

Multivariate Characterization of “Argentinean Mistletoe”, *Ligaria cuneifolia* (Loranthaceae) According to Their Mineral Nutrient Composition

[Caracterización multivariable de “muérdago criollo”, *Ligaria cuneifolia* (Loranthaceae) de acuerdo a su composición mineral]

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Abstract

The aim of this study was to characterize samples of *Ligaria cuneifolia* collected from three areas of the north-east region of Argentina, during two sampling periods. In this work, the authors propose a mathematical model for searching associations among mineral contents and other factors such as geographic origin or sampling period. Mineral monitoring as a pattern recognition method is a promising tool in the characterization and/or standardization of phytomedicines. In the present work measurable amounts of Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr, and Zn were detected in phytomedicinal samples of *L. cuneifolia* by inductively coupled plasma optical emission spectrometry (ICP-OES). Finally, this methodology allows reliable determinations of mineral content in pharmaceutical quality control of medicinal plants.

Keywords: *Ligaria cuneifolia*, mineral composition; ICP-OES; Principal component analysis; Pattern recognition

Resumen

El objetivo de este estudio fue caracterizar muestras de *Ligaria cuneifolia* recolectadas en tres zonas de la región noreste de Argentina, durante dos períodos de muestreo. En este trabajo los autores proponen un modelo matemático para la búsqueda de asociaciones entre el contenido mineral y otros factores como el origen geográfico o el período de muestreo. El monitoreo de las concentraciones de los elementos minerales, como método para el reconocimiento de patrones, es una herramienta prometedora en la caracterización y / o estandarización de fitofármacos. En el presente trabajo se pudieron detectar cantidades medibles de Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr y Zn que fueron detectadas en muestras fitomedicinales de *L. cuneifolia* por espectroscopía de emisión óptica de plasma acoplado inductivamente (ICP-OES). Finalmente, esta metodología permitió realizar determinaciones confiables del contenido mineral en el control de calidad farmacéutica de plantas medicinales.

Palabras Clave: *Ligaria cuneifolia*; composición mineral; ICP-OES; Análisis de componentes principales; Reconocimiento de patrones.

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List of abbreviations: ICP-OES - Inductively coupled plasma optical emission spectrometry; PCA – Principal component analysis; PC – Principal component

INTRODUCTION

Ligaria cuneifolia (R. et P.) Tiegh. is an epiphytic, hemiparasitic southern South American subshrub popularly named “liga” or “liga roja” (Del Vitto *et al.*, 1997; Fernández *et al.*, 1998) (also “Argentinean mistletoe”), that belongs to the Loranthaceae family. It is widely used in Argentinean folk medicine because of its cardiovascular effects (antihypertensive) (Cortella and Pochettino, 2011), as in the case (or in substitution) of “European mistletoe” (*Viscum album* L., Viscaceae) (Varela and Gurni, 1995; Fusco *et al.*, 2003; Fusco *et al.*, 2004). It grows on woody stems of a lot of native and exotic trees and shrubs, from Ulmaceae to Asteraceae, but predominantly on tree Fabaceae. This hemiparasitic specie, take advantage of the xylem flow of their host, extracting water and minerals through haustoria (modified root structures) that provide vascular continuity from parasite to hosts (Rustán *et al.*, 2003). However, they are photosynthetic organisms, producing their own nutritive carbohydrates.

Like others herbal medicines, plant material from *L. cuneifolia* is a complex system of mixtures. The quality of the raw drugs depends on many factors that influence on the content of chemical constituent, which affects the features of the final product. The factors that could influence this quality could be of the different nature (genetic, seasonal, geographical, nutritional, manufacturing procesess, etc.). So, it is therefore essential to set up internationally recognized guidelines, to improve the control trade in medicinal plants and their products, by modern and suitable technologies. Currently, the methods of choice for identification of ‘botanical drug’ are mainly intended to get both morphoanatomical and chemotaxonomical characteristics of the genuine plant (Applequist, 2006; Ahmad *et al.*, 2010), as well as a characteristic fingerprint of quality defining chemical constituents, i.e. bioactive markers (Huang *et al.*, 2004).

In this context, the nutritional and medicinal properties of *L. cuneifolia* may be inter-link through phytochemicals, both nutrient and non-nutrient. Several studies have been carried out on edible wild plants. But, limited studies were carried out on mineral composition of medicinal and aromatic plants growing

in Argentina. Therefore, mineral element contents in botanical samples could give a signal of environmental pollution and herewith also a mark of the geographical origin. Besides, there are no reports about the major, minor and, trace elements in *L. cuneifolia*, although such knowledge is significant considering the wide use of this specie in South America.

The aim of this study was to characterize samples of *L. cuneifolia* collected from three areas of the north-east region of Argentina, searching associations among chemical variables and other factors such us geographic origin or sampling period. Thus, we evaluated several 14 mineral element contents. Besides, the potential relationship between the complex chemical constituent, the contents of some essential elements were also studied.

MATERIALS AND METHODS

Sample collection

Aerial parts of *L. cuneifolia* were collected from three cities/towns spread over two provinces (Chaco and Corrientes) on the northeastern region of Argentina. Samples were identified according to its procedence as follow: CTE (Corrientes, Capital zone); SP (Chaco, Saenz-Peña zone) and CH (Chaco, Resistencia zone). Sampling periods were also coded as S (September 2010 to December 2010) and A (December 2010 to March 2011). On average, 50 g of plant material was collected from the very bottom to the top of the shrub, mostly from the residential areas, and kept in polyethylene bags. These were first wiped with tissue paper to remove any dirt and then thoroughly washed with distilled water to avoid any surface contamination. All the specimens obtained came from subshrubs grown on cultivated host *Ulmus pumila* L., “Siberian elm” (Ulmaceae), and were authenticated by herbarium samples deposited at the Herbarium of the Universidad Nacional de San Luis (acronym: UNSL; voucher specimens: L.A. Del Vitto #9245-b, #9245-c, #9245-d).

Sample preparation

All samples of crude drug were dried in a heater of forced air at 40° C until reach to the stage of hygroscopic moisture. Later were milled with a Wiley mill series 3379 with a stainless steel container, and a sieve up to 0.50 mm diameter; for each sample was followed this procedure: 0,5 g of plant material was put in a porcelain crucible, loosely covered with a lid, and carbonized during 1 h by gentle ignition at 500° C;

after cooled, was added 15 ml of HCl, 10 ml of HNO₃, and 5 ml of HClO₄; leading up to a final volume of 50 ml, and shaking strongly.

Analytical procedures

The concentrations of 14 selected minerals (Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr and Zn) were determined using an ICP-OES spectrometer (Varian Vista-PRO[®]), with a Czerny-Turner monochromator, holographic diffraction grid and a VistaChip charge coupled device (CCD) array detector. The wavelength calibration was periodic and automatic (based on Argon and emission lines). Calibration curves for each element were constructed in triplicate using five different concentrations. The regression coefficient (*r*²) values of such curves ranged from 0.995 to 0.999.

Statistical analysis

Principal Component Analysis.

PCA was used to achieve a reduction of dimension and to observe a primary evaluation of the between-class similarity. PCA is a projection method that allows an easy visualization of all the information contained in a data set. In addition, PCA helps to find out differences of one sample with respect from another and which variables contribute mostly to these disparities. This chemometric tool is used to observe similarities among different plant material samples, reducing the dimension of the original variables at only two or three principal components, keeping most information of the original data set. Finally, it quantifies the amount of useful information, as opposed to noise or meaningless variation, contained in the data set. One important step on the application of PCA is the previous preparation of the original data. Generally, in a raw chemical composition dataset, it is possible that individual data points are missing or below a detection threshold, so that the dataset is not complete. One way to cope with this problem is to fill in missing data with the mean of the other measurements for the variable in question. As a rule of thumb, this procedure can be used if < 30% of the data are missing for a variable; otherwise, that variable should be removed from the dataset. All each variable in the original dataset is often autoscaled by subtracting its mean and dividing by its standard deviation. This process allows avoiding problems with dimensionality of raw data. The new series of generated autoscaled data has zero mean and unit standard deviation. It should be noted that the

principal disadvantage of standardization pretreatment may amplify noise associated with minor variables that may have relatively larger analytical error.

All data analyses were performed using R software version 2.14.1 (R_Development_Core_Team, 2012) with ade4 package for PCA (Thioulouse and Dray, 2007).

RESULTS AND DISCUSSION

Mineral contents of 27 *L. cuneifolia* samples obtained from three regions of Argentina were achieved using ICP-OES. Fourteen minerals were determined in all samples. The average results and standard deviations of three replicate analyses of macro-mineral and microelements are shown in Table 1 and 2 respectively. Samples are grouped according to its geographic origin and sampling period.

Published data on chemical composition of *L. cuneifolia* are limited, and in the few available studies we could not find reported data with regard to mineral composition. In general, the mineral contents of the botanical material that we studied were similar to the literature values published for other medicinal herbs from Argentina (Cantarelli *et al.*, 2010; Petenatti *et al.*, 2011) or worldwide (Başgel and Erdemoğlu, 2006; Kalny *et al.*, 2007; Musa Özcan *et al.*, 2008; Karadaş and Kara, 2012). Some discrepancies may be due to the differences in the species, locations, soil properties, harvesting times, geographic parameters and analytical processes. In this work, the mineral contents were determined to vary widely depending on the geographic origin and sampling period.

From the point of view of macro-minerals, similar mineral abundance profiles were observed among samples under study. Ca, K and P contents of samples in this study were established higher than those of other minerals. Al, Na and Sr, were present at levels under 5 mg g⁻¹. In the same manner, Mg is not only essential, but it is also a constitutive element of chlorophyll. The Mg content of the samples ranged from 10.07 to 11.92 mg g⁻¹.

For all the determined microelements, the highest concentration was found for iron. The content of this element in plant material was in the range of 135.5–289.5 mg kg⁻¹. Fe concentration found for all of the herbs was higher than those reported by Łozak *et al.*, (2002) who determined 239 and 107 mg kg⁻¹ Fe in mint and nettle raw materials, respectively (Łozak *et al.*, 2002).

Table 1
Concentrations (mg g⁻¹ dry weight basis) of the macro-minerals analyzed in *Ligaria cuneifolia*, according to the geographic origin and sampling period.

Element	Sampling period	Origin		
		CTE	CH	SP
Al	Set10 – Dec10 (S)	1.42 ± 0.53	1.48 ± 0.49	0.82 ± 0.17
	Dec10 – Mar11 (A)	1.12 ± 0.70	1.02 ± 0.27	0.87 ± 0.39
Ca	Set10 – Dec10 (S)	118.50 ± 1.72	119.52 ± 3.22	116.20 ± 7.65
	Dec10 – Mar11 (A)	97.18 ± 44.40	105.65 ± 31.90	106.75 ± 33.23
K	Set10 – Dec10 (S)	230.62 ± 22.24	231.35 ± 15.56	222.42 ± 22.72
	Dec10 – Mar11 (A)	176.92 ± 76.42	188.62 ± 59.54	212.68 ± 63.50
Mg	Set10 – Dec10 (S)	11.28 ± 1.05	11.03 ± 0.89	10.97 ± 1.20
	Dec10 – Mar11 (A)	10.07 ± 4.43	10.28 ± 2.97	11.92 ± 3.57
Na	Set10 – Dec10 (S)	1.98 ± 0.19	1.92 ± 0.22	1.87 ± 0.89
	Dec10 – Mar11 (A)	1.87 ± 0.90	1.83 ± 0.50	2.14 ± 0.66
P	Set10 – Dec10 (S)	25.32 ± 2.12	24.25 ± 1.92	25.84 ± 2.95
	Dec10 – Mar11 (A)	22.04 ± 0.97	22.13 ± 0.64	28.65 ± 0.87
Sr	Set10 – Dec10 (S)	1.24 ± 0.59	0.78 ± 0.53	1.10 ± 0.65
	Dec10 – Mar11 (A)	0.79 ± 0.25	0.32 ± 0.27	0.13 ± 0.12

Table 2
Concentrations (µg g⁻¹ dry weight basis) of the micro-minerals analyzed in *Ligaria cuneifolia*, according to the geographic origin and sampling period

Element	Sampling period	Origin		
		CTE	CH	SP
Co	Set10 – Dec10 (S)	1.75 ± 0.11	1.87 ± 0.44	1.70 ± 0.06
	Dec10 – Mar11 (A)	1.79 ± 0.80	1.78 ± 0.80	1.78 ± 0.82
Cr	Set10 – Dec10 (S)	2.98 ± 0.19	2.95 ± 0.25	2.74 ± 0.30
	Dec10 – Mar11 (A)	2.34 ± 1.05	3.30 ± 1.73	2.50 ± 1.10
Cu	Set10 – Dec10 (S)	35.32 ± 2.40	36.32 ± 1.60	35.30 ± 1.60
	Dec10 – Mar11 (A)	34.65 ± 18.10	37.90 ± 16.15	39.60 ± 16.65
Fe	Set10 – Dec10 (S)	1520 ± 905	1835 ± 250	1355 ± 678
	Dec10 – Mar11 (A)	1820 ± 985	1540 ± 820	2895 ± 703
Li	Set10 – Dec10 (S)	14.80 ± 7.00	9.30 ± 6.27	20.90 ± 9.45
	Dec10 – Mar11 (A)	15.28 ± 12.20	6.30 ± 6.10	4.80 ± 2.85
Mn	Set10 – Dec10 (S)	158.8 ± 8.7	155.10 ± 10.3	161.7 ± 14.4
	Dec10 – Mar11 (A)	139.0 ± 61.7	144.6 ± 42.9	190.3 ± 57.8
Zn	Set10 – Dec10 (S)	214.6 ± 14.9	211.6 ± 9.4	203.6 ± 12.2
	Dec10 – Mar11 (A)	171.5 ± 79.1	242.0 ± 125.1	193.2 ± 77.2

Co and Cr concentrations in the raw material indicated similar maximum levels (under 5.0 µg g⁻¹); however Cr level of all herbs are relatively higher than Co level. They were determined in the range of 2.34 - 3.30 µg g⁻¹ and 1.70 - 1.87 µg g⁻¹, respectively. On the other hand, both Cu and Zn are essential elements for plant growth and human nutrition. They were determined in the range of 34.6 - 39.6 µg g⁻¹ and 171.5 - 242.0 µg g⁻¹, respectively.

Another interesting element with beneficial pharmacological properties, detected on samples was Li. There is evidence to suggest that lithium is also an

essential element. Li content on samples ranged from 4.8 to 20.9 µg g⁻¹. These values are in accordance with the findings on mineral composition of five medicinal plants, reporting studies of samples from non-polluted areas in Argentina (Petenatti *et al.*, 2011). Finally, the Mn concentration fluctuated from 139 to 190 µg g⁻¹.

Statistical analysis

Each botanical sample (object) was considered an assembly of fourteen variables represented by the Al, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Na, P, Sr and Zn concentration in aerial parts. These variables formed a

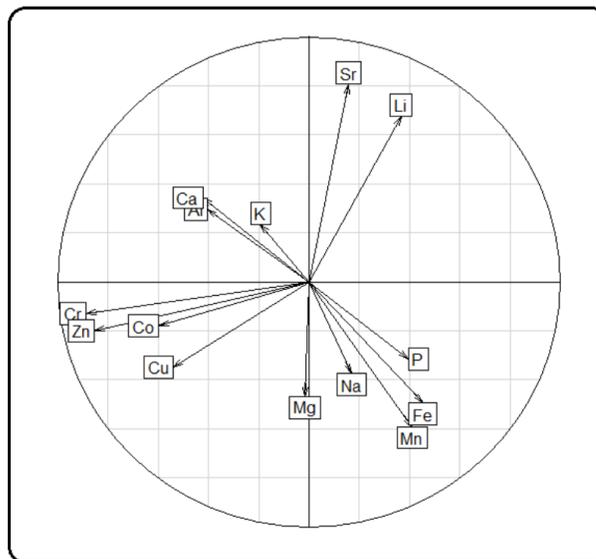
data vector which represented an herb sample. Data vectors belonging to the same group (geographic origin or sampling period) were analyzed. The group was termed a category. The results of analysis were arranged in a matrix of 27 rows and 14 columns (samples _ variables). Geographic origin were coded as CH (Chaco, Resistencia zone), CTE (Corrientes, Capital zone), and SP (Chaco, Saenz Peña zone); sampling periods were also coded as S (Set10 - Dec10) and A (Dec10 - Mar11).

A previous study of the means corresponding to each variable showed significant differences between geographic origin (except for K and Ca) and sampling period (except Zn); apparently, the data were appropriate to be used in a chemometric analysis. Macromineral concentrations were markedly greater than that of any other element. So, the data were autoscaled in order to achieve independence in the different scale factors of element concentrations. PCA was applied to this standardized matrix.

Then, on the basis of eigenvalues > 1 , PCA evolved three principle components explaining about 65.5% of the total variance. According to the eigenvalue criterion, only the PCs with eigenvalues greater than one are considered important. This criterion is based on the fact that the average eigenvalues of the autoscaled data is just one.

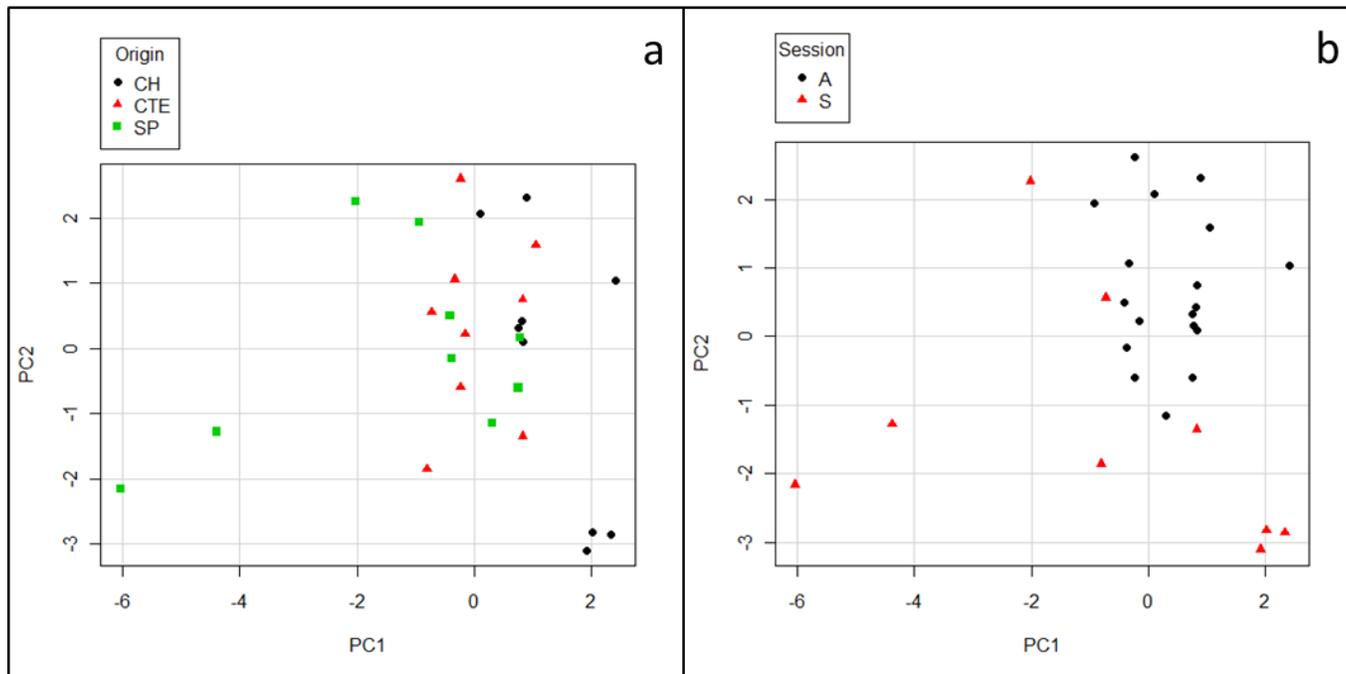
The first PC accounting for 32% of the total variance follow by the second PC accounting for 17% of total variance. The corresponding correlation coefficients of the original variables, standardized by the variance within groups are shown on Figure 1 represented in a graph commonly named 'correlation circle'. In this graph, the closer a variable is to the circle of correlations, the better we can reconstruct this variable from the represented components. Thus the most outstanding contribution to the first PC was obtained from Sr, Li and Mn whereas for the second, it was obtained from Cr, Zn and in minor proportion from Co and Cu. Applying these functions to the mineral composition of the different botanical samples, the corresponding scores of each function may be calculated. A plotting of them versus the first two PCs allows visualizing their ability to discriminate among the studied grouping factors.

Figure 1
Correlation circle of standardized variables included in principal component analysis.



The score plot (Figure 2a) for the first two principal components assumes a very widespread distribution values considering the geographic origin of samples as grouping factor. Visual inspection of the obtained plot indicates a poor separation between groups. However, the samples from CH region assumes high values on PC2 with regard to the other groups producing a slightly cluster on the right of the figure. On Figure 2b it could be watched the scores of samples classified by sampling period. Samples obtained on spring period (S: Set10 - Dec10) were characterized by negative values on PC1 (approximately from -1 to -3), however two samples were situated in the region of positive values of the same PC. Scores corresponding to summer period (A: Dec10 - Mar11) could be slightly grouped from the rest.

Figure 2
Score plot (two-dimensional) for minerals and trace elements analysed (PC1 vs. PC2) grouped by geographic origin (a) and by sampling session (b). PCA was calculated on 14 variables data matrix.



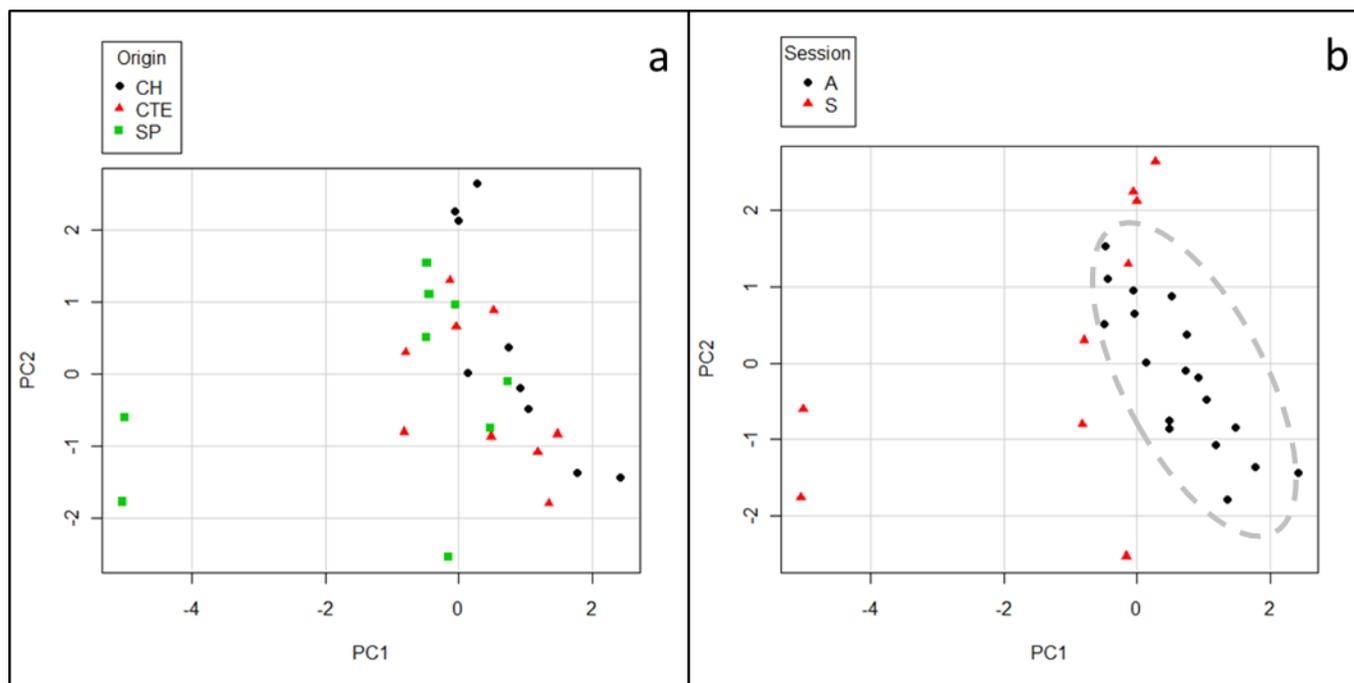
Then, a final model was obtained using as variables only six elements (Sr, Li, Mn, Cr, Zn and Co) present in the 27 samples, since their influence were higher than that of the other elements. Figure 3a shows the score plot obtained with geographic origin as grouping factor. It could be observed that not is possible limit zones that correspond exclusively to each geographical region; this fact could indicate that the mineral composition of the studied samples is poorly influenced by the geographic region of collection. However when the session sampling was consider as grouping factor (Figure 3b), samples collected on summer period (A: Dec10 - Mar11) form an interesting group, closed to the origin of coordinates of bi-dimensional plot.

CONCLUSIONS

This work provides the mineral composition (some elements commonly not included in the nutrition labeling) of a widely consumed medicinal herb in some countries of South America. There are many factors influencing the mineral and trace element concentrations, such as cultivar or sampling period. All these factors are acting at the same time, and therefore, it is difficult to come to definitive conclusions. The chemometric evaluation of the obtained data shows that there exist relationships between the analyzed factors. The results of this study clearly demonstrate the potentiality and versatility of these methodologies, which could be applied to routine monitoring metal content in phyto-pharmaceutical formulations. However, even if these results are quite encouraging, it must be pointed out that its future extensions require the construction of major databases to complete the information about.

Figure 3

Score plot (two-dimensional) for minerals and trace elements analysed (PC1 vs. PC2) grouped by geographic origin (a) and by sampling session (b). PCA was calculated on 6 selected variables (Sr, Li, Mn, Cr, Zn and Co) data matrix.



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