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Original Article

MINERAL COMPOSITION OF LEAVES, ETHANOLIC LEAF EXTRACT AND INFUSIONS OF A. OCCIDENTALE L. FROM AMAZON IN NORTHERN BRAZIL

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ABSTRACT

Objective: The present study aimed to evaluate the content of mineral elements of leaves, ethanolic extract and a tea infusion leaf of Anacardium occidentale L., known as cashew, has economic importance and is used for the treatment of diseases by the population. Thus the mineral study shows become of interest as far agricultural as medicinal. **Methods:** All the elements were determined using Flame Atomic Absorption Spectrometry (FAAS). The samples were divided into two groups according to the leaves used: young and adult. **Result:** Determination of elements showed that the higher concentration of Ca (73.74±0.47 mg/L), Mg (57.45±8.04 mg/L), and Fe (22.75±0.36 mg/L) occurred in the mature ones. In the young, the highest concentrations observed were Ca (12.08±0.11 mg/L), Mg (35.24±0.13 mg/L), and Fe (10.65±0.72 mg/L). Zn showed no significant difference and the Cu concentration was higher in young leaves. During the infusion, no significant differences were observed between adult and young, where Mg was the compound with the highest concentration (4.35±0,07 mg/L and 4.35±0,01 mg/L for mature and young leaves, respectively). In the extract, the element with higher concentration was Ca (74,85±1,26 mg/L), followed by Mg (30.75± 1.5mg/L) and Fe (21.28±1.62mg/L).

Keywords: Cashew, leaves, tea infusion, ethanolic extract.

INTRODUCTION

Anacardiaceae comprises about 70 genus with approximately 875 species widely distributed in tropical regions. In Brazil, some can be highlighted, mainly *Anacardium, Mangifera, Spondias* and *Schinus* genus [1]. *Anacardium occidentale* L. species, popularly known as cashew (Fig. 1), is a tropical plant from Brazil, which has spread throughout the country [2]. This plant is a tree that can up to 15 m height and has a thick and tortuous trunk; the fruit is the nut and the other part that is used to make juices is, called a pseudocarp or false fruit. Several parts of this plant (bark, heartwood, leaves) are used for the treatment of diseases, such as urogenital and gastrointestinal infections, sore throat, gingivitis, dental and oral infections, as well as an antidiabetic and hypertensive agent [3-6]. In Brazil, leaves are used to treat genital and skin diseases and gastric problems [7].

Fig.1: A cashew tree. In detail, a yellow cashew with its nut.

The Amazon is the largest tropical forest in the world and spans across nine countries, including Brazil. Known for its biological diversity in the world, has a considerable traditional knowledge that includes a vast collection of information about the management and use of biodiversity, mainly about plants. The plants are an important source of biologically active natural products, which constitute models for the synthesis of substances for several purposes because they have a variety of structures and physicochemical and biological properties and thus have caused a significant increase in the use of herbs with pharmacological properties [8]. The use of leaves, teas and plant extracts in the treatment of diseases is a practice used by people since antiquity. Several researchers have shown that medicinal herbs have many classes of compounds such as tannins, carotenoids, polyphenols, alkaloids, terpenoids, flavonoids and phenolic acids, which exhibiting pharmacological properties such as anticarcinogenic, blood vessel protection, antiviral, anti-inflammatory and anti-allergic [9, 10].

Associated with compound classes are the mineral elements, the fundamentals in the biochemical and physiological functions of the human body [11, 12], as components of enzymes and hormones, the synthesis of vitamins and for bone formation [13]. It is also important, however, to note that high levels of minerals can be toxic [14,15]. The level of minerals, which plants are able to absorb depends, for instance, on the chemical composition and fertility of the soil, accumulating them in the leaves, barks, and other parts [16].

Therefore, it is very important to analyze quantitatively the mineral composition of plants, both for medical use as agriculture. The monitoring of the minerals in the plants allows the identification of the deficiency, adequacy or excesses that can optimize production [17]. In vegetable, extracts have great importance since it is from dried plant extracts which are manufactured in many herbal medicines [18, 19]. For the mineral study, many techniques are used, among them the flame atomic absorption spectrometry (FAAS) is one of the techniques most extensively used for determining several trace metals with a significant precision and accuracy, fairly low operation cost, high analytical frequency and good selectivity [20]. This study aimed to establish the mineral content of young and adult leaf calcinations, tea infusion, and ethanolic extract of *A. occidentale* L. leaves by FAAS, comparing their metal concentration.

MATERIALS AND METHODS

Collection plant material

The leaves were collected from a cashew tree that was growing into the Campus of the Federal University of Amapá (0°0'22,9212", S 51°5.5896' W, altitude 6.0 m), northern Brazil, close to the French Guiana (Fig. 2). A voucher specimen was deposited in the Amapaense Herbarium (HAMAB) located at IEPA (Instituto de Pesquisa Científica e Tecnológica do Estado do Amapá), for proper identification of the species under registration nº 018684. For the mineral analysis, the leaves were divided into two groups: young and adults. The adult leaves were collected from the fourth node, while young leaves of the second node. Samples of fresh leaves were selected and thoroughly washed with running water for to remove particles and dust, and then were washed with distilled to remove any residues on the surface.

Fig. 2: Map of the study area showing the site of sample collected.

To determinate the concentration of metal ions in leaves was performing the procedure of wet digestion. It were weighed 1 g of each leaves group (young and adults), carbonized in a Bunsen burner until the complete release of the fumes, and calcined in a muffle furnace for 8h at 500 º C. After that, samples were solubilized in 3 mL of HCL 1:1v/v and transferred to a 50 mL flask [16].

Ethanolic extract

To obtain the alcohol extract from the leaves, they were separated, cleaned and dried in a circulating air oven at 40° C for 72 hours. Then, they were milled and the resulting powder, stored. The extract was prepared in the Pharmacognosy Laboratory of the Federal University of Amapá, were used 500g of leaf and like extractor liquid ethyl alcohol, at a ratio of 1:4 (powder/solvent) by cold maceration during 72 h, at room temperature, and that process was repeated three times and subsequently filtered using No.1 Watt-man filter paper [23]. The filtrate was concentrated using a rotary evaporator at 50º C (Quimis®). To determinate the concentration of metal ions in the ethanolic extract was weighed 0,01 g of sample, which was solubilized in 1 mL of ethanol [16].

Infusion

The infusions (teas) were prepared from samples of young and adults leaves, chopped into small pieces (± 0.5 x 0.5 cm), totaling 2 samples. Posteriorly was weighed 2g and added 100 ml of boiling distilled water and the flask was capped, to reduce water losses. After letting to cool for 10 min, the infusion was filtered with a filter paper [22].

Instrumental

To obtain the analytical determinations, it was used a flame atomic absorption spectrophotometer (Shimadzu AA-6300). Acetylene gas was used as fuel and air as a support in FAAS. An oxidizing flame was used in all the cases and a hollow cathode lamp of each metal was used as a radiation source. Detailed instrumental analytical conditions for analyses of selected heavy metals are given in Table 1. The extracts were analyzed for six metals, namely, Ca, Cu, Fe, Mg, Mn and Zn.

Device calibration and detection limit

The calibration curve used for the determination of metals in leaves, infusion and ethanolic extract samples by FAAS was established using the following procedure. To determine detection limit, replicate analysis of ten blank samples was performed, and the pooled standard deviation of the ten reagent blanks was done. The detection limits were obtained by multiplying the pooled standard deviation of the reagent blank by three. The limits of detection (LOD) and quantification (LOQ) presented in Table 1 were determined according to Equations (1) and (2).

$$LOD = \frac{3xs}{m} \quad (1)$$

$$LOQ = \frac{10xs}{m} \quad (2)$$

where "s" is the standard deviation of the measures referring to the blank (n = 10) and "m" is the inclination of the analytical curve.

Table 1. Instrumental analytical conditions for analysis of selected metals in the samples.

Metals	Wavelength (nm)	Lamp current (mA)	Slit width (nm)	Gas flow (L min^{-1})	LOD (μg L^{-1})	LOQ (μg L^{-1})
Ca	422. 7	10	0.7	2.0	14	48
Cu	324.8	6	0.7	1.8	8.1	20
Fe	248.3	12	0.2	2.2	70	234
Mg	285.2	8	0.7	1.8	1.3	4.3
Mn	279.5	10	0.2	2.0	0.9	3.1
Zn	213.9	8	0.7	2.0	140	40

Table 2: Mineral analysis of *A. occidentale* (mg/L). YL: Young Leaves; AL: Adult Leaves; IAL: Infusion of Adult Leaves; IYL: Infusion of Young Leaves; EE: Ethanolic Extract.

	YL	AL	IYL	IAL	EE
Ca	12.08±0.11	73.74±0.47	3.06±0.39	3.42±0.18	74.85±1.26
Cu	2.03±0.02	1.91±0.04	0.11±0.01	0.05±0.01	2.25±0.08
Fe	10.65±0.72	22.75±0.36	0.56±0.01	0.57±0.03	21.28±1.62
Mg	35.24±0.13	57.45±8.04	4.35±0.01	4.35±0.07	30.75± 1.5
Mn	2.79±1.15	5.97±0.29	0.27±0.01	0.36±0.01	0.27±0.09
Zn	1.50±0.25	1.89±1.70	0.19±0.07	0.42±0.36	2.33±2.62

RESULTS AND DISCUSSION

The mineral concentrations of six metals (Ca, Cu, Fe, Mg, Mn, Zn) of the Anacardium occidentale leaves (young and adult), infusion and ethanolic extract samples are listed in table 2.

Comparing the results of the elements determination between young and adult leaves presented in table 2, it is possible to note that the higher concentration of Ca, Mg and Fe occurred in the adult ones. In the young, the highest concentrations observed were Ca, Mg, and Fe. Zn and Cu showed the larger concentration, this can be related to the age of the plant and bioavailability elements in their different parts, and this effect is dependent on many factors such as the species, soil, climatic conditions, among others [23].

In the case of infusion, as can be seen in table 2, Mg was observed to have the highest concentration followed by Ca for both IYL and IAL. The level of metals decreases in the order Mg >Ca> Fe > Zn >Mn> Cu to IAL samples and Mg >Ca> Fe > Zn > Cu >Mn to IYL samples. By comparison with the calcined leaves (YL and AL), it can be seen that the concentrations obtained by the infusion method were lower because minerals extracted from the infusion does not reflect the total content of minerals in medicinal plants, only a small fraction is extracted in this process. It was shown that temperatures above than 60ºC tend to reduce the transfer rate of Cu and Fe from leaves because of the insolubility of the polyphenolic complexes of these elements [23].

The mineral composition was also evaluated in the ethanolic extract to verify the content of elements which may possibly be linked to organic compounds. For this sample, the highest concentration was for Ca and the lowest was for Mn. The order of metal concentration was Ca> Mg > Fe > Zn > Cu >Mn, as shown in table 2.

Among the different elements present in tests carried out in this work, Ca, Mg and Fe were the major constituents of *A. occidentale* leaves. In plants, calcium is an essential nutrient, which is required for structural roles in the cell wall and membranes, neutralizes toxic acids and increases resistance to pests and diseases. Magnesium is related to photosynthesis; since this element is part of the chlorophyll molecule and also has an active role in DNA and RNA synthesis. Iron has high redox capacity, mainly in respiration, photosynthesis and enzyme reactions. In general, the role of Fe is similar to the role of Mg [24-27].

In human bodies, calcium is one of the essential minerals needed in the composition of bones and teeth and in the activations of a large number of enzymes, having also a vital role for the functioning of muscles. A calcium deficiency can lead to diseases such as tachycardia, rickets and osteoporosis, among others [28]. Magnesium is extremely relevant in the processes of the human body including activation of enzymes like myokinase, creatine kinase, and many others [29]. However, the excess of magnesium causes problems in the nervous system and peripheral vasodilatation [30]. Iron is crucial to delivering oxygen to each cell of the body and is present in red blood cells, is present in the liver, bone marrow, spleen and muscles, and acts as an essential component of various processes that occur in the body and is also engaged in immune function and cognitive performance [31].

CONCLUSION

The mineral contents of *A. occidentale* leaves (young and adult), infusion and ethanolic extract were determined by flame atomic absorption spectroscopy. The results of this study have revealed that the samples contain higher levels of Ca, Fe and Mg and lower of Zn, Mn and Cu. Of the analyzed materials, the leaves and extract had higher mineral concentrations than those prepared by infusion. This studying is essential for assurance and safe use of medicinal herbs.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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