Nuclear Analytical Methods in the Life Sciences

Edited by

Rolf Zeisler

National Institute of Standards and Technology,
Gaithersburg, MD

and

Vincent P. Guinn

University of California,
Irvine, CA

Humana Press • Clifton, New Jersey

This work also appears as volumes 26 and 27 of the Humana Press journal,
Biological Trace Element Research Editor-in-Chief: Gerhard N. Schrauzer

ISBN: 0-89603-202-7

Crescent Manor
PO Box 2148
Clifton, NJ 07015 USA

Printed in the United States of America.

This publication is printed on acid-free paper. ∞

Determination of Inorganic Components in Brazilian Medicinal Plants by Neutron Activation Analysis

M. SAIKI,[1,*] M. B. A. VASCONCELLOS,[1] AND J. A. A. SERTIÉ[2]

Instituto de Pesquisas Energéticas e Nucleares, IPEN—CNEN/SP, Radiochemistry Division, CEP 05508, São Paulo-SP, Brasil; and Universidade de São Paulo, Instituto de Ciências Biomédicas, Department of Pharmacology, CEP 05508, São Paulo, SP Brasil

Received April 17, 1989; Accepted December 11, 1989

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}–10^{13} n/cm^{2}s in the IEA-R1 nuclear reactor. The NBS Tea Leaves (1572) and NIES Pepperbush (1) reference materials were analyzed simultaneously with the plant extracts.

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

Index Entries: Neutron activation analysis; medicinal herbs; analysis of plant extracts; *Cordia Verbenacea; Folidago Microglossa; Petiveria Alliacea.*

*Author to whom all correspondence and reprint requests should be addressed.

Biological Trace Element Research Editor: G. N. Schrauzer

INTRODUCTION

The use of medicinal herbs is widespread in Brazil. In the last years there has been an increasing interest in using natural medicines, in order to avoid the risks of side effects and the rising cost of medicines obtained from synthetic chemical products.

Although the efficiency of some natural drugs for curing diseases has been proven, the specialists in this subject have warned against the indiscriminate use of these products because they can cause serious intoxications.

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

It is very important to determine the elemental composition of medicinal plants or 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 the 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 also depend on the region where it was cultivated.

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 studies of their toxicity, medicinal action, or active component.

MATERIALS AND METHOD

Extract Samples Obtained from Medicinal Herbs

Medicinal extracts obtained from three species of herbs were analyzed by INAA in this work. *Cordia Verbenacea DC*, from the family of Boraginaceae, is a perennial bush plant used for many centuries by Brazilian Indians to treat infections and rheumatisms. The bioflavonoids produced by their leaves show antiinflammatory 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, so 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 antiinflammatory and cicatrizing drug. *Petiveria Alliacea* (Phytolaccaceae), known in Brazil by the name "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 pipeted onto a small sheet of Whatman No. 42 filter paper and dried in a dessicator at room temperature. The amounts of elements in these standards were about 10 times higher than those found in the samples. The sheets of filter paper were also put into polyethylene bags or capsules.

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

The occurence of the interfering reactions ^{27}Al (n,p) ^{27}Mg, ^{31}P (n,α) ^{28}Al and ^{28}Si (n,p) ^{28}Al in the determinations of Mg and Al was also examined by irradiation of the samples with epithermal neutrons using 1.0-mm-thick cadmium containers.

The irradiated samples and standards were counted using an ENER-TEC hyperpure Ge detector coupled to an EG & G ORTEC 4096 channel plus height analyzer connected to a 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

Table 1
Experimental Conditions and Nuclear Data of the Nuclides Used in This Study

Irradiation Time	Thermal Neutron Flux $n.cm^{-2}.s^{-1}$	Decay Time	Radioisotopes (Gamma ray energies measured in keV)
1 min	3.7×10^{11}	2 min	^{28}Al (1778) ; ^{27}Mg (1014) ; ^{80}Br (617);
		60 min	^{38}Cl (1642) ; ^{56}Mn (1810)
30 min	1.7×10^{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)

^{57}Co. Samples and standards were measured in the polyethylene capsules or bags used for their irradiations. The presence of impurities in these involucres 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 the 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 No. 1 from the National Institute for Environmental Studies (NIES), Japan, were analyzed 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 analyzed on a dried basis.

RESULTS AND DISCUSSION

Table 2 shows the results of elemental concentrations obtained for *Cordia Verbenacea* and *Folidago Microglossa* extracts, and Table 3 presents 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 <10%, were obtained for most of the elements analyzed. 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 because of 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 analyzed, 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 little 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, since Cl is generally found in higher concentration in the green parts (leaves) of the plant than in the roots.

Table 2
Concentrations of Elements in Extracts Obtained from Leaves of *Cordia Verbenacea* and *Folidago Microglossa* by INAA

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

[a]Values in parentheses are relative standard deviations.

It is important to note that the medicinal extracts analyzed here have shown a high concentration of K, varying from 3–10%, 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 analyzing Citrus Leaves and Pepperbush reference materials. Table 4 shows the results obtained

Table 3
Concentrations of Elements in Extracts from *Petiveria Alliacea* (Guiné) Obtained by INAA

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

[a]Values in parentheses are relative standard deviations.

in these analyses, together with certified values presented by NBS and NIES. We have determined 15 elements; 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 $<10\%$ were obtained. Also in these samples, the least precise results were obtained for Co and Cs.

ACKNOWLEDGMENTS

This work was partially supported by the Brazilian National Research Council (CNPq) and Financial Agency for Studies and Projects (FINEP).

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

[a] Values in parentheses are relative standard deviations.
[b] Information values from Ref (*8*).

REFERENCES

1. Ch. L. Ndiokwere, *J. Radioanal. Nucl. Chem.*, **85**, 325 (1984).
2. S. Mukhammedov, Kh. Tillaeva, and N. B. Badalov. *Sov. At. Energy* **61(6)**, 1043 (1987).
3. M. Koyama, M. Shirakawa, J. Takada, Y. Katayama, and T. Matsubara, *J. Radioanal. Nucl. Chem.*, **112(2)** 489 (1987).
4. S. B. Aidid, *J. Radioanal. Nucl. Chem.*, **120(2)**, 335 (1988).
5. L. Marichkova and O. Kostarova, *Proceedings of Balkan Conference on Activation Analysis*, Varna, Bulgaria May 6–8, 1985, in INIS-mf-11126, 121–3, 1985.
6. J. A. A. Sertié, A. C. Basile, S. Panizza, A. K. Matida, and R. Zelnik, *Planta Medica*, **1**, 7 (1988).
7. F. W. Lima and L. T. Atalla, *J. Radioanal. Chem.*, **20**, 769 (1974).
8. NBS Certificate of Analysis for SRM 1572 Citrus Leaves, Office of Standard Reference Materials, US Dept. of Commerce, Washington DC 1982.
9. NIES Certificate of Analysis for NIES Pepperbush No. 1 Reference Material, National Institute for Environmental Studies, Japan, 1980.