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Elemental analysis of leaves and extracts of *Casearia* medicinal plants by instrumental neutron activation analysis

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Instrumental neutron activation analysis (INAA) was applied to determine the inorganic composition of leaves and extracts from three different species of *Casearia* genus plant (*C. sylvestris, C. decandra* and *C. obliqua*). Statistical analysis of the analytical data of leaf showed that the three *Casearia* species present similar elemental composition. Extract from *C. sylvestris* showed significantly different inorganic content in comparison with the two other species. Certified reference material NIST SRM-1515 Apple Leaves was analyzed for quality control.

Introduction

Macro, micro and trace elements are known to have important biological functions in plants and in human metabolic reactions. In medicinal plants, these elements make up active compounds or participate in reactions which lead to the formation of these compounds.[1] In the human body, the elements play vital role in many physiological reactions and their deficiency or excess can affect human health.[2] Particularly in the past twenty years, the popularity and consumption of medicinal plants leaves and of their preparations have increased worldwide, thus, scientific confirmation about quality, efficacy and safety of these plants is required.

Brazil has a great potential for development of drugs derived from medicinal plants due to the variety of plant species found in its forests. The Brazilian Atlantic Forest is represented by 20,000 vascular plant species, but not even 1% of these plants have been investigated for their pharmacological properties.[3]

In this study, elemental concentrations in three species of the medicinal plants *Casearia* genus (*Casearia sylvestris*, *Casearia decandra* and *Casearia obliqua*) were determined. *Casearia* plants are commonly found in American tropical countries and their leaves and extracts are popularly used as cicatrizing, antiseptic, and topical anesthetic agent. Besides the traditional uses, *C. sylvestris* can neutralize snake and bee venoms[4,5] and its antiulcer[6] and antitumor activities[7–9] have already been scientifically confirmed. A recent study with plant species belonging to four different genus of *Flacourtiaceae* family showed that *Casearia* genus is the most promising one in terms of antioxidant, cytotoxic and antimicrobial activities.[10] This fact suggests that further researches on the chemical composition of *Casearia* plants, as well as its relation with the biological properties presented by the plant, are needed.

Instrumental neutron activation analysis (INAA), a sensitive, fast, nondestructive and multielemental technique, was applied in this study. INAA has been frequently used to evaluate inorganic contents of medicinal plants,[1,11–15] though other analytical techniques such as atomic absorption spectrophotometry,[16] atomic emission spectrometry,[17] X-ray emission,[18] X-ray fluorescence,[19] and mass spectrometry,[20] are also being routinely employed for such studies.

For quality control of the results obtained in this study, the certified reference material SRM-1515 Apple Leaves[21] provided by the National Institute of Standards and Technology (NIST) was analyzed under the same experimental conditions. Hierarchical cluster analysis (HCA), a multivariate statistical method, was used to examine the similarities of the analyzed plant samples. The proximity of the groups in the obtained dendrogram reflects the similarity of their characteristics.[22] In this study, HCA was performed using the Ward's minimum variance method as linkage type and squared Euclidean distance for measuring proximity of the samples.

Experimental

C. sylvestris, *C. decandra* and *C. obliqua* leaves collected from Reserva do Morro Grande located in the Atlantic Forest, São Paulo, Brazil, were provided by the Instituto de Ciências Biomédicas (ICB) of the São Paulo University. The plant species were identified by the Departamento de Botânica of ICB. The leaves were washed using Milli-Q water and freeze-dried for 12 hours under a pressure of about $5 \cdot 10^{-2}$ mbar using a Micro Modulyo lyophilizer. The dried leaves were ground using Fritsch micro vibrator pulverisette to obtain a fine powder. For the preparation of the ethanolic extracts, the plants leaves were broken into

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small pieces, subjected to maceration and percolation processes for approximately 5 days and extracted using 75% ethanol at 50 °C. The obtained viscous liquid was then freeze-dried.[23]

Synthetic standards irradiated along with the samples 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.

About 150 mg of each leaf or extract samples were weighed in previously cleaned polyethylene bags. These samples were irradiated at the nuclear research reactor IEA-R1 of Instituto de Pesquisas Energéticas e Nucleares-CNEN/SP, under two irradiations schemes, short and long irradiations. Short irradiations (5 minutes) were carried out at the pneumatic facility with a thermal neutron flux of approximately 10^{11} n·cm^{-2}·s^{-1}. Long irradiations (16 hours) were carried out under a thermal neutron flux of $5 \cdot 10^{12}$ n·cm^{-2}·s^{-1}. The gamma-activities of the samples and standards were measured using a hyperpure Ge detector Model GX2020 coupled to Canberra Integrated Signal Processor and System 100 MCA Card. The system had a resolution of 1.0 keV for 121 keV ^{57}Co gamma-ray energy and of 1.80 keV for 1332 keV ^{60}Co gamma-ray energy. In the short irradiation scheme, samples and synthetic standards were measured for 300 or 600 seconds depending on their activities. The concentrations of Cl, K, Mg, Mn and Na could be determined. In the long irradiation scheme, samples and synthetic standards were measured after 4, 11 and 20 days of cooling. Synthetic standards were measured for 5,400 seconds and the samples for 20,000 to 50,000 seconds. Br, Ca, Co, Cr, Cs, Fe, La, Na, Rb, Sc and Zn elements were determined in the long irradiation scheme.

Canberra S100 and VERSAO2 softwares were used to obtain the gamma spectra and for their processing, respectively. The radioisotopes were identified by their half-lives and gamma-ray energies. The radioisotopes measured in this study were: ^{76}As, ^{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, ^{122}Sb, ^{46}Sc, ^{75}Se and ^{65}Zn. Comparative method was used for calculating the elemental concentrations.

Results and discussion

In order to evaluate the precision and accuracy of the results, the certified reference material SRM-1515 Apple Leaves was analyzed. As recommended in its certificate, elemental concentrations in the reference material were calculated on a dry weight basis. A sub-sample of the reference material was separated to determine the moisture content by drying at 85 °C for 24 hours. The weight loss obtained was used to correct the results.

Results obtained in the analyses of certified reference material SRM-1515 Apple Leaves, as well as its certified values are shown in Table 1. For most elements, relative standard deviations (RSD) were lower than 12% confirming the precision of the results obtained by this analytical methodology. For As and Se, the RSD were high due to the low concentrations of these elements in the samples. The accuracy of the results was satisfactory, with relative errors lower than 7.7% for elements presenting certified values. Concentrations of Br, Co, Cr, La, and Sc in Apple Leaves, are not certified in this reference material. The results obtained for these elements in this study may represent a contribution for their certification. The *Z*-score values[24] calculated for the elements determined in the reference material are shown in Fig. 1. For all elements the *Z*-score values were $|Z|<3$, which means that the results obtained are in the 99% confidence interval of the certified values.

The elemental concentrations obtained for the leaves and extracts of the three *Casearia* species are presented in Table 2. As can be seen in this table, Ca, K and Mg are the most abundant elements in both leaves and extracts presenting concentrations at the mg·g^{-1} levels. In the human metabolism, Ca is known to be an important constituent of bones and teeth, to be participated in the biochemical blood clotting process and to be responsible for proper nerve and muscle function. K is responsible for regulating osmotic pressure of body fluids and for maintaining cardiac rhythm. Mg is known to catalyze important reactions related to muscle contraction and its deficiency in human metabolism can cause neuromuscular dysfunctions.[25]

The concentrations for Br, Fe, Mn, Na, Rb and Zn were found at the µg·g^{-1} level, and for Co, Cr, Cs, La, Sc and Se at the µg·kg^{-1} level. These elements are also known to play vital role in human metabolism. The toxic elements As and Sb were found in some samples but at very low concentrations while Cd and Hg were not detected in any of the analyzed samples.

Leaves of *C. sylvestris*, *C. decandra* and *C. obliqua* present concentrations of comparable magnitude for most elements. The exceptions were Cl, Co, Mn and Na since *C. decandra* presented slightly higher concentrations of these elements. In the case of the extracts, *C. sylvestris* shows the highest concentrations for elements such as Ca, Cl, Co, Cr, La, Mg, Na, Sc, Se and Zn.

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

Element	This work				Certified value
	n[a]	X[b] ± SD[c]	RSD,[d] %	Er,[e] %	(NIST, 1995)[f]
As, $\mu g \cdot g^{-1}$	4	0.036 ± 0.006	16.7	5.2	0.038 ± 0.007
Br, $\mu g \cdot g^{-1}$	4	2.13 ± 0.01	0.5		(1.8)
Ca, $\mu g \cdot g^{-11}$	4	15.5 ± 0.4	2.6	1.6	15.3 ± 0.2
Co, $\mu g \cdot g^{-1}$	5	0.107 ± 0.003	2.8		(0.09)
Cr, $\mu g \cdot g^{-1}$	5	0.85 ± 0.02	2.4		(0.3)
Fe, $\mu g \cdot g^{-1}$	4	81.7 ± 1.7	2.1	1.2	83 ± 5
K, $mg \cdot g^{-1}$	4	16.2 ± 0.1	0.6	0.6	16.1 ± 0.2
La, $\mu g \cdot g^{-1}$	5	20.19 ± 0.07	0.3		(20)
Mg, $mg \cdot g^{-1}$	3	2.5 ± 0.3	12	7.7	2.71 ± 0.08
Mn, $\mu g \cdot g^{-1}$	3	50.2 ± 1.0	2.0	7.4	54 ± 3
Rb, $\mu g \cdot g^{-1}$	4	9.8 ± 0.2	2.0	3.9	10.2 ± 1.5
Sc, $\mu g \cdot g^{-1}$	5	0.031 ± 0.003	1.0		(0.03)
Se, $\mu g \cdot g^{-1}$	3	0.05 ± 0.01	20	0.0	0.050 ± 0.009
Zn, $\mu g \cdot g^{-1}$	3	12.7 ± 0.2	1.6	1.6	12.5 ± 0.3

[a] Number of determinations.
[b] Mean value.
[c] Standard deviation.
[d] Relative standard deviation.
[e] Relative error.
[f] Informative values are in parentheses.

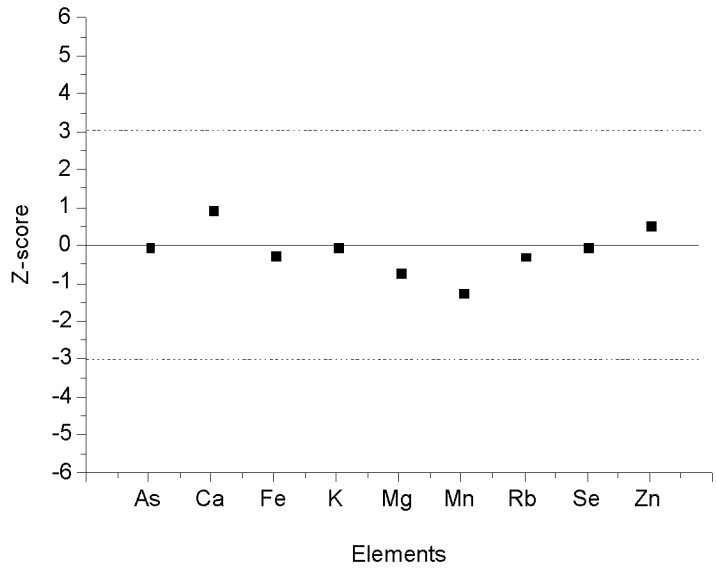

Fig. 1. Z-score values obtained for the elements determined in reference material SRM-1515 Apple Leaves

Considering that 1 mg of the lyophilized extract was obtained from 13.91 mg of dry leaves, it was possible to evaluate to what percentage of elements are transferred from the leaves to the extract. Table 3 shows that the elements have different extractabilities to the ethanolic solution and that Br, Cl and Ca were the elements with the highest extraction efficiencies. Due to the high RSD, results obtained for Cr and Sb were not considered in this table.

The dendrogram presented in Fig. 2 was obtained from hierarchical cluster analysis (HCA) applied to the results obtained for leaf and extract samples. Leaves and extracts are each clustered together in two well distinguishable groups (A and B). The results obtained for the three *Casearia* species leaves showed similarity. In the case of extract results, *C. sylvestris* extract formed a separate group from the other two *Casearia* species extracts.

Table 2. Elemental concentrations of leaves and extracts from different species of ***Casearia*** plant

Element	*C. sylvestris*						*C. decandra*						*C. obliqua*					
	Leaf			Extract			Leaf			Extract			Leaf			Extract		
	n[a]	X[b] ± SD[c]	RSD,[d] %	*n*	X ± SD	RSD, %	*n*	X ± SD	RSD, %	*n*	X ± SD	RSD, %	*n*	X ± SD	RSD, %	*n*	X ± SD	RSD, %
As, $\mu g \cdot kg^{-1}$	1	10.1 ± 1.8[e]		1	35 ± 7			ND[f]			ND			ND		1	14 ± 3	
Br, $\mu g \cdot kg^{-1}$	2	3.60 ± 0.01	0.3	2	15.99 ± 0.05	0.3	2	3.77 ± 0.01	0.3	2	24.34 ± 0.07	0.3	3	4.86 ± 0.02	0.4	2	8.90 ± 0.03	0.3
Ca, $mg \cdot g^{-1}$	2	4.8 ± 0.1	2.1	1	12.8 ± 1.7		3	5.6 ± 0.1	1.8	1	3.22 ± 0.04		4	2.12 ± 0.07	3.3	1	5.1 ± 1.3	
Cl, $mg \cdot g^{-1}$	3	1.48 ± 0.06	4.1	3	7.1 ± 0.2	2.8	3	1.88 ± 0.08	4.3	3	3.15 ± 0.08	1.9	3	0.44 ± 0.02	4.6	3	5.5 ± 0.1	1.8
Co, $\mu g \cdot kg^{-1}$	3	65.4 ± 1.4	2.1	2	74.6 ± 1.9	2.6	3	187 ± 4	2.1	2	35.6 ± 1.4	3.9	4	77 ± 2	3.1	2	58.0 ± 1.7	2.9
Cr, $\mu g \cdot kg^{-1}$	3	114 ± 12	10	2	1150 ± 20	1.7	3	58 ± 11	19	1	1000 ± 20		2	58 ± 12	20	2	880 ± 20	2.3
Cs, $\mu g \cdot kg^{-1}$	3	80 ± 3	3.6	2	59 ± 3	5.3	3	113 ± 2	1.8	2	121 ± 3	2.5	4	148 ± 4	2.7	2	56.4 ± 2.5	4.4
Fe, $\mu g \cdot kg^{-1}$	3	86.4 ± 1.5	1.7	2	16.8 ± 0.7	4.2	3	72.7 ± 1.3	1.8	2	9.8 ± 0.6	6.1	4	56.3 ± 1.1	2.0	2	18.9 ± 1.2	6.4
K, $mg \cdot g^{-1}$	3	14.9 ± 0.7	4.7	4	11.9 ± 0.6	5.0	4	16.5 ± 0.5	3.0	4	11.5 ± 0.5	4.3	5	14.5 ± 0.6	4.1	4	11.2 ± 0.6	5.3
La, $\mu g \cdot kg^{-1}$	3	59.4 ± 1.3	2.2	2	4.9 ± 1.8	37	4	80.0 ± 1.7	2.1	2	2.6 ± 0.8	31	4	36.8 ± 1.5	4.1	1	1.4 ± 1.1	
Mg, $mg \cdot g^{-1}$	3	2.2 ± 0.3	14	1	1.2 ± 0.4		3	2.3 ± 0.2	8.7	2	0.64 ± 0.09	14	3	2.3 ± 0.1	4.3	1	0.4 ± 0.2	
Mn, $\mu g \cdot kg^{-1}$	3	342 ± 7	2.0	3	16.3 ± 0.4	2.4	3	510 ± 20	3.9	3	26.4 ± 0.5	1.9	2	193 ± 4	2.1	3	10.5 ± 0.3	2.9
Na, $\mu g \cdot kg^{-1}$	4	40 ± 4	9.9	4	647 ± 14	2.1	4	244 ± 16	6.6	4	98 ± 3	3.3	4	14.39 ± 0.01	0.1	4	247 ± 8	3.2
Rb, $\mu g \cdot kg^{-1}$	3	79.0 ± 0.8	1.0	2	59.7 ± 0.5	0.8	3	98.4 ± 0.8	0.8	2	113 ± 2	1.8	3	149 ± 2	1.3	2	52.0 ± 1.5	2.9
Sb, $\mu g \cdot kg^{-1}$	3	16.9 ± 1.5	8.9	2	217 ± 3	1.4	3	9.4 ± 1.5	16	2	198 ± 4	2.0	4	11.3 ± 1.1	9.7	2	237 ± 3	1.3
Sc, $\mu g \cdot kg^{-1}$	3	10.2 ± 0.1	1.0	2	1.5 ± 0.1	6.7	3	5.8 ± 0.1	1.7	2	0.82 ± 0.09	11	4	4.8 ± 0.2	4.2	2	1.0 ± 0.1	10
Se, $\mu g \cdot kg^{-1}$		ND		1	42 ± 8			ND		1	32.2 ± 13			ND			ND	
Zn, $\mu g \cdot kg^{-1}$	3	17.3 ± 0.1	0.6	2	34.8 ± 0.2	0.6	3	28.8 ± 0.2	2.4	2	12.1 ± 0.6	5.0	4	29.2 ± 0.2	2.3	2	27.4 ± 0.2	0.7

[a] Number of determinations.
[b] Mean value.
[c] Standard deviation.
[d] Relative standard deviation.
[e] Result of one determination and the uncertainty estimated by statistical counting errors of the sample and standards.
[f] Not detected.

Table 3. Elemental tranferrence (in percents) from leaves to ethanolic extract

Element	*C. sylvestris*	*C. decandra*	*C. obliqua*
As	24.9	–	–
Br	31.9	46.4	13.2
Ca	19.2	4.1	17.3
Cl	34.5	12.0	89.9
Co	8.2	1.4	5.4
Cs	5.3	7.7	2.7
Fe	1.4	1.0	2.4
K	5.7	5.0	5.6
La	0.6	0.2	0.3
Mg	3.9	2.0	1.3
Mn	0.3	0.4	0.4
Na	116	2.9	123
Rb	5.4	8.3	2.5
Sc	1.1	1.0	1.5
Zn	14.5	3.0	6.7

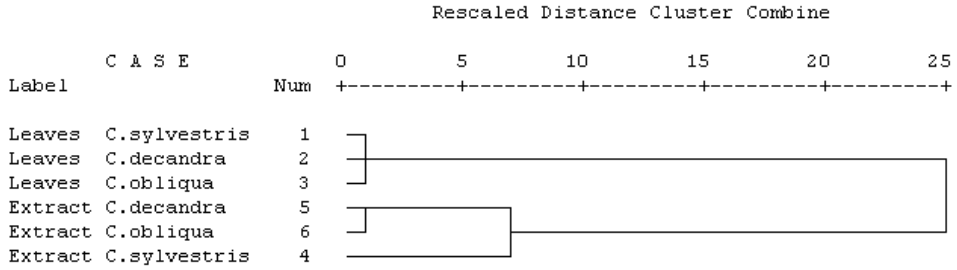

Fig. 2. Hierarchical cluster analysis dendrogram of leaf and extract samples of *Casearia* plants

Conclusions

Instrumental neutron activation analysis (INAA) has proved to be an efficient analytical technique to determine a variety of elements in a wide range of concentrations. In this study 18 elements were determined in leaves and extracts from three different species of *Casearia* medicinal plant. Ca, K and Mg were determined at the $mg \cdot g^{-1}$ levels in both leaf and extract samples. These samples also presented many other elements with important biological function in human metabolism. Statistical analysis of the results showed that the elemental concentration of leaf of the three *Casearia* plant species were similar to each other, while in the case of *C. sylvestris* the extract showed a dissimilarity from the other two species. Analyses of certified reference material SRM-1515 Apple Leaves assured the accuracy and precision of the results obtained.

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