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Characterization of trace elements in *Casearia* medicinal plant by neutron activation analysis

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Abstract

Leaves of *Casearia sylvestris*, *Casearia decandra* and *Casearia obliqua* plant species, collected at the Atlantic Forest in Brazil, were analyzed by using instrumental neutron activation analysis (INAA). Short and long irradiations using thermal neutron flux of the IEA-R1 nuclear reactor were carried out for these analyses. Concentrations of Ca, K and Mg were found in these samples at the percentage levels, Br, Cl, Fe, Mn, Na, Rb and Zn at the $\mu g\,g^{-1}$ levels and Co, Cr, Cs, La, and Sc at the $\mu g\,kg^{-1}$ levels. Comparisons were made among the element concentrations obtained in these three *Casearia* species and significant differences were found for the elements Cl, Co, Cs, Cr, La, Mn, Na and Sc. The precision and the accuracy of the results were evaluated by analyzing the certified reference materials NIST-1515 Apple Leaves and NIST-1573a Tomato Leaves.

Keywords: Neutron activation analysis; INAA; Medicinal plants; *Casearia* plant; Trace elements

1. Introduction

Medicinal plants have been used for many years to cure a great variety of diseases. Recently, according to the World Health Organization, the use of traditional herbal medicine has spread not only in the developing countries but also in the industrialized ones, as a complementary way to treat and to prevent illnesses (WHO, 2003).

The pharmacological properties of the medicinal plants have been attributed to the presence of active constituents which are responsible for important physiological function in living organisms. It has been reported that trace elements play an important role in the reactions which will lead to the formation of these active constituents (Serfor-Armah et al., 2001). However, a correlation between elemental composition of medicinal plants and their curative properties have not been established yet. Besides, element concentrations present in medicinal plants are of great importance to understand their pharmacological actions (Serfor-Armah et al., 2002).

Inorganic constituents of several medicinal plants have been determined by applying several techniques such as atomic absorption spectroscopy (Chizzola et al., 2003) atomic absorption spectrophotometry (Caldas and Machado, 2004; Ajasa et al., 2004), flame atomic emission spectrometry (Razic et al., 2003), flame atomic absorption spectrometry (Razic et al., 2003; Gomez et al., 2004), electrothermal atomic absorption spectrometry (Gomez et al., 2004), X-ray emission (Mohanta et al., 2003), X-ray fluorescence (Obiajunwa et al., 2002; Salvador et al., 2003), inductively coupled plasma mass spectrometry (Falcó et al., 2003) and instrumental

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neutron activation analysis (Naidu et al., 1999; Serfor-Armah et al., 2001, 2002; Dim et al., 2004).

In this work, instrumental neutron activation method was applied in order to compare inorganic constituents present in three species of *Casearia* genus medicinal plant collected at Reserva do Morro Grande located at the Atlantic Forest, São Paulo State, Brazil. Leaves of *C. sylvestris* are traditionally used as an antiseptic, cicatrizant and topical anesthetic agent and have been widely studied for its antiulcer property (Sertié et al., 2000), snake and bees venoms neutralization capacity (Borges et al., 2000, 2001) and antitumor activity (Itokawa et al., 1988, 1990; Oberlies et al., 2002). There are no reports about the traditional use of *C. decandra* and *C. obliqua* yet; however, researches about their pharmacological properties are being carried out.

The precision and the accuracy of the results were also evaluated by analyzing the certified reference materials NIST-1515 Apple Leaves (NIST, 1995) and NIST-1573a Tomato Leaves (NIST, 1993) both provided from National Institute of Standard and Technology, USA.

2. Experimental

2.1. Sampling and sample preparation

Adult leaves of three species of *Casearia* genus medicinal plant, namely *C. sylvestris*, *C. decandra* and *C. obliqua*, were provided by the Instituto de Ciências Biomédicas (ICB) of Universidade de São Paulo (USP). These samples were collected at Reserva do Morro Grande located at the Atlantic Forest, São Paulo, Brazil, and the species were identified by the Departamento de Botânica of USP. The leaves were washed using Milli-Q water and dried for 12 h under a pressure of about 5×10^{-2} mbar using Micro Modulyo lyophilizer. The dried leaves were ground using Fritsch micro vibrator pulverisette to obtain a fine powder.

About 150 mg of each sample were weighed in polyethylene bags which were previously cleaned using diluted nitric acid solution and Milli-Q water.

2.2. Irradiation

The samples were irradiated at the nuclear research reactor IEA-R1 of IPEN-CNEN/SP along with synthetic standards of the elements. These synthetic standards were prepared by pipetting 50 μL of the solutions containing one or more elements onto pieces of Whatman No. 41 filter paper. These solutions were prepared from certified standard solutions provided by Spex Chemical, USA. The quantities of the elements used for irradiation were (in μg): Br = 5.0; Ca = 1001.2; Cl = 200; Co = 0.15; Cr = 2.0; Cs = 0.60; Fe = 350.1; K = 1002.4; La = 0.61; Mg = 1000; Mn = 1.49; Na = 100.2; Rb = 10.0; Sc = 0.07; Zn = 35.0.

Irradiations were carried out under two experimental conditions. Short irradiations of 5 min at the pneumatic station with thermal neutron flux of approximately $10^{11}\,n\,cm^{-2}\,s^{-1}$ were used for determining the elements Cl, K, Mg, Mn and Na. Longer irradiations of 16 h under thermal neutron flux of $5\times10^{12}\,n\,cm^{-2}\,s^{-1}$ were used for Br, Ca, Co, Cr, Cs, Fe, La, Na, Rb, Sc and Zn determinations. After appropriate decay times, the gamma activities of the samples and elemental standards were measured using a hyperpure Ge detector Model GX2020 coupled to Model 1510 Integrated Signal Processor and System 100 MCA Card, both from Canberra. The system had a resolution of 1.0 keV for 121 keV ^{57}Co gamma-ray energy and of 1.8 keV for 1332 keV ^{60}Co gamma-ray energy. In the case of short irradiations, samples and synthetic standards were measured for 300 or 600 s depending on their activities. Samples and standards from long irradiations were measured after 4, 11 and 20 days of decay times. Synthetic standards were counted for 5400 s and samples were counted for a period of 20000–50000 s. For first measurements, samples and synthetic standards were placed 3 or 6 cm from the detector depending on the activity of the radioisotopes. After 11 days of decay time they were measured on the detector. Canberra S100 software was used for acquisition of the gamma spectra and VERSAO2 for processing the obtained spectra. The identification of radioisotopes was carried out by its half-life and its gamma-ray energies. The radioisotopes measured in this study were: ^{82}Br, ^{47}Ca, ^{38}Cl, ^{60}Co, ^{51}Cr, ^{134}Cs, ^{59}Fe, ^{42}K, ^{140}La, ^{27}Mg, ^{56}Mn, ^{24}Na, ^{86}Rb, ^{46}Sc and ^{65}Zn. Comparative method was used for calculating the concentrations of the elements present in the samples.

2.3. Analysis of certified reference materials

The certified reference materials NIST-1515 Apple Leaves and NIST 1573a Tomato Leaves were analyzed in the same experimental conditions used in the sample analyses in order to evaluate the precision and accuracy of the results. As recommended in their respective certificates, the elemental concentrations in the reference materials were evaluated on a dry weight basis. The moisture was determined in a separate sub-sample (not taken for analyses) that was dried at 85 °C during 24 h. The following values (in percent) of weight loss were obtained: 3.70% for Apple Leaves and 5.05% for Tomato Leaves. These values were used to correct the final results.

3. Results and discussion

Tables 1 and 2 show the results obtained in the analyses of certified reference materials NIST-1515

Apple Leaves and NIST-1573a Tomato Leaves, respectively. Certified values reported by NIST are also shown in these tables for comparison. The precision of the results was satisfactory with relative standard deviations varying from 0.2% to 11.4%. The relative errors were lower than 9.2% confirming the accuracy of the results obtained by this analytical methodology. Some elements determined in these reference materials are not certified. Thus, the results obtained for Br, Co, Cr, La, and Sc in Apple Leaves and for Br, Cl, Cs, La, Mg and Sc in Tomato Leaves, constitute a contribution for their certification in these reference materials. Fig. 1 shows the Z score values calculated for the elements determined in the reference materials (Bode, 1996). The Z score values for all elements were $|Z|<3$, which means that the results obtained are in the 99% confidence interval of the certified values.

Table 3 presents the arithmetic mean values of the elemental concentrations obtained in three different *Casearia* species collected in the Reserva do Morro Grande located at the Atlantic Forest, Brazil. Ca, K and Mg were the most abundant elements present in *Casearia* samples, and were found in % levels. These results are in agreement with those presented for other plant species (Serfor-Armah, et al., 2001, 2002; Razic et al., 2003; Dim et al., 2004). The elements Br, Cl, Fe, Mn, Na, Rb and Zn were found at $\mu g\,g^{-1}$ levels and Co, Cr, Cs, La and Sc presented the lowest concentrations and were found at $\mu g\,kg^{-1}$ levels. Concentrations of Br, Ca, Fe, K, Mg, Rb and Zn were of the same magnitude for the three species; however, for Cl, Co, Cs, Cr, La, Mn, Na and Sc their concentrations indicated significant differences. These differences may be attributed to factors such as preferential absorbability of a plant species for a specific element (Serfor-Armah et al., 2001) and elemental composition of the soil where the plants were cultivated.

The elements Ca, Mg, Na, K and Cl are some of the macronutrients which are essential to human health and nutrition. Ca is the main constituent of the skeleton and is important for regulating many vital cellular activities such as nerve and muscle function, hormonal actions, blood clotting and cellular mortility (Martin et al., 1985). The highest concentration of this element was found in *C. decandra*. Mg is an important electrolyte also responsible for proper nerve and muscle function. It also works as co-factor in more than 300 metabolic reactions (Berdanier, 1994). All three species presented comparable levels of Ca and Mg. Ca concentrations ranged from 0.22% to 0.56% and Mg concentrations ranged from 0.17% to 0.23%. Due to their high Ca and Mg content, preparations from medicinal plant leaves can be used to neutralize the excess of stomach acidity, avoiding lesions in the stomach inner walls. Na, K and Cl are important elements for the maintenance of acid–base equilibrium and of osmotic pressure of body fluids (Martin et al., 1985). Highest concentration of Na was found in *C. decandra* and the lowest one was in *C. obliqua*. K concentrations were also comparable for all plant species, ranging from 1.1 to 1.5%. K participates actively in the maintenance of the cardiac rhythm (Martin et al., 1985) and in constipation. Cl

Table 1
Elemental concentrations obtained for NIST-1515 Apple Leaves reference material

Element	This work				Certified value (NIST, 1995)
	n^a	$X^b \pm SD^c$	RSD[d] (%)	Er[e] (%)	
Br ($\mu g\,g^{-1}$)	4	2.13±0.01	0.5		NR[f]
Ca (%)	3	1.49±0.07	4.7	2.4	1.526±0.015
Co ($\mu g\,kg^{-1}$)	4	107±3	2.8		NR
Cr ($\mu g\,kg^{-1}$)	3	770±20	2.6		NR
Fe ($\mu g\,g^{-1}$)	3	81.8±2.1	2.6	1.4	83±5
K (%)	4	1.58±0.01	0.6	1.9	1.61±0.02
La ($\mu g\,g^{-1}$)	4	19.65±0.06	0.3		NR
Mg (%)	3	0.246±0.028	11.4	9.2	0.271±0.008
Mn ($\mu g\,g^{-1}$)	3	51.3±1.9	3.7	5.0	54±3
Rb ($\mu g\,g^{-1}$)	3	9.9±0.2	2.0	2.9	10.2±1.5
Sc ($\mu g\,kg^{-1}$)	4	30.8±0.2	0.6		NR
Zn ($\mu g\,g^{-1}$)	2	13.4±0.3	2.2	7.2	12.5±0.3

[a]number of determination.
[b]mean value.
[c]standard deviation.
[d]relative standard deviation.
[e]relative error.
[f]not reported.

Table 2
Elemental concentration obtained for NIST-1573a Tomato Leaves reference material

Elements	This work				Certified value (NIST, 1995)
	n^a	$X^b \pm SD^c$	RSD[d] (%)	Er[e] (%)	
Br ($mg\,g^{-1}$)	3	1.089 ± 0.002	0.2		NR[f]
Ca (%)	3	4.94 ± 0.17	3.4	2.2	5.05 ± 0.09
Cl ($mg\,g^{-1}$)	4	5.85 ± 0.21	3.6		NR
Co ($\mu g\,g^{-1}$)	2	0.60 ± 0.02	3.3	5.3	0.57 ± 0.02
Cr ($\mu g\,g^{-1}$)	3	2.06 ± 0.02	1.0	3.5	1.99 ± 0.06
Cs ($\mu g\,kg^{-1}$)	3	54.3 ± 2.9	5.3		NR
Fe ($\mu g\,g^{-1}$)	3	367 ± 9	2.4	0.3	368 ± 7
K (%)	3	2.93 ± 0.15	5.1	8.5	2.70 ± 0.05
La ($\mu g\,g^{-1}$)	3	2.16 ± 0.02	1.0		NR
Mg ($mg\,g^{-1}$)	4	11.5 ± 1.0	8.7		NR
Mn ($\mu g\,g^{-1}$)	3	236 ± 6	2.5	4.1	246 ± 8
Na ($\mu g\,g^{-1}$)	3	145 ± 14	9.6	6.6	136 ± 4
Rb ($\mu g\,g^{-1}$)	3	14.7 ± 0.4	2.7	1.3	14.89 ± 0.27
Sc ($\mu g\,kg^{-1}$)	3	104 ± 1	1.0		NR
Zn ($\mu g\,g^{-1}$)	3	30.3 ± 0.5	1.7	1.9	30.9 ± 0.7

[a]number of determination.
[b]mean value.
[c]standard deviation.
[d]relative standard deviation.
[e]relative error.
[f]not reported.

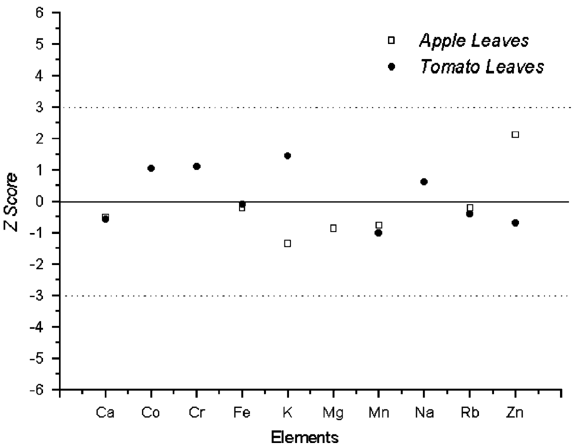

Fig. 1. *Z* score values obtained for the elements determined in reference materials.

concentrations were significantly higher in the species *C. decandra* and *C. sylvestris* than those found in *C. obliqua*.

Br, Co, Cr, Cs, Fe, La, Mn, Rb, Sc and Zn obtained at trace levels in the samples may participate in several physiological processes. Br is considered a non-essential element for living organisms (Berdanier, 1994) and the concentrations of this element in the three species were of the same magnitude, varying from 3.6 to 5.0 $\mu g\,kg^{-1}$.

Table 3
Mean values of element concentrations determined in three different *Casearia* species

Elements	Plant species		
	C. obliqua	*C. decandra*	*C. sylvestris*
Br ($\mu g\,g^{-1}$)	5.04 ± 0.01 (2)[a]	3.773 ± 0.008 (2)	3.60 ± 0.01 (2)
Ca (%)	0.22 ± 0.01 (2)	0.56 ± 0.01 (3)	0.48 ± 0.02 (2)
Cl ($\mu g\,g^{-1}$)	425 ± 22 (3)	2067 ± 76 (3)	1450 ± 43 (2)
Co ($\mu g\,kg^{-1}$)	66 ± 2 (3)	187 ± 4 (3)	65 ± 2 (3)
Cr ($\mu g\,kg^{-1}$)	43 ± 10 (2)	64 ± 1 (3)	114 ± 1 (3)
Cs ($\mu g\,kg^{-1}$)	142 ± 4 (3)	107 ± 4 (2)	83 ± 3 (3)
Fe ($\mu g\,g^{-1}$)	58.0 ± 1.7 (3)	72.7 ± 1.3 (3)	88.8 ± 1.5 (3)
K (%)	1.061 ± 0.003 (3)	1.466 ± 0.003 (3)	1.104 ± 0.004 (3)
La ($\mu g\,kg^{-1}$)	36.6 ± 1.6 (3)	80.5 ± 1.7 (3)	59.4 ± 1.3 (3)
Mg (%)	0.23 ± 0.02 (3)	0.23 ± 0.02 (2)	0.17 ± 0.03 (2)
Mn ($\mu g\,g^{-1}$)	193 ± 4 (2)	508 ± 15 (3)	357 ± 9 (2)
Na ($\mu g\,g^{-1}$)	12.6 ± 0.1 (3)	183.0 ± 0.3 (3)	32.9 ± 3.0 (3)
Rb ($\mu g\,g^{-1}$)	149 ± 2 (3)	98.4 ± 0.8 (3)	79.1 ± 0.8 (3)
Sc ($\mu g\,kg^{-1}$)	5.1 ± 0.1 (3)	5.79 ± 0.12 (3)	10.2 ± 0.1 (3)
Zn ($\mu g\,g^{-1}$)	29.9 ± 0.2 (3)	28.8 ± 0.2 (3)	17.4 ± 0.2 (3)

[a]Mean values and standard deviation. Values in parenthesis indicate number of determinations.

Leaves of *C. obliqua* presented a slightly high concentration of Br. Co is an important component of vitamin B_{12} which participates as a coenzyme in important enzymatic reactions (Berdanier, 1994). The highest concentration of this element was obtained for *C. decandra* species. Cr is thought to regulate carbohydrate, nucleic acid and lipoprotein metabolism and it also potentiates insulin action (Kaplan et al., 2003). The highest concentration of this element was found in *C. sylvestris*. Fe concentrations found in these three species ranged from 58.0 to 88.8 $\mu g\, g^{-1}$. This element is an important hemoglobin component responsible for oxygen transport in human body (Martin et al., 1985). The highest concentration of Mn was found in *C. decandra* species and this element is thought to aid in the maintenance of epithelial tissue (Martin et al., 1985). Rb is also considered a non-essential element for human organism (Underwood, 1971). Its highest concentration was found in *C. sylvestris* species. Concentrations of Sc varied from 5.1 to 10.2 $\mu g\, kg^{-1}$. *C. obliqua* and *C. decandra* samples presented quite similar levels of Sc concentrations, while in *C. sylvestris* this element concentration was slightly higher. Zn is the component of more than 270 enzymes (Zinpro Corporation, 2000) and its deficiency in the organism is accompanied by multisystem dysfunction. Besides, Zn is responsible for stimulating growth of epidermal and epithelial cells (Kaplan et al., 2003), sperm manufacture, fetus development and proper function of immune response (Serfor-Armah et al., 2001). Zn concentrations found in the plants varied from 17.4 to 29.9 $\mu g\, g^{-1}$.

Toxic elements such as Cd, Hg and Sb were not detected in the samples. As and Cd was detected in some species but at very low concentrations. The detection limit values determined according to Currie criterion (Currie, 1968) for the elements As, Cd and Sb were (in $\mu g\, kg^{-1}$) 10.4, 185.9 and 5.0, respectively. From the pharmacological and toxicological points of view the concentrations of these elements present in the samples analyzed are very low to cause any kind of effect.

4. Conclusions

In this study, concentrations of 15 elements were determined in leafy samples of three *Casearia* genus plant species using INAA. The results obtained showed differences among the elemental concentrations determined for three species of *Casearia* plant. This fact suggests the analysis of extracts of these plants to verify if there is a correlation between their elemental concentrations and pharmacological effects.

Determinations of the element composition of the soil in which the plants were grown are in development in our laboratory in order to evaluate its influence in the element concentration present in the leaves.

Results obtained in the analysis of certified reference materials indicated good accuracy and precision of the method applied in this work.

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