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CHEMOMETRIC EVALUATION OF TRACE ELEMENTS IN BRAZILIAN MEDICINAL PLANTS

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ABSTRACT

The growing interest in herbal medicines has required standardization in order to ensure their safe use, therapeutic efficacy and quality of the products. Despite the vast flora and the extensive use of medicinal plants by the Brazilian population, scientific studies on the subject are still insufficiency. In this study, 59 medicinal plans were analyzed for the determination of As, Ba, Br, Ca, Cl, Cs, Co, Cr, Fe, Hf, K, Mg, Mn, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr by neutron activation analysis and Cu, Ni, Pb, Cd and Hg by atomic absorption. The results were analyzed by chemometric methods: correlation analysis, principal component analysis and cluster analysis, in order to verify whether or not there is similarity with respect to their mineral and trace metal contents. Results obtained permitted to classify distinct groups among the analyzed plants and extracts so that these data can be useful in future studies, concerning the therapeutic action the elements here determined may exert.

1. INTRODUCTION

The demand for herbal medicines is growing worldwide. The expansion of interest has required the standardization of the sector with implementation and constant review of technical standards for production and marketing of these medicines, ensuring the safe use, therapeutic efficacy and quality of the products [1,2]. According to data from the World Health Organization, approximately 80% of world population has resorted to the benefits of certain herbs with therapeutic action popularly recognized [3]. Also, the determination of major, minor and trace elements and the research of metabolic processes, with their impacts on human health, are of great importance due to the growth of environmental pollution, affecting directly the plants and, therefore, the phytoterapics [4].

The presence of elements in plants in trace, micro or macro amounts, while playing an essential role may also pose a risk to human health, especially for the fact that herbal drugs are consumed indiscriminately, without a quantity or a quality control, age or gender of the user. Studies have shown that the absorption of metals, either toxic or not, may vary widely depending on age (children and elderly are more susceptible to the accumulation of Cd and Pb), and sex (premenopausal women accumulates Cd more than men). Pregnant women are generally considered the highest-risk group for exposure of metals, since this fact results in exposure of the fetus.

Despite the vast flora and the extensive use of medicinal plants by the population, it is a consensus that scientific studies on the subject are insufficient. Therefore, it is necessary to stimulate such studies in view of the importance of the results of both, individual and social fields.
Chemometric methods, which involve multivariate statistics, mathematical modeling and computing have been a useful tool in evaluating the quality and the identity patterns in medicinal plant element concentrations [5,6]; Pearson coefficient correlation, principal component analysis (PCA) and hierarchical cluster analysis (HCA) are among the procedures that provide the most interesting and promising approach to these goals.

Pearson Coefficient Correlation is a statistical measure of the relationship between two variables, in which the correlation coefficient (r) is used to measure the strength of the association. A correlation coefficient near the unity indicates that the variables are correlated. When the correlation coefficient is equal to +1.0 or −1.0, it indicates a perfect correlation, positive or negatively, respectively. Typically, correlation coefficients from ±0.8 to ±1.0 indicate a strong correlation, while values from ±0.5 to ±0.8 indicate a moderate correlation and values less than ±0.5 indicate a weak correlation [7]; nevertheless, most of the statistics packages establish a significant correlation based on the measurement numbers for a given significance p-level. Pearson Coefficient Correlation allows the pairwise comparison of samples and is, therefore, independent of the total number of samples [8].

The Principal component analysis is a multivariate technique that quantifies the significant variation in a data set and aims at reducing the number of variables to a small number of indices, retaining a maximum amount of the variance in the data set [9,10] and resulting in the retention of only the important characteristics of the original data, from which natural clusters of similar samples are identified. Analysis using the PCA requires the data set to be mean-centered to provide normalization over the entire data set, adjusting the magnitude of the data. An eigenvector (PC1), which accounts for the maximum amount of variance in the data, is established. Next, an orthogonal vector to the first vector is chosen, (PC2), which describes the maximum amount of variance remaining in the data. The total number of eigenvectors, or principal components, is based on the number of degrees of freedom in the data. However, only the principal components that describe 80–90% of the total variance are typically selected to describe the data [8].

Hierarchical cluster analysis is a group of multivariate techniques whose primary purpose is to assemble objects based on the characteristics of their similarities, by sorting cases into groups, or clusters, what results in a strong association between members of the same cluster and a weak association between members of different clusters. The object clusters found should, then, exhibit high internal similarity and high external dissimilarity [9]. The Euclidean distance is the geometric distance in a multidimensional space that usually gives the best similarity between two samples [11]. The amalgamation method generally used is the Ward's method that attempts to minimize the sum of squares of any two (hypothetical) clusters that can be formed at each step [12].

The objective of this study was to apply chemometric approaches to the element concentration data, obtained by instrumental neutron activation analysis (INAA), inductively coupled plasma - atomic emission spectrometry (ICP-OES) and cold vapor atomic absorption spectrometry (CV AAS) in Brazilian medicinal plant and their extracts, commonly used as medicines by Brazilian folks, in order to verify the elementary pattern distribution, both in plants and extracts, as well as their correlations. The elements determined were As, Ba, Br, Ca, Cl, Cs, Co, Cr, Fe, Hf, K, Mg , Mn, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr (INAA) Cu, Ni, Pb, Cd (ICP-OES) and Hg (CS AAS).

2. MATEIRALS AND METHODS

The plants analyzed in this study were selected from the list of herbs regulated for production and marketing by ANVISA – AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA, in 2010 (DOU, 10/03/2010). The samples were obtained in specialized pharmacies and drugstores. From the 66 herbs recommended, 59 were obtained. In Table 1, the plants selected for this study, their used parts and applications are shown.

For the analyses, the plant samples were dried at approximately 45º C till constant weight and, then, ground with the help of a porcelain mortar and a pestle to a 180-mesh particle size. The aqueous extracts were obtained as established in ANVISA resolution for each type of plants: the processes involved were infusion, decoction and maceration.

For the element concentration determination by neutron activation analysis, the plant samples and reference materials (USGS - RGM and STM, and synthetic standards prepared by pipetting convenient aliquots of standard solutions onto small filter paper sheets) were weighed, approximately 150 mg of each, and packed in polyethylene bags. The extract solutions were dried, transferred to filter paper sheets as well as the subsequent water used to wash the beaker. In the IEA-R1 nuclear reactor at IPEN, the plant samples, reference materials and synthetic standards were irradiated for 8 h under a thermal neutron flux of 1 – 5 x 10^{12} n cm^{-2} s^{-1}. The radiation counting was performed by Gamma Spectrometry, using an Ortec Gamma Hyperpure Ge detector and associated electronics, with a resolution of 0.88 keV and 1.90 keV for ^{57}Co and ^{60}Co, respectively, one and two weeks after irradiation. The elements As, Ba, Br, Ca, Co, Cr, Cs, Fe, Hf, K, Na, Rb, Sb, Sc, Se, Ta, Th, U, Zn and Zr were determined. The analysis of the data was done by using in-house gamma ray software, VISPECT program to identify the gamma-ray peaks. The methodology precision and accuracy were verified by using the reference materials apple leave (SRM 1515), peach leave (SRM1547) and tomato leave (SRM1573a).

For the ICP-OES and CV-AAS, approximately 300 mg of dried powdered plant samples were weighed in Teflon tubes and HNO_3, HCl, $HClO_4$ and H_2O_2 were added. Subsequently, the samples were completely dissolved in a Mars 5-CEM microwave system. After cooling, the samples were filtered and the volume completed to 50 ml with Milli-Q ® water. The extracts preparation followed the same procedures described for INAA. The ICP-OES analysis was performed in a Spectro, Spectro Flame M120E model. The CV-AAS was performed by using a Perkin Elmer FINS 100 (flow injection mercury system). Detailed methodology, concerning these methods, was given elsewhere.

3. RESULTS AND DISCUSSION

In Table 2, the statistical results for the obtained concentrations: mean, geometric mean, median, minimum and maximum values, lower and upper quartiles and the standard deviation of the mean are presented. In the Table 3, these parameters are showed for the element concentration in the extract samples. Maximum and minimum concentrations indicate high variation in the values obtained. This variation must be related to the differences in the analyzed species, parts of the plant or in the mechanisms of the element absorption by the plant. On the other hand, environmental pollution and anthropogenic activities may also be factors affecting these observed values [13, 14]. It can, also, be observed that for almost all the elements, the geometric mean and median is lower than the arithmetic mean, indicating that at least one of the samples possesses higher values of concentrations, both for the plants and extracts.

Table 1: Plants analyzed in this study: scientific name, part of the plat used for extract preparation, medicinal indication according to ANVISA.

Scientific name	Used part	Indication
Achillea millefolium	Shoots	Lack of appetite, fever, inflammation and cramping
Achyrocline satureioides	Inflorescence	Poor digestion and intestinal cramps, mild sedative, and anti-inflammatory
Aesculus hippocastanum	Seeds in shell	Capillary fragility, venous insufficiency (varicose veins and hemorrhoids)
Ageratum conyzoides	Shoots without flowers	Joint pain (arthritis, arthrosis) and rheumatism
Allium sativum	Bulb	High cholesterol, as expectorant
Anacardium occidentale	Under bark	Noninfectious diarrhea
Arctium lappa	Roots	Dyspepsia, diuretic and anti-inflammatory such as the joint pain
Arnica montana	flowers	Trauma, bruises, sprains, swelling due to fractures and sprains
Baccharis trimera	Shoots	Dyspepsia
Bidens pilosa	Leaves	Jaundice
Calendula officinalis	Flowers	Inflammations and injuries, bruises and burns
Caesalpinia ferrea	Beans	Injuries as hemostatic astringent and antiseptic healing
Casearia sylvestris	Leaves	Pain and injuries, as an antiseptic and healing topic
Cinnamomum verum	Bark	Lack of appetite, mild cramping, flatulence and feeling of fullness
Citrus aurantium	Flowers	Mild cases of anxiety and insomnia, sedative
Cordia verbenacea	Leaves	Inflammation in bruises and pain
Urcuma longa	Rhizomes	Dyspepsia, Anti-inflammatory
Cymbopogon citratus	Leaves	Intestinal and uterine cramping, mild anxiety cases, insomnia, sedative
Echinodorus macrophyllus	Leaves	Edema by fluid retention and inflammation
Equisetum arvense	Shoots	Edema by fluid retention and inflammation
Erythrina verna	Bark	Mild cases of anxiety and insomnia, sedative
Eucalyptus globulus	Leaves	Colds and flus to clear airway as an adjunct in the treatment of bronchitis and asthma
Eugenia uniflora	Leaves	Noninfectious diarrhea
Glycyrrhiza glabra	Root	Coughs, colds and flus
Hamamelis virginiana	Bark	Skin inflammations and mucous membranes, hemorrhoids
Harpagophytum procumbens	Root	Joint pain (arthritis, arthrosis, arthralgia)
Illicium verum	Fruit	Bronchitis, expectorant
Lippia sidoides	Leaves	Gargles, mouthwashes and rinses
Malva sylvestris	Leaves and flowers	Respiratory expectorants
Matricaria recutita	Flowers	Intestinal cramps, mild anxiety cases, mild tranquilizer
Maytenus ilicifolia	Leaves	Dyspepsia, heartburn and gastritis, adjuvant ulcer prevention
Melissa officinalis	Inflorescence	Abdominal cramps, mild anxiety and insomnia cases, mild tranquilizer
Mentha x piperita	Leaves and inflorescence	Colic, flatulence, liver problems

Mentha pulegium	Shoots	Respiratory expectorant, appetite stimulant, digestive disturbances, gastrointestinal spasms
Mikania glomerata	Leaves	Colds and flus, allergic and infectious bronchitis, expectorant
Momordica charantia	Fruit, and seeds	Dermatitis and scabies
Passiflora alata	Leaves	Mild anxiety and insomnia cases, mild tranquilizer
Passiflora incarnata	Shoots	Mild anxiety and insomnia cases, mild tranquilizer
Paullinia cupana	Seeds	Fatigue, stimulant
Peumus boldus	Leaves	Dyspepsia, choleretic and cholagogue
Phyllanthus niruri	Shoots	Elimination of small kidney stones
Pimpinela anisum	Fruit	Dyspepsia, gastrointestinal cramps
Plantago major	Leaves	Inflammations of the mouth and pharynx
Polygonum punctatum	Shoots	Varicose veins and varicose ulcers
Table 1: continuation		
Psidium guajava	Young leaves	Noninfectious diarrhea
Punica granatum	Fruit peel	Inflammation and infection of the mouth and pharynx anti-inflammatory
Rhamnus purshiana	Bark	Eventual intestinal constipation
Rosmarinus officinalis	Leaves	Circulatory disorders, antiseptic and healing
Salvia officinalis	Leaves	Dyspepsia and excessive sweating
Sambucus nigra	Flowers	Colds and flus
Schinus terebinthifoia	Bark	Vaginal inflammation, leucorrhea, hemostatic, astringent and healing
Senna alexandrina	Fruit and folioles	Eventual intestinal constipation
Solanum paniculatum	Whole plant	Dyspepsia
Stryphnoden dromadstrigens	Bark	Injuries, healing and topical antiseptic on the skin, oral mucosa and genital
Taraxacum officinale	Whole plant	Dyspepsia, appetite stimulant and as a diuretic
Uncaria tomentosa	Bark	Joint pain (arthritis and osteoarthritis) and acute muscle anti-inflammatory
Vernonia condensata	Leaves	Pain and dyspepsia
Zingiber officinale	Rhizome	Sickness, nausea and vomiting of pregnancy, postoperative motion, dyspepsia

In Table 4, the Pearson correlation coefficients for the elements analyzed in the samples of medicinal plants selected for this study are shown; significant correlations at $p < 0.05$ are in bold. Observing these results, the trace metals elements Co, Cr, Fe, Hf, Ni, Pb, Sc, Th and Zr generally show good correlations to each other. Another group exhibiting good correlation is formed by the elements Br, Cs, Rb, Sb and Zn. The element pairs Ba/Ca, Ba/Pb were, also, well-correlated. Among the elements considered as toxic, it can be seen that As presents significant correlation only with Co; Cd only with Zn; Pb with Ba, Co, Cr, Ni. Mercury is not correlated with anyone of the other elements. Significant negative correlations are observed only for the pairs Se/U and Se/Ni.

In Table 5, the Pearson correlation coefficients for the samples of extracts and significant correlations at $p < 0.05$, in bold, are shown. The correlations observed in the extracts are not the same of those for plants. Among the trace metals, significant correlations were observed for Co, Fe and Sc and for these elements with Ni, Th and Zn. Among the toxic elements, good correlations were observed for As with Ba and Co; Cd with Ba, Hf, Ni and Pb; and Pb with Cr; Na and Cu correlate well with only with Cr. Mercury was below the detection limits in all samples and, therefore, it was not included in the statistics.

The way in which the elements are correlated can best be visualized by means of a cluster analysis. The results of the analyses for the samples of plants are shown in Figure 1a, where the formation of four main groups can be observed. Group 1 was formed by Th, Hf, Sc, Fe and Co; group 2, by Zn, Rb, Sb, Cs, K and Br; group 3, by Pb, Ni and Cr; and group 4, by Na, Ca, Ba, Hg, Cu, Cd, Se, U, Zr, Ta and As.

It could be seen that the groups formed agree with what was observed in the analysis of coefficient correlation: group 1 contains the essential elements Fe and Co. The fact that these elements were grouped with Sc, Th and Hf may indicate a similar mechanism of uptake by plants. The second group consists, predominantly, of alkali metals and halogens, which are characterized by their high solubility. Group 3 formation should be also related to the mechanisms of absorption of these elements by the plants; however, such mechanisms should be different from those that govern the absorption of elements present in group 1 [15]. Group 4 contains alkaline earth metals, toxic elements, such as Cd and Hg and the elements that were determined in a relatively small number of plants.

Table 2: Statistics of the obtained results (in μg g^{-1}, except for Hg, in ng g^{-1}) in the plant samples.

	Valid N	Mean	Geometric	Median	Minimum	Maximum	Lower	Upper	Std.Dev.
As	10	0.25	0.18	0.21	0.052	0.59	0.069	0.31	0.19
Ba	55	75.2	45.8	47.9	2.16	390	20.4	112.7	73.2
Br	59	32.7	16.7	17.8	0.72	168.2	7.12	41.6	37.7
Ca	59	10065	7637	10736	347.4	23795	4881	13549	6126
Co	59	0.75	0.42	0.38	0.069	9.12	0.22	0.66	1.33
Cr	59	22.2	9.54	11.2	0.50	256.5	4.53	29.6	38.3
Cs	47	0.31	0.12	0.12	0.014	4.29	0.051	0.22	0.70
Fe	58	754	319	310	26	12259	154	520	1696
Hf	52	0.32	0.108	0.07	0.014	2.63	0.047	0.20	0.62
K	59	20325	13360	17623	113	81850	7236	31990	17246
Na	56	448.9	115.3	87.9	8.02	7718	51.9	234	1229
Rb	57	38.5	22.1	25.3	2.6	229	10.1	49.1	43.4
Sb	37	0.03	0.024	0.02	0.005	0.09	0.013	0.04	0.02
Sc	58	0.21	0.052	0.05	0.0012	3.7	0.02	0.11	0.56
Se	27	0.33	0.245	0.20	0.031	1.08	0.17	0.55	0.25
Ta	10	0.04	0.025	0.04	0.003	0.09	0.010	0.05	0.03
Th	50	0.23	0.087	0.06	0.012	1.99	0.034	0.21	0.43
U	19	0.40	0.226	0.32	0.013	1.16	0.092	0.71	0.35
Zn	56	32.6	25.5	23.81	7.44	169.4	17.4	39.7	28.8
Zr	12	35.9	17.8	22.9	2.34	109	5.8	66.7	38.1
Hg	57	85.1	55.97	62.9	2.51	434	36.4	101	80.2
Cd	30	0.14	0.098	0.07	0.026	0.55	0.049	0.20	0.14
Cu	59	8.30	7.24	7.89	1.90	24.5	4.92	10.9	4.27
Ni	59	5.62	3.11	2.86	0.36	53.1	1.23	6.7	7.96
Pb	47	7.50	3.22	2.32	0.50	76.9	1.20	7.04	13.9

In Figure 1b, dendrogram for the measured elements in the extracts of medicinal plants is presented. The formation of three main groups was observed and the elements that were grouped together should, probably, share characteristics such as solubility and concentration in the extract. Group 1 is composed by Zn, Th, Sc and Fe and group 2 contains the elements Ni, Cd, Hf, Co and Ba. Group 3 can be subdivided into a) Rb and Cs; b) Pb, Na and Cr; c) Cu, Sb, K, and Ca; d) Br and; e) Zr, U, Ta, Se and As. Groups 1 and 2 contain, basically, transition metals except Ba. In group 3, membership 3a was probably grouped due to solubility characteristics, while the group 3e was formed by the elements determined only by a small number of samples. It may also be noted, in group 3, the individual behavior for elements such as Pb and Cu. The differences observed between the results of the correlation analysis and the grouping elements indicate that complex factors must be involved in the extraction processes, such as, for example, the bound type of each element in different parts of the plants used for the extracts preparation [16].

Table 3: Statistics of the obtained results (in µg g^{-1}) in the extract samples.

	Valid N	Mean	Geometric	Median	Minimum	Maximum	Lower	Upper	Std.Dev.
As	23	0.05	0.03	0.03	0.006	0.45	0.015	0.05	0.09
Ba	53	6.36	3.98	3.42	0.46	25.7	2.14	8.66	6.40
Br	44	1.77	0.54	0.46	0.001	21.89	0.20	1.43	4.10
Ca	56	936	582	647	37.9	3977	269	1305	906
Co	58	0.12	0.06	0.06	0.008	0.83	0.033	0.10	0.17
Cr	57	0.78	0.59	0.55	0.078	2.71	0.33	1.01	0.63
Cs	55	0.14	0.03	0.02	0.002	3.00	0.013	0.07	0.45
Fe	58	19.20	15.60	15.67	2.99	75.88	10.30	24.34	13.81
Hf	21	0.01	0.00	0.01	0.001	0.03	0.002	0.01	0.01
K	29	17387	11500	10320	2044	84502	7145	21479	17487
Na	56	380	205	212	0,20	3124	117	460	500
Rb	58	25.35	14.08	14.64	0.86	169.54	6.33	32.55	31.23
Sb	47	0.02	0.01	0.01	0.001	0.28	0.006	0.02	0.04
Sc	57	0.007	0.004	0.004	0.0003	0.034	0.002	0.007	0.008
Se	10	0.05	0.04	0.04	0.011	0.15	0.018	0.09	0.05
Ta	2	0.001	0.001	0.001	0.0007	0.0014	0.0007	0.0014	0.0005
Th	44	0.01	0.01	0.01	0.002	0.11	0.004	0.01	0.02
U	9	0.02	0.02	0.02	0.006	0.05	0.016	0.02	0.01
Zn	51	8.79	6.82	7.32	1.09	27.68	3.99	13.41	6.28
Zr	22	2.40	1.30	1.13	0.31	19.24	0.53	2.60	4.00
Cd	58	12.22	0.07	0.03	0.003	101	0.017	0.05	33.18
Cu	58	1.71	1.14	1.17	0.091	6.74	0.54	2.38	1.47
Ni	58	5.52	0.27	0.20	0.037	101	0.11	0.37	22.50
Pb	58	0.52	0.39	0.39	0.061	2.35	0.238	0.66	0.42

Figure 2 presents the dendrogram obtained for the analyzed plants. Group 1 is characterized by having high concentrations of all elements, except Cd. In this group, it is verified that the part of the plant recommended for use is, predominantly, shoots and leaves except for *Momordica charantia* and *Aesculus hippocastanum plants* whose indication for use are leaves, fruits and seeds and only seeds, respectively indicating that the aerial parts of the plants of this group are enriched in virtually all elements.

Group 2 is characterized by having high Ca, Cs, Rb, Sb, Se, Ta, Zn, Hg, Cd, and Cu concentrations; intermediate As, Ba, Br, Co, Cr, Fe, hf, K, Na, Sc, Th and Pb concentrations and low U, Zr and Ni concentrations. This group includes, mainly, plants whose use indication is the leaves, except *Senna alexandrina*, with the indication use of fruits and leaflets. The leaves of the plants of this group, although rich in essential elements such as Ca, Se and Zn are, also, rich in toxic elements, such as Hg and Cd.

Table 4: Pearson correlation coefficient obtained for the concentrations of the determined elements in plant samples.

	As	Ba	Br	Ca%	Co	Cr	Cs	Fe	Hf	K%	Na	Rb	Sb	Sc	Se	Ta	Th	U	Zn	Zr	Hg	Cd	Cu	Ni	Pb
As	1.00																								
Ba	-0.11	1.00																							
Br	0.49	-0.01	1.00																						
Ca%	-0.38	**0.39**	0.05	1.00																					
Co	**0.72**	0.20	0.16	0.02	1.00																				
Cr	-0.01	0.03	**0.32**	-0.15	**0.33**	1.00																			
Cs	0.52	-0.17	**0.38**	-0.03	-0.01	-0.07	1.00																		
Fe	0.50	0.08	0.16	0.14	**0.88**	0.25	0.01	1.00																	
Hf	0.58	0.18	**0.30**	0.19	**0.49**	**0.30**	-0.04	**0.67**	1.00																
K%	0.39	0.03	**0.32**	0.09	0.11	0.10	0.07	0.04	-0.01	1.00															
Na	0.17	-0.12	-0.03	0.23	0.03	0.02	0.00	0.07	0.12	0.04	1.00														
Rb	0.47	-0.03	**0.35**	0.04	0.01	-0.13	**0.61**	-0.07	-0.13	**0.33**	0.01	1.00													
Sb	0.09	0.03	**0.57**	0.05	0.18	0.12	**0.73**	0.19	0.17	0.19	-0.09	**0.38**	1.00												
Sc	0.48	0.07	0.12	0.15	**0.87**	0.13	-0.01	**0.96**	**0.59**	0.05	0.05	-0.07	0.12	1.00											
Se	-0.12	-0.20	0.33	0.05	-0.05	**0.39**	0.15	0.31	0.18	0.11	0.09	0.15	0.16	0.29	1.00										
Ta		0.23	0.55	-0.23	0.52	**0.68**	0.18	0.54	**0.86**	-0.11	-0.08	-0.21	0.37	0.54	0.16	1.00									
Th	0.51	0.21	0.26	0.26	**0.59**	0.18	0.09	**0.80**	**0.91**	0.03	0.02	-0.12	0.28	**0.74**	0.17	**0.90**	1.00								
U	0.06	0.07	0.07	0.22	0.00	-0.18	-0.21	-0.07	-0.06	-0.03	-0.00	-0.19	0.19	-0.07	**-0.62**	-0.57	0.11	1.00							
Zn	0.48	0.10	**0.26**	0.17	0.09	0.01	**0.58**	0.19	**0.35**	0.18	0.00	**0.51**	**0.42**	0.14	0.17	0.64	**0.51**	0.00	1.00						
Zr		0.39	0.16	0.47	**0.68**	0.10	-0.06	**0.77**	**0.98**	-0.03	0.22	-0.39	0.14	**0.76**	0.24	0.69	**0.92**	0.49	0.58	1.00					
Hg	-0.32	0.00	-0.03	-0.01	-0.11	-0.15	-0.05	-0.09	-0.06	-0.05	-0.05	-0.00	0.01	-0.07	0.25	0.28	-0.07	-0.22	0.04	-0.20	1.00				
Cd	-0.95	0.11	-0.16	-0.02	-0.17	-0.21	-0.15	-0.10	-0.16	-0.12	-0.20	0.11	-0.22	-0.09	0.22	0.01	-0.14	-0.21	**0.39**	-0.60	0.04	1.00			
Cu	-0.25	-0.05	-0.05	0.04	0.21	0.14	0.00	**0.24**	0.33	0.10	0.12	0.11	-0.04	**0.26**	-0.05	0.12	**0.31**	0.08	0.37	**0.30**	0.07	-0.01	1.00		
Ni	-0.21	0.10	0.06	-0.07	**0.29**	**0.67**	-0.01	0.14	0.07	0.25	-0.02	-0.08	-0.01	0.10	**-0.45**	-0.29	0.06	-0.01	0.03	0.15	-0.12	-0.21	0.24	1.00	
Pb	-0.39	**0.38**	0.02	-0.14	**0.37**	**0.45**	-0.09	-0.00	-0.01	0.08	-0.05	0.06	0.25	-0.05	-0.15	0.10	-0.06	0.32	0.09	0.04	-0.03	-0.10	0.10	**0.52**	1.00

Table 5: Pearson correlation coefficient obtained for the concentrations of the determined elements in the extract samples.

	As	Ba	Br	Ca	Co	Cr	Cs	Fe	Hf	K	Na	Rb	Sb	Sc	Se	Th	U	Zn	Zr	Cd	Cu	Ni	Pb
As	1.00																						
Ba	**0.47**	1.00																					
Br	-0.14	0.05	1.00																				
Ca	-0.01	0.26	-0.18	1.00																			
Co	**0.91**	**0.46**	0.22	0.15	1.00																		
Cr	0.08	0.22	0.03	**0.28**	0.08	1.00																	
Cs	-0.04	-0.11	-0.03	-0.06	-0.05	-0.09	1.00																
Fe	0.06	0.06	**0.34**	-0.07	**0.47**	**0.34**	-0.04	1.00															
Hf	0.08	**0.52**	0.24	0.01	0.32	-0.19	-0.06	0.11	1.00														
K	0.58	0.02	-0.13	**0.46**	-0.02	**0.50**	0.15	0.25	-0.32	1.00													
Na	0.25	0.09	-0.13	0.07	-0.01	**0.60**	-0.01	0.15	-0.10	0.36	1.00												
Rb	-0.17	0.09	0.11	0.07	0.09	0.06	**0.60**	0.05	0.10	0.35	0.07	1.00											
Sb	0.45	0.08	-0.07	0.21	-0.01	0.34	0.22	0.00	0.14	0.36	-0.01	0.25	1.00										
Sc	-0.12	-0.03	-0.00	**0.29**	**0.44**	0.13	-0.06	**0.49**	0.12	-0.05	0.06	-0.08	-0.03	1.00									
Se	0.05	-0.39	-0.25	0.32	-0.26	0.64	-0.08	-0.19	0.30	-0.10	0.63	-0.24	0.05	-0.33	1.00								
Th	-0.22	-0.11	0.17	0.07	0.06	0.12	0.20	**0.36**	0.08	0.14	-0.03	0.08	**0.41**	**0.58**	-0.29	1.00							
U	0.03	0.66	**0.93**	-0.09	**0.89**	**0.71**	0.01	0.66	0.77		0.18	**0.72**	-0.32	-0.12	0.63	-0.10	1.00						
Zn	0.06	-0.02	-0.14	0.14	0.18	0.18	0.02	**0.36**	-0.00	**0.42**	-0.06	0.04	-0.01	**0.50**	0.54	**0.54**	-0.41	1.00					
Zr	-0.26	0.03	0.09	-0.21	-0.01	0.15	-0.16	0.41	-0.43	0.26	-0.17	-0.14	0.09	-0.12	-0.49	-0.08	**-0.90**	0.09	1.00				
Cd	-0.06	**0.43**	-0.06	0.04	-0.04	0.02	-0.04	-0.04	**0.77**	0.15	0.08	-0.09	0.02	-0.07	0.44	-0.01	-0.00	0.13	0.27	1.00			
Cu	-0.02	-0.13	-0.06	0.00	-0.12	**0.26**	0.01	-0.02	-0.29	0.34	0.10	0.10	-0.03	-0.07	0.16	-0.05	0.36	0.10	0.21	-0.07	1.00		
Ni	-0.01	**0.31**	-0.05	0.24	**0.30**	-0.07	**0.28**	-0.02	**0.65**	-0.12	-0.03	-0.01	0.17	**0.31**	0.36	0.04	0.11	0.23	-0.03	**0.57**	-0.05	1.00	
Pb	-0.22	-0.01	-0.00	-0.05	-0.15	**0.35**	-0.05	0.23	0.38	-0.02	**0.28**	-0.10	-0.06	-0.00	**0.71**	0.23	0.17	0.11	0.24	**0.36**	0.17	0.14	1.00

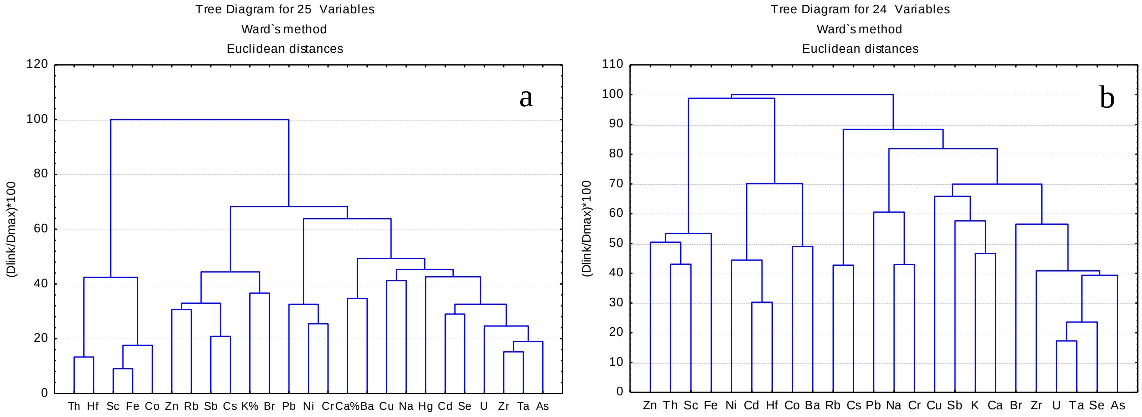

Figure 1: a) dendrogram obtained for the elements in the plant samples and b) dendrogram obtained for the elements in the extract samples.

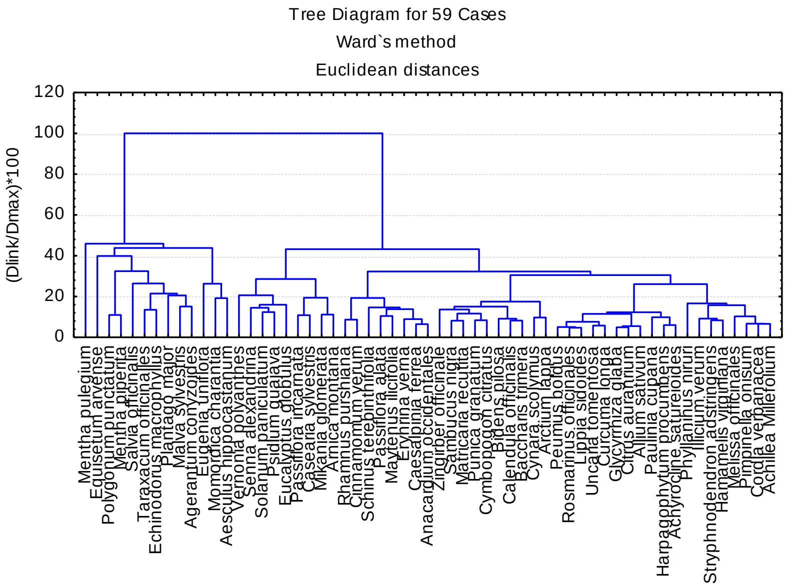

Figure 2: Dendrogram obtained for the cluster analysis of the plants.

Group 3 is characterized by having high Ba, Ca and U concentrations; intermediate K, Rb, Sb, Cd and Ni concentrations, and low Br, Co, Cr, Cs, Fe, Hf , Na, Sc, Se, Ta, Th, Zn, Hg, Cu and Pb concentrations. This group contains the majority of plants in which the use indication is the bark, which is enriched mainly in Ca. It can be also noted that metal elements show low ability to accumulate in the bark of these plants, since they are found in intermediate or low concentrations.

Group 4 is characterized by higher K, Rb and Se concentrations; intermediate As, Br, Co, Cr, Cs, Fe, Na, Sb, Zn, Hg, Cd, Cu and Pb concentrations, and low Ba, Ca, Hf, Sc, Ta, Th, U, Zr and Ni concentrations. In this group, there is not a predominance of a specific part of the plant indicated for use and the used parts are leaves, rhizome, flower, fruit rind, aerial parts and roots, with concentrations varying from intermediate to low for both major and trace elements.
Group 5 is characterized by higher U concentration, intermediate As, Cs, Fe, Hf, Ta, Zr and Ni concentrations and low Ba, Br, Ca, Co, Cr, K, Na, Rb, Sb, Sc, Se, Th, Zn, Hg, Cd, Cu and

Pb concentrations. Plants in this group also shows a large variation among the indicated used parts of plant, such as inflorescences, bulbs, flowers, rhizomes, roots, leaves, seeds and barks and, again, it can be seen that these plant parts have low or intermediate concentrations of major and trace elements.

Group 6 is characterized by higher Cd and Cu concentrations, intermediate As, Co, Cr, Cs, Fe, K, Sb, Sc, Ta, Zn, Zr, Hg and Ni concentrations, and low Ba, Br, Ca, Hf, Na, Rb, Se, Th and Pb concentrations. The plant parts indicated for use in this group have wide variation, as in group 5, including shoots, fruits, barks, inflorescences, fruits and leaves; however, it can be observed, in these plants, that they have high concentrations of toxic or potentially toxic elements, such as Cd and Cu.

Figure 3 presents the dendrogram obtained for the extracts obtained from the plants analyzed. It can be seen that five main groups were formed. The extracts grouped in group 1 are characterized by relatively high concentrations of all the elements, except Zn, Cu and Pb. This group is characterized by relatively higher concentrations of Ca, Cr, Fe, Sc, Se, Th, Zn, Zr, and Pb, intermediate concentrations of As, Ba, Br, Co, Hf, K, Na, Rb, sb, Cd, Cu and Ni, and relatively lower concentrations of Cs and U.

Group 3 is characterized by having intermediate Ca concentrations and relatively low concentration of the other elements. The extracts of group 4 are characterized by relatively high Cu and Cr concentrations, intermediate K, Rb, U, Cd, Pb and Ni concentrations and relatively lower As, Ba, Br, Ca, Co, Cs, Fe, hf, Na, Sb, Sc, Th, Zn and Zr concentrations. The extracts of group 5 are characterized for having relatively high Br, K, Rb and Cd concentrations, intermediate Ba, Ca, Co, Cs, Hf, Na, Cu, Ni and Zr concentrations and low As, Cr , Fe, Sb, Sc, Th, U, Zn and Pb concentrations.

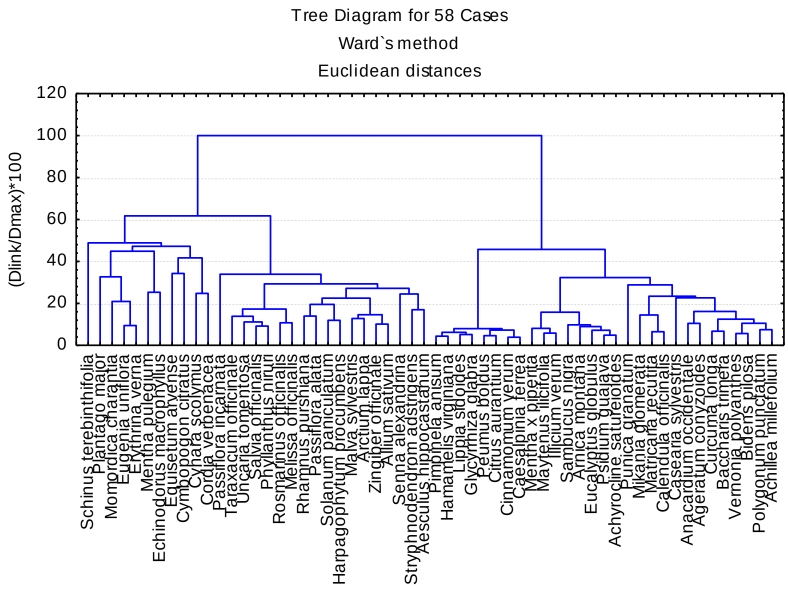

Figure 3: Dendrogram obtained for the cluster analysis of the extract.

The application of the principal component analysis to the data obtained from the plant analysis led to the formation of 8 factors, with an explained variance of 74.62%: the five first factors were responsible for 54.93% of the explained variance. Factors 1 and 2 are shown in Figure 4 and factors 3 to 5 are shown in Figure 5. In these figures it can be seen that factor 1 has high loading for the elements Co, Fe, Hf, Sc, Ta, Th and Zr; factor 2, for the elements Br, Cs, Rb, Sb and Zn, all of them positively correlated. The high loading in factor 3 was obtained for the elements Cr, Ni and Pb, positively correlated; in factor 4, for the elements Se and U, with negative correlation, and in factor 5, there was a high loading only for Ca and Ba.

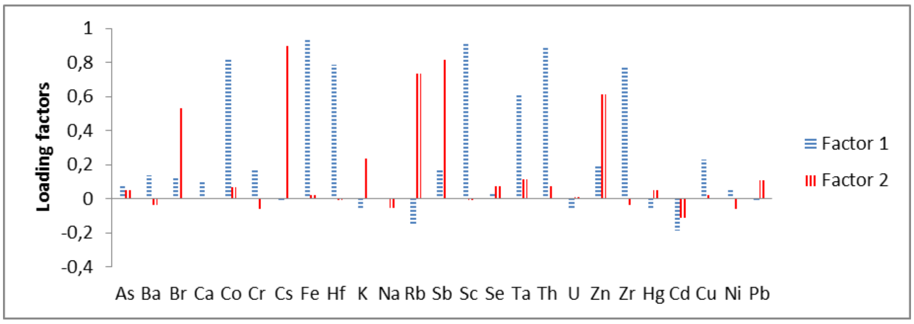

Figure 4: Loading factors, for the factors 1 and 2, obtained in the PCA applied to the results of the plant concentrations.

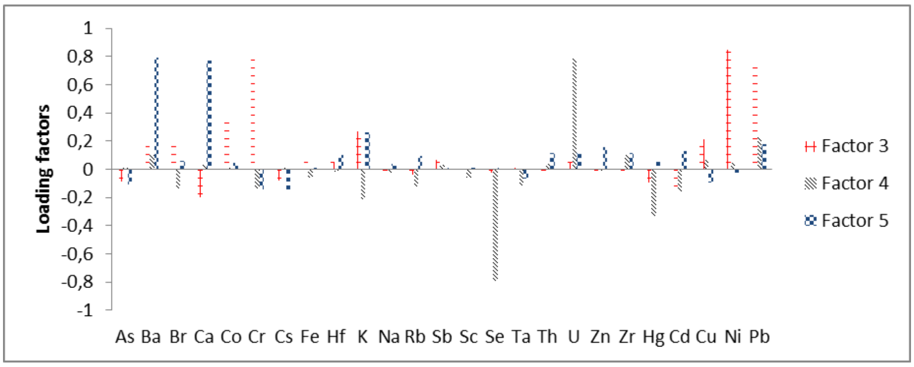

Figure 5: Loading factors, for the factors 3 to 5, obtained in the PCA applied to the results of the plant concentrations.

The application of the principal component analysis to the data obtained from the plant analysis led to the formation of 10 factors, with an explained variance of 78.51%: the five first factors were responsible for 51.90% of the explained variance. In Figure 6, it is shown the loading factor for factors 1 and 2; in Figure 7, the loading factor for factors 3 to 5. It can be seen in the figures that factor 1 has high loadings for the elements Hf, Cd, Ni and Ba, and factor 2, for the elements Cr, Na, K and Pb, all of them positively correlated. Factor 3 has high loadings for the elements Sc, Th, Fe and Zn, positively correlated, while factor 4 shows high loadings for the elements Br and U, negatively correlated with the other factors. As to factor 5, the elements Cs and Rb were those with higher loadings.

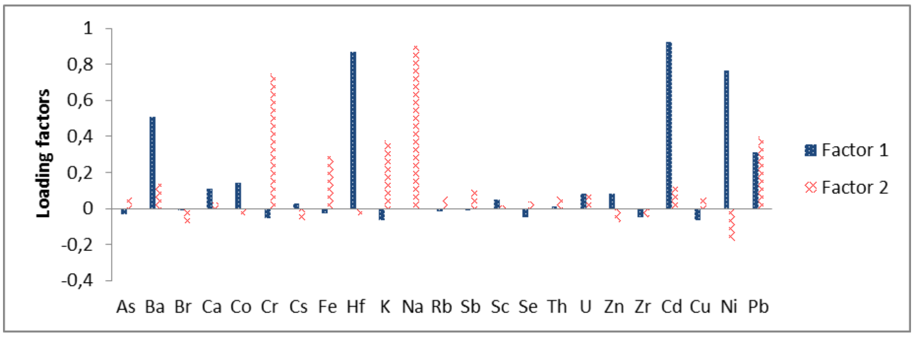

Figure 6: Loading factors, for the factors 1 and 2, obtained in the PCA applied to the results of the extract concentrations.

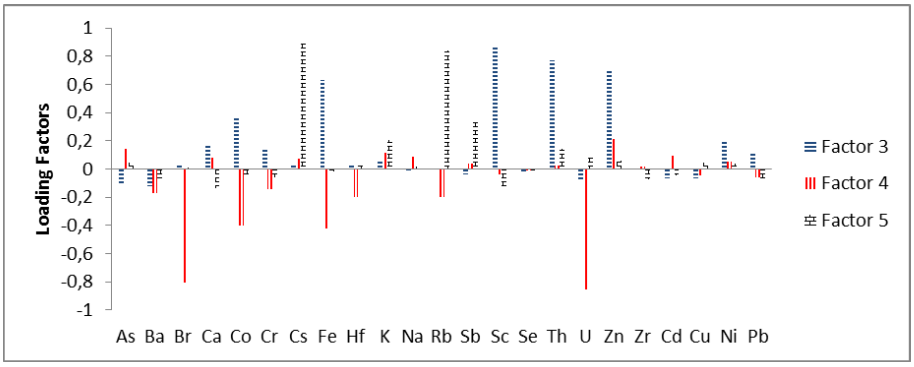

Figure 7: Loading factors, for the factors 3 to 5, obtained in the PCA applied to the results of the extract concentrations.

4. CONCLUSIONS

The results presented for the chemometric study of the plants analyzed in this paper showed good agreement among the statistical analyses performed (PCC, HCA and PCA), then, it can be concluded that these medicinal plants may be characterized by the variability of trace metals, such as Co, Fe, Hf, Sc and Zr, highly soluble elements Br, Cs and Rb well correlated with Zn, the variability of the potentially toxic elements Cr, Ni and Pb and, yet, by the correlation between Ca and Ba. The results obtained in the statistical analysis for the extract indicates that the variability of the elements in the plant extract is somewhat similar to that found in the plants but not the same, since the presence of these elements in the plants depends on the availability of the elements to be transferred to the plant from the soil, water or air, while the distribution of the elements in the extract depends on factors like their solubility in the extract preparation conditions.

The chemometric analysis applied permitted, also, to classify distinct groups among the analyzed plants and extracts, so that these data may be useful in future studies concerning the therapeutic action that the elements here determined may exert.

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