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Correlation of Metal Ions and Phenolic Compounds in Tea Infusions of Medicinal Plants

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Abstract: A quantitative analysis of phenolics, flavonoids and mineral species of Al, Cu, Fe, Mn, Zn, Pb, Cd, Ca, Cr, Na, K and Mg was performed in aqueous infusions from medicinal plants using the spectrophotometric techniques in the visible and atomic absorption spectrometry, respectively. Principal component analysis was performed to identify groups of samples and correlations between variables. The mineral content in herbal infusions showed variability among the types of plants. However, distribution of the elements in the infusions is not high and it is nil especially for Cd, Cr and Pb. The aqueous infusions do not show antimicrobial activity againt some microorganisms of importance in food. The concentrations of organic compounds ranged from 0.66-2.33 mg g^{-1} for flavonoids and 3.35-9.00 mg g^{-1} for phenolic acids.

Key words: Medicinal plants, infusions, mineral, trace elements, phenolic compounds, Brazil

INTRODUCTION

The use of plants for medicinal purposes has become a powerful therapeutic alternative of wide acceptance by the population and it has been developing with the medical community, ever since the plants biological activities have been scientifically verified, confirming its efficacy and safety.

Many researchers have been accomplishing studies on the virtues of the medicinal plants whether for food, cosmetic or medicinal (Basgel and Erdemoglu, 2006; Katalinic *et al*., 2006; Kumar *et al*., 2005; Lozak *et al*., 2002). The World Health Organization (WHO, 1995) estimates that 80% of world population uses medicinal plants as the main resource in primary health care. The use of medicinal plants, traditional practice that still exists between the peoples of the world has been receiving incentives of the own world health organization.

Several investigations have shown that medicinal plants contain several classes of compounds such as polyphenols, alkaloids, tannins, carotenoids and terpenoids, etc. (Arabbi *et al*., 2004; Hamburger *et al*., 2003; Leite *et al*., 2001; Nossack *et al*., 2000). Among these classes are the flavonoids and phenolic acids which are known to exhibit various pharmacological properties as vasoprotective, anti-carcinogenic, antiviral, anti-inflammatory and antiallergic, some of these properties have been related to the action of these compounds as antioxidants (Katalinic *et al*., 2006; Hamburger *et al*., 2003; Carbajal *et al*., 1989; Puatanachokchai *et al*., 2002; Runnie *et al*., 2004).

Besides organic compounds, attention has been given to the macro and micro nutrients and heavy metals in plants, both medicinal and aromatic (Kumar *et al*., 2005; Ajasa *et al*., 2004; Chizzola *et al*., 2003; Kalny *et al*., 2007; Malik *et al*., 2008; Razic *et al*., 2003). Plants can absorb heavy metals from soil and under certain conditions; high levels can be accumulated in the leaves and other edible parts of the plant. Among the minerals and trace elements essential for human beings, Ca, Na, K, Mg and Mn are present in medicinal plants in the order of g kg^{-1} while Cr, Fe, Co, Ni, Cu, Zn are present in order mg kg^{-1}. The Al, most of its forms presents no harm to living organisms. However, under certain conditions such as low pH, tends to form toxic species. These then are potentially toxic to all living beings including humans. Pb and Cd, both in its elemental form as in organic and inorganic compounds present a risk of poisoning to exposed organisms. Possible poisoning caused by Pb in the human population currently occurs through occupational exposure.

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Yemane *et al.* (2008), analyzed the content of metals (K, Ca, Mg, Fe, Mn, Cu, Zn, Na, Cd and Pb) in five samples of tea *Camellia assamica* by atomic absorption spectrometry with flame, among the macronutrients studied, K was the most abundant element in the teas (17.7-24.8 mg g^{-1}), the toxic metals Pb and Cd were below the detection limit of the method used.

This study highlights three medicinal plants grown and used by the population of Guarapuava-PR, Brazil: marigold (*Calendula officinalis* L.), espinheira-santa (*Maytenus ilicifolia* Martius Reissek ex) and lemon grass (*Cymbopogon citratus* (DC) Stapf). These plants are widely cultivated in Guarapuava and used by local population and worldwide to treat several diseases. The main objective was the quantification of metal ions essential and not essential to the human organism in aqueous infusions of medicinal plants cited by the technique of atomic absorption spectrometry in flames since, metal ions are essential to the human body. It was determined the total phenolics and flavonoids for UV-vis spectroscopy because these substances are considered antioxidants. It was also evaluated the antimicrobial activity of infusions opposite to some microorganisms of importance in food. At the end of the study became the principal components analysis of data in order to evaluate a possible correlation between the results as well as similarities among the samples.

MATERIALS AND METHODS

Instrumentation: Determinations of Cd, Pb, Cr, Fe, Ca, Mg, Mn, Cu, Zn, Al, Na and K were performed using a Flame Atomic Absorption Spectrometer-FAAS (Varian modelo AA 220), equipped with Varian brand hollow cathode lamps (Table 1). All of the absorbance measured was carried out in area integration mode. Samples were prepared in triplicate and their signals subtracted from their blanks. Determinations of the total phenols and flavonoids were performed using a Varian Cary 50 Bio UV-Vis spectrophotometer. For the phenolic acids, a wavelength of 760 nm was used and for the flavonoids, a wavelength of 425 nm (Naczk and Shahidi, 2004; Ikawa *et al.*, 2003).

Samples and reagents: The samples of *Calendula officinalis* L., *Maytenus ilicifolia* Martius and *Cymbopogon citratus*, dried and packaged were provided by CERCCOPA (Central Regional de Comercializacao do Centro Oeste do Parana). This local cooperative produces and commercializes dried medicinal plants and bee products as an alternative to improve the income of rural producers. The reagents and solvents used in the spectrophotometric analyses were of analytic grade. All standards used for the analyses of metal ions by FAAS were obtained from J.T. Baker Instra-Analysed® (1000 μg mL L^{-1}). All solutions were prepared with ultrapure water (HUMAN UP 900®). In the determination of the total phenols were used: a stock solution of gallic acid (VETEC 99%) 1000 μg mL L^{-1} in methanol, a saturated solution of sodium carbonate (20 g) and sodium tartarate (1.2 g) in 100 mL of ultrapure water and the colorimetric reagent Folin-Ciocalteu® (Biotec). For the determination of flavonoids were used: a stock solution of quercetin (Sigma 98%) 1000 μg mL L^{-1} in methanol and a solution of dihydrated aluminum chloride 5% m/v in methanol. For the antimicrobial testes, the following pathogenic microorganisms were used: Gram-positive bacteria *Staphylococcus aureus* (American Type Culture Collection) ATCC 25923, *Listeria monocytogenes* ATCC 19111; Gram-negative bacteria *Pseudomonas aeruginosa* ATCC 27853 and clinically isolated *Klebsiella pneumonia* and the clinically isolated yeast *Candida albicans* which were cultivated in Tripticase Soy Agar (TSA).

Table 1: FAAS operating conditions for determination of some elements in the herbs and their infusions

Metal	λ (nm)	LD (mg L^{-1})	Slit (nm)	i (mA)	Flame
Cd	228.8	0.03	0.5	4	ar/C_2H_2
Pb	217.0	0.28	0.1	5	ar/C_2H_2
Cr	357.9	0.05	0.2	6	ar/C_2H_2
Fe	248.3	0.06	0.2	5	ar/C_2H_2
Ca	239.9	0.02	0.2	10	C_2H_2/N_2O
Mg	202.6	0.01	0.1	4	ar/C_2H_2
Mn	279.5	0.09	0.2	5	ar/C_2H_2
Cu	324.7	0.14	0.5	4	ar/C_2H_2
Zn	213.9	0.18	0.1	5	ar/C_2H_2
Al	309.3	0.17	0.2	10	C_2H_2/N_2O
Na	330.3	0.01	0.1	-	ar/C_2H_2
K	766.5	0.03	0.1	-	ar/C_2H_2

LD = Detection Limit

Determination of metal ions: To determine the total concentration of metal ions in plants was used the procedure by wet digestion. Weighed 1 g of each plant, carbonized in a Bunsen burner until the complete release of the fumes, calcined in a muffle furnace for 8 h at 500°C. After, solubilized with approximately 3 mL of HCl 1:1 v/v and transferred to 50 mL flask. For the determination of metal ions in aqueous infusion of each plant were weighed 2 g sample into a beaker of 50 mL was added to water at 90°C to cover the plant and the infusion was filtered after 10 min. The infusions were being raised to 100 mL flasks.

Determination of total phenolic compounds and flavonoids: The total phenols were measured from a calibration curve using standard solutions prepared by adding known aliquots of gallic acid in methanol

(5-70 mg mL^{-1}), 500 mL of Folin-Ciocalteau and 500 mL of saturated solution of carbonate sodium tartrate and sodium in 5 mL volumetric flask. The solutions were allowed to rest for 2 h before each measurement. Flavonoids were quantified by constructing calibration curves with solutions of quercetin in methanol (5-60 mg mL^{-1}) prepared from the stock solution. For the preparation of these solutions it was used 250 mL of solution of aluminum chloride dihydrate 5% w/v, swelled with methanol to 5 mL flask and let in rest for 30 min.

The total content of phenolic compounds was also quantified in ethanol extracts obtained from commercial samples of plants in order to have a comparison parameter with the content extracted in aqueous infusions. It was transferred 25 g of each plant to flask of 1000 mL and added to 400 mL of ethanol 70% v/v in each container; they remained under magnetic stirring for 24 h. The solvent was evaporated by rotary evaporator with water bath temperature up to 60°C under reduced pressure to obtain the dried extracts.

Antimicrobial activity: The microorganisms were tested against the water infusion (2 g/100 mL) of the plants previously sterilized by filtration through membrane 0.22 mm. All materials used for antimicrobial testing was autoclaved for 15 min for 120°C. The procedure for inoculation of microorganisms and the addition of infusions was carried out in flow chamber. The microorganisms were transferred into tryptic soy broth (Tryptic Soy Broth-TSB) (Difco) and maintained for 24 h at 35°C in a bacteriological incubator. These microbial suspensions were standardized in sterile saline (0.9%) to a concentration of 10^6 CFU mL^{-1} (colony forming units), compared with a 0.5 McFarland scale, according to Clinical and Laboratory Standards Institute. The microbial suspensions were sown with the aid of sterile swab on the surface of Petri dishes containing 18 mL of tryptic soy agar TSA (Difco). Then holes of 8 mm were made with Duran tubes in the TSA media already inoculated with the microbial suspensions and 100 μL of the infusions were added to each hole. Water was used as a negative control. The plates were incubated at 37°C for 24 h for bacteria and 25°C for 48 h for yeast.

Analyses of correlations between the parameters evaluated: In order to evaluate possible correlations between variables (concentration of metal ions, phenols and flavonoids) and between samples (similarities between the samples) was applied a chemometric study with the obtained results. The statistical tool used was Principal Component Analysis (PCA). The PCA allows evaluating the data set, reducing the size of them, saving the most useful statistical information present in the original data. The statistical operations were performed using the Statistic program.

RESULTS AND DISCUSSION

The amount of essential and nonessential metals extracted in the tea infusion prepared from the plant doesn't reflect the total content of metals in medicinal plants since, for some metals only a small fraction is extracted in the infusion process. Therefore, a study of the total concentration of metals was performed by spectroscopic measurements after calcination plant in furnace. The ash content obtained after calcination of each plant was determined to quantify the mineral present. The sample of *Cymbopogon citratus* had the highest ash content (9.5%), followed by *Calendula officinalis* L. (7.4%) and after *Maytenus ilicifolia* Martius (4.3%).

The Table 2 shows the distribution of the metals in plants and their infusions. It was found that the metals Cr, Pb and Cd are present in the plant but not in water infusion which may be considered satisfactory since, the individual will not eat these toxic metals in a tea. It was also found that these plants contained significant

Table 2: Metal content (μg g^{-1}) extracted in the process of infusion of medicinal plants in relation to the total content of metals contained in each plant (%), n = 3

	Maytenus ilicifolia Martius			*Calendula officinalis* L.			*Cymbopogon citratus*		
Metal	Infusion	Total	%	Infusion	Total	%	Infusion	Total	%
Cu	2.30±0.01	17.75±0.13	12.9	7.20±0.01	12.50±0.11	57.6	1.35±0.01	7.02±0.02	19.2
Zn	5.08±0.01	23.02±0.12	22.0	19.14±0.03	43.91±0.23	43.5	5.37±0.01	25.29±0.03	21.2
Mn	20.88±0.04	82.08±0.13	25.4	12.32±0.02	30.95±0.03	39.8	43.87±0.04	109.0±0.17	40.2
Fe	6.35±0.05	12.95±0.02	49.0	6.23±0.01	89.55±0.29	6.9	5.00±0.01	80.35±0.14	6.2
Na	47.87±0.01	104.28±0.18	45.9	428.37±0.06	700.82±0.16	61.1	50.95±0.01	137.46±0.18	36.8
Cr	ND	0.77±0.01	0.0	ND	0.47±0.01	0.0	ND	0.02±0.01	0.0
Al	148.67±0.26	372.50±0.1	39.9	153.83±0.39	528.67±0.54	29.1	139.50±0.12	548.50±0.47	25.4
Mg	1,510.75±0.17	5,201.00±0.06	29.0	1,170.88±0.05	3,102.50±0.03	37.7	711.50±0.02	2,277.25±0.01	31.2
Ca	489.00±0.01	6,455.00±0.05	7.5	1,797.00±0.40	6,680.00±0.09	26.9	1,206.83±0.06	3,851.65±0.02	31.3
K	8,766.80±0.62	12,846.15±0.06	68.2	17,850.80±0.63	28,465.00±0.02	62.7	19,425.88±0.54	34,451.25±0.29	56.3
Pb	ND	13.17±0.03	0.0	ND	9.84±0.02	0.0	ND	9.17±0.02	0.0
Cd	ND	ND	0.0	ND	ND	0.0	ND	ND	0.0

ND = Not Detected

amounts of essential minerals such as Mg, Ca and K. Of the nine essential metals, the infusion of *Calendula officinalis* L. plant showed a higher amount of minerals Cu, Zn, Na, Ca and a large quantity of K present.

Knowing the total amount of metals present in each plant and the amount extracted by the aqueous infusions, it can be estimated the percentage that is administered in a diet using teas from these plants (Table 2). It can be observed that some metals are better extracted from the infusion of *Calendula officinalis* L. It is noteworthy that the portion of the *Calendula officinalis* L. plant that are used is the flowers and not the leaves while the other two, *Cymbopogon citratus* and *Maytenus ilicifolia* Martius leaves are used. The extraction of Fe was significant in *Maytenus ilicifolia* Martius. Potassium was the element with the highest percentage of extraction this occurs because the K is an element of high concentration and mobility in plants since, it doesn't bind to organic chelates. When there is too much Mg in a plant, this may affect the amount of Ca and K absorbed. This was observed in *Maytenus ilicifolia* Martius that showed high levels of Mg and lower Ca and K. Cu is an element with a great affinity to organic matter, binding to humic and fulvic acids, nearly 98% of Cu is chelated to organic compounds.

The displacement and the solubility of Al in plants can be quite influenced by the presence of complexing organic acids as natural soil humic acid, citric acid, oxalic acid and especially by fulvic acids (Schnitzer, 1969) for coming forward in relation to humic acid, a greater amount of functional groups (for example $COOH^-$) and higher values of stability constants (Stevenson, 1982). Al, from soil cultivation is present in significant amounts in plants. There are numerous studies in the literature indicating the negative effects of Al and yet others show beneficial effects in some species of plants such as the need for a small amount of Al in soil solution to stimulate the development of some plants (Kovacik *et al*., 2010). When Al is present in high concentrations in the soil, the plant develops a defense mechanism able to accumulate high amounts of Al in their tissues as a means of detoxification. Several compounds may complexing Al and other metals as a form of detoxification on the plan. Phenolic compounds show high stability in their complexes with metals and antioxidant present in conditions that can cause changes in plant growth.

There is no confirmed evidence that Al has some essential function in animals or humans. The main consideration related to Al and health is its potential toxicity when exposure is excessive. Toxicological aspects of Al consumed orally are less well defined. Al interacts with a number of other elements including Ca, F, Fe, Mg, P and Sn and when ingested in excess can reduce their absorption. Considerably, the most important contribution to the intake comes from Al antacid medications that can provide several grams of metal per day. These amounts interfere with the absorption of other elements can also lead to a gradual accumulation of Al in the skeleton. Locally increased concentrations of Al occur in the brain of patients with Alzheimer's but if the metal has a causative role in the pathogenesis of this disease has not been established (Molloy *et al*., 2007).

To evaluate the toxic effects of Al, it is important to consider not only the total concentration of soluble Al in a sample, it is also necessary to investigate the distribution of its species since, the bioavailability and toxicity of an element depend o1n the way in which it's found in the food. The free species of Al seems to be primarily responsible for its toxicity (Erdemoglu *et al*., 2000). Furthermore, natural ligands such as phenolic compounds present in plants contribute to reducing their toxicity through the formation of stable complexes (Flaten, 2002).

The Table 3 shows the results of the amounts of phenolic acids and flavonoids present in aqueous infusions and ethanolic extracts of plants. The *Calendula officinalis* L. was the plant that had the highest content of organic compounds in the infusions. Related to the total content of phenolics and flavonoids *Cymbopogon citratus* was the richest in these constituents. It is important to know that many factors can interfere with the amount of phenolic acids in plants like the period of the year that the plant was harvested, new tissues generally have higher amount of phenolic acids, lower amount of phenolic compounds are produced when there is too much nitrogen in the soil.

The infusions were evaluated in antimicrobial activity *in vitro* however, showed no inhibition of the microorganisms tested. According to Da Silva Silveira *et al*. (2007) the use of plants to treat diseases is a very common practice especially in

Table 3: Determination of phenolic acids and flavonoids of aqueous infusion and ethanolic extracts by UV-Vis (mg g^{-1}), n = 3

	Phenolic acids			Flavonoids		
Plants	Infusion	Total (ethanolic extracts)	%	Infusion	Total (ethanolic extracts)	%
Calendula officinalis L.	9.00±0.67	92.35±0.50	9.7	2.23±0.50	13.55±0.55	16.9
Cymbopogon citratus	3.35±0.36	285.97±0.84	1.2	0.66±0.08	41.54±2.69	1.6
Maytenus ilicifolia Martius	4.36±0.62	230.44±0.40	1.9	0.89±0.73	20.60±1.25	4.3

underdeveloped countries because of the difficulty of immediate access to the service of public health. Thus, the use of teas or infusions by the population should be reviewed carefully, depending on the goal because many times the infusion of *Calendula officinalis* L. is used to treat candidiasis. On the other hand, the researchers must stress that often molecules such as polysaccharides from mushrooms have no positive effect *in vitro* tests however, *in vivo*, stimulate the immune system and provide beneficial effects. Therefore, the results of absence of the antimicrobial activity of the infusions should be interpreted with caution and they only indicate the need for further studies to clarify whether there are real benefits in the body after ingestion of these teas.

The principal component analysis was applied to the experimental data of self-tiered levels of metals, phenols and flavonoids obtained from infusions of medicinal plants to determine the similarities and differences between the samples related to these variables and the possible correlation between these variables.

The Fig. 1 shows the graphs of scores of samples and weights of variables. The first and second principal components together capture 100% of variance of experimental data. In 72.09% of variance captured by the first principal component, samples were divided into three groups (Fig. 1a). Figure 1b shows the weights of the variables. By factor 1 (X axis) is observed that most metals are strongly correlated with *Calendula officinalis* L. Only the Mn is the mineral of highest concentration in the *Cymbopogon citratus*. The phenolic acids and flavonoids are also present in higher concentrations in the infusion of *Calendula officinalis* L. The PCA (factor 1) showed a positive correlation between Al, phenols and flavonoids, indicating the complexation of toxic metal ions with organic compounds. The elements with more mobility in plants such as Na and K are present in greater concentrations in plants of *Calendula officinalis* L. and *Cymbopogon citratus*. According to the second principal component (27.91% variance captured, Y axis), one can still distinguish the plant *Maytenus ilicifolia* Martius of the remaining *Calendula officinalis* L. and *Cymbopogon citratus*, respectively.

The *Maytenus ilicifolia* Martius is located on the Y axis positive and then presents the highest values for the variables located on this axis i.e., contents of Mg, Fe and Al. *Calendula officinalis* L. and *Cymbopogon citratus* contain higher levels for the other variables (Ca, K and Mn). The variables Cu, Zn, Na, phenolic acids and flavonoids were not significant by factor 2. Despite the lower content of the compounds in teas, important information is that the infusions contain compounds that can be absorbed quickly by the body because they are more soluble in water. Another relevant fact is that heavy metals were not found in teas and may be secured by less soluble constituents of the plant.

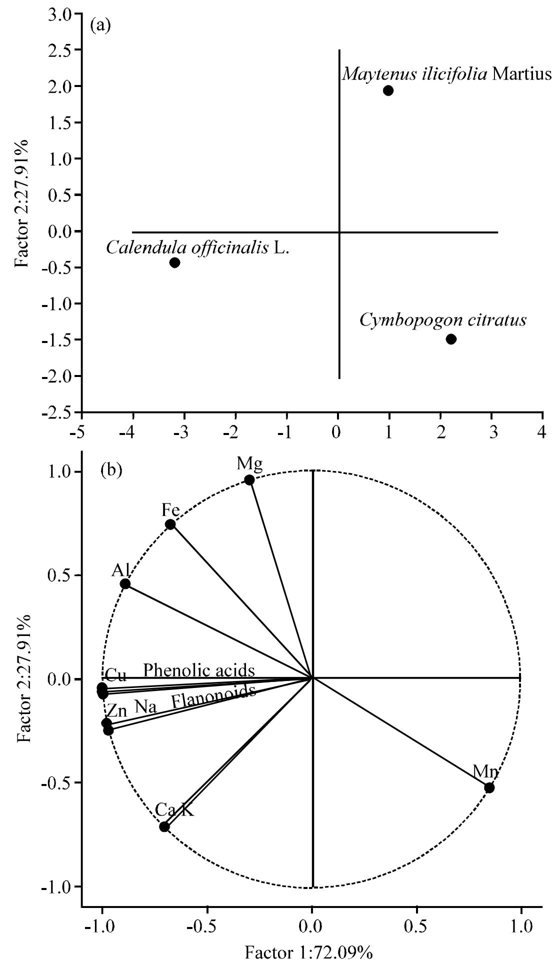

Fig. 1: Scores (a) and weights (b) of the first and second principal components for infusions aqueous

CONCLUSION

The conclusion of this study is that the tea (water infusion) of medicinal plants are important sources of essential minerals like magnesium, potassium and calcium, it is clear also that the nonessential metals, Pb, Cr and Cd are weakly extracted in infusions causing no harm to humans. Al is present in significant amounts in infusions but it is toxic only if the person suffers from kidney problems, the small amounts absorbed in normal diets are excreted by healthy kidneys so that no accumulation occurs considerably. As there was a strong correlation of this metal with organic compounds it is suggested that a significant percentage is complexed with phenolic acids and flavonoids with Al leaving less reactive to the body.

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