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NEUTRON ACTIVATION ANALYSIS OF MEDICINAL PLANT EXTRACTS

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Instrumental neutron activation analysis was applied to the determination of the elements Br, Ca, Cl, Cs, Fe, K, La, Mg, Mn, Na, Rb and Zn in medicinal extracts obtained from *Centella asiatica, Citrus aurantium L., Achyrolcline satureoides DC, Casearia sylvestris, Solano lycocarpum, Zingiber officinale Roscoe, Solidago microglossa* and *Stryphnondedron barbatiman* plants. The elements Hg and Se were determined using radiochemical separation by means of retention of Se in HMD inorganic exchanger and solvent extraction of Hg by bismuth diethyldithiocarbamate solution. Precision and accuracy of the results were evaluated by analyzing biological reference materials. The therapeutic action of some elements found in plant extracts analyzed is briefly discussed.

In Brazil, the use of medicinal plants is very popular and therefore considerable efforts have been made for their chemical and pharmacological characterization.

The great interest in using phytopharmaceuticals is to provide alternative to imported drugs as well as to avoid the risks of side effects caused by synthetic drugs. Besides, our tropical forests constitute a resource of biodiversity for pharmacological industries interested in using medicinal plants as raw materials for the preparation of medicines. The determination of trace elements in medicinal plants or in their extracts has become of increasing interest since several elements can play an important role in human health and disease.

Some elements are components of active constituents or they can affect the plant metabolism and consequently the formation of active constituents. Therefore studies about elemental concentrations in medicinal plants have been presented in several recent papers[1-7].

Following our studies[2] of determination of trace elements in medicinal extracts, this paper deals with the application of neutron activation analysis of extracts obtained from eight plants used in Brazilian popular medicine. The purpose of this work is to contribute to the studies of identification and availability of active compounds responsible for the pharmacological effects of medicinal plants.

Both instrumental and radiochemical neutron activation analyses were applied to the trace element determinations in medicinal plant extract samples and biological reference materials. Radiochemical procedure was required for the determination of Hg and Se. Determination of these two elements by instrumental neutron activation analysis was seriously hampered by high activities of several radionuclides such as ^{24}Na, ^{42}K, ^{82}Br and ^{32}P. Besides the 279.6 keV γ-rays of ^{75}Se interfered in measuring the 279.1 keV γ-rays of ^{203}Hg, which is the most important analytical line of ^{203}Hg.

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Materials and Methods

Medicinal Plant Extracts: The plant extracts analyzed were provided by the Department of Pharmacology of the Biomedical Science Institute of University of São Paulo. The extracts were obtained using a 70% or 65% alcoholic solution or acetone at room temperature[8]. After the filtration the solution was concentrated under reduced pressure at 50°C and then the aqueous viscous material was freeze-dried. The freeze-dried extract was stored in a dessicator. The characteristics of plants studied are presented in Table 1. In case of Casearia sylvestris plant, the extracts were obtained using dried and fresh leaves separately.

Table 1
Characteristics of the Brazilian medicinal plants analyzed.

Scientific plant name	Family name	Popular name	Popular use	Part of the plant used
Casearia sylvestris	Flacourtiaceae	guaçatonga, guaça, erva de lagarto	antiseptic, cicatrizant, topical anaesthetic, anti-ulcer drug	dried and fresh leaves
Zingiber officinale Roscoe	Zingiberaceae	gengibre, gengivre, mangaratiá	antioxidant, antispatic, cicatrizant, carminative, diaphoretic	fresh rhizomes
Stryphnondedron barbatiman	Leguminosae	barbatimão, uabati-mão,barba deTimão	antihaemorrhagic, tonic, depurative, cicatrizant	barks and leaves
Solano lycocarpum	Solanaceae	fruto do lobo, lobeira	sedative, diuretic	fruits
Achyrolcline satureoides DC	Compositae	macela, marcela, paina	analgetic, sedative, sudorific, bactericide	flowers
Citrus aurantium L.	Rutaceae	laranja amarga, laranja azeda	against stomach acidity, antioxidant	bark
Solidago microglossa	Compositae	arnica-do-Brazil	anti-inflamatoriy	leaves and roots
Centella asiatica	Umbelliferae	cairuçú, pé-de-cavalo	for slimming, to treat varicosevein, cicatrizant	leaves

Instrumental Neutron Activation (INAA): About 80 mg of the powdered extracts were weighed into clean plastic envelopes and irradiated at the IEA-R1 nuclear research reactor, together with the elemental synthetic standards. The synthetic standards were prepared by pippeting the elemental standard solutions onto pieces of Whatman No.41 filter paper. Stock solutions of standards were prepared by dissolving high purity metals, oxides or salts in appropriate reagents. Dilute solutions containing one or more elements were prepared from these stock solutions.

Short and long time irradiations were carried out in order to determine as many elements as possible using the following conditions:

a) short irradiations for 3 minutes at a thermal neutron flux of 3.72×10^{12} n.cm^{-2}.s^{-1} for determination of Cl, Mg and Mn;

b) long irradiations for 8 or 16 hours at a thermal neutron flux from 10^{12} to 10^{13} n.cm^{-2}.s^{-1} for Br, Ca, Cs, Fe, K, La, Na, Rb and Zn determination.

After adequate decay time varying from 2 minutes to 10 minutes in case of short irradiations, and from 2 days to 2 weeks after long irradiations, gamma ray measurements were carried out using a HPGe detector. The counting system had a resolution (FWHM) of 1.15 keV for 122 keV gamma-rays of ^{57}Co and of 2.5 keV 1332 keV gamma-rays of ^{60}Co. The gamma ray spectra were processed using a VISPECT computer program. This program evaluates a peak area (count rate) and gamma-ray energies of radioisotopes. The radioisotopes measured in this study (^{82}Br, ^{47}Ca, ^{38}Cl, ^{134}Cs, ^{59}Fe, ^{42}K, ^{140}La, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{86}Rb and ^{65}Zn) were identified according to their half-lives and gamma-ray energies. The comparative method was used for calculating the content of the respective elements.

The accuracy of the results was checked by analyzing certified (standard) reference materials such as CRM No.1 Pepperbush from the National Institute for Environmental tudies (NIES) of Japan and SRM 1572 Citrus Leaves from the U. S. National Institute of Standards and Technology (NIST). Elemental concentrations in these reference materials were evaluated on a dry weight basis determined as recommended in their respective certificates.

Radiochemical Neutron Activation Analysis (RNAA): The procedure applied was based on a procedure developed by Greenberg[9]. About 130 mg of the sample weighed in a quartz ampoule were irradiated together with standard solutions of selenium and mercury also placed in quartz ampoules. The samples and standards were irradiated in the IEA-R1 nuclear research reactor for 16 hours at a thermal neutron flux of about 10^{13} n.cm^{-2}.s^{-1}. After approximately 15 days of decay time the sample was transferred to a Teflon beaker, to which 3 ml of conc. nitric acid, 3 ml of distilled water and 20 μg of Hg and 47 μg of Se carriers were added and wet-ashed in a closed system (Teflon bomb) in an oven at 100°C during 8 hours. The resulting solution was diluted with distilled water to 15 ml.

A HMD column was prepared as follows. About 3 ml of the HMD inorganic exchanger, were pre-conditioned for at least one night in a 1 mol/l nitric acid solution and the fine particles of HMD were discarded. In the column, HMD was preconditioned using a solution containing 1 mol/l nitric acid and 1 mol/l phosphoric acid as described by Greenberg[9].

After the sample solution has passed through the HMD column, the HMD was washed twice with 15 ml of a solution containing 1 mol/l nitric acid and 0.0025 mol/l phosphoric acid and later the HMD with the Se retained was transferred to a scintillation vial and centrifugated. The supernatant was discarded. Selenium was measured using the 264.6 keV gamma-rays of ^{75}Se.

The effluent solution was transferred to a separation funnel and its pH was adjusted to amount to 0.3 - 1.0. Extraction was performed with a solution of 5.10^{-3} mol/l bismuth diethyldithiocarbamate in chloroform by manual agitation for 5 minutes. The phases were separated by decantation and the organic phase containing mercury was

drained into a glass (penicillin) vial for counting. Mercury was determined by means of the 279.2 keV gamma-rays of ^{203}Hg.

Standards of Hg and Se were prepared in two ways: by pippeting directly known aliquots of standard solutions into the scintillation vials or by processing the aliquots added to the unirradiated extract sample by the same procedure as for the extract samples analyzed. This was done to determine the chemical yield of the separations employed.

For counting, the aliquots of non-processed standards (2.07 μg and 7.78 μg of Hg and Se, respectively) were diluted with distilled water containing few drops of conc. nitric acid to the same volume as the test samples to obtain the same counting geometry.

For quality assurance purposes, Hg and Se were determined in two reference materials (RMs) of the International Atomic Energy Agency (IAEA): IAEA-MA-1/TM Copepod and IAEA-MA-2/TM Fish Flesh. The weight losses of 5.67% and 4.96% for Copepod and Fish Flesh, repectively, which were determined according to recommendations in the respective IAEA certificates[10,11] were used to correct the Hg and Se contents determined to a dry weight basis.

Results and Discussion

Table 2 shows the results obtained by INAA for NIES CRM No.1 Pepperbush and NIST SRM 1572 Citrus Leaves. The results obtained in these RMs are in good agreement with the respective certified values[12,13]. The relative errors (deviations from the mean certified values) are lower than 11%. Also a good precision, expressed as relative standard deviations varying from 0.2% to 8%, was obtained. Table 3 shows the results for Br, Ca, Cl, Cs, Fe, K, La, Mg, Mn, Na, Rb and Zn obtained by INAA in *Solidago microglossa, Achyrolcline satureoides DC, Centella asiatica, Stryphnondedron barbatiman, Solano lycocarpum* extracts and Table 4 presents the results for *Casearia sylvestris, Citrus aurantium L.* and *Zingiber officinale Roscoe* extracts. In these analyses also acceptable precision as obtained for most of elements, with relative standard deviations varying from 1% to 11%, which is generally considered as a good result in trace analysis. The precision was not so good for Mg in *Citrus aurantium L.* extract and for Cl in *Solano lycocarpum* and *Citrus aurantium L.* extracts. Determination of Mg is interferred by the presence of Al in the samples, because ^{27}Al forms by the (n,p) nuclear reaction ^{27}Mg, the same radioisotope used for Mg determination. Also the 847 keV gamma-rays of ^{56}Mn can interfere in Mg determination when measuring the 843 keV gamma-rays of ^{27}Mg. No reason was found for slightly high standard deviations obtained in Cl determinations in some samples.

In order to evaluate the applicability of the radiochemical procedures employed, the chemical separation yields were determined. Chemical yields of (99.9 + 6.7)% and (102.2 + 2.2)% were obtained respectively for Hg and Se in five determinations.

Table 5 shows the results for Hg and Se obtained by RNAA in the medicinal plant extracts and RMs analyzed. The results obtained for the RMs compare well with the

Table 2
Analysis of Pepperbush No.1 and 1572 Citrus Leaves by instrumental neutron activation method.

	PEPPERBUSH No.1			1572 CITRUS LEAVES		
ELEMENTS	This work mean + s	NIES value.(12)	relative error	This work mean + s	NIST value(13)	relative error
Br (μg/g)	1.5 ± 0.1 (7%)*			8.1 ± 0.4 (5%)	8.2**	
Ca (%)	1.44 ± 0.07 (5%)	1.38 ± 0.07	4%	3.2 ± 0.1 (3%)	3.15 ± 0.10	2%
Cl (μg/g)	ND			434 ± 37 (8%)	414**	
Cs (μg/kg)	1198 ± 65 (5%)	1200**		97 ± 3 (3%)	98**	
Fe (μg/g)	208 ± 1 (0.5%)	205 ± 17	1%	84 ± 2 (2%)	90 ± 10	7%
K (%)	1.5 ± 0.1 (7%)	1.51 ± 0.06	0.7%	1.8 ± 0.1 (5%)	1.82 ± 0.06	1%
La (μg/kg)	317 + 24 (8%)			171 ± 3 (2%)	190**	
Mg (μg/g)	4320 ± 178 (4%)	4080 ± 200	6%	6227±177(3%)	5800 ± 200	7%
Mn (μg/g)	1799 ± 119 (7%)	2030 ± 170	11%	21.5 ± 0.3 (1%)	23 ± 2	6%
Na (μg/g)	102 ± 5 (5%)	106 ± 13	4%	153 ± 12 (8%)	160 ± 21	4%
Rb (μg/g)	75 ± 2 (3%)	75 ± 4	0%	4.9 ± 0.1 (2%)	4.84 ± 0.06	1%
Zn (μg/g)	337.6 ± 0.8(0.2%)	340 ± 20	0.7%	30 ± 2 (7%)	29 ± 2	3%

* numbers in parenthesis are relative standard deviations
** information values
ND non detected
Results obtained from at least four determinations

certified values given by Toro et at[14]. The precision of the results obtained for extract samples and reference materials was also satisfactory within the limits expected for trace element analysis (less than 1 μg/g). To check accuracy of RNAA procedure, the NIES CRM no.1 Pepperbush was not used because Hg and Se are not certified in this material and in the case of the NIST SRM Citrus Leaves, Se is not certified.

From the results presented in Table 3, 4 and 5 it can be seen that several elements found in the medicinal extracts are essential for human beings, such as Ca, Fe, K, Mg, Mn, Rb, Se and Zn[15]. Elements Ca (in some extracts), K and Mg were found at the highest concentration in the extracts analyzed. This high concentration of K in medicinal extracts could be related to the diuretical action of the drugs prepared from the plant materials. K is presented in natural diuretics as well as in drugs used for eliminating phlegm and invigorating stomach[6]. Besides it is known that potassium salts can regulate body fluids and also participate in cardiac muscle contraction.

Table 3
Results for *Solidago microglossa, Achyrolcline satureoides DC, Centella asiatica, Stryphnondedron barbatiman* and *Solano lycocarpum* extracts obtained by INAA.

Elements	*Solidago microglossa*	*Achyrolcine satureoides DC*	*Centella asiatica*	*Stryphnondedron barbatiman*	*Solano lycocarpum*
Br (μg/g)	39 ± 2 (a)	2.8 ± 0.1	23.0 ± 0.5	7.4 ± 0.4	21 ± 1
Ca (%)	0.36 ± 0.01	0.15 ± 0.03	1.6 ± 0.1	0.084 ± 0.004	0.059 ± 0.007
Cl (μg/g)	8.80 ± 0.03	0.41 ± 0.03	3.4 ± 0.3	4.6 ± 0.5	5.9 ± 1.0
Cs (μg/kg)	1127 ± 17	121 ± 1	228 ± 7	420 ± 4	265 ± 12
Fe (μg/g)	164 ± 17	468 ± 41	431 ± 18	30 ± 2	(c)
K (%)	3.1 ± 0.2	0.84 ± 0.05	4.4 ± 0.1	0.67 ± 0.02	2.35 ± 0.08
La (μg/kg)	174 ± 19	(c)	749 ± 17	(b)	220 ± 27
Mg (μg/g)	4328 ± 230	990 ± 55	2345 ± 129	1053 ± 140	1174 ± 27
Mn (μg/g)	57 ± 2	0.84 ± 0.05	195 ± 2	11.0 ± 0.7	8.7 ± 0.8
Na (μg/g)	169 ± 7	75 ± 5	1404 ± 13	156 ± 7	208 ± 16
Rb (μg/g)	138 ± 2	548 ± 12	134 ± 2	24 ± 1	60 ± 2
Zn (μg/g)	118 ± 7	133 ± 8	577 ± 36	16.9 ± 0.5	59 ± 3

(a) mean and standard deviation from at least four determinations
(b) indicates that the element was not detected
(c) indicates that the element was detected but not quantified

The high concentrations of Ca and Mg present in medicinal extracts can explain the absence of side effects as regard stomach lesions. Also Ca and Mg salts are recommended in the prevention of diseases like osteoporosis.

Fe is an important component of hemoglobin in human body, and it is present in the extracts at the levels of μg/g. Besides this element can present the effect of promoting diuresis eliminating phlegm and strenghthening the function of the stomach[6].

The elements Zn and Se are used as antioxidants in the orthomolecular medicine field. Drugs containing Zn are used in the treatment and prevention of the ulcer[6] and to heal wounds.

Concentrations of Hg found in medicinal extracts in the present work were too low and other toxic elements such as Cd, As and Ni were not detected, as well.

It can be concluded that the very good agreement of our results with the certified values of RMs analyzed proved the accuracy of determinations carried out by the

Table 4
Results for *Casearia sylvestris, Citrus aurantium L.* and *Zingiber officinale Roscoe* extracts obtained by INAA.

Elements	*Casearia sylvestris* (fresh leaves)	*Casearia sylvestris* (dried leaves)	*Citrus aurantium L.*	*Zingiber officinale Roscoe*
Br (μg/g)	889 ± 37 (a)	124 ± 4	577 ± 36	18 ± 1
Ca (%)	0.202 ± 0.006	(b)	0.30 ± 0.02	(b)
Cl (μg/g)	2.2 ± 0.1	3.2 ± 0.3	6.8 ± 1.4	1.80 ± 0.09
Cs (μg/kg)	313 ± 7	413 ± 10	1927 ± 60	31 ± 3
Fe (μg/g)	91 ± 3	1742 ± 105	7.4 ± 0.4	115 ± 12
K (%)	4.70 ± 0.08	8.2 ± 0.8	9.4 + 0.8	4.7 ± 0.4
La (μg/kg)	112 ± 7	(c)	(b)	328 ± 29
Mg (μg/g)	2431 ± 325	4394 ± 380	4407 ± 735	2082 ± 55
Mn (μg/g)	206 ± 24	133 ± 3	297 ± 24	259 ± 6
Na (μg/g)	2071 ± 109	3576 ± 188	1039 ± 40	465 ± 19
Rb (μg/g)	108 ± 7	132 ± 7	533 ± 15	29 ± 2
Zn (μg/g)	85 ± 3	113 ± 2	131 ± 9	75 ± 3

(a) mean and standard deviations from at least four determinations
(b) indicates that the element was not detected
(c) indicates that the element was detected but not quantified

INAA and RNAA procedures employed in this work. Neutron activation analysis can be used succesfully for the determination of the elemental levels in medicinal extracts and these analyses can bring new information for their pharmacological studies.

Table 5
Results for mercury and selenium in medicinal plant extracs and reference materials obtained by radiochemical neutron activation analysis.

MATERIAL	SELENIUM	MERCURY
Centella asiatica	122 ± 12 (a)	108 ± 6
Solano lycocarpum	12.7 ± 1.4	5.9 ± 0.5
Casearia sylvestris (dried)	27.7 ± 7.5	19.0 ± 3.0
Casearia sylvestris (fresh)	32.0 ± 5.1	251 ± 52
Achyrolcline satureoides DC	29.8 - 35.0 (b)	509 - 1020 (b)
Citrus aurantium L.	22 - 151 (b)	469 ± 88
Copepod	2700 ± 200 (3000 ± 201) (c)	270 ± 12 (280 ± 10) (c)
Fish Flesh Homogenate	1500 ± 200 (1700 ± 300) (c)	473 ± 40 (470 ± 20) (c)

Results are given in μg/kg of dried materials
(a) mean and standard deviations from at least three determinations
(b) individual results
(c) numbers in parenthesis are certified values from ref. (14)

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