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Major and Trace Elements Contents in Crude Drug and Infusions of Two South American species of *Achyrocline* (Asteraceae) named "Marcelas"

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***SUMMARY*. Multielement analysis of crude drug and infusions from *Achyrocline satureioides* and *A. tomentosa* (Asteraceae), "marcelas", were carried out by ICP-OES, to know both mineral composition and safety. These plants are used as herbal remedies and extracts for bitter beverages in southern South America. Twenty seven major and trace elements were determined. Crude drugs contained valuable amounts of K, Ca, Mg, and P and essential trace elements (Fe, Mn, Zn, and Cu). Variability in the passage of minerals towards infusions was observed, too, and concentrations of K, Ca, P, and Mg were higher than those of Na, Mn, Zn, Cu, Al, Fe, Ni, Li, Hg and Mo, while other minerals were not detected. The infusions would be safe for human consumption because remain within the limits of the recommended daily intake or tolerable upper intake, and contribute to the daily intake in the case of some essential minerals.**

INTRODUCTION

Metals and their compounds as well as other mineral substances play a crucial role in the development of biologic systems [1] and in the environment as a whole [2]. Plants accumulate a lot of minerals that are considered essentials from the standpoint of its growth, both macronutrients and oligoelements, as well as other minerals (*e.g.* Se and Co) considered not essentials for all plants [3].

When plants or plant products prepared from them entering the human body as foods, beverages, medicines, nutraceuticals or in any other form, their mineral content can be useful or dangerous. Approximately 25 elements are essential in human nutrition [4,5] for the maintenance of human health, each one in an optimal degree of concentration, and their excess or poverty can induce symptoms or signs characteristics of intoxication or nutrient deficiency, respectively. Traces of Cd and Pb that has long detected in nearly all the plants and foodstuffs [6] can turn unsafe the use of some natural products [7-10], and in some cases has shown that process for the production of some beverages and licorice can increase the concentration of elements such as arsenic and copper at levels that often exceed those established by restrictive legislation [11]. Moreover, a strong interaction between minerals and organic compounds often cause a low bioavailability of the extractable forms of some minerals [12].

Many of the herbal remedies from traditional medicines are becoming more popular worldwide in the last decades, in despite to their poor regulations [13]. Some of these plants have importance as raw material for the pharmaceutical or alimentary industry, especially from the point of view of their mineral content. This is the case of two South American native plants that belong to the genus *Achyrocline* (*A. satureioides* and *A. tomentosa,* Family Asteraceae, Tribe Inuleae),

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widely spread in Argentina, Brazil, Bolivia, Uruguay and Paraguay [14]. They are commonly well-known as "marcelas" (Spanish) or "macelas" (Portuguese) [15], and have long been used in folk medicine in South America, mainly as a digestive, carminative and antispasmodic, both as herbal medicinal crude drugs or phytotherapic preparations, in infusions, tinctures and glycolic extracts [16]. From the point of view of their nourishing value, these species are widely used for the preparation of bitter traditional beverages, called "amargos" [15].

Achyrocline satureioides was included in lists of herbs approved for human use in Argentina, both as an herbal drug [17] and for bitter drinks in the Argentinean food code [18]. Also, it is an official drug in Brazil [19], and in Uruguay has been included in a permitted herbal list [20].

A. satureioides has been studied phytochemically; it is rich in polyphenols and flavonoids [21], lactones and polysaccharides [22], coumarine [23], dibenzofurans [24], and essential oils [25]. Some of its popular uses have been validated by *in vivo* and *in vitro* pharmacological studies: cytoprotection against the oxidative stress [26-28]; hepatoprotection [29] increasing the bile flow; anti-inflammatory and analgesic [30]; antimicrobial [31], antitumor [32] and antiviral properties [33], including HIV-1 virus [34], and also a strong immunostimulant activity [35] that appears to be exerted, at least in part, through metallic ion-containing polysaccharides [36].

This work was carried out as part of studies initiated on the mineral composition of plant drugs and other natural products in Argentina [37-40]. The aim of the present study was to determine the content of essential and non-essential minerals of *A. satureioides* and *A. tomentosa,* which is not yet known, establishing their availability in both crude drug and infusions, the more frequently used form of pharmaceutical administration. Also, their mineral compositions were compared with previously established limits for herbal medicines and products derived from them [10, 41-45] to determine their safety for human consumption.

MATERIAL & METHODS

Plant material

Samples of aerial parts from each of the two plant species (eight samples of *Achyrocline satureioides* and six samples of *A. tomentosa)* were harvested in the mountains to the northeast of San Luis City, Argentina, at 850-950 m above sea level, in the late summer, 2007, and authenticated by means of herbarium samples, preserved at Herbario de la Universidad Nacional de San Luis (acronym: UNSL) and identified as follows: *Achyrocline satureioides* (Lam.) DC., voucher specimen: L.A. Del Vitto *et al.* # 8603 ; *Achyrocline tomentosa* Rusby, voucher specimen: L.A. Del Vitto *et al.* # 6765.

Sample preparation

The samples of crude drug of each species 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 HNO_3, and 5 ml of $HClO_4$; leading up to a final volume of 50 ml, and shaking strongly. Moreover, infusions have been obtained from each sample according to the Argentinian Pharmacopoeia [45], *i.e.* 5% W/V (to 5 g sample was added 100 ml of deionized water, stirring for 5 min at 90 °C), the liquid obtained was dried and carbonized, and then treated with the same procedure as in the case of crude drugs. All reagents used were of analytical quality (Merck).

Analytical procedures

The concentrations of 27 selected minerals were determined using an inductively coupled plasma optic emission spectroscope (ICP-OES) "Varian" model "Vista-PRO™, SL.ICP.04", radial type, solid state detector, 167-785 nm, software version v3.1b 394, firmware version 2.15; their wavelength calibration was periodic and automatic (based on Argon and emission lines); their detection limits for each element and treatment are showed in Tables 1 and 2.

Statistical analysis

Concentration of minerals in the samples are represented by each arithmetic mean (average) and standard deviation (SD) expressed in %, based on air- dried material.

RESULTS AND DISCUSSION

During this search, analytical data were obtained on 27 elements contained in samples of the studied species, both from crude drugs and their infusions. The major and trace elements that are useful in the nutrition and maintenance

Elements		Limit of Detection (mg/kg)	*Achyrocline satureioides* (n = 8)		*Achyrocline tomentosa* (n = 6)	
			Average (mg/kg DW)	relative SD in %	Average (mg/kg DW)	relative SD in %
Major essential minerals	K	0.5	11454	27.8	10469	35
	Ca	0.5	7415	29	6204	32.5
	Mg	0.03	1353	39.5	1677	21.4
	P	1	1157	32.1	1658	43
	Na	0.1	146	44.2	144	25
Trace essential minerals (oligoelements)	Fe	0.5	585	96	475	76
	Mn	0.01	147	46	107	39
	Zn	0.05	28	39	38	48
	Cu	0.05	11	48	15	36
	Cr	0.01	3	34	4	40.5
	Ni	0.05	2	43	3	52
	V	0.01	1.3	36	0.9	27
	Mo	0.03	0.8	48	0.8	32
	Se	0.5	<0.5	-	<0.5	-
Other minerals	Al	0.05	579	33	304	36
	Ba	0.01	56.7	57.5	47.88	41
	Ti	0.1	46.65	28.8	22.11	44.3
	Sr	0.01	40.67	35	33.75	41.5
	Li	0.03	10.5	42	7.9	31
	Pb	0.05	1.9	45	2.1	33
	As	0.1	0.4	51	0.6	36
	Co	0.006	0.32	43	0.71	28
	Sb	0.5	<0.5	-	4.8	31.8
	Sn	0.5	<0.5	-	<0.5	-
	Ag	0.2	<0.2	-	<0.2	-
	Cd	0.01	0.04	41	0.05	65
	Hg	0.05	<0.05	-	<0.05	-

Table 1. Concentration of Elements in Raw Herbal Material of Two *Achyrocline* Species. References: DW = dry weight; SD = standard deviation.

of human health are listed in Table 1, along with some elements whose importance is still being investigated [4] or that have proven harmful properties.

Major minerals

Among the major elements considered essentials for the human nutrition and health, the studied crude drug samples contain valuable amounts of K, Ca, Mg, P and Na, in the same sequence in both plant species (Table 1). In fact, the more abundant element is K (ranged from 6,500 to 15,000 mg/kg), while Ca concentrations show variations between 4,000 to 10,000 mg/kg, followed by Mg (from 800 to 2,100 mg/kg) and P (from 750 to 2,400 mg/kg); by last, Na was present from 80 to 225 mg/kg. It was established that many samples of *A. satureioides* surpasses *A. tomentosa* in the content of K and Ca, whereas in the case of Mg and P, the respective concentrations of most samples of *A. tomentosa* are greater than those of *A. satureioides;* moreover, the mean values and the distribution of Na is very close between the samples of both species.

Trace elements (oligoelements)

Among the trace elements that are considered essential, the studied crude drugs contain Fe, Mn, Zn, Cu, Cr, Ni, V, Mo, and Se, in the same sequence in both plant species (Table 1). While Fe, Mn, Zn and Cu were found in significant quantities (more than 10 mg/kg in average), the concentrations of Cr, Ni, and V achieved lower rates (from 1 to 5 mg/kg in average); finally, Mo, and Se were detected in very small quantities (less than 1 mg/kg in average).

Fe was abundant in many samples of crude drug, but it was very variable (20-1,200 mg/kg), while Mn oscillated between 75 to 225 mg/kg;

Elements		Limit of Detection (mg/L)	*Achyrocline satureioides* (n = 8)		*Achyrocline tomentosa* (n = 6)	
			Average (mg/L)	% release to the infusion	Average (mg/L)	% release to the infusion
Major essential minerals	K	0.45	314.74	54.95	285.574	54.56
	Ca	0.021	32.752	8.83	28.235	9.10
	Mg	0.06	10.411	15.39	13.180	15.71
	P	0.2	29.934	51.74	22.677	27.35
	Na	0.06	3.718	50.93	3.270	45,42
Trace essential minerals (oligoelements)	Fe	0.012	0.227	0.77	0.194	0.82
	Mn	0.003	0.534	7.26	0.431	8.06
	Zn	0.0	0.457	32.64	0.346	18.21
	Cu	0.009	0.322	58.54	0.258	34,4
	Cr	0.012	nd	-	nd	-
	Ni	0.03	0.042	42	0.03	20
	V	0.009	nd	-	nd	-
	Mo	0.015	nd	-	<0.015	-
	Se	0.15	nd	-	nd	-
Other minerals	Al	0.06	0.275	0.95	0.172	1.13
	Ba	0.03	0.092	3.25	0.075	3.13
	Ti	0.006	nd	-	nd	-
	Sr	0.0009	0.105	5.16	0.096	5.69
	Li	0.006	0.008	1.52	0.007	1.77
	Pb	0.1	nd	-	nd	-
	As	0.105	nd	-	nd	-
	Co	0.005	0.005	31.25	0.008	22.53
	Sb	0.063	nd	-	nd	-
	Sn	0.051	nd	-	nd	-
	Ag	0.021	<0.0021	-	<0.0021	-
	Cd	0.03	nd	-	nd	-
	Hg	0.03	<0.03	-	<0.03	-

Table 2. Mean Concentration of Minerals in Herbal Infusions and Percent of Elements Released to Infusions in Two *Achyrocline* Species; *nd* = not detected.

Zn varied from 15 to 60 mg/kg; Cu was present from 5 to 22 mg/kg; Cr was found between 1.7 to 4 mg/kg; Ni from 1 to 5 mg/kg; V from 0.75 to 1.15 mg/kg; Mo was detected in concentrations from 0.2 to 1.2 mg/kg; and finally Se was present but in amounts below the limit of detection of the instrument (0.5 mg/kg).

Achyrocline satureioides shows averages higher than *A. tomentosa* in the cases of Fe, Mn and V, while *A. tomentosa* has shown higher levels of Zn, Cu, Cr and Ni; for Mo, and Se have found little differences between samples of the two species.

Other minerals

They were also obtained analytical data on other minerals, which are toxic or whose essentiality is still under discussion [4]. Their abundance in the studied plants is shown in descending order (Table 1): Al (190-775 mg/kg), Ba (22-93 mg/kg), Ti (12-61 mg/kg), Sr (18-56 mg/kg), Li (5-15 mg/kg), Pb (1-3 mg/kg), As (0.2 to 1.2 mg/kg), Co (0.2-1 mg/kg), Cd (0,02-0,08 mg/kg), and Sb (0-6.5 mg/kg); while the three others, *i.e.* Sn (<0.5mg/kg), Ag (<0.2 mg/kg), Hg (<0.05mg/kg), were found below the limit of detection of the instrument in both species.

In general, most of crude drug samples of *A. satureioides* have shown higher levels of Al, Ba, Ti, Sr and Li, while many of the samples of *A. tomentosa* were richer in Pb, Co and Cd. In reference to Sb, the samples of *A. tomentosa* have a significative level (3-6.5 mg/kg), while the samples of *A. satureioides* have shown concentrations below the limit of detection (0.5 mg/kg).

Releasing of the Minerals to the infusions

In both species were found a great variability in the passage of minerals from crude drug towards infusions, from 55% (for K, in samples of

A. satureioides) to virtually 0% (Cr, V, As, Se, Sn, Ti , Pb, Cd, in samples of both species). This variability may be due primarily to the diverse solubility of minerals in boiling water, the formation of complex stable structures, or their incorporation into organic compounds.

The more soluble minerals were Cu, K, P, Na and Ni (>40% up to 55%), following Zn, Mg, and Co (15-32%), Ca, Mn, Sr, and Ba (3-9%), Al and Li (1-1.7%) and Fe (0.7-0.8%). Other minerals studied (Cr, Pb, V, Mo, As, Se, Sn, Ti, Cd, Sb, Ag, and Hg) were not detected at all in infusions or were present below the instrumental limit of detection of each one.

Table 2 shows that samples of infusions of *A. satureioides* have greater average concentration on 13 elements (K, Ca, P, Na, Fe, Mn, Zn, Cu, Ni, Al, Ba, Sr, and Li), while *A. tomentosa* shows greater values only in two of them (Mg and Co); other 9 elements (Cr, V, Mo, Se, Ti, Pb, As, Sb, Sn, and Cd) were not detected in any of the infusions of the two species; lastly, Mo was not detected in *A. satureioides* but was present in infusions of *A. tomentosa* (at amounts lesser than the limit of detection); two others (Cd and Sb) were present in infusions of both species, but at concentrations below the limit of detection.

Elements		*A. satureioides* (n = 8)		*A. tomentosa* (n = 6)		Recommended/ Upper Intake (b) (mg/day)	Contribution of the infusions to the daily intake (%)
		Mean content in infusion (mg/L)	Calculated average ingested per day (a)	Mean content in infusion (mg/L)	Calculated average ingested per day (a)		
Major essential minerals	K	314.74	141.633	285.574	128.508	4,700AI	2-4
	Ca	32.752	14.738	28.235	12.706	1,000-1,200AI; 2,500UL	0.8-1.6
	Mg	10.411	4.684	13.18	5.931	310-320RDA; 350UL	1-2.2
	P	29.934	13.470	22.677	10.205	700RDA; 4,000UL	0.8-2.3
	Na	3.718	1.673	3.27	1.471	1,500AI; 2,300UL	0.05-0.15
Trace essential minerals (oligo-elements)	Fe	0.227	0.102	0.194	0.087	8-18RDA; 45UL	0.05-1.1
	Mn	0.534	0.240	0.431	0.194	1.8-2.3AI; 11UL	5-15
	Zn	0.457	0.206	0.346	0.156	8-11RDA; 40UL	0.25-0.64
	Cu	0.322	0.145	0.258	0.116	0.9RDA; 10UL	1-22
	Cr	nd	0	nd	0	0.025AI	0
	Ni	0.042	0.019	0.03	0.013	1UL	1.3-1.9
	V	nd	0	nd	0	1.8UL	0
	Mo	nd	0	<0.015	0-<0.007	0.045RDA; 2UL	0-<15.5
	Se	nd	0	nd	0	0.055RDA; 0.4UL	0
Other minerals	Al	0.275	0.124	0.172	0.077	2-10 d	0.5-8.5
	Ba	0.082	0.037	0.075	0.034	8-11RDA; 40UL	1-3
	Ti	nd	0	nd	0	unk	0
	Sr	0.105	0.047	0.096	0.043	unk	nc
	Li	0.008	0.004	0.007	0.003	0.2-0.6 d	1
	Pb	nd	0	nd	0	0.007-0.0126 c; 0.015-0.1 d	0
	As	nd	0	nd	0	0.105-0.406 c	0
	Co	0.005	0.002	0.008	0.004	unk	nc
	Sb	nd	0	nd	0	unk	0
	Sn	nd	0	nd	0	1-40 d	0
	Ag	nd	0	<0.021	<0,001	unk	nc
	Cd	nd	0	nd	0	0.0084-0.0147 c	0
	Hg	<LOD	<0,013	<LOD	<0.013	0.0021-0.0091 c; 0.01-0.02 d	nc

Table 3. Calculated Contribution by Herbal Infusions of Two *Achyrocline* Species to the Recommended Intake of Minerals. (a) calculated on the basis of a consumption of 450 mL of infusion/day, *i.e.* 3 cups of 150 mL each per day; (b) on the basis of the requirements or tolerance of male or female between 19-50 years old, according to the Dietary Reference Intakes (DRIs) and/or Tolerable Upper Intake, in mg/day, where: (RDA) = Recommended Dietary Allowance, (AI) = Adequate Intake, and (UL) = Tolerable Upper Intake Level (from www.nap.edu 46). Exceptionally, the data proceed from other sources indicated as follows: (c) = Estimated Dietary Intakes (from COT 47), and (d) = typical daily intake 48. LOD = Limit of detection; nc = not calculated; nd = not detected; unk = unknown.

There was a correlation between the values of minerals found in infusions in both species. The elements detected followed a descending order, with more concentration in infusions for samples of *A. satureioides* to that of *A. tomentosa,* reaching the following average values: K (315 mg/L in *A. satureioides* and 286 mg/L in *A. tomentosa),* Ca (33 and 28 mg/L), P (30 and 23 mg/L), Na (4 and 3 mg/L), Mn and Zn (between 0.5 and 0.3 mg/L), Cu, Al and Fe (0,3 and 0,2 mg/L), and Ni, Li, Hg and Mo (below 0.05 mg/L); only in Mg the infusions of *A. tomentosa,* with an average of 13 mg/L, ahead of those of *A. satureioides,* which only reached 10.4 mg/L on average.

The amount of each element in herbal infusions was calculated to assess both the contribution to the daily intake and their potential hazard to health. The data from Table 3 shows the daily intake of each element, and the contribution being made by infusions to the recommended daily intake of essential minerals and trace (or the maximum permitted level of toxic elements), in all cases considering the established recommendations and limits [10,13,40-43,47,49-52], calculated on the basis of the mineral concentration in the infusion and the assumption that the average consumption of herbal tea for a single person is three cups a day (150 ml per cup) of an infusion of 5 g of crude drug % mL of boiling water.

Referring to the mineral contribution to the diet, have been identified three groups of elements: up 1% contribution to the daily intake, are presented Na (0.05-0.15%), Fe (0.05-1%), Zn (0.25-0.64%), and Li (1%); up to 5%, are included K (2-4%), Ca (0.8-1.6%), Mg (1-2.2%), P (0.8-2.3%) and Ni (1.3-1.9%); and more than 5%, are Mn (5-15%), Cu (1-22%), and Al (0.5-8.5%). Regarding Mo, their contribution could range from 0 (in samples of *A. satureioides)* to less than 15.5% (in *A. tomentosa)*. For other elements was impossible to estimate their contribution to the daily diet because they have not yet been established its requirements.

CONCLUSIONS

The crude drug of these plants are relatively rich in some minerals, especially K, Ca, Mg, P, and Na, and some trace elements as Fe, Mn, Zn, Cu, Cr, Ni, and V. On the other hand, some of these minerals release to the infusions, and can make interesting the consumption of these herbs from the perspective of its contribution to the recommended daily intake of each one as well as a possible remineralizing action for the human body. The infusions of Achyrocline satureioides show a major level of essential minerals than *A. tomentosa* ones, with the exception of Mg.

It has been found that none of the elements exceeds the maximum permitted levels from the point of view of a daily dietary intake. In the case of heavy metals, the major toxic ones (Cr, Pb, Cd, As, and Hg) do not pass at all to the infusions, while Ni, Zn, Cu and Co release in a scarce proportion. However, it is always essential make a good quality control of plant raw materials and determine the presence of some pollutants, particularly toxic elements, to avoid excessive consumption and its potential toxicity in case of accumulation in the long-term use.

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