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Evaluation of different extractants for copper, manganese and zinc in medicinal plants

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ABSTRACT

The extraction of copper, manganese and zinc by different organic solvents and aqueous solutions from powdered and droughts of nine medicinal plants were investigated as a possible way of accomplishing the speciation of these metals in such samples. The extractants were 75 g L^{-1} HCl, 50 g L^{-1} NaCl, 50 g L^{-1} NaOH, 50 g L^{-1} Na_2CO_3, (1 : 1) glicero-alkali solution, pure water, ethanol and ether. None of the organic solvents extracted significant amounts of the three elements. For Cu, only the HCl solution has shown significant extraction efficiency. On average, Zn was more efficiently extracted when H_2O, NaOH, and Na_2CO_3 solutions were used while Mn was more efficiently extracted with the HCl and NaCl solutions. The differentiated extraction behavior of the studied elements in relation to the different aqueous extractants is an indicative of different pools of these elements in the plant substrate.

Keywords: medicinal plants, phytochemistry, copper, manganese, zinc

INTRODUCTION

The therapeutic use of medicinal plants in several diseases has been scientifically known as phytotherapy (Fisher and Ward, 1994). The knowledge of the chemical composition of the phytotherapeutical products remains almost bound to its organic components, which normally represent the active principles (secondary metabolites) of the plant, such as alkaloids and flavonoids, among others (Miller, 1993; Stace, 1984). In this context, the knowledge of the trace element contents found in the phytotherapeutical products may enlarge its indication since certain metals, in its ionic form or as a chelate, have therapeutic effects, acting as anti-inflammatory and anti-viral agents (Sorenson, 1982; Perrin and Stunzi, 1982). Furthermore, one has to keep in mind the positive dietetic of metals that are essential for the human health, such as Zn, Cu and Mn (WHO, 1996), in a situation of a previous nutritional deficiency.

Most of the studies of microconstituents and trace elements in plants are frequently associated to their total content (Pais and Jones, Jr., 1997; Kabata-Pendias and Pendias, 2001). However, many publications on the analysis of trace metals were based on selective extraction (Miyazawa *et al*., 1984; Farago, 1994; Edashige and Ishii, 1996; Jona and Fronda, 1997). In this context, the chemical extractions may be systemized in the following groups: (i) extractants with ion exchange properties; (ii) extractants with carbonate dissolution properties; (iii) extractants with acid reduction properties; (iv) extractants that weaken the bond between metal and organic compounds and sulfates; (v) extractants represented by strong acids for the total dissolution

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Table 1 Pharmacological properties of the nine medicinal plants investigated in the present study

Common name	Scientific name	Main active principles	Herbal properties and actions	Part used
Avocado	*Persea gratissima* Gaertn.	Abacatina (bitter principle); flavonoids (quercetin, B-sitosterol, D-perseitol); essential oils (estragol, anetol)	Diuretic, carminative, emmenagogue (promotes menstruation), antidiarrheic	Leaves
Bhuiaonla	*Phyllanthus niruri* L.	Phyllanthin; hypophyllanthin; lignansniranthin; nirteralin; phyltetralin; ellagic acid	Liver protective; expels stones; antspasmodic; antiviral and antibacterial propoerties; diuretic; hypoglicemic; analgesic	Leaves
Boldo	*Peumus boldus* Mold.	Alkaloids (boldine)	Stimulates digestion; diuretic; vermifuge; reduces rheumatism	Leaves
Carqueja	*Baccharis genistelloides* Pers.	Flavonoids (quercetin, luteolin, nepetin, apifenin, hispidulin)	Liver protective; antiacid; antiulcer; antidiabetic; antiviral properties	Whole plant
Cascara sagrada	*Rhamnus purshianus* DC	Anthraquinones	Mild laxative; promotes gastric digestion and appetite	Bark
Catuaba	*Anemopaegma mirandum* DC	Alkaloids; tannins; aromatic oils; fatty resins; phytoterols; cyclolignans; sequiterpenes; flavonois	Antibacterial and antiviral properties; aphrodisiac; analgesic; vasodilator; vasorelaxant	Bark
Centella asiatica	*Hydrocotile asiatica* L.	Terpenes; asiatic and modecassic acid	Antipsoric; stimulates collagen synthesis	Stem and leaves
Ginko biloba	*Ginkgo biloba* L.	Flavonoid (quercetin, kaempferol); diterpenes (gingksolides, bilobalide)	Improves blood flow	Leaves
Valerian	*Valeriana officinalis* L.	Valerianic, formic and acetic acids; alkaloids (chatarine, valerianine)	Stimulant; carminative (Gas expel); antiespasmodic; sedative.	Root and rhizome

Sources: Penna (1946); Grieve (1971); Quer (1975); Corrêa (1984); Vieira (1992); Rehm (1994); Taylor (2005).

of what remained from the sample, after the previous extractions.

The present paper intends to study the behavior of different solutions for the extraction of copper, manganese and zinc from some medicinal herbs. The choice of these elements is justified by their importance for the biological systems.

METHODOLOGY

The plants selected for this analysis were the avocado (*Persea gratissima* Gaertn.), boldo (*Peumus boldus* Mold.), carqueja (*Baccharis genistelloides* Pers.), californian buckthorn or cascara sagrada (*Rhamnus purshianus* DC.), catuaba (*Anemopaegma mirandum* DC.), centella asiatica (*Hydrocotile asiatica* L.), ginko biloba (*Ginkgo biloba* L.), bhuiaonla (*Phyllanthus niruri* L.) and valerian (*Valeriana officinalis* L.). This choice has been due to the fact that these species are frequently used in phytotherapy as phytopharmaceutics, in the form of teas, tablets, tinctures and capsules. Table 1 presents their main known active principles and some phytotherapeutical uses for these plants. The studied samples were in their powder form, which is the most important form of raw material used for the manufacture of capsules and tablets by the phytopharmaceutical industry and manipulation pharmacies. The suppliers were the Brazilian pharmaceutical distributors Galena, Spectrum, Química e Naturalpharma.

The determination of the total concentration of Cu, Mn e Zn in the samples has been carried out in triplicate, making use of the parallel analysis of a certified reference material (NIST SRM 1572, ‘‘citrus leaves’’) for the validation of the procedure and quality control of the results. In all cases, concentrations of Cu, Mn and Zn found in the present work (17 ± 1, 24 ± 1, and 31 ± 1 µg g^{-1}, respectively) were within the ranges of the certified reference material (16.5 ± 1, 23 ± 2, and 29 ± 2 µg g^{-1}, respectively). The procedure consisted, basically, in the digestion of 1 g of sample by a 1 : 1

Table 2 Manganese, zinc, and copper concentrations ($\mu g\ g^{-1}$) found in the different extractants investigated[a,b]

Plant	HCl	NaOH	Na_2CO_3	NaCl	H_2O	Total
Manganese						
Avocado	452 (30)	19 (0.1)	12 (0.1)	435 (20)	27 (1)	587 (10)
Boldo	109 (5)	21 (2)	5 (0.1)	48 (1)	4 (0.1)	132 (2)
Carqueja	712 (30)	50 (2)	10 (1)	292 (30)	18 (0.1)	746 (10)
Cascara	264 (10)	58 (4)	7 (0.1)	212 (2)	20 (4)	268 (10)
Catuaba	86 (8)	19 (2)	5 (1)	63 (0.1)	11 (3)	86 (2)
Centella	328 (20)	16 (1)	3 (0.1)	118 (3)	24 (3)	402 (30)
Ginko	61 (3)	11 (0.1)	11 (1)	29 (0.1)	6 (1)	75 (1)
Bhuiaonla	148 (2)	36 (2)	3 (1)	186 (15)	23 (1)	214 (10)
Valerian	169 (3)	8 (0.1)	8 (1)	67 (4)	7 (0.1)	301 (10)
Zinc						
Avocado	21 (0.1)	7 (1)	1.1 (0.1)	4 (0.1)	4 (0.1)	39 (1)
Boldo	11 (0.1)	7 (1)	1.3 (0.1)	11.0 (0.1)	2 (1)	11 (1)
Carqueja	30 (0.1)	13 (1)	5.1 (0.1)	6 (1)	4 (0.1)	39 (3)
Cascara	3 (0.1)	3 (0.1)	1.0 (0.1)	2 (1)	1 (0.1)	4 (1)
Catuaba	3 (0.1)	<LD	1.3 (0.1)	2.4 (0.1)	2 (0.1)	11 (1)
Centella	14.4 (0.1)	4 (0.1)	0.2 (0.1)	2 (0.1)	6 (1)	38 (5)
Ginko	4 (0.1)	1.3 (0.1)	0.3 (0.1)	<LD	1 (0.1)	12 (2)
Bhuiaonla	24 (2)	8 (1)	1.0 (0.1)	11 (1)	18 (1)	45 (3)
Valerian	13 (0.1)	4 (0.1)	3.3 (0.1)	4 (1)	3 (0.1)	27 (1)
Copper						
Avocado	9 (1)	<LD	<LD	<LD	<LD	22 (1)
Boldo	4.9 (0.1)	<LD	<LD	<LD	<LD	4.5 (0.1)
Carqueja	6 (1)	<LD	<LD	<LD	<LD	14 (2)
Cascara	1.2 (0.1)	<LD	<LD	<LD	<LD	2 (1)
Catuaba	3.2 (0.1)	<LD	<LD	<LD	<LD	3.0 (0.1)
Centella	4 (1)	<LD	<LD	<LD	<LD	11 (1)
Ginko	1.5 (0.1)	<LD	<LD	<LD	<LD	6.6 (0.1)
Bhuiaonla	7 (1)	<LD	<LD	<LD	<LD	17 (1)
Valerian	4 (0.1)	<LD	<LD	<LD	<LD	16 (1)

[a] In parenthesis, the standard deviation of the metal determination.
[b] LD, limit of detection: 0.2, 0.2, and 0.75 $\mu g\ g^{-1}$ for Mn, Zn, and Cu, respectively.

mixture of nitric acid and hydrogen peroxide in a digestion block at 85°C, for a period of 12 hours. The digestion was carried out in screwed cap conical ended 50 mL plastic tubes (Sarsted, Germany), and the final volume was made up in the plastic tubes themselves with ultrapure water. The selective extraction studies consisted on mechanically shaking (for a period of 15 minutes) 1 g of sample with 15 mL of the following solutions: hydrochloric acid (75 g L^{-1}); sodium hydroxide (50 g L^{-1}); sodium carbonate (50 g L^{-1}); sodium chloride (50 g L^{-1}) and a 1 : 1 glycerin-alcohol solution. These experiments were performed in duplicate. The following pure solvents were also used: water, ethanol and ether. The extractants used in the present work were selected based on the extractant grouping presented in the Introduction section and on literature (Miyazawa *et al.*, 1984; Farago, 1994; Edashige and Ishii, 1996; Jona and Fronda, 1997). The 15 minutes shaking period was used throughout the experiments since previous work had demonstrated that readsoprtion of the studied metals could occur in larger periods. After the extraction all the solutions obtained were filtered through quantitative filter paper (Schwarzband no. 589). The studied elements were determined by flame atomic absorption spectrometry, using both a model 1100B Perkin Elmer as well as model AA5 Varian Techtron atomic absorption spectrometers. Cu, Mn and Zn hollow cathode lamps were used as line sources and the instruments were set according to their respective manufacturer manuals. Calibrations were performed by external analytical curves prepared in the blanks of the specific extraction and digestion media final solutions, in order to minimize interferences.

All glass and plasticware was previously decontaminated by immersion in Dextrin, for a minimum period of 2 hours and, after that, in 200 mL L^{-1} nitric acid, for a minimum period of 6 hours.

RESULTS AND DISCUSSION

The investigated extractants act on a particular way, according to their chemical properties. According to the literature (Farago, 1994), the sodium hydroxide and the hydrochloric acid solutions probably act by weakening the bond between metal and organic compounds, such as hemicellulose, in the case of NaOH, and protopectates, in the case of HCl. The sodium carbonate (50 g L^{-1}, pH 12) and sodium chloride (50 g L^{-1}, pH 6) solutions would act as ion exchangers whereas water and the organic solutions would lead to speciation in relation to their different solubilizing properties due to their respective polarities. However, the use of ethanol, ether and the glycerin-alcohol solution led to no noticeable metal extraction. Therefore, only the other extractants will be discussed in continuation. The results are displayed in Table 2.

Copper extraction

According to Table 2, copper is significantly extracted from the plants only when the extraction is carried out by the hydrochloric acid solution. This suggests that this metal is found mainly strongly bonded to the plant's substratum and that these bonds are weakened only by this strong extractor. It can also be observed that this extractor was able to extract from 22 to 107% of the total copper content, depending on the plant (Table 3). Boldo and catuaba were the most effectively extracted plants, while Ginko and Valerian presented the smaller extraction yield.

Manganese extraction

Manganese extraction has occurred in all plants and extractants investigated (Table 2). The 75 g L^{-1} hydrochloric acid solution led to extraction yields greater than 70% of the total plant content, in most plants (Table 3). It is also possible to observe that the fraction of manganese extracted by the sodium chloride solution was greater than 30% for most plants. Some values were greater than 70% as in the case of the avocado, cascara sagrada, catuaba and bhuiaonla (Table 3). The extractions with pure water and the sodium carbonate solution have led to low yields, when compared to the total content and have generally shown values below 10%. The extraction with the sodium hydroxide solution presented quite variable values.

The extraction of zinc

The extraction of Zn has also occurred in all extractants for all plants studied (Table 2). However, only boldo, carqueja and cascara sagrada have presented extractions yields with the hydrochloric acid solution greater than 70% in relation to the total content. In the case of the extraction with sodium chloride, only boldo and cascara sagrada have presented yields greater than 30%. Yet the extractions with pure water and sodium hydroxide have generally presented yields greater than 10%. In the case of sodium carbonate five, of the nine plant species studied, have also led to extraction yields greater than 10% (Table 3).

General evaluation

The results displayed in Table 3 suggest some sort of differential pattern of the three metals studied in relation to their extracting behavior and consequently, its bioavailability, in the materials studied.

The element that seems to have the strongest bond to the plant substrate of the nine samples studied is Cu. This element was only extracted by the HCl solution, which is a very strong extractant for plant tissues (Miyazawa *et al*., 1984). In respect to the other two metals, Zn was the most efficiently extracted by the NaOH and Na_2CO_3 solutions and by H_2O, as well. In contrast, Mn was more efficiently extracted by the HCl and NaCl solutions.

The Cu results are in agreement with the literature that reports this element as forming stronger and more stable complexes than the majority of the other elements (Kochian, 1991; Laurie and Manthey, 1994; Kabata-Pendias and Pendias, 2001).

In respect to the other two metals, different chemical forms are expected, depending on their location in the plant. A great part of Mn happens to be in its ionic form in the plant's fluids, while Zn is found mainly complexed to organic compounds of low molecular weight (Kabata-Pendias and Pendias, 2001). Nevertheless, in the vegetable tissues, especially in leaves, they assume different forms. According to the literature (Burnell, 1988), most of the Mn (about 70%) is found, in this context, associated to biochemical systems activated by metals, from where it can be substituted by ion exchange for another metal. Mn can also be found strongly bonded to chlorophyll (about 30%) and, in a smaller proportion, to enzymes, particularly, the superoxide dismutase. This is in agreement with the results found in the present study, which indicate

Table 3 Metal recovery (%) in relation to the total metal content relatively to the different extractants investigated. Some other ratios are also displayed

Plant	HCl	NaOH	Na_2CO_3	NaCl	H_2O	NaCl/HCl	H_2O/HCl
Manganese							
Avocado	77	3.2	2	74	4.6	96	6
Boldo	83	16	3.8	36	3	44	3.7
Carqueja	95	7	1.3	39	2.4	41	2.5
Cascara	98	22	2.6	79	7.5	80	7.6
Catuaba	100	22	5.8	73	13	73	13
Centella	82	4	0.7	29	6	36	7.3
Ginko	81	15	15	39	8	47	9.8
Bhuiaonla	69	17	1.4	87	11	126	15
Valerian	56	3	2.7	22	2.3	40	4.1
Average	82.4	12	3.9	53.2	6.4	64.9	7.7
SD	14.3	7.9	4.3	24.7	3.7	31.2	4.3
Zinc							
Avocado	54	18	2.8	10	10	19	19
Boldo	100	64	12	100	18	100	18
Carqueja	77	33	13	15	10	20	13
Cascara	75	75	25	50	25	67	33
Catuaba	27	1.8	12	22	18	80	68
Centella	38	10	0.5	5.3	16	14	42
Ginko	33	11	2.5	1.7	8.3	5	25
Bhuiaonla	53	18	2.2	24	40	46	75
Valerian	48	15	12	15	11	31	23
Average	56.2	27.3	9.1	27.1	17.5	42.4	35
SD	23.7	25.4	7.9	30.8	10.0	33	22.1
Copper							
Avocado	41	—	—	—	—	—	—
Boldo	109	—	—	—	—	—	—
Carqueja	43	—	—	—	—	—	—
Cascara	60	—	—	—	—	—	—
Catuaba	107	—	—	—	—	—	—
Centella	37	—	—	—	—	—	—
Ginko	28	—	—	—	—	—	—
Bhuiaonla	41	—	—	—	—	—	—
Valerian	25	—	—	—	—	—	—
Average	53.8	—	—	—	—	—	—
SD	32.4	—	—	—	—	—	—

SD, standard deviation.

that the HCl and NaCl extractors have been more efficient in the Mn extraction. Zn in leaves is more easily translocated than Mn, especially in the senescence stage (Loneragan, 1988; Longnecker and Robson, 1993). It is an indication that this element is found less bonded to insoluble compounds. Actually, the literature (Brown *et al*., 1993) suggests that most of the Zn is found in complexes with organic compounds of low molecular weight. It is worth mentioning that the same authors report that free ionic Zn responds for 5.8 to 6.5% of the total Zn content. Thus, the good efficiency in the extraction of Zn by H_2O and NaOH is easily understood. In contrast, the extraction with the Na_2CO_3 solution does not show the expected results, that is those related to ionic exchange: no significant correlation was observed between these results and those associated to the extraction with the NaCl solution ($r = 0.449$ for Mn and $r = 0.202$ for Zn).

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