Inorganic profile of some Brazilian medicinal plants obtained from ethanolic extract and " *in natura*" samples

Manuel Octávio Marques Ferreira[1], Paulo Teixeira de Sousa[2],
Vera Lúcia Ribeiro Salvador[1] , Ivone Mulako Sato[1]
[1]Instituto de Pesquisas Energéticas e Nucleares – IPEN–CNEN/SP
Centro de Química e Meio Ambiente
Av. Prof. Lineu Prestes 2.242, 05508-900, São Paulo, SP, Brazil
[2]Universidade Federal de Mato Grosso – UFMT
Departamento de Química
Av Fernando Corrêa da Costa S/N, 78060-900, Cuiabá, MT, Brazil

lawless@usp.br

ABSTRACT

The *Anadenathera macrocarpa, Schinus molle, Hymenaea courbaril, Cariniana legalis, Solidago microglossa and Stryphnodendron barbatiman,* were collected "*in natura"* samples (leaves, flowers, barks and seeds) from different commercial suppliers. The pharmaco-active compounds in ethanolic extracts had been made by the Mato Grosso Federal University (UFMT). The energy-dispersive x-ray fluorescence (ED-XRF) spectrometry was used for the elemental analysis in different parts of the plants and respective ethanolic extracts. The Ca, Cl, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr and Zn concentrations were determined by the fundamental parameters method. Some specimens showed a similar inorganic profile for "*in natura*" and ethanolic extract samples and some ones showed a distinct inorganic profile. For example, the *Anadenathera macrocarpa* showed a similar concentration in Mg, P, Cu, Zn and Rb elements in "*in natura*" and ethanolic extract samples; however very different concentration in Na, S, Cl, K, Ca, Mn, Fe and Sr was observed in distinctive samples. The *Solidago microglossa* showed the K, Ca, Cl, S, Mg, P and Fe elements as major constituents in both samples, suggesting that the extraction process did not affect in a considerable way the "in *natura*" inorganic composition. The elemental composition of the different parts of the plants (leaves, flowers, barks and seeds) has been also determined. For example, the *Schinus molle* specimen showed P, K, Cl and Ca elements as major constituents in the seeds, Mg, K and Sr in the barks and Mg, S, Cl and Mn in the leaves, demonstrating a differentiated elementary distribution. These inorganic profiles will contribute to evaluate the quality control of the Brazilian herbaceous trade and also will assist to identify which parts of the medicinal plants has greater therapeutic effect.

KEYWORDS

X-ray fluorescence analysis, medicinal plants, inorganic profile

INTRODUCTION

Medicinal plants are prescribed by traditional healers for diseases like as common cold, malaria, ulcers and infection across the world since ancient time.[1] In the recent years, the use of the phytoterapics has been focused as safer and more congenial to the human body. Medicinal plants come into preparation of various modern drugs or even have been used as the principal source of raw materials for conventional drugs.[2] In the development countries, these plants are easily found in local market. Non-prescription phytoterapics drugs are in almost their totality. They are available in the mixtures of medicinal plants, extracts and capsules forms; therefore are easy to use and the industry promises more quick effects.[3] There is a great interesting in trace and essential elements composition in the medicinal science; it is believed that the great majority of elements act as key components of essential enzyme systems or vital biochemical functions.

In this work the energy-dispersive X-ray fluorescence (ED-XRF) technique was used to multi-elemental determination of Ca, Cl, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr and Zn elements in the ethanolic extracts and parts of medicinal plants (leaves, roots, flowers, leaves). The quantitative inorganic profile these parts of plants showed a different distribution for each part. This information could be used to identify which part of plant is more effective for therapeutic use.

EXPERIMENTAL

Sample preparation

The specimens their scientific and popular name, distinctive parts studied and the principal therapeutic use are listed in Table 1. The ethanolic extracts had been made by the Mato Grosso Federal University (UFMT). The medicinal plants were collected from different commercial suppliers. The "*in natura*" samples were separated into leaves, fruits, seeds, flowers and barks parts.

Methodology

The elemental analysis was performed by an energy dispersive X-ray fluorescence spectrometer (ED-XRF) from Shimadzu Co., model EDX-900, with a silicon drift chamber and semiconductor detector. The instrumental measurement parameters like voltage, current, filter and counting fixed time were established for each characteristic $K\alpha$ X-ray emission line.

The Ca, Cl, Cu, Fe, K, Mg, Mn, Na, Ni, P, Rb, S, Sr and Zn concentrations were determined by the fundamental parameters (FP) method. This method is considered to be all-round analytical procedure. However, corrections for the absorption and inter-elemental excitation of coexisting elements have to be made.[4] Instrumental sensitivity curve was made with certified material NIES N° 9 (Sargasso), NIES N° 10a (Rice Flour) and NIES N° 3 (Chlorella). The accuracy of the method (precision, exactness and repeatability) was evaluated by certified material NIST N° 1547 (Peach Leaves). A total of 42 fluorescent intensity measurements for each characteristic line emission were made.

RESULTS AND DISCUSSION

The NIST 1547-Peach Leaves reference material data were treated previously by Chauvenet and Cochrane statistical tests to identify “outliers” and ANOVA test to verify homogeneity variance.[5] The method validation was performed using Z-Score test.[6] A comparison of determined and certified values is listed in Table 2. The analytical methodology could be approved by following Z-score values: $-1<z<1$value for Sr, Rb, Fe, Mg, Mn, P and Cu and $-2<z<2$ value for Ca and Cl determination (Figure 1).

The plants data were treated previously using Chauvenet test to eliminate “outliers”. The average and standard deviation of major and trace elements are presented in Table 3 and Table 4.

The Ca, Cl, Cu, Fe, K, Mg, Mn, P, Rb, S, Sr and Zn were found in all herbaceous but in different concentrations. All the plants showed Mg, K and Ca as major elements (Mg: 860±294 to 3320±888; K: 2368±30 to 26011±924; Ca: 1670±44 to 6967±41 μg G^{-1}), except for specimen *Solidago microglossa* flower, that showed a concentration in (Ca: 701±134μg G^{-1}). Different distribution of the Mg, P, S, Cl, K and Ca were observed in distinctive part of the *Schinus molle* and *Solidago microglossa* specimens. For example, for first one, the K content ranged 6105± 28, 3885 ±112 and 12151± 35 μg G^{-1}, respectively for leaves, bark and seed parts. And for second one, the K content ranged 26011±230, 5302±1142 and 3559±51 μg G^{-1} for same parts of plants.

The Mn, Fe, Ni, Cu, Zn, Rb and Sr were presented in all plants at trace level (less than 300 μg G^{-1}). Exception was observed for Fe and Mn content in the *Carinlana leralis* (respectively: 1991±140 and 700±55μg G^{-1}). Ni was detected only in the *Solidago microglossa* (bark: 12.2±3.5 μg G^{-1}), the *Stryphnodendron barbatiman* (bark: 10.7±0.4 μg G^{-1}) and the *Cariniana legalis* (bark: 90±1 μg G^{-1}). Na was detected only in the *Anadenathera macrocarpa* (bark: 2638±810 μg G^{-1}) and in the *Solidago microglossa* (flower: 1223±75 μg G^{-1}). All the ethanolic extracts showed Na content in around 3000 μg G^{-1}, demonstrating that Na is proceeding from extracting method. Also, it could be observed that extracting method affect Ca content for the all specimens, verifying significant decrease in its content.

The *Schinus molle* specime has anti- inflammatory, antiseptic and diuretic therapeutic indication and its seed is widely used as seasoning. The specimen showed high concentration in Mg, Cl and K demonstrating a relation between elements content and symptoms. Moreover, the K content is high in seeds in relation to other parts of the plant. The *Solidago microglossa* is used for pain relief and as anti-inflammatory and antiseptic. The specimen showed high concentration in Cl, S, Mg, P and K elements. Also, those elements were presented in high level in leaves compared to bark and flower parts.

CONCLUSION

The established analytical method using ED-XRF technique could be used for inorganic profiles determination of medicinal plants. The high values in standard deviation in some elements could be attributed to samples heterogeneity.

The inorganic profile could contribute for the elaboration of a quality and security guide to assure the Brazilian phytotherapics trade.

REFERENCES

[1] K. Chen, C. Tseng, T. Lin, J. Radional. Nucl. Chem., 170 (1993) 265
[2] G. D. Kanias. E. Tsita, A. Loukis, V. Kilikoglou, J. Radianal. Nucl. Chem., 169 (1993) 97
[3] E. I. Obiajunwa, A. C, Adebajo, O. R. Omobuwajo, J, Radioanal. Nucl. Chem., 252 (2003) 473
[4] O. Juichi, H. Khono, H. Toda, Analytical Chimica Acta 307 (1995) 117
[5] Chatfield, C. Statistics for Technology. Chapman and Hall, London, (1979)
[6] Bode, P. Instrumental and organizational aspects of a neutron activation analysis laboratory. Delft, University of Technology, The Netherlands. (1996)

LIST TABLE AND FIGURE CAPTIONS

Table 1 – Scientific and common names of the medicinal plants. Therapeutic use and their distinctive parts analyzed.

Table 2 - Comparison of determined and certified values from
NIST 1547 Peach Leaves certified material

Table 3 – Concentrations of inorganic elements in medicinal plants (parts and ethanolic extracts) analyzed by EDXRF technique (I, II, III)

Table 4 – Concentrations of inorganic elements in medicinal plants (parts and ethanolic extracts) analyzed by EDXRF technique (IV, V, VI)

Figure 1 – Z-Score values determined to K, Sr, Ca, Rb, Fe, Mg, Mn, P, Cu, Cl and Zn elements using certified material NIST 1547 Peach Leaves.

Table 1 – Scientific and common names of the medicinal plants. Therapeutic use and their distinctive parts analyzed.

Scientific name	Popular name	Therapeutic use	Leave	Bark	Flower	Seed	Ethanolic extract
Anadenathera macrocarpa	Angico (Angico)	Cough, bronchitis, expectorant		X			X
Schinus molle	Brazillian peppertree (Aroeira)	Anti-inflammatory, antiseptic, diuretic	X	X		X	X
Hymenaea courbaril	Brazilian copal (Jatobá)	Anemia, fungicide, vermifuge		X			X
Solidago microglossa	Arnica (Arnica)	Pain relief, anti-inflammatory, brain health	X	X	X		X
Stryphnodendron barbatiman	Amazon sword (Barbatimão)	Hemorrhage, ulcer, wounds		X			X
Carinlana leralis	Jequitibá (Jequitibá)	Gargle, tonsillitis, laryngitis		X			X

Table 2 – Comparison of determined and certified values NIST 1547 Peach Leaves certified material

Element µg G^{-1}	$x_{cert} \pm \sigma_{cert}$	$\overline{x}_{\det} \pm \sigma_{\det}$
Ca	15600±200	13974±1432
Cl	360±9	444±40
Cu	3,7±0,4	5,4±2,1
S	(2000)	1500±98
Sr	53±4	39±4
Fe	218±14	215±9
P	1370±70	1366±166
Mg	4320±80	4122±871
Mn	98±3	101±8
Ni	0,69±0,09	<1
K	24300±300	20972±671
Rb	19,7±1,2	18,7±1,6
Zn	17,9±0,4	25,5±1,2

$\overline{x}_{\det} \pm \sigma_{\det}$: average and standard deviation determinated

$x_{cert} \pm \sigma_{cert}$: cetified values

Table 3 – Concentrations of inorganic elements in medicinal plants (parts and ethanolic extracts) analyzed by EDXRF technique (I, II, III)

Element	Plant codes							
µg G^{-1}	I(N_b)	I(E_x)	II(N_l)	II(N_b)	II(N_s)	II(E_x)	III(N_b)	III(E_x)
Na	2638±810	4029±1700	<50	<50	<50	2296±757	<50	3244±1042
Mg	860±294	890±60	3200±500	1776±172	1335±217	2062±250	1160±536	533±156
P	552±115	692±39	1000±50	417±51	1803±44	172±28	466±125	90±12
S	693±87	183±17	1954±38	533±85	989±12	155±28	500±66	117±17
Cl	300±91	689±52	1982±42	398±66	1241±17	801±36	297±98	268±35
K	2442±238	1163±28	6105±28	3885±112	12151±35	2827±12	5022±235	2237±41
Ca	4488±750	29,4±4,5	2837±43	5851±49	1729±19	291±15	3779±41	75,4±7,3
Mn	37,3±7,5	7,7±1,1	28,5±3,8	35,9±5,6	24,3±2,5	3,9±1,3	121±15	17,3±2,3
Fe	55,9±4,5	4,9±0,5	21,0±2,2	65,7±5,1	81,9±3,4	3,3±1,7	49,2±6,5	8,7±0,6
Ni	<1	<1	<1	<1	<1	<1	<1	<1
Cu	1,7±0,8	1,9±0,7	1,6±0,6	2,6±0,8	2,1±1,2	2,2±0,8	2,3±0,4	1,9±0,8
Zn	8,7±2,5	8,3±0,7	11,8±1,2	7,1±1,8	18,2±2,0	6,8±1,1	23,1±2,2	6,6±1,6
Rb	3,9±1,4	2,5±0,4	4,4±0,9	3,2±1,1	12,4±0,6	4,5±0,6	2,1±0,5	3,4±0,7
Sr	77,8±2,5	3,0±1,0	6,7±0,3	220±71	17,8±1,1	3,9±1,2	12,7±7,2	3,2±0,9

Plant codes: **IN_b** *Anadenathera macrocarpa* (bark); **IE_x** *Anadenathera macrocarpa* (ethanolic extract); **IIN_l** *Schinus molle* (leaves); **IIN_b** *Schinus molle* (bark); **IIN_s** *Schinus molle* (seed); **IIE_x** *Schinus molle* (ethanolic extract); **IIIN_b** *Hymenaea courbaril* (bark); **IIIE_x** *Hymenaea courbaril* (ethanolic extract)

Table 4 – Concentrations of inorganic elements in medicinal plants (parts and ethanolic extracts) analyzed by EDXRF technique (IV, V, VI)

Element	Plant codes							
µg G^{-1}	IV(N_l)	IV(N_b)	IV(N_f)	IV(E_x)	V(N_b)	V(E_x)	VI(N_b)	VI(E_x)
Na	<50	<50	1223±75	2790±1132	<50	3210±70	<50	3094±31
Mg	3332±888	2540±337	593±81	1370±132	1187±138	644±55	2612±382	613±36
P	2138±117	741±222	553±56	473±56	243±50	141±14	516±35	587±15
S	1399±45	390±22	357±16	386±10	444±49	136±16	927±21	230±8
Cl	4019±94	2453±38	907±25	641±49	1555±57	1877±3	787±15	1358±4
K	26011±924	5302±1142	3559±51	6377±107	2368±30	2424±2	5816±16	933±2
Ca	6498±230	4200±520	701±13	136±11	1670±44	41,7±7,5	6967±41	26,4±3,2
Mn	287±9	176±29	7,6±0,4	58,3±3,7	54,7±2,5	7,8±2,8	700±55	6,6±1,8
Fe	237±7	601±10	28,9±1,6	180±6	527±10	3,1±0,9	1991±140	4,9±0,3
Ni	<1	12,2±3,5	<1	1,3±0,2	10,7±0,4	<1	90,0±1,2	<1
Cu	4,7±1,6	2,2±0,5	1,2±0,3	2,4±1,2	2,6±1,4	3,3±1,6	2,3±0,7	2,4±0,9
Zn	72,1±3,4	8,8±1,7	3,8±0,3	17,9±0,9	5,5±1,6	6,8±1,2	10,9±2,8	9,7±1,0
Rb	8,3±1,3	6,9±1,1	1,1±0,1	3,7±0,7	5,8±1,0	3,3±0,7	4,5±1,4	2,3±0,5
Sr	17,7±1,6	17,6±3,5	<1	3,3±1,1	5,4±0,5	2,9±1,1	23,5±4,1	2,2±0,7

Plant codes: **IVN_l** *Solidago microglossa* (leaves); **IVN_b** *Solidago microglossa* (bark); **IVN_f** *Solidago microglossa* (flower); **IVE_x** *Solidago microglossa* (ethanolic extract); **VN_b** *Stryphnodendron barbatiman* (bark); **VE_x** *Stryphnodendron barbatiman* (ethanolic extract); **VIN_b** *Carinlana leralis* (bark); **VIE_x** *Carinlana leralis* (ethanolic extract)

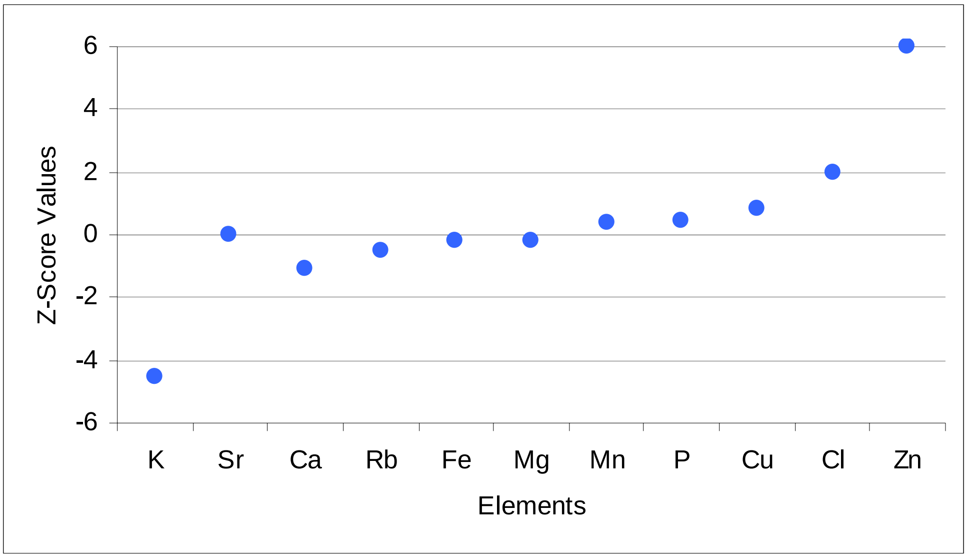

Figure 1 – Z-Score values to K, Sr, Ca, Rb, Fe, Mg, Mn, P, Cu, Cl and Zn determination using certified material NIST 1547 Peach Leaves.