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MEDICINAL PLANTS BY NEUTRON ACTIVATION ANALYSIS

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DETERMINATION OF INORGANIC COMPONENTS IN BRAZILIAN MEDICINAL
PLANTS BY NEUTRON ACTIVATION ANALYSIS

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ABSTRACT

Instrumental neutron activation analysis (INAA) has been applied to multielemental determinations of medicinal extracts obtained from the plants: Cordia Verbenacea DC , Folidago Microglossa DC and Petiveria Alliacea.

Concentrations of the elements Al, Br, Ca, Cl, Co, Cs, Fe, K, La, Mg, Mn, Na, Rb, Sb and Zn have been determined in dried extracts of these herbs by short and long irradiations under a thermal neutron flux of 10^{11} to 10^{13} n.cm⁻².s⁻¹ in the IEA-R1 nuclear reactor. The NBS ^{Citrus} Tea Leaves (1572) and NIES Pepperbush (Nº 1) reference materials were also analysed simultaneously with the plant extracts.

The results obtained in these analysis have shown a good accuracy and reproducibility of the method. The relative errors and the relative standard deviations were lower than 10% for most of the elements analysed.

INDEX ENTRIES: Neutron activation analysis, Medicinal herbs, Analysis of plant extracts, Cordia Verbenacea, Folidago Microglossa, Petiveria Alliacea.

INTRODUCTION

Brazil is a country in which the use of medicinal herbs is widely spread. In the last years there has been an increasing interest in using natural medicines as an alternative to the risks of side effects and to the rising cost of the medicines obtained from synthetic chemical products.

Although the efficiency of some natural drugs for curing diseases has been proved, the specialists in this subject have adverted about the indiscriminate

use of this kind of products because they can cause serious intoxications.

The belief that the medicinal herbs do not present side effects has taken people to use them without any medical orientation. Besides this, people have usually used medicinal plants for a long period to treat chronic diseases or many times they start using them just to follow a trend.

It is very important to determine the elemental composition of medicinal plants or of their extracts for pharmacological assays as well as to study the effect of the environment on changes in their composition. The analysis of inorganic components in these kinds of materials is essential for the evaluation of their usefulness in relation to their medicinal value. The chemical elements may take part in the active component of medicinal plant as a healing agent. Besides this, some elements can be closely related to human health or disease when their excess or deficiency induce physiological and metabolic changes.

On the other hand, the concentration of elements in plants depends on many factors such as the oxidation state of the element, type and pH of the soil, species and age of the plant and also on the fertilizer used. Consequently, the efficiency of a medicinal plant may depend on the region where it was cultivated too.

Many papers have been published about the determination of elements in several types of plants⁽¹⁻⁴⁾ and few about herb extracts⁽⁵⁾.

The purpose of this work was to apply the method of instrumental neutron activation analysis (INAA) to determine inorganic components in herb extracts for further contribution to study their toxicity, medicinal action or the active component of them.

The INAA method has been applied in the present paper to the analysis of extracts obtained from three species of herbs: Cordia Verbenacea DC, Folidago Microglossa DC and Petiveria Alliacea.

MATERIALS AND METHOD

Extract Samples Obtained From Medicinal Herbs

Medicinal extracts obtained from three species of herbs were analyzed in this paper. Cordia Verbenacea DC from the family of Boraginaceae, is a perennial bush plant known since many centuries ago by Brazilian Indians to treat infections and rheumatisms. The bioflavonoids produced by their leaves show anti-

-inflammatory activity and can be used as a medicine without causing side effects⁽⁶⁾ such as stomach lesions, hormonal changes and liquid retention in the body. Besides this, the leaves of this herb produce essential oils that contain substances responsible for their peculiar smell. Certain insects and beetles that destroy the orchard leaves are attracted by this smell and then this herb is planted among the orange and lemon trees to be used as a "trap plant". Folidago Microglossa DC (Compositae) is known in Brazil by the name of Brazilian Arnica and the aerial parts of this plant are used as an anti-inflammatory and cicatrizing drug. Petiveria Alliacea (Phytolaccaceae) known in Brazil by the name of "Guiné" is a plant widely used in popular medicine "to calm nerves" and as an anesthetic, diuretic, analgesic and abortive drug too.

The extracts of these plants were provided by the Faculty of Pharmaceutical Sciences of the University of the State of São Paulo. The fresh material of these herbs was extracted using a 70% alcoholic solution at room temperature as described in ref(6). After the filtration, the dark green solution was concentrated under reduced pressure at 50 C to obtain an aqueous viscous material that was lyophilized afterwards. The dried extracts are stored in a dessicator.

Instrumental Neutron Activation Analysis of the Samples

Dried extracts of the herbs (50-100mg) were weighed and heat-sealed in clean polyethylene bags or in polyethylene capsules imported from the Free University of Amsterdam for irradiation together with synthetic standards prepared in our laboratory using standard solutions of the elements to be determined.

These standard solutions were prepared by dissolving high purity metal, oxide or salt of elements with adequate reagents. An appropriate volume of these standard stock solutions was pipetted in a small sheet of Whatman N° 42 filter paper and dried in a dessicator at room temperature. The amounts of elements in these standards were about ten times higher than those found in the samples. The sheets of filter paper were also put in polyethylene bags or capsules.

Irradiations were performed in the IEA-R1 nuclear research reactor. The irradiation conditions, decay times and the nuclear data of the nuclides used in this study are shown in Table 1.

The occurrence of the interfering reactions $^{27}\text{Al} (n,p) ^{27}\text{Mg}$, $^{31}\text{P} (n,\alpha) ^{28}\text{Al}$ and $^{28}\text{Si} (n,p) ^{28}\text{Al}$ in the determinations of Mg and Al was also examined by irradiation of the samples with epithermal neutron flux using 1,0 mm thick cadmium container.

The irradiated samples and standards were counted using an ENERTEC hyperpure Ge detector coupled to an EG & G ORTEC 4096 channel pulse height analyzer connected to Monydata PC 200 Plus microcomputer. The counting system had a resolution (FWHM) of 2.5 keV for the 1332 keV gamma-ray of ^{60}Co and 1.4 keV for the 122 keV gamma-ray of ^{57}Co . Samples and standards were measured in the polyethylene capsules or bags used for their irradiations. The presence of impurities in these involucre was previously examined and it was found to be negligible in comparison with the amounts of elements present in the sample. The gamma-ray spectra were processed by using the modified version of FALA⁽⁷⁾ program, written in Pascal language. This program locates peak positions and calculates gamma-ray energies and net areas.

Two certified reference materials: Citrus Leaves (1572) provided by the National Bureau of Standards (NBS) USA and Pepperbush N^o 1 from the National Institute for Environmental Studies (NIES), Japan, were analysed in this work for the evaluation of the accuracy of the method. These reference materials were dried as recommended in their respective certificates^(8,9). With the drying process there was a weight loss of 6.47% for Citrus Leaves and of 7.97% for Pepperbush. These values were used to calculate the weights of materials analysed on dried basis.

RESULTS AND DISCUSSION

Table 2 shows the results of elemental concentrations obtained for Cordia Verbenacea and Folidago Microglossa extracts and in Table 3 are presented the results for extracts obtained from the roots and aerial parts of Petiveria Alliacea herb. These reported values are the averages and standard deviations from at least four determinations. Acceptable precisions, with relative standard deviations lower than 10%, were obtained for most of the elements analysed. The less precise results were obtained for Co, Cs, Sb and Mg. The induced activity of ^{60}Co , ^{134}Cs and ^{122}Sb was not high due to the small concentrations of these elements in the samples (< 1 ppm). Magnesium is present in relatively high concentration. However, the 1014 keV gamma-ray photopeak of ^{27}Mg is not very pronounced, resulting in poor counting statistics.

Concerning the elements analysed, Al, Br, Cl, Cs, Fe, K, Mg, Mn, Na, Rb and Zn were found in all the extracts. Calcium was not detected in the extracts obtained from the roots and aerial parts of Petiveria Alliacea. Table 2 shows there is not much difference in the concentrations of Al, Br, Ca, Cl, Co, Cs, Fe,

K, Mg, Mn, Rb and Zn found in Cordia Verbenacea and Folidago Microglossa extract.

Also the results obtained for Petiveria Alliacea extract (Table 3) show that the concentrations found for Al, Br, Cl, Co, Fe, K, Mg, Mn, Na, Rb and Zn in the extracts from roots are practically the same as those found in the extracts from aerial parts. Especially for Cl, this was not expected because Cl is generally found in higher concentration in the green parts (leaves) of the plant rather than in the roots.

Table 3
It is important to note that the medicinal extracts analysed here have shown high concentration of K, varying from 3 to 10% and that potassium salts participate actively in the cardiac rhythm and in constipation. Also Mg present in these medicinal extracts can neutralize the acidity and avoid side effects of stomach lesions when these drugs are taken orally by patients.

From the pharmacological and toxicological point of view the concentration of the elements present in the three species studied are too low to cause any kind of effect.

The accuracy of the method was checked by analysing Citrus Leaves and Pepperbush reference materials. Table 4 shows the results obtained in these analyses together with certified values presented by NBS and NIES. We have determined 15 elements and most of them are in reasonably good agreement with these certified values. For the elements Ca, Cs, K, Mn, Na, Rb and Zn, relative errors lower than 10% were obtained. Also in these samples, the less precise results were obtained for Co and Cs.

Table 4
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EXPERIMENTAL CONDITIONS AND NUCLEAR DATA OF THE NUCLIDES USED IN THIS STUDY

Irradiation Time	Thermal Neutron Flux n.cm ⁻² .s	Decay Time	Radioisotopes (Gamma ray energies measured in keV)
1 min	3.7 x 10 ¹¹	2 min 60 min	²⁸ Al (1778) ; ²⁷ Mg (1014) ; ⁸⁰ Br (617) ; ³⁸ Cl (1642) ; ⁵⁶ Mn (1810)
30 min	1.7 x 10 ¹²	15 h	⁴² K (1525) ; ⁵⁶ Mn (846 , 1810) ; ⁸² Br (776) ; ²⁴ Na (1368)
8 h	10 ¹³	5 - 20 d	⁸² Br (776) ; ⁴⁷ Ca (1296) ; ⁶⁰ Co (1173) ; ¹³⁴ Cs (796) ; ⁵⁹ Fe (1099) ; ¹⁴⁰ La (1596) ; ²⁴ Na (1368) ; ⁸⁶ Rb (1076) ; ¹²² Sb (564) ; ⁶⁵ Zn (1115)

T A B L E 2

CONCENTRATIONS OF ELEMENTS IN EXTRACTS OBTAINED FROM LEAVES
OF CORDIA VERBENACEA AND FOLIDAGO MICROGLOSSA BY INAA

Element	<u>Cordia Verbenacea</u> Extract	<u>Folidago Microglossa</u> Extract
Al (ppm)	240 \pm 24 (10.0) ^a	192 \pm 21 (10.9)
Br (ppm)	56 \pm 4 (7.1)	34 \pm 3 (8.8)
Ca (%)	0.84 \pm 0.08 (9.5)	0.31 \pm 0.02 (6.4)
Cl (%)	3.19 \pm 0.08 (2.5)	1.06 \pm 0.04 (3.8)
Co (ppb)	135 \pm 35 (25.9)	112 \pm 11 (9.4)
Cs (ppb)	940 \pm 180 (19.1)	964 \pm 124 (12.8)
Fe (ppm)	175 \pm 16 (0.9)	140 \pm 9 (6.4)
K (%)	4.6 \pm 0.2 (4.3)	2.95 \pm 0.18 (6.2)
La (ppb)	-	130 \pm 14 (10.9)
Mg (ppm)	4107 \pm 1230 (30.9)	2746 \pm 576 (20.9)
Mn (ppm)	19.5 \pm 1.4 (7.1)	58 \pm 3 (5.2)
Na (%)	1.4 \pm 0.1 (7.1)	0.020 \pm 0.002 (10.0)
Rb (ppm)	109 \pm 7 (6.4)	135 \pm 5 (4.0)
Sb (ppb)	-	740 \pm 100 (13.5)
Zn (ppm)	63 \pm 4 (6.0)	112 \pm 5 (4.5)

a - Values in parentheses are relative standard deviations.

T A B L E 3

- CONCENTRATIONS OF ELEMENTS IN EXTRACTS FROM PETIVERIA ALLIACEA (GUINÉE)
OBTAINED BY INAA

Element	Extract from Roots	Extract from Aerial Parts
Al (ppm)	424 \pm 28 (6.6) ^a	118 \pm 15 (13.0)
Br (ppm)	325 \pm 33 (10.1)	160 \pm 8 (5.0)
Cl (%)	1.38 \pm 0.16 (11.6)	1.60 \pm 0.09 (5.6)
Co (ppb)	756 \pm 102 (13.5)	840 \pm 43 (5.1)
Cs (ppb)	552 \pm 65 (11.8)	721 \pm 46 (6.4)
Fe (ppm)	69 \pm 2 (2.9)	14.8 \pm 0.9 (6.1)
K (%)	10.4 \pm 1.5 (1.4)	9.2 \pm 0.5 (5.4)
Mg (ppm)	1558 \pm 564 (36.2)	1525 \pm 525 (34.4)
Mn (ppm)	270 \pm 36 (13.3)	334 \pm 28 (8.4)
Na (%)	0.61 \pm 0.04 (6.5)	0.19 \pm 0.01 (5.3)
Rb (ppm)	320 \pm 20 (6.2)	373 \pm 11 (2.9)
Sb (ppb)	241 \pm 49 (20.3)	1109 \pm 121 (10.9)
Zn (ppm)	134 \pm 7 (5.2)	451 \pm 19 (4.2)

a - Values in parentheses are relative standard deviations.

T A B L E 4

- CONCENTRATIONS OF ELEMENTS IN NBS CITRUS LEAVES (1572) AND IN NIES PEPPERBUSH (NO 1) REFERENCE MATERIALS OBTAINED BY INAA

Element	Citrus Leaves		Pepperbush	
	This work	Ref (8)	This work	Ref (9)
Al (ppm)	137 \pm 16 (11.6) ^a	92 \pm 15	705 \pm 17 (2.4)	
Br (ppm)	8.0 \pm 0.5 (6.2)	8.2 (b)	1.4 \pm 0.2 (12.5)	
Ca (%)	3.30 \pm 0.27 (8.2)	3.15 \pm 0.10	1.41 \pm 0.04 (2.9)	1.38 \pm 0.07
Cl (ppm)	660 \pm 102 (15.4)	414 (b)	-	
Co (ppb)	33 \pm 6 (18.7)	20 (b)	172 \pm 49 (28.5)	230 \pm 30
Cs (ppb)	107 \pm 16 (15.4)	98 (b)	1274 \pm 88 (6.9)	1200
Fe (ppm)	111 \pm 11 (9.9)	90 \pm 10	220 \pm 12 (5.3)	205 \pm 17
K (%)	1.84 \pm 0.09 (4.9)	1.82 \pm 0.06	1.53 \pm 0.07 (4.7)	1.51 \pm 0.06
La (ppb)	228 \pm 36 (15.7)	190 (b)	252 \pm 30 (11.9)	
Mg (%)	0.46 \pm 0.06 (13)	0.58 \pm 0.03	0.393 \pm 0.04 (10.4)	0.408 \pm 0.02
Mn (ppm)	24 \pm 3 (12.5)	23 \pm 2	2228 \pm 110 (4.9)	2030 \pm 170
Na (ppm)	170 \pm 11 (6.7)	160 \pm 20	109 \pm 7 (6.3)	106 \pm 13
Rb (ppm)	4.94 \pm 0.17 (3.5)	4.84 \pm 0.06	75 \pm 3 (4.6)	75 \pm 4
Sb (ppb)	45 \pm 4 (8.9)	40 (b)	-	
Zn (ppm)	30 \pm 3 (10.0)	29 \pm 2	342 \pm 24 (7.0)	340 \pm 20

a - Values in parentheses are relative standard deviations.

b - Information values from Ref (8).