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NUTRIÇÃO / NUTRITION

Multi-element analysis in aerials parts of *Verbena minutiflora* and infusions

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ABSTRACT. This study evaluated the mineral content in the infusions and aerial parts (leaves, flowers and stems) of Gervão (*Verbena minutiflora*) for four consecutive years using the atomic spectrometry. The different parts of air *V. minutiflora* showed significant variability between certain mineral levels. The leaves and flowers showed the highest concentrations of all minerals, except for K, regardless of year of collection: stem (Fe = 50; Ca = 20, Zn = 770; Mg = 7.5; Cu = 4450; Al = 110; Na = 350, K = 13520 mg kg^{-1}), flowers (Fe = 150, Ca = 33, Zn = 1.110, mg = 15, Cu = 7510, Al = 480; Na = 260, K = 10620 mg kg^{-1}), leaves (Fe = 340, Ca = 35; 1930 = Zn, mg = 20, Cu = 12600; Al = 1.160; Na = 420, K = 12180 mg Kg^{-1}). Notably, the concentrations of toxic elements (Pb, Cd and Cr) were below the limits of detection and quantification of the analytical technique in any shoot of Verbena. Minerals determined in aqueous infusions showed proportions ranging from 1.7 to 57.1% of the total value of minerals found in the leaves of Verbena.

Keywords: medicinal plants, mineral levels, gervão, verbenaceae, atomic absorption.

Análise multi-elementar das partes aéreas da *Verbena minutiflora* e infusões

RESUMO. Este estudo objetivou determinar o teor de minerais nas infusões e partes aéreas (folhas, flores e caules) do gervão (*Verbena minutiflora)* durante quatro anos consecutivos usando a espectrometria atômica. As diferentes partes aéreas da *V. minutiflora* apresentaram variabilidade significativa entre os teores de minerais determinados. As folhas e flores apresentaram as maiores concentrações de todos os elementos avaliados, exceto para o K, independentemente do ano de coleta: caule (Fe = 50; Ca = 20; Zn = 770; Mg = 7,5; Cu = 4450; Al = 110; Na = 350; K = 13520 mg kg^{-1}), flores (Fe = 150; Ca = 33; Zn = 1,110; Mg = 15; Cu = 7510; Al = 480; Na = 260; K = 10620 mg kg^{-1}), folhas (Fe = 340; Ca = 35; Zn = 1930; Mg = 20; Cu = 12600; Al = 1,160; Na = 420; K = 12180 mg Kg^{-1}). Notavelmente, as concentrações dos elementos tóxicos (Pb, Cd e Cr) ficaram abaixo dos limites de detecção e quantificação da técnica analítica, em qualquer parte aérea da *Verbena*. Os minerais determinados nas infusões aquosas apresentaram proporções que variaram de 1,7 a 57,1% do valor total de minerais encontrados nas folhas da referida espécie de *Verbena*.

Palavras-chave: plantas medicinais, teores minerais, gervão, verbenaceae, absorção atômica.

Introduction

Medicinal plants had a key role in worldwide health system by the reason that they serve as a starting point for the development and advancement of modern drugs (Hosseinzadeh, Jafarikukhdan, Hosseini, & Armand, 2015; Shakya, 2016). The use of plants, parts of plants and isolated phytochemicals for the prevention and treatment of various health ailments has been in practice from time immemorial. It is estimated that about 25% of the drugs prescribed worldwide are derived from plants and 121 such active compounds are in use. Of the total 252 drugs in WHO's essential medicine list, 11% is exclusively of plant origin (Sahoo, Manchikanti, & Dey, 2010). The human body requires a number of minerals in order to maintain good health (Gupta & Gupta, 2014). The analysis of the levels of minerals in plants is important because they are essential in the human diet, however, there is often an inadequate intake of these nutritional components (Rehecho et al., 2011). Present in medicinal plants, trace elements play a significant role in the formation of active chemicals such as Zn, Cu and Fe constituents and only become toxic at high concentrations, while others such as Pb and Cd are considered dangerous to the body while in low concentrations (Abugassa et al., 2008; Nookabkaew, Rangkadilok, & Satayavivad, 2006).

Recently, plant species have been identified that contain nutrients displaying new beneficial medicinal or therapeutic properties trace elements have both a curative and a preventive role in combating diseases (Fernandes, Casal, Pereira, Saraiva, & Ramalhosa, 2017). It is therefore of major interest to establish the levels of some metallic elements in common herbal plants because, at elevated levels, these metals can also be dangerous and toxic (Nischwitz et al., 2017). Tea is the most widely consumed drink after water, due to its refreshing and mildly stimulant effects and plays a major role in the intake of a number of nutritional and toxic trace elements in humans (Malik, Szakova, Drabek, Balik, & Kokoska, 2008). Tea drinking is associated with the reduction of serum cholesterol, prevention of low density lipoprotein oxidation, decreased risk of cardiovascular disease and cancer. The regular consumption of tea can contribute to the daily dietary requirements of some of these elements (Karak & Bhagat, 2010).

The Verbenaceae family comprises about 32 genera and 840 species in the floras of North and South America, especially in tropical and subtropical region (O´Leary et al., 2012). In Brazil, thirty-one species were found, of which eleven are endemic of south region of country (O´Leary & Thode, 2016). They used in folk medicine for the treatment of several pathologies (Calvo, 2006). Several species of the genus Verbena are traditionally used in many countries to treat fever, diarrhea, gastrointestinal disorders, diuretic, expectorant, anti-rheumatic and anti-inflammatory topical applications such as sexually transmitted diseases (Calvo, 2006). The Verbenaceae is popularly known as Gervão, Gervão-roxo, Rinchão, Gervão do campo, and Vassourinha de botão; and is frequently used as infusion (tea) in the treatment of various diseases (Schapoval et al., 1998). However, at present few studies have been found on chemical and pharmacological validation of this plant (Soares et al., 2016; Peloi, Bovo, Messias-Reason, & Perez, 2016).

The mineral contents of some herbal teas have been determined in several previous publications (Gallaher, Gallaher, Marshall, & Marshall, 2006; Nookabkaew et al., 2006; Özcan & Akbulut, 2008; Özcan, Ünver, Uçar, & Arslan, 2008). These studies usually used univariate methods such as analysis of variance (ANOVA), compared with the concentration of one element with another or one sample with another. However, multivariate methods such as principal component analysis (PCA) can provide further interpretation. PCA is a data reduction technique that aims to explain most of the variance in the data whilst reducing the number of variables to a few uncorrelated components (Kara, 2009). This method enables us to identify groups of variables or individuals. PCA is used to identify groups of variables, based on the loadings, i.e. correlations between the variables and the principal components, and groups of individuals based on the principal component scores. Generally the output of a PCA package is a graph which are called "scores" (equivalent to the variables) that are estimated in bilinear modeling methods where information carried by several variables is concentrated onto a few underlying variables. Each sample has a score along each model component. The scores show the locations of the samples along each model component, and can be used to detect sample patterns, groupings, similarities or differences. One of the other graphs produced using PCA are called "loadings" that are estimated in bilinear modeling methods where information carried by several variables is concentrated onto a few components. Each variable has a loading along each model component. The loadings show how well a variable is taken into account by the model components. They can be used to understand how much each variable contributes to the meaningful variation in the data, and to interpret variable relationships (Mingoti, 2005; Johnson & Wichern, 1999). They are also useful for interpreting the meaning of each model component. Principal component analysis was used to evaluate teas (collected from different parts of the world and their metal contents (Kara, 2009).

The aim of this study was to evaluate the mineral content in aerial parts of *V. minutiflora* and infusions and monitor minerals variability over consecutive years, in order to determine the contribution of infusions from aerial part to the human diet. Consequently, metal ions, both essential and non-essential to the human organism (Cu, Fe, Zn, Mg, Na, K, Al, Ca, Pb, Cd and Cr), were quantified in aerial parts of *V. minutiflora* and its infusions. The analysis was performed using flame atomic absorption spectrometry and atomic emission spectrometry. Data were statistically analyzed by a two way Analysis of Variance (ANOVA) and by Principal component analysis (PCA) to evaluate possible correlations among metallic ions, year of collection and plant parts, as well as, similarities among the samples.

Material and methods

Samples and reagents

The plant *V. minutiflora* (Figure 1) was collected in Guarapuava, Paraná State, Brazil in the same geographic area (25º 23'08.57" S, 51º 26'50. 63" W; altitude 1115 m) and over four consecutive years since 2009 to 2012. Wild vegetable samples were collected in November, during spring season, when the plant has plenty on flowers and leaves. *V. minutiflora* was identified and classified by the Herbarium of the Botanical Museum Hall of Curitiba, Paraná State, Brazil (Registration No. 359683).

Figure 1. Aerial parts of *V. minutiflora,* (A) dried vegetable specimen, (B) flower, (C) leaf . (Source: Kelly C. N. Soares)

The incidence of solar radiation and precipitation of collection site ranged from 11.918 - 21.093 cal/cm2/d and 84.3 - 152.2 mm, respectively, between the years 2009-2012. The climatic and meteorological data were supplied by Agronomic Institute of Paraná (IAPAR) from Guarapuava, Paraná State, Brazil. The vegetable material was dried at room temperature in the shadow. After that, the aerial parts were separated and stored in a dry, dark and cooled room. All reagents used in the spectrometric analyses were of analytic grade. All standards used for the analyses of metal ions by flame atomic absorption spectrometer - FAAS and atomic emission spectrometer - ICP-OES were obtained from J.T. Baker Instra-Analysed® (1000 μg mL^{-1}). Ultrapure water from a TKA Gen-Pure system (German) was used to prepare all solutions.

Instrumental

Determinations of Fe, Ca, Mg, Cu, Zn, Al, Na and K were performed using a FAAS (Varian, AA 220), equipped with Varian brand hollow cathode lamps. Determinations of Cd, Pb, Cr were performed using an ICP-OES (PerkinElmer, Optima 8000 DV).

Determination of the concentration of metallic ions

Minerals were separately determined in leaves, flowers and stems of *V. minutiflora* by dry digestion. The samples used in the determinations were *V. minutiflora* samples collected in the years 2009, 2010, 2011 and 2012 respectively. To determine the total concentration of metals in the plants digestion procedure dry method, calcination was used. It was weighed between 0.5 and 1.0 g of each sample (leaves, stems and flowers) in preweighed crucibles, is carbonized in a bunsen burner until the complete release of fumes was taken after calcination in a muffle furnace for 4h at 550°C. After cooled in a desiccator, it was weighed ash, solubilized in 3 mL of the solution of nitric acid (HNO3) 1: 3 (v /v), transferred to a 50 mL flask and completed with deionized water. Additionally, infusions were prepared from 10 g of manually cut leaves using 200 mL of boiling deionized water. After 20 min. the infusion was filtered, frozen and lyophilized. The analytical procedure is summarized in Figure 2.

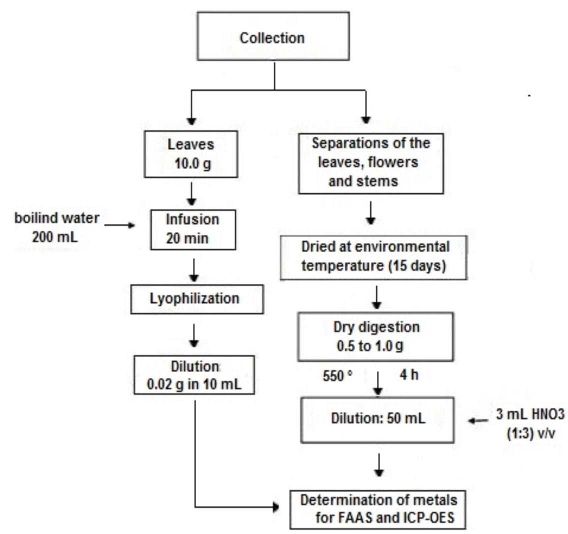

Figure 2. Analytical procedure for the preparation of plant samples.

Statistical analyses

The differences between experimental groups were compared by ANOVA followed by Fisher and Tukey tests ($p < 0.05$). A two way ANOVA was carried out to evaluate first and second-order effects of the factors aerial parts and year of collection on the mineral content of *V. minutiflora*. The statistical tool used was principal components analysis (PCA). PCA allow the evaluation of the data set, reducing its dimension, conserving most of the useful statistical information present in the original data. The data can be the original (scaled or centered on the mean) or the scores generated by the PCA. The statistical operations were performed using the MATLAB program, version 6.0.

Results and discussion

Metal ions in aerials parts and infusions of *Verbena minutiflora*

Total content of 11 elements (Cu, Fe, Zn, Mg, Na, K, Al, Ca, Pb, Cd and Cr) was determined in both, infusion of plant and dry matter (leaves, flowers, steams). The results are given in Table 1. Element concentrations in rude material were expressed in mg kg^{-1} of dry weight. Pb, Cd and Cr were below the limit of detection in all samples.

The most abundant elements in *V. minutiflora* collected between the years 2009 to 2012 showed the following descending order of concentration: Ca > K > Mg > Al > Na > Fe > Zn > Cu. The leaves had a higher contents of Ca, Mg, Al, Zn and Cu in the plant, reaching reasons leaf/stem of the order of 1.7 (Zn) to 10.5 (Al), followed by flowers and stems. The presence of higher concentration of elements in the leaves is important because it is the part of the plant most commonly consumed by population in the preparation of tea (infusion), being an important source of food supplement. For the Na and K, it was found that the magnitude of these elements in the aerial parts of the plant were not significant, since they have high mobility, since it does not bind to organic chelates. The high Ca content found in the samples demonstrated that *V. minutiflora* may be considered appropriate for maintaining biological function in mediating the vascular contraction supply in nerve transmission and muscle contraction (Abugassa et al., 2008). The ratio K/Na was 33.4 (leaves), indicating major advantage of the nutritional point of view, since the higher intake of K relative to Na has been related to a lower incidence of hypertension (Abugassa et al., 2008). The high Al content in the leaves (1160 mg kg^{-1}) was not surprising, since the plants are normally known to be accumulating Al from soil (Mehra and Baker, 2007, Ducat et al., 2011). The main consideration related to Al and health is its potential toxicity when exposure is excessive. Al interacts with a number of other elements, including Ca, Na, Fe, Mg, P and Sn, and when it ingested in excess, can reduce the absorption of essential elements (Molloy et al., 2007). The human body needs a number of minerals in order to maintain good health, macro and micro-elements that influence biochemical processes in the human organism. For example calcium and magnesium are the major mineral components of bones, they are necessary for normal growth and physiological function (Arceusz, Radecka, & Wesolowski, 2010). Concentrations of metallic ions in infusions are given in Table 1. These results show that solubility of metals in infusion were highly significant. Average extraction rates were highest for the Na (57.1%), followed by Mg (47.6%), Ca (36.2%), Zn (32.8%), Cu (10%), Al (8.6%), Fe (3.8%) and K (1.7%). These results exhibited that percentage of elements that was transferred into the tea liquor varied widely for the different metals ions in the Verbena. Under the Prevention of Food Adulteration Act (PFA) of India the tolerance limits have been fixed only for Cu and Pb. It was evident from this study the average Cu content in all tea samples was around 2 mg kg^{-1}, which is below the maximum allowed limit of 150 mg kg^{-1} established by PFA (Seenivasan, Manikandan, Muraleedharan, & Selvasundaram, 2008). The percentage of Al transferred to the infusion was 8.6 %, which can be a cause of concern in terms of metal intake from drinking tea. In our sample, the average content of Al in tea infusion was 10 mg kg^{-1}. Several studies demonstrated, in spite of the high level of Al in tea samples, bioavailability of Al is very low, probably because it is linked to phenolic compounds (Powell, Greenfield, Parkes, & Thompson, 1993). Made the speciation of Al in infusions of black tea and green tea and did not detect species of free Al^{3+}. Despite the low content of the element in teas, it is not worthy that the ions present in the infusions can be quickly absorbed by the body because they are more soluble in water (Street, Drabek, Szakova, & Mladkova, 2007). It is essential to have good quality control of plant raw materials and to determine the presence of some contaminants, especially toxic elements to avoid over consumption and their cumulative toxicities. Table 2 present the calculated results compared to the average daily dietary intakes of each element (Powell et al., 1993). The daily intake of all these elements in the infusions are below the average recommended daily intake.

Table 1. Statistics of average concentrations (mg Kg^{-1}) of metals in the *V. minutiflora* samples examined in this study (n = 3; referring to four collections: 2009-2012).

V. minutiflora mg Kg^{-1}	Statistics	Fe	Zn	Mg	Cu	Ca	Al	Na	K
Stems	mean	50	20	770	7.5	4450	110	350	13520
	minimum	37	16	576	4	3739	90	233	12840
	maximum	56	26	1016	10	5158	140	583	14390
Flowers	mean	150	33	1110	15	7510	480	260	10620
	minimum	110	31	908	13	6716	371	232	9547
	maximum	192	38	1342	18	8247	595	364	12830
Leaves	mean	340	35	1930	20	12600	1160	420	12180
	minimum	264	31	1197	19	10520	1078	321	11230
	maximum	363	38	2522	25	13640	12380	513	13960
Infusion (leaves)	mean	13	11	920	2	4570	10	240	210
	minimum	1	10	727	1.8	3600	7	200	157
	maximum	21	15	1075	3.5	5258	17	283	283
Extraction %		3.8	32.8	47.6	10.0	36.2	8.6	57.1	1.7

Table 2. Daily intake of elements through tea infusion (leaves) of *V. minutiflora* (three cups a day with one packet of 2 g each).

Element	[mg] 100 mL^{-1}	Daily intake with infusion [mg day^{-1}]	Dietary reference (Powell et al., 1993)
Fe	0.026	0.078	15 (10-18)
Zn	0.023	0.069	15
Mg	1.84	5.52	330-350
Cu	0.004	0.012	2.5
Ca	9.14	27.42	1000-1200
Al	0.02	0.06	5
Na	0.48	1.44	1200-1500
K	0.42	1.26	4700

Statistical analysis

Possible correlations among minerals were investigated and significant ($p < 0.05$) Pearson positive correlations were observed among all of them (Table 3). Results indicated that Fe content showed a correlation extremely high with Mg (r = 0.97), Cu (r = 0.94), Ca (r = 0.99) and Al (r = 1.00); Zn with Cu (r = 0.98); Mg with Ca (r = 0.99) and Al (r = 0.97); Cu with Al (r = 0.94) and Ca with Al (r = 0.99).

Table 3. Correlation matrix (Pearson) between element concentrations in leaves of the *V. minutiflora* (figures in bold indicate significant strong correlations between two metals).

	Fe	Zn	Mg	Cu	Ca	Al	Na	K
Fe	1							
Zn	0,87	1						
Mg	0,97	0,72	1					
Cu	0,94	0,98	0,83	1				
Ca	0,99	0,83	0,99	0,91	1			
Al	1	0,86	0,97	0,94	0,99	1		
Na	0,73	0,52	0,68	0,64	0,68	0,72	1	
K	0,51	0,7	0,29	0,68	0,41	0,49	0,69	1

For statistical analysis the parameters analyzed was aerial parts of the plant, year of collection and The two way ANOVA demonstrated the specific aerial part used to quantify metals was the most statistically significant first-order effect to affect the amount of all minerals. Leaves, followed by flowers, had the highest average content of all elements regardless the year of collection. Only the average content of K was higher in stems. The year of collection also had a statistically significant effect on the content of Fe, Zn, Cu, Mg, Al, K and Na, although in a lesser extension than the type of aerial parts. Second order interaction effects were also observed between the type of aerial parts and year of collection for all metals with exception of Cu, indicating that the sources of metal variability could not be analyzed independently.

Chemometric study

Principal components analysis (PCA) was applied to the schedule experimental data of the levels of metal ions from the *V. minutiflora* to verify possible similarities and differences among the samples and their correlation with the variables. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on. After development of the PCA, the first and second principal components captured 75.50 and 12.36% of the variance of the experimental data, respectively, together totaling 87.86% of variance captured. The first and second principal components (Figure 3 A) separated samples of plant parts, and the leaves and flowers were placed in the negative quadrant of the x-axis (PCA factor 1), indicating a strong correlation with all the metallic ions. While the stems and infusions showed low concentrations (positive quadrant of the x-axis). The concentrations of metals ions didn´t varied with year of collection of the plant.

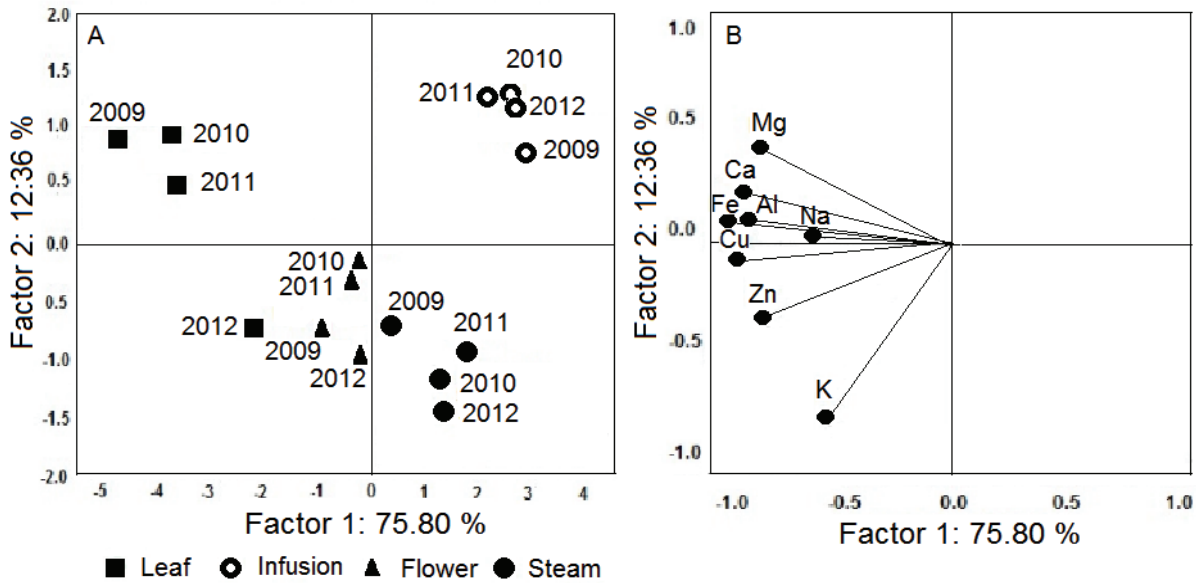

Figure 3. Scores of samples (A) and loadings of variables (B) on the plane defined by the principal components of PCA analysis for metal concentrations in parts of the plant.

Conclusion

The most abundant elements in *V. minutiflora* were K followed by Ca, Mg and Na. It was found that considering only the aerial parts of the plant, the leaves had the highest concentration of inorganic elements followed by the flower and stem. Statistical data (ANOVA) indicated that parts of the plant are evaluated by the main source of variation in the mineral content. The year of collection, as well as the interaction between the factors year of collection and parts of the plant was less mean to explain the variability in the chemical composition of the plant.

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