

Direct Solid Sample Analysis of Medicinal Herbs for Determination of Ba, Cu, Ni, V and Zn by EDXRF

Sidnei O. Souza,^{a,b} Dayara V. L. Ávila,^a Carolina M. O. Prado,^c Silvânio S. L. Costa^d and Rennan G. O. Araujo^{id}*,^{a,e}

^aDepartamento de Química Analítica, Instituto de Química, Universidade Federal da Bahia, 40170-115 Salvador-BA, Brazil

^bDepartamento de Farmácia, Universidade Federal de Sergipe, 49400-000 Lagarto-SE, Brazil

^cInstituto Tecnológico e de Pesquisa do Estado de Sergipe (ITPS), 49020-380 Aracaju-SE, Brazil

^dNúcleo de Petróleo e Gás, Universidade Federal de Sergipe, 49100-000 São Cristóvão-SE, Brazil

^eInstituto Nacional de Ciência e Tecnologia do CNPq-INCT de Energia e Ambiente, Universidade Federal da Bahia, 40170-115 Salvador-BA, Brazil

In this work, an analytical method for determination of Ba, Cu, Ni, V, and Zn, in medicinal herbs, through direct solid sample analysis by energy dispersive X-ray fluorescence spectrometry (EDXRF), was applied. Accuracy and precision (intra and inter day) of the analytical method were confirmed through analysis of certified reference material, tomato leaves (CRM NIST 1573a). Principal component analysis (PCA) and hierarchical cluster analysis (HCA) allowed to observe the formation of two groups. Group I was composed by highest levels of Cu and Zn, and the group II with highest concentrations of Ba, V, and Zn. Group I also was subdivided into two subgroups. The concentration of Ni in some samples exceeded the maximum allowed limit. In all samples, Cu and Zn concentration were below the maximum limit allowed by Brazilian legislation. However, for Ba and V, the evaluation was not possible, because there are no maximum limits in legislation.

Keywords: medicinal herbs, direct solid sample analysis, EDXRF, PCA, HCA

Introduction

Medicinal herbs are widely consumed by many peoples in various countries due to easy access, low cost and their applications for prevention, treatment and cure of a variety of diseases. They have in their composition flavonoids, amino acids, proteins, enzymes, vitamins, minerals and others substances, which form the aroma, volatile oils, and carbohydrates. However, undesirable substances such as pesticide residues, mycotoxins, and potentially toxic elements, that could affect human health, may be derived from the geographical origin, depending on the water and soil used on the planting process, through fertilizers, fungicides and pesticides, of the environment, through the time of exposure of plants to atmospheric particulate material, and finely from transportation and storage.¹⁻⁵

The literature reports the determination of different inorganic constituents in medicinal herbs and/or their

infusions, including essential and non-essential elements, being timely their quantification and evaluation to ensure the quality prior their consumption.^{4,6-14}

The World Health Organization (WHO) sets maximum limit allowed for inorganic contaminants in medicinal herbs.¹⁵ In Brazil, Decree No. 55871 of March 26, 1965¹⁶ and Resolution of the Collegiate Board of Directors (RDC) No. 42, of August 29, 2013,¹⁷ established maximum allowable limits of inorganic contaminants in foods, including herbs, for potentially toxic elements such as As, Pb, Cd, Cu, Cr, Hg, Ni, Se, and Zn.

For determination of inorganic constituents in medicinal herbs spectroanalytical techniques are been used, such as inductively coupled plasma mass spectrometry (ICP-MS),^{5,8,13,18-23} inductively coupled plasma optical emission spectrometry (ICP OES),^{9-12,23-27} electrothermal vaporization-ICP OES (ETV-ICP OES),³ flame atomic absorption spectrometry (F AAS),^{7,9,28} flame atomic emission spectrometry (F AES),²⁸ graphite furnace atomic absorption spectrometry (GFAAS),^{10,14} hydride generation

*e-mail: rgoa01@terra.com.br, rennan@ufba.br

atomic absorption spectrometry (HG-AAS),¹⁴ and high resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GF AAS).²⁹

However, chemical analysis of medicinal herb samples has often required to convert them into a mineralized form through destruction of the organic matrix, using mineral acids or mixing of high purity oxidizing agents under aggressive conditions, in addition to a long time sample preparation.³⁰⁻³³

As alternative, total reflection X-ray fluorescence spectrometry (TRXRF)⁴ and energy dispersive X-ray fluorescence spectrometry (EDXRF) are attractive, due to the low cost and possibility of the direct solid sample analysis, when compared with many spectroanalytical techniques. EDXRF is a non-destructive technique that allows the simultaneous multielementary determination between sodium ($Z = 11$) and uranium ($Z = 92$), through direct solid sample analysis, making it possible to eliminate the decomposition step, being an alternative to spectroanalytical techniques.³⁰⁻³⁴

On the other hand, the EDXRF technique also presents low sensitivity, matrix interference, due to high background, spectral interferences from line overlap and spectrum emission, and physical and matrix interferences caused by homogeneous samples that difficult achieve homogeneous irradiation.³¹⁻³⁶ However, these effects can be minimized by decreasing particle size as well as by matrix simulation using certified standards of similar composition to the samples for the construction of calibration curves, mathematical corrections and the use of chemometric tools.^{31,35}

Multivariate data analysis applying principal component analysis (PCA) and hierarchical cluster analysis (HCA) based on chemical composition contents are used to evaluate trends in medicinal herbs.^{12,19,37-40} The PCA and HCA also allow the visualization of the entire data set in a bi- or three-dimensional plot, expressing information about the possible interrelationships or similarities that may exist between the variables, being pattern recognition techniques used to confirm results.^{12,19,37-40}

In this context, direct solid sample analysis of medicinal herbs for determination of Ba, Cu, Ni, V, and Zn by EDXRF, was applied. These elements were determined because when consumed in excess can cause damages to the organism, according to the Substance Priority List of the Agency for Toxic Substances and Disease Registry (ATSDR) in positions 134, 118, 57, 200, and 75, respectively.⁴¹ After analysis, these chemical element concentrations were used to evaluate classification or similarity tendencies between the samples of different medicinal herbs by multivariate data analysis: (PB) *Peumus boldus* (boldo), (BT) *Baccharis trimera* (carqueja), (PA) *Pimpinella anisum* (fennel), (MP) *Mentha x piperita* (mint), (PE) *Passiflora edulis*

(passion fruit), (MR) *Matricaria recutita* (chamomile), (CSw) *Camellia sinensis* (white), (CSg) *Camellia sinensis* (green), (CSb) *Camellia sinensis* (black), (IP) *Ilex paraguariensis* (mate), (MO) *Melissa officinalis* (lemon balm), (ZO) *Zingiber officinale* (ginger), (HS) *Hibiscus sabdariffa* (hibiscus), and (CA) *Cassia angustifolia* (sene).

Experimental

Instrumentation

For grinding of the medicinal herb samples a Wiley-type knife mill with 4 special fixed steel knives of high hardness with anti-oxidant treatment (TECNAL, model TE-650/1, São Paulo, Brazil) was used.

For the chemical analysis of medicinal herb samples, it was employed an energy dispersive X-ray fluorescence spectrometer (EDXRF, model EDX-720, Shimadzu, Tokyo, Japan) with a rhodium X-ray tube, silicon detector, 10 mm collimator, and a liquid nitrogen-based cooling system. The spectrometer was operated at a voltage of 15 kV and the measurement time was 100 s.

Collection and treatment of samples

Thirty-one medicinal herbal samples were analyzed. Nine samples of natural medicinal herbs were collected at the free market in the city of Itabaiana, Sergipe, and twenty-two samples of medicinal herbs in sachets were collected from supermarkets in the cities of Itabaiana and Aracaju, Sergipe, Northeast, Brazil.

The natural samples were taken to the laboratory and placed for drying in an air circulation oven at 40 °C for 72 h. After this time, they were ground in a Wiley-type knife mill with a 20 mesh sieve to obtain a particle size similar to the sachet samples.

A mass of approximately 1.0 g of the samples, natural and sachet medicinal herbs, and certified reference material (CRM NIST 1573a, tomato leaves), were pressed at a pressure of 15 tons using a hydraulic press to obtain a 20 mm diameter pellet, having as based on the pellet, a mass of 2.0 g of boric acid (H_3BO_3) of analytical grade previously pressed. The pellets were stored in a desiccator until the analysis by EDXRF.

Direct solid sample analysis of medicinal herbs using EDXRF

The external calibration curves for the elements were adjusted using linear regression. For the realization of the external calibration curves, certified reference

materials of mixed polish herbs (INCT-MPH-2), peach leaves (NIST 1547), trace and minor elements in lichen (IAEA-336), tea leaves (INCT-TL-1), chlorella (NIES 3), and apple leaves (NIST 1515) were used to simulate the sample matrix. A pellet with boric acid without addition of the sample was used as an analytical blank to obtain the limits of detection and quantification.

Multivariate data analysis

The concentrations of Ba, Cu, Ni, V, and Zn in medicinal herb samples were evaluated using principal component analysis (PCA) and hierarchical component analysis (HCA). For construction of the 31×5 data matrix (31 samples and 5 concentrations of the trace metals), the samples were organized as rows and trace element concentrations as columns. All data were auto scaling, because of the great variation in the concentration of the trace metals, in order to obtain the same relevance for all concentrations. The data were processed using the Statistica® software.⁴² For the HCA, the Euclidian distance and Ward's method of linkage were used to evaluate the similarity of the medicinal herb samples.

Results and Discussion

Figures of merit

The parameters obtained from the external calibration curve are summarized in Table 1. The values of linear correlation coefficients obtained for all inorganic constituents were better than 0.999, being in agreement with the standards established by Brazilian Sanitary Surveillance Agency (Agência Nacional de Vigilância Sanitária, ANVISA) and National Institute of Metrology, Quality and Technology (Instituto Nacional de Metrologia, Qualidade e Tecnologia, INMETRO), with a minimum acceptance criterion $r \geq 0.99$ and $r \geq 0.90$, respectively.^{43,44}

The limit of detection (LOD) and limit of quantification (LOQ) were obtained by multiplying by 3 and 10 times,

respectively, the standard deviation of 10 measures of the analytical blank, divided by slope of the linear equation obtained by external calibration curve. In this work, boric acid, used as support, was considered as analytical blank. Values of LOD ranged from 0.07 mg kg^{-1} for Ni to 2 mg kg^{-1} for Ba, and the LOQs ranged from 0.2 mg kg^{-1} for Ni to 7 mg kg^{-1} for Ba, as shown in Table 1. The LODs are in agreement with values reported in the literature for the determination of inorganic constituents in medicinal herb samples.¹⁻¹²

The accuracy and precision of the analytical method were evaluated by analysis of the certified reference material of tomato leaves (CRM NIST 1573a), according to Table 2. The accuracy, expressed through the agreement between the found and certified values, varied between 87 ± 2 (Zn) and 99 ± 2 (Cu).

The linear regression analysis from CRM resembles ideality, where the intercept value must be equal to zero ($b = 0$), while the slope of the equation of the line and the correlation coefficients equal to the unit ($a = r = 1$).⁴⁵ It is possible to notice from linear regression analysis that there is a good correlation between the found and certified values showing that the proposed method was accurate, obtaining a slope value of $0.96 (\pm 0.09)$ for line equation and intercept value of $0.36 (\pm 2.94)$, at 95% confidence level. There is also a good correlation between the found and certified values, through the correlation coefficient (r) of 0.999.

The precision was expressed as relative standard deviation (RSD, in percentage) and evaluated in two ways. First, the precision was verified on the same day (intraday), and later evaluated on another day (inter day), where the intermediate precision were less than 5% ($n = 4$) and less than 6% ($n = 4$), respectively. Therefore, the proposed analytical method presented good accuracy and precision for determination of Ba, Cu, Ni, V, and Zn in medicinal herb samples using EDXRF.

Analytical application

The analytical method was applied to the analysis of 31 samples of medicinal herbs for the determination

Table 1. Figures of merit obtained for determination of Ba, Cu, Ni, V, and Zn in medicinal herb samples by EDXRF

Element	Line equation for external calibration curve	Correlation coefficient (r)	LOD / (mg kg^{-1})	LOQ / (mg kg^{-1})
Ba	$I = 0.0016[\text{Ba}] - 0.0165$	0.999	2	7
Cu	$I = 0.0354[\text{Cu}] + 0.2405$	0.999	0.6	2
Ni	$I = 0.0085[\text{Ni}] + 0.0588$	0.999	0.07	0.2
V	$I = 0.0755[\text{V}] + 1.9909$	0.999	0.08	0.3
Zn	$I = 0.0313[\text{Zn}] - 0.2257$	0.999	2	6

I: intensity of fluorescence obtained for each chemical element; LOD: limit of detection; LOQ: limit of quantification.

Table 2. Evaluation of the accuracy, precision (intraday) and intermediate precision (inter day) for the determination of Ba, Cu, Ni, V, and Zn in CRM tomato leaves (NIST 1573a) by EDXRF

Element	Certified value / (mg kg ⁻¹)	Found value / (mg kg ⁻¹)	Agreement / %	RSD intra day / %	RSD inter day / %
Ba	63 ^a	61 ± 5	98 ± 8	5	5
Cu	4.70 ± 0.14	4.67 ± 0.11	99 ± 2	1	3
Ni	1.582 ± 0.041	1.549 ± 0.022	97 ± 1	1	2
V	0.835 ± 0.034	0.786 ± 0.050	94 ± 6	4	6
Zn	30.94 ± 0.55	26.98 ± 0.39	87 ± 2	1	2

^aInformed value. All results were expressed as mean ± confidence interval at 95% (n = 4). RSD: relative standard deviation.

of Ba, Cu, Ni, V, and Zn by EDXRF. Table 3 shows the found concentrations for the five inorganic constituents. In the *Peumus boldus* samples, the mean concentrations were like to Cu, Ni and V, and 4.4 times higher to Zn in sachet samples, and like to Cu, Ni and V, and 6.3 times higher to Zn in natural samples, when comparing with Martins *et al.*,²⁶ which found concentrations (in mg kg⁻¹) of 4.00 ± 0.12 (Cu), 0.40 ± 0.03 (Ni), 0.60 ± 0.04 (V), and 3.80 ± 0.08 (Zn) in samples from South region of Brazil. And 1.3 times higher (Cu) and like to Zn in sachet samples, and 1.4 times higher (Cu) and 1.6 times higher (Zn) in natural sample, when comparing with Gomes *et al.*,⁴⁰ which found concentrations (in mg kg⁻¹) of 2.7 (Cu) and 15.8 (Zn) in samples from Southwest of Bahia State, Brazil.

For *Pimpinella anisum*, in sachets and natural samples, the mean concentrations found were in agreement with those found by Ababneh,¹¹ that were 13.9 ± 4.5 (Cu), 3.98 ± 4.90 (Ni), and 72.3 ± 21.1 (Zn) in samples from Jordan, and by Gomes *et al.*⁴⁰ of 7.2 (Cu) and 35.3 (Zn) in samples from Southwest of Bahia State, Brazil.

For *Matricaria recutita*, sachet and natural samples, were found mean concentrations equivalent to those quantified by Ababneh,¹¹ that were 12.6 ± 1.3 (Cu), 3.05 ± 2.80 (Ni), and 49.8 ± 3.3 (Zn) in samples from Jordan, by Martins *et al.*²⁶ for Cu (12.90 ± 0.50), Ni (0.83 ± 0.03), V (1.93 ± 0.11), and Zn (10.00 ± 0.72) in samples from South region of Brazil. Gomes *et al.*⁴⁰ found concentrations (in mg kg⁻¹) of 6.7 (Cu) and 23.3 (Zn), in samples from Southwest of Bahia State, Brazil.

In the *Camellia sinensis* (white tea in sachet) sample and in the natural white tea, the mean concentrations found were in accordance with the concentrations reported by Martins *et al.*²⁶ being 17.70 ± 0.60 (Cu), 4.10 ± 0.20 (Ni), < 0.1 (V), and 12.55 ± 1.10 (Zn) in samples from South region of Brazil.

In *Camellia sinensis* (green tea in sachet), the mean concentrations obtained were also in agreement with those found (in mg kg⁻¹) in the literature, where Martins *et al.*²⁶

quantified concentrations of 14.10 ± 0.80 (Cu), 2.86 ± 0.17 (Ni), 0.83 ± 0.08 (V), and 6.85 ± 0.50 (Zn) in samples from South region of Brazil. Han *et al.*⁸ obtained mean concentrations of 3.08 (Cu) and 12.07 (Ni) in samples from China; Szymczycha-Madeja *et al.*⁹ verified concentrations of 40.2 (Ba), 12.5 (Cu), 5.32 (Ni), and 23.5 (Zn) in packaged samples and 17.8 (Ba), 13.0 (Cu), 3.63 (Ni), and 25.5 (Zn) in leaves samples of Poland markets; Martín-Domingo *et al.*¹⁴ found concentrations of 14.2 (Cu) and 26.4 (Zn) in samples from South region of Brazil; Dalipi *et al.*⁴ found concentrations of 17.5 (Ba), 12.4 (Cu), 5.7 (Ni), and 41 (Ba) in samples from Sri Lanka, Kenya, China, India and Korea. Gomes *et al.*⁴⁰ found concentrations (in mg kg⁻¹) of 9.4 (Cu) and 26.3 (Zn) in samples from Southwest of Bahia State, Brazil.

Chen *et al.*⁴⁶ reported Cu, Ni and Zn concentrations ranging from 5 to 500 mg kg⁻¹ in tea leaves. The authors showed that Ni and Zn are accumulated in tender leaves and Cu (12-18 mg kg⁻¹) concentration can be changed with the cultivation region and by fungicides application. Vázquez *et al.*⁴⁷ reported Cu (2.38-9 µg g⁻¹), Ni (4.4 mg kg⁻¹) and Zn (2.3-37.7 µg g⁻¹) concentrations in different tea leaves. In addition, the authors informed that concentrations may vary considerably between the numerous cultures that consume infusions.

In the *Zingiber officinale* were found mean concentrations 4 times lower (Cu), 3 times lower (Zn) and 6 times higher for Ni than those reported in the literature by Tokalioğlu¹⁹ 17.6 ± 2.7 (Cu), 2.26 ± 0.12 (Ni), and 26.6 ± 0.9 (Zn) in samples from Turkey, and lower (Cu), 3 times lower (Zn) and 6 times higher for Ni than those reported by Ababneh,¹¹ which found concentration values of 6.70 ± 1 (Cu), 2.4 ± 1.6 (Ni), and 26.3 ± 4.9 (Zn) mg kg⁻¹, in samples from Jordan.

In *Melissa officinalis* samples were found mean concentrations half for Cu, 1.5 lower (Zn) and like to Ni in sachet, and 1.6 lower (Cu), 2.5 higher (Zn) and like to Zn in *natura* sample, than those reported in the literature by Tokalioğlu¹⁹ (in mg kg⁻¹) of Ni (2.60 ± 0.13), Cu (13.9 ± 1.2),

Table 3. Results of the concentrations of Ba, Cu, Ni, V, and Zn in medicinal herb samples, after direct analysis by EDXRF

Botanical name	Popular name	Type	Sample code	Concentration / (mg kg ⁻¹)				
				Ba	Cu	Ni	V	Zn
<i>Peumus boldus</i>	boldo	sachet	PB 1	71 ± 8	3.1 ± 0.2	< 0.2	0.76 ± 0.04	16.0 ± 0.9
<i>Peumus boldus</i>	boldo	sachet	PB 2	65 ± 9	3.7 ± 0.8	< 0.2	0.77 ± 0.04	19.0 ± 1.2
Average of sachet samples				68	3.4	n.d.	0.77	17
<i>Peumus boldus</i> ^a	boldo natural	natural	PB n	59 ± 10	3.7 ± 0.3	< 0.2	0.77 ± 0.06	24.0 ± 1.7
<i>Baccharis trimera</i>	carqueja	sachet	BT 1	72 ± 9	7.7 ± 0.7	< 0.2	0.77 ± 0.07	26.0 ± 1.2
<i>Baccharis trimera</i>	carqueja	sachet	BT 2	69 ± 5	8.2 ± 0.5	< 0.2	0.82 ± 0.09	36.0 ± 1.9
Average of sachet samples				70	8.0	n.d.	0.80	31
<i>Baccharis trimera</i> ^a	carqueja natural	natural	BT n	78 ± 7	8.1 ± 1.4	< 0.2	0.79 ± 0.11	33.0 ± 2.2
<i>Pimpinella anisum</i>	fennel	sachet	PA 1	76 ± 6	7.8 ± 0.7	< 0.2	0.80 ± 0.13	36.0 ± 3.0
<i>Pimpinella anisum</i>	fennel	sachet	PA 2	74 ± 10	7.0 ± 0.4	5.8 ± 0.6	0.77 ± 0.06	31.0 ± 1.8
<i>Pimpinella anisum</i>	fennel	sachet	PA 3	49 ± 13	9.4 ± 1.7	< 0.2	0.81 ± 0.02	36.0 ± 1.9
Average of sachet samples				65	8.1	5.8	0.79	34
<i>Pimpinella anisum</i> ^a	fennel	natural	PA n	75 ± 4	8.2 ± 1.1	5.80 ± 0.17	0.77 ± 0.05	28.0 ± 0.70
<i>Mentha x piperita</i>	mint	sachet	MP 1	38 ± 8	5.8 ± 0.9	< 0.2	0.80 ± 0.04	19.0 ± 1.2
<i>Mentha x piperita</i>	mint	sachet	MP 2	48 ± 5	6.6 ± 0.8	< 0.2	0.84 ± 0.02	20.0 ± 1.8
Average of sachet samples				43	6.2	5.8	0.82	19
<i>Mentha x piperita</i> ^a	mint natural	natural	MP n	19 ± 4	4.7 ± 0.3	11.0 ± 1.6	0.86 ± 0.06	24.0 ± 1.4
<i>Passiflora edulis</i> ^a	passion fruit	sachet	PE	73 ± 6	4.6 ± 0.7	< 0.2	0.75 ± 0.04	23.0 ± 2.5
<i>Matricaria recutita</i>	chamomile	sachet	MR 1	79 ± 16	3.6 ± 0.3	14.0 ± 1.4	2.10 ± 0.08	8.7 ± 0.6
<i>Matricaria recutita</i>	chamomile	sachet	MR 2	74 ± 3	4.6 ± 0.7	15.0 ± 1.7	2.10 ± 0.10	9.3 ± 0.7
Average of sachet samples				76	4.1	14	2.1	9.1
<i>Matricaria recutita</i> ^a	chamomile natural	natural	MR n	77 ± 8	3.2 ± 0.3	8.1 ± 1.3	2.50 ± 0.11	10.0 ± 0.6
<i>Camellia sinensis</i> ^a	white	sachet	CS 1	74 ± 11	5.2 ± 0.4	11.0 ± 1.3	1.80 ± 0.14	7.6 ± 1.2
<i>Camellia sinensis</i> ^a	black	sachet	CS 2	78 ± 6	4.2 ± 0.1	10.0 ± 0.8	2.20 ± 0.10	9.0 ± 1.3
<i>Camellia sinensis</i> ^a	green	sachet	CS 3	59 ± 9	12.0 ± 0.2	4.60 ± 0.89	0.84 ± 0.03	26.0 ± 1.6
<i>Camellia sinensis</i> ^a	white natural	natural	CS n	80 ± 10	3.0 ± 0.2	10.0 ± 0.6	1.90 ± 0.80	9.0 ± 1.1
<i>Ilex paraguariensis</i> ^a	mate	sachet	IP	77 ± 4	5.8 ± 0.2	19 ± 2.5	2.70 ± 0.27	10.0 ± 0.8
<i>Melissa officinalis</i>	lemon balm	sachet	MO 1	69 ± 9	4.6 ± 0.7	< 0.2	0.75 ± 0.05	21.0 ± 1.5
<i>Melissa officinalis</i>	lemon balm	sachet	MO 2	55 ± 8	11.0 ± 0.9	< 0.2	0.78 ± 0.08	25.0 ± 1.6
<i>Melissa officinalis</i>	lemon balm	sachet	MO 3	76 ± 6	3.8 ± 0.1	7.6 ± 0.49	1.80 ± 0.13	7.5 ± 0.7
Average of sachet samples				67	6.5	2.5	1.8	18
<i>Melissa officinalis</i> ^a	lemon balm natural	natural	MO n	63 ± 8	8.4 ± 0.6	6.6 ± 1.0	0.78 ± 0.07	27.0 ± 1.8
<i>Maytenus ilicifolia</i> ^a	holy thorn natural	natural	MI n	74 ± 5	3.8 ± 0.5	< 0.2	2.20 ± 0.18	8.8 ± 1.0
<i>Zingiber officinale</i> ^a	ginger	sachet	ZO	71 ± 10	4.3 ± 0.7	14.0 ± 0.3	2.20 ± 0.07	9.2 ± 0.9
<i>Hibiscus sabdariffa</i> ^a	hibiscus	sachet	HS 1	78 ± 6	3.1 ± 0.2	14.0 ± 2.1	1.90 ± 0.04	8.8 ± 0.6
<i>Hibiscus sabdariffa</i> ^a	hibiscus natural	natural	HS n	77 ± 6	4.5 ± 0.7	12.0 ± 1.0	2.00 ± 0.13	7.8 ± 1.2
<i>Cassia angustifolia</i> ^a	sene	sachet	CA	71 ± 10	4.7 ± 1.5	14.0 ± 1.7	2.40 ± 0.14	8.9 ± 0.8

^aConcentrations in unit value. Results expressed as mean ± deviation standard (n = 3). n.d.: not determined.

and Zn (27.7 ± 1.3) in samples from Turkey, and 1.5 lower (Cu), 2.4 lower (Ni) and 1.4 lower (Zn) in sachet, and like to Cu and Ni, and 1.4 lower (Zn) comparing with Ababneh,¹¹

which found concentration values of 9.79 ± 1.30 (Cu), 6.01 ± 3.30 (Ni), and 38.9 ± 15.1 (Zn) mg kg⁻¹, in samples from Jordan.

In *Hibiscus sabdariffa* samples were found mean concentrations 4.7 times lower (Cu), like to Ni, and 16 times lower (Zn) in sachet, and 3 times lower (Cu), 1.2 times lower (Ni), and 17 times lower (Zn) in natural sample, comparing with reported by Ababneh,¹¹ which found concentrations of 14.7 ± 3.1 (Cu), 12.7 ± 4.3 (Ni), and 135 ± 8 (Zn) mg kg^{-1} , in samples from Jordan.

In *Cassia angustifolia* samples were found concentrations like to Cu and 2 times lower (Zn) in comparing with Martín-Domingo *et al.*,¹⁴ which found concentrations of 3.92 and 16.4 mg kg^{-1} for Cu and Zn, respectively, in samples from Spain.

In the *Ilex paraguariensis* samples were found concentrations 1.2 times higher (Ba), 2 times lower (Cu), 8.0 times higher (V) and 6.4 times lower (Zn) than those obtained by Marcelo *et al.*,²⁰ which found concentrations of 61.5, 11.9, 0.377, and 63.6 mg kg^{-1} for Ba, Cu, V, and Zn, respectively, in samples from Brazil, Argentina, Paraguay and Uruguay.

In the *Baccharis trimera* samples the found mean concentrations in sachets and natural sample are in agreement with Pedro *et al.*,⁴⁸ which found concentrations of 10 and 30 mg kg^{-1} for Cu and Zn, respectively, in samples from Central-West region of Brazil.

In the *Mentha x piperita* samples the found mean concentrations in sachets and natural sample are in agreement with Rubio *et al.*,⁴⁹ which found concentrations in mg kg^{-1} , of 17.63 ± 1.96 (Ba), 12.41 ± 0.87 (Cu), 1.34 ± 0.23 (Ni), and 27.05 ± 3.00 (Zn) in samples from Spain.

Evaluating the concentrations of the elements found in the samples related to the maximum limit established by Brazilian legislation, it was possible to notice that the concentration values of Ni in the samples PA, MR, CSb, CSw, ZO, MO natural, HS, CA, IP, and MP natural, exceeded the maximum allowed limit (5.0 mg kg^{-1}). For Cu and Zn concentrations, all samples were below the maximum limit allowed by legislation, being 30 and 50 mg kg^{-1} , respectively. However, for Ba and V, the evaluation was not possible since there are no maximum limits allowed in Brazilian legislation.

The factors responsible for the presence of the elements in medicinal herbs can be of natural origin: type of soil, climatic factors, among others; or anthropogenic origin: use of pesticides and fertilizers, water used in the irrigation, among others.⁵⁰ For example, in India, Priya *et al.*⁵¹ found nickel contamination in groundwater from textile mill effluent. Other studied regions in this work, where the water used for irrigation comes from Marcela Reservoir, Santos *et al.*,⁵² evaluated Cu, Ni and Zn in sediment core, and showed that Cu and Zn were derived from

anthropogenic sources, and Ni was of natural origin, however, Ni probably cause frequent effects in the biota of the reservoir. Thus, it is very complex to establish the exact origin of these found chemical elements, and there is no significant difference between the found values for tea samples in sachet and tea leaves *in natura*.

Multivariate data analysis

Principal component analysis

The PCA was performed based on scores graphical analysis (represented by the objects = medicinal herbs) and loadings (represented by variables = analyte concentrations), being applied with purpose of evaluating grouping trends among the medicinal herb samples. The first three principal components (PCs) described 93.89% of the total data variance. In PC1 was explained 63.74%, PC2 described 16.32%, and PC3 dominated 13.83% of the data variability. The values of loadings for variables in the first three principal components and variance explained by each component are shown in Table 4.

Table 4. Values of loadings for variables for the first three PCs

Variable	PC1	PC2	PC3
Ba	0.525	0.846	0.069
Cu	-0.736	0.067	0.641
Ni	0.801	-0.256	0.460
V	0.933	-0.035	0.212
Zn	-0.926	0.169	0.141
Total variance / %	63.74	16.32	13.83
Cumulative variance / %	63.74	80.06	93.89

The PC1 was influenced by Ba, Cu, Ni, V, and Zn concentrations, with Cu and Zn contributing with negative loadings and Ba, V, and Zn with positive loadings. In PC2, there is the influence of Ba, with positive loadings, and in the PC3, Cu influenced negatively.

Figures 1a and 1b show the scores and loadings graphics of PC2 versus PC1 for medicinal herb samples and chemical elements, being possible to observe a tendency in the formation of two groups. Group I composed by samples that obtained the highest concentrations of Cu and Zn, and group II with highest concentrations of Ba, Ni and V, formed by samples of MR 1, MR 2, MR natural, HS 1, HS natural, ZO, CA, IP, CS 1, CS 2, CS natural, MO 3, and MI. The MO 3 sample distanced itself from the other samples of MO because it presented higher levels of Ni, V and Zn, probably because of the production process, since it is a sachet sample, or soil contamination.^{53,54}

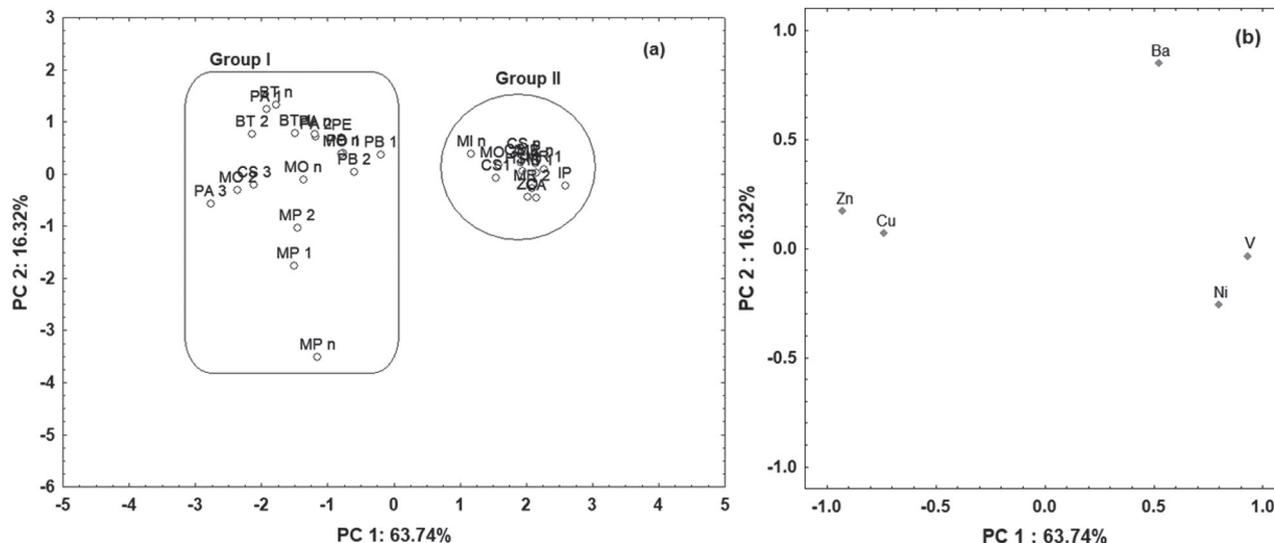


Figure 1. Scores and loadings graphs of PC1 versus PC2 for the samples (a) (MR: *Matricaria recutita*, HS: *Hibiscus sabdariffa*, ZO: *Zingiber officinale*, CA: *Cassia angustifolia*, IP: *Ilex paraguariensis*, CS: *Camellia sinensis*, MO: *Melissa officinalis*, MI: *Maytenus ilicifolia*, PB: *Peumus boldus*, PE: *Passiflora edulis*, MP: *Mentha x piperita*, BT: *Baccharis trimera*, PA: *Pimpinella anisum*), and for the elements (b).

Figures 2a and 2b show the scores and loadings graphics of PC3 versus PC1. It is possible to observe that there are two subgroups in group I, subgroup I formed by samples of PB 1, PB 2, PB natural, PE, MO 1, and MP 1, MP 2, MP natural, and the subgroup II formed by samples BT 1, BT 2, BT natural, PA 1, PA 2, PA 3, PA natural, MO 2, MO natural, and CS 3. The sample CS 3 distanced itself from the other samples of *Camellia sinensis* because it presented higher concentrations of Cu and Zn, probably because of the production process, since it is a sachet sample, or soil contamination.^{53,54}

Hierarchical cluster analysis

Hierarchical cluster analysis (HCA) was applied to standardized data using the Euclidian distance and Ward's linkage method, to verify the existence of similarities between samples. In Figure 3, there is the dendrogram obtained for the 31 medicinal herb samples, evidencing what was observed in PCA, where there was a trend of formation of two groups, being group I tended to subdivide into two subgroups, the subgroup I, formed by PB 1, PB 2, PB n, PE, MO 1, MP 1, MP 2 samples, and MP n, and the subgroup II, formed by the BT 1, BT 2, BT n, PA 1, PA 2, PA 3, PA n, MO 2, MO n, and CS 3 samples.

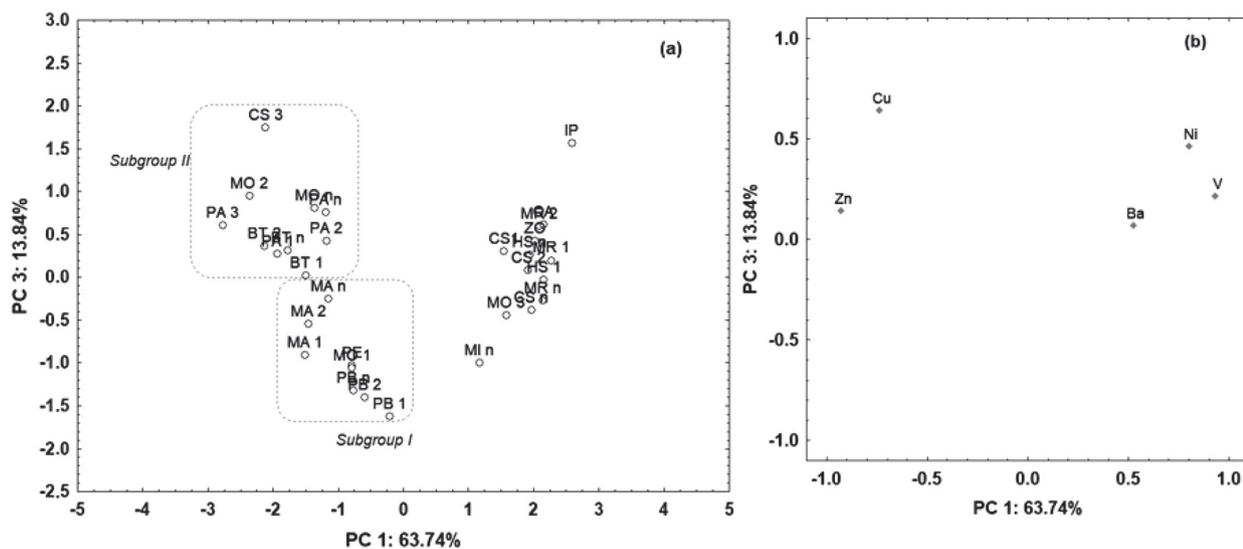


Figure 2. Scores and loadings graphs of PC1 versus PC3 for the samples (a) (MR: *Matricaria recutita*, HS: *Hibiscus sabdariffa*, ZO: *Zingiber officinale*, CA: *Cassia angustifolia*, IP: *Ilex paraguariensis*, CS: *Camellia sinensis*, MO: *Melissa officinalis*, MI: *Maytenus ilicifolia*, PB: *Peumus boldus*, PE: *Passiflora edulis*, MP: *Mentha x piperita*, BT: *Baccharis trimera*, PA: *Pimpinella anisum*), and for the elements (b).

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