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INORGANIC CONSTITUENTS IN HERBAL MEDICINE BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

The demand for herbal medicines is growing worldwide. The expansion of interest has required the standardization of the sector with implementation and constant review of technical standards for production and marketing of these medicines in order to ensure the safe use, therapeutic efficacy and quality of the products. According to data from the World Health Organization, approximately 80% of world population has resorted to the benefits of certain herbs with therapeutic action popularly recognized. Despite the vast flora and the extensive use of medicinal plants by the population, it is a consensus that scientific studies on the subject are insufficiency. Therefore, it is necessary to stimulate such studies in view of the importance of the results of both individual and social field. The determination of major, minor and trace elements and the research of metabolic processes and their impacts on human health are of great importance due to the growth of environmental pollution that directly affects the plants and therefore the phytoterapics. Therefore, the objective of this work was to determine the content of inorganic constituents in herbal medicine: moisture, total ash and the elements As, Ba, Br, Ca, Cs, Co, Cr, Fe, Hf, K, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr by neutron activation analysis in order to verify the quality of the products. It was observed that the elemental concentrations varied in a wide range from plant to plant and elements with higher concentrations were Ba, Fe, Cr and Zn.

1. INTRODUCTION

The use of medicinal plants in therapeutics or as dietary supplements goes back beyond recorded history. Observing animals feeding them-selves, men started to taste plants and realized that they were sometimes relieved of headaches, stomachaches and muscular aches after external or internal use of medicinal plants [1]. Nowadays medicinal plants play a significant role due to the damaging effects of food processing and over medication that have assumed alarming proportions and their use has increased substantially in the last decades [2–4].

Generally, the studies related with therapeutic plants aim at characterizing the active component of the plant for scientific evidence of its therapeutic properties [5–7]. However, plants can contain heavy metals from their presence in the soil (including contamination of the plant material with soil), water or air [8]. High levels of toxic metals can occur in medicinal preparations when they are used as active ingredients. To be used as a raw material for pharmaceutical formulations the plant must have low or zero toxicity. The presence of some elements, even in trace amounts, can pose a potential threat for the patient. Therefore, the knowledge of the elemental concentration of these elements can be useful to verify possible interferences in therapeutic activity; depending on their concentration they also can

represent some threat for human being [7]. On the other hand, the elemental composition of the plants is also important because some of these elements are vital for normal growth and maintaining good health. The imbalance of these elements in various organs and body fluids can cause physiological disorders [9, 10]. In order to establish a direct link between elemental content and its curative capability, the elemental composition monitoring of the plants has become essential. Despite the increasing use of herbal medicines, there is still a significant lack of research data in this field.

Since the concentration of mineral elements constitutes a minute fraction in plant samples, a sensitive, selective and reliable instrumental method is necessary to collect precise and accurate data. The instrumental neutron activation analysis (INAA) occupies a prominent position among the various analytical methods [11] due to its relative simplicity, inherent selectivity and sensitivity in the analysis of various biological [12] and environmental samples [13, 14].

The objective of this work was to determine the content of inorganic constituents in herbal medicine samples of *Eucalyptus globulus*, *Eugenia uniflora*, *Calendula officinalis*, *Rosmarinus officinalis*, *Psidium guajava*, *Phyllanthus niruri*, *Casearia sylvestris*, *Passiflora alata*, *Cynara scolymus* and *Solanum paniculatum*: moisture, total ash and the elements As, Ba, Br, Ca, Cs, Co, Cr, Fe, Hf, K, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr by neutron activation analysis in order to verify the quality of products.

2. SAMPLING COLLECT AND PROCESSING METHOD

Samples were acquired in the regular commerce. Before the analysis, a search for impurities was done to determine and separate the foreign matter present in the sample plant. Then samples were weighted, left to dry in a furnace at 40ºC till constant weigh and transferred to a mortar previously decontaminated with HNO_3. Samples were then pulverized to 100 mesh size particle, homogenized and approximately 200 mg were packed in polyethylene bags for irradiation. Reference Standard Material Montana Soil (NIST 2710) and Estuarine Sediment (NIST 1646a) were packed in the same way of the samples. In the IEA-R1 nuclear reactor at IPEN the samples and reference materials were irradiated for 8 h and counted after 7 to 15 days depending on the radionuclide half-live produced in the irradiation, under a thermal neutron flux of 1 to 5x 10^{12} n cm^{-2} s^{-1}. Gamma spectrometry was performed using a Canberra gamma X hyperpure Ge detector and associated electronics, with a resolution of 0.88 keV and 1.90 keV for ^{57}Co and ^{60}Co, respectively. The data analysis was done by using in-house gamma ray software, VISPECT program to identify the gamma-ray peaks [15].

The concentrations were obtained by comparing the photopeak area of the interest element in the sample spectrum with that of the standard reference material using the following expression:

$$C_{ai} = \frac{(A_{ai}\, w_p\, C_{pi})\, e^{-\lambda\,(ta\,-\,tp)}}{Ap_i\, w_a}$$

Were C_{ai} is the *i* element concentration in the sample (in mg kg^{-1}); C_{pi} is the *i* element concentration in the standard (in mg kg^{-1}); A_{ai} is the activity of the element *i* in the sample (in counts per second); A_{mi} is the activity of the element *i* in the standard (in counts per second); w_a e w_p are the weighs of the sample and standard (in g), respectively; λ is the element

decay constant and t_a and t_p is the difference of the counting time between the sample and standard.
The method precision and accuracy were verified by using the measurement of the standard reference material IAEA-336 (lichen).

3. RESULTS AND DISCUSSION

To verify the methodology precision and accuracy, samples of the reference material IAEA 336 (lichen) were analyzed and the results are shown in table 1 for the elements with certified values. The results indicate good precision and accuracy for the method.

Table 1: Measured (M), certified (T) and range (R), in mg kg^{-1}, values for reference material IAEA 336, n= 4.

	Br	**Ce**	**Co**	**Cr**
M	14±1	1.02±0.3	0.25±0.03	1.77±0.4
R	11.2-14.6	1.11-1.46	0.24-0.34	0.89-1.23
T	12.9	1.28	0.29	1.06
	Cs	**Eu**	**Fe(%)**	**La**
M	0.09±0.02	0.025±0.01	0.042±0.002	0.62±0.09
R	0.097-0.123	0.019-0.027	0.038-0.048	0.56-0.76
T	0.11	0.023	0.043	0.66
	Lu	**Rb**	**Sb**	**Sc**
M	0.006±0.002	1.75±0.2	0.09±0.05	0.17±0.02
R	0.0064-0.0068	1.54-1.98	0.06-0.08	0.15-0.19
T	0.007	1.76	0.07	0.17
	Se	**Sm**	**Tb**	**Th**
M	0.2±0.01	0.11±0.02	0.012±0.016	0.14±0.04
R	0.18-0.26	0.09-0.12	0.012-0.016	0.12-0.16
T	0.22	0.11	0.014	0.14
	Yb	**Zn**	**As**	**Sb**
M	0.085±0.02	25±5	0.6±0.2	0.05±0.01
R	0.025-0.049	27-33.8	0.55-0.71	0.063-0.083
T	0.037	30.4	0.63	0.073

M = measured value
R = reference material range of concentration
T = reference material true value of the element

In table 2 are presented the part of the plant used in the preparation of the infusion, the percentages of impurity, moisture and ash in the samples analyzed in this study. According to the WHO [16] medicinal plants should be entirely free from visible signs of contamination by moulds or insects and other animal contamination, including animal excreta and no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. In the samples analyzed in this study the foreign matter varied in a wild range of impurities from 0.4 to 60.2% and the plant with the higher content of impurities was *Eugenia uniflora*.

The moisture determination is necessary since an excess of water can encourage microbial growth, presence of fungi or insects and deterioration following hidrolisys. The percentages of moisture in the analyzed plants varied from 4.9 to 8.5%. Samples with higher moisture

content deserve special care in its storage. The total ash determination indicates the content of insoluble constituents of the medicinal plant samples. For the plants here considered the higher inorganic content were observed for *Cynara scolymus*, *Solanum paniculatum* and *Calendula officinalis*.

Table 2: Used part of each medicinal plant and percentages of impurities, moisture and total ash.

Samples		*Used part*	*% of impurity*	*% of moisture*	*% of ash*
Eucalyptus globulus	Euc	Leaves	30.4	5.5	1.1
Eugenia uniflora	Pit	Leaves	60.2	7.4	2.8
Calendula officinalis	Cal	Flowers	0.4	8.0	6.4
Rosmarinus officinalis	Ale	Leaves	15.7	4.9	3.9
Phyllanthus niruri	QP	Arial parts	0.6	5.6	3.9
Psidium guajava	Goi	Leaves	45.2	7.4	4.0
Casearia sylvestris	Gua	Leaves	10.1	7.4	5.4
Passiflora alata	Pas	Leaves	9.1	8.2	5.1
Solanum paniculatum	Jur	Whole plant	1.1	7.6	6.8
Cynara scolymus	Alc	Leaves	6.6	8.5	7.4

In table 3 are shown the results for the concentrations of the elements measured by neutron activation analysis in the samples of this study. The data presented shows that different medicinal plants contain the determined elements in various proportions. The variation of elemental content from plant to plant is mainly attributed to the differences in botanical structure, as well as in the mineral composition of the soil in which the plants was cultivated. Other factors responsible for a variation in elemental content are preferential absorbability of the plant, use of fertilizers, irrigation water and climatological conditions [17].

A number of trace elements play an important role in the metabolism. These elements are called essential. Among then, higher concentrations were observed for Ba, Fe and Zn. The concentrations found for the present samples are in the same order of that observed by Sussa [18].

Enhance body resistance against many diseases, such as cold and cough, could be related to elements Fe, Mn, Zn, Rb, V, Se, Cu, etc [19]. Besides, the element Fe, whose concentrations varied from 190 to 1390 mg kg^{-1}, also is an important mineral that enters into the vital activity of blood and glands and Zn, varying from 19 to 61 mg kg^{-1}, is especially important for several enzymatic processes that helps in eliminating fatigue and reduce nervous irritability. The lowest and highest content of Ba were found in *Rosmarinus officinalis* and *Eugenia uniflora*, for Fe, in *Casearia sylvestris* and *Phyllanthus niruri* and for Zn in *Eucalyptus globulus* and *Calendula officinalis*, respectively.

Another essential element, chromium plays an important role in the maintenance of normal glucose metabolism. The function of chromium is directly related to the function of insulin, which plays a very important role in diabetes. Chromium is found in the pancreas, which produces insulin [20]. In the analyzed samples Cr varied from 1.6 to 56 mg kg^{-1}.

Table 3: Concentrations, in mg kg^{-1}, and uncertainty for the analyzed samples, (-) not determined.

	As		Ba		Br		Ce		Co	
Euc	-		13	±3	-		2.0	±0.1	0.43	±0.02
Pit	-		211	±14	-		1.38	±0.01	4.23	±0.05
Cal	-		20	±3	-		0.5	±0.1	0.31	±0.01
Ale	-		19	±3	-		0.7	±0.1	0.32	±0.02
Q P	0.26	±0.07	50	±3	7.7	±0.7	1.84	±0.05	0.62	±0.01
Goi	-		81	±7	-		1.7	±0.1	0.32	±0.02
Gua	-		44	±4	34.3	±3	0.43	±0.06	0.27	±0.01
Pas	-		-		86	±6	1.3	±0.1	0.79	±0.02
Jur	-		102	±6	82	±6	4.14	±0.05	0.95	±0.01
Alc	-		52	±5	33	±2	1.6	±0.1	0.62	±0.02
	Cr		**Eu**		**Fe**		**Hf**		**La**	
Euc	3.1	±0.2	0.05	±0.01	470	±10	0.10	±0.01	1.59	±0.22
Pit	2.5	±0.1	0.021	±0.001	190	±4	0.054	±0.005	1.00	±0.08
Cal	5.9	±0.3	-		150	±10	-		-	
Ale	5.2	±0.2	-		350	±10	0.07	±0.01	-	
Q P	6.9	±0.2	-		1390	±20	0.16	±0.01	1.15	±0.01
Goi	1.6	±0.2	-		160	±10	-		0.42	±0.09
Gua	5.0	±0.2	-		130	±10	0.04	±0.01	0.21	±0.02
Pas	56	±2	-		930	±20	0.42	±0.03	1.05	±0.04
Jur	50	±2	-		570	±10	0.50	±0.02	4.74	±0.04
Alc	30	±1	-		780	±20	0.15	±0.02	1.24	±0.03
	Nd		**Rb**		**Sb**		**Sc**		**Se**	
Euc	-		28	±2	-		0.13	±0.003	-	
Pit	0.5	±0.1	75	±4	0.051	±0.011	0.04	±0.001	0.031	±0.008
Cal	-		91	±5	-		0.03	±0.001	-	
Ale	-		-		-		0.11	±0.002	0.07	±0.01
Q P	0.48	±0.09	-		0.024	±0.004	0.412	±0.002	-	
Goi	-		12	±1	-		0.02	±0.001	-	
Gua	2.02	±0.22	-		0.11	±0.01	0.047	±0.001	-	
Pas	2.50	±0.28	-		0.08	±0.01	0.239	±0.003	-	
Jur	3.73	±0.25	-		0.03	±0.01	0.088	±0.001	-	
Alc	-		-		-		0.134	±0.002	-	
	Sm		**Th**		**U**		**Yb**		**Zn**	
Euc	0.30	±0.04	0.11	±0.01	-		0.06	±0.02	19	±1
Pit	0.09	±0.01	0.044	±0.004	-		0.010	±0.003	26.8	±0.7
Cal	-		-		-		-		61	±2
Ale	-		0.10	±0.01	-		-		22	±1
Q P	0.193	±0.004	0.17	±0.01	-		-		44	±1
Goi	-		0.04	±0.01	-		-		46	±2
Gua	0.062	±0.005	0.03	±0.01	0.11	±0.04	-		30	±1
Pas	0.120	±0.008	0.61	±0.02	-		0.10	±0.01	50	±2
Jur	0.71	±0.01	0.26	±0.01	-		0.075	±0.005	26.9	±0.6
Alc	0.138	±0.010	0.14	±0.01	-		-		26	±1

Cobalt is essential for the B12 vitamin and the thyroid metabolism and its concentrations in the present samples varied from 0.27 to 4.23 mg kg^{-1}. The lower and higher content were observed for *Casearia sylvestris* and *Eugenia uniflora*, respectively.

4. CONCLUSIONS

The concentration of the elements As, Ba, Br, Ca, Cs, Co, Cr, Fe, Hf, K, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr were determined by neutron activation analysis in samples of the medicinal plants *Eucalyptus globulus*, *Eugenia uniflora*, *Calendula officinalis*, *Rosmarinus officinalis*, *Psidium guajava*, *Phyllanthus niruri*, *Casearia sylvestris*, *Passiflora alata*, *Cynara scolymus* and *Solanum paniculatum*. It was observed that the concentration varies considerably from plant to plant. For various elements the concentrations could not be determined in the majority of the samples. The essential elements Ba, Co, Cr, Fe and Zn were measured in almost all the samples and the determination of their concentration can be compared with the limits of intake for safety of their use.

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