarian medicine and have been reported to display healing properties [5], [6], [7]. It is a common practice among people living in rural areas to gather the plants from their natural habitat and prepare teas and other remedies. All the plants could be found in the Bulgarian peninsula, or could be bought from the commercial network (they are used as a compound in various pharmaceutical products). **Table 1** present the main botanical and medical characteristics of the studied plants. The samples studied in the present work were collected from regions that are ecologically clean, in order to avoid contamination due to anthropological activities.

The plant material was dried first at room temperature and then in an oven at 40° C until constant weight. The dry material was then homogenized using ceramic homogenizer, any contact with metal surfaces was avoided. The homogenized material was then palletized in a special press to create samples with exact shape, size and weight, as a requirement for the irradiation geometry. The pallets were packed in two ways – in aluminum foil for long irradiation, and in plastic for short irradiation. From each plant three subsamples were prepared to achieve statistical significance of the results and to avoid errors in the measurements. After packing, the samples were sent for irradiation at the IBR-2 reactor at FLNP.

Special Pneumatic channels were used to transport the samples to the irradiation channels. Two types of irradiation were carried out- for short lived isotope with duration of 180s, and for long lived isotopes with duration of 3 days 23 hours and 57 minutes. After the irradiation the induced activity of the samples was measured on HPGe detectors. The gamma spectra were analyzed using the Genie-2000 software. Special software developed for the purposes of INAA at the REGATA facility [8] was used to calculate the elemental

concentrations of the samples. The method is based on the relative standardization using high quality standard reference materials. The samples and the SRMs were irradiated together under the same conditions. In that way to calculate the concentration in the samples the following relation could be used:

$$C_{sample} = \frac{A_{sample}}{A_{standard}} . C_{standard}$$

Here
C_{sample} is the concentration of the element in the sample,
$C_{standard}$ is the concentration of the element in the standard,
A_{sample} is the activity of the sample,
$A_{standard}$ is the activity of the standard.

Table 1 Botanical and medicinal properties of the studied plants

Botanical name	**Family**	**Main biologically active substances**	**Actions**	**Medicinal use**
Valeriana officinalis	Valerianaceae	Volatile oil valepotriates	Tranquillizer antispasmodic, expectorant, diuretic, reduces blood pressure, carminative	Used for anxiety and insomnia, reduce high blood pressure, for muscle cramp
Salvia officinalis	Lamiaceae	Volatile oil	Anti-inflammatory, antispasmodic, astringent, antiseptic, relaxes peripheral blood vessels, uterine stimulant, antibiotic	Menopause problems, Liver stimulant, weaning problems, respiration
Rhodiola rosea	Crassulaceae	Salidrosids Tannins Volatile oil	Adaptogen, tonic, stimulant	Physical and mental stimulant, anticancer

The SRMs used in the present work are NIST– 1515 Apple leaves, 1572 Citrus leaves, 1566b Spinach leaves, 1577 Bovine liver; IAEA 407 Trace elements in fish tissue. For long-lived isotopes: by NIST-2710 Montana soil, 1632 Trace elements in coal; By JCR-BCR667.

RESULTS

Total of 34 (*Na, Al, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Ni, Co, Zn, As, Se, Br, Rr, Sr, Mo, Cd, Sb, Ba, Cs, La, Sm, Tb, Tm, Yb, Hf, Ta, W, Au, Th* and *U*) elements were detected in the samples. Among them there are 13 essential, shown in **Table 2,** and 4 toxic elements shown in **Table 3**. The results were obtained with very good uncertainty varying from 1% to 30% for different elements. A comparison was made with the "Reference plant by Markret" [9] in

order to establish high elemental concentrations. The concentrations of the toxic elements were compared with maximal permissible values given by regulatory documents [10], [11]. Valeriana officinalis and Salvia officinalis displayed unusually high mas fractions of As and Cd which is an indicator for potential threat for the health. It could also be seen that Valeriana officinalis, Rhodiola Rosea and Silivia officialis displayed high concentration of many essential elements (*Ca, Na, Fe, Zn, Cr, Al*) .Since most of them are metals, further investigation is necessary in order to establish if this behavior is due the medicinal nature of the plants or due to anthropological contamination.

Table 2 Essential elements detected in the studied samples. Results presented in form C(σ%). C is the mass fraction in mg/kg and σ% is the relative uncertainty in percent

mg/kg (σ,%)	Ca	K	Cl	Na	Mg	Fe	Zn
Valeriana Officialis	2650 (6)	24700 (8)	699 (6)	457 (3)	2270 (3)	1990 (8)	127 (4)
Rhodiola Rosea	4610 (6)	12000 (8)	163 (6)	237 (3)	2140 (3)	1100 (8)	26 (4)
Salvia Officialis	14500 (6)	16400 (8)	2430 (6)	567 (3)	4250 (3)	1050 (8)	123 (4)
Refference plant	10000	19000	2000	150	2000	150	50
mg/kg (σ,%)	Cr	Mn	Al	Mo	V	Se	
Valeriana Officialis	9 (15)	56 (4)	3280 (2)	1,7 (30)	7,4 (10)	0,145 (17)	
Rhodiola Rosea	6 (15)	47 (4)	2790 (2)	1,3 (30)	4,4 (10)	0,164 (17)	
Salvia Officialis	7 (15)	56 (4)	1830 (2)	2,8 (30)	3,3 (10)	0,207 (17)	
Refference plant	1,5	200	80	0,5	0,5	0,02	

Table 3 Toxic elements detected in the studied samples. Results presented in form C (σ %). Where C is the mass fraction in mg/kg and σ % is the relative uncertainty in percent. Last column show the maximal permissible concentrations according to [10], [11]

mg/kg (σ,%)	As	Sb	Ba	Cd
Valeriana Officialis	1,5 (3)	0,37 (10)	62 (5)	12 (30)
Rhodiola Rosea	0,7 (3)	0,02 (10)	77 (5)	0,236 (30)
Salvia Officialis	2,1 (3)	0,60 (10)	84 (5)	0,238 (30)
Refference plant	**0,1**	**0,1**	**40**	**0,05**
Mximal permisable conc.	**0,26**	**No data**	**No data**	**0,2**

CONCLUSIONS

INAA is an excellent method for studying the elemental composition of medicinal plants, especially in respect to minerals and toxic elements. It gives the opportunity to detect the presence of wide variety of elements even if they are present in minute quantities. Although high elemental mass fractions of important elements were established, a definite relation to the medicinalproperties of the plants could not be established. The results however give a good direction for further investigation. It is necessary to examine the bioavailability of the detected elements since there are factors that influence theabsorption and functional

activity of those elements in the human organism. Never the less the ecological factors, under which the plants have been grown, should be studied as well (soils, anthropological activity in nearby regions etc.).

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