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Energy Dispersive X-Ray Fluorescence Analysis of Amapá Milk (*Brosimum ssp.*)

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Abstract

Latex from *Brosimum* species has several indications in folk medicine and is also largely ingested at the Amazon Region with food purposes, to replace cow's milk and as base for chewing gum. The presence of inorganic substances with potential to be irritating or toxic were analysed by Energy Dispersive X-Ray Fluorescence multi-element technique for the three main latex producing species: *Brosimum potabile, Brosimum utile subsp ovatifolium* and *Brosimum parinarioides subsp parinarioides*. From 26 elements detected, including toxic metals as chromium and manganese, nine elements were quantified: Na, Mg, Al, P, Cl, K, Ca, Se and Rb. They reflect some specific patterns from Amazonian soil and are species fingerprint. Some elements, as calcium, essential to the human diet, were observed in large amounts, but with great variation among species, as also within species. The inter and intra-species variation on the essential elements and the presence of toxic metals, indicates that the use as food should not be recommended and the regulation and control is strongly suggested.

Keywords: *Brosimum*; Moraceae; Amazon; Inorganic composition; EDXRF

Abbreviations: EDXRF: The energy dispersive fluorescence X-ray method; BUT: *Brosimum utile subsp ovatifolium*; BPO: *Brosimum potabile*; BPA: *Brosimum parinarioides*; WHO: World Health Organization

Introduction

Brosimum is an important genus from Moraceae botanical family, popularly known in the Amazon Region as Amapá or milkmaid amapá, in reference to the latex exuded by the trunk of the trees, similarly to rubber tree. *B. potabile*, *B. utile subsp ovatifolium* and *B. parinarioides subsp parinarioides* are the main latex producing species [1-6], with colours ranging from yellow, red or black [7] and milky consistency [8,9].

This genus is widely known at the Amazon Region since several different popular applications have been described [6,10,11]. Its latex is used in the food, as chewing gum [12] and in replacement to cow's milk; and in folk medicine, as treatment for diverse illnesses, but mainly to treat respiratory diseases [9]. The leaves, twigs and bark of their species have several substances that present pharmacological effects [5,13,14], such as to prevent vitiligo and skin diseases [15].

Several plant species in the Amazon region are characterized by the production of latex, assigned to important functions such as transportation and storage of nutrients [16]. *Himatanthus sucuuba (Spruce) Woodson* (Apocynaceae), popularly known as *sucuúba*, is one of these species that, according to Galuppo [9], presents some similarities with *B. parinarioides subsp parinarioides*, for instance their physical aspects and folk medicine use for the same purposes.

Despite the extensive use, there are few reports on the organic chemical composition of Amapá latex [7,17] and, to the best of our knowledge, only one report of inorganic composition [9]. In spite of the importance to human health that the accumulation of metals by plants denotes, especially those ingested in high amounts, as teas and food, few studies about the inorganic chemical composition of plants can be observed. Liu et al. [18] and Melquiades et al. [19] are some examples using that use X-ray methods. The energy dispersive fluorescence X-ray method (EDXRF) has been applied efficiently not only for detection but also for quantification of all chemical components between fluorine and uranium [20]. This methodology allows a quick review of most of the elements present in the sample, whether they are essential for the plant or toxic metals absorbed soil, for example. Its application in food analysis is especially important, since the technique is non-destructive, allows analysis of the fresh samples and does not require sample preparation or digestion, maintaining its original characteristics, use and avoiding contact with acids and toxic solvents, ensuring the accuracy and sensitivity of the technique as well as reducing the analysis time [21-23].

Rudger et al. [24] used EDXRF to analyse Amazonian oleoresins similar to incense and myrrh, obtained from the same botanical family Burseraceae, performing a qualitative study of inorganic elements. They were able to show the similarity between species of the same genus based on the presence or absence of certain elements and also the presence of some elements few investigated.

Faced to the medicinal importance of the *Brossimun* latex and its use as a base for chewing gum [25] and substitute for cow's milk, the inorganic composition of 34 latex samples of the three major species was studied by EDXRF.

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Materials and Methods

Samples

Botanical material was collected (34 samples of all species) from *B. utile subsp ovatifolium* (3 latex samples, named BUT), *B. potabile* (8 latex samples, named BPO) and *B. parinarioides* (23 latex samples, named BPA). Samples were obtained from adult trees, with trunk diameter at breast height above 30 cm, located in different properties in the municipality of Presidente Figueiredo city (Amazonas, Brazil), in the rural settlement Cristo Rei do Uatumã, located at Km 28 of the road to the municipality of Balbina (AM 140), coordinates (2° 02' 54.79" S, 60° 01' 39.63" W). Voucher samples were deposited in the INPA herbarium under numbers 18,042, 164,991 and 175,895, respectively.

Elemental analysis

Qualitative and quantitative analysis were performed using X-ray Energy Dispersive fluorescence spectrometer of Shimadzu, model EDX700, with analysis of elements with atomic number between 11 and 92. The data was obtained using a rhodium tube, detector of Silicon-Lithium, time of 200 s of exposure and with a voltage of 40 keV. Elements were detected in the scanning range of the device [20,26], identified by their Ka energies and Kβ [27]. Quantifications were based on their relative intensities (cps/uA), using external standards, and the OriginPro 7.5 program [28].

Results and Discussion

Comparative studies were performed among the three species, as well as comparisons with literature data, qualitative and quantitatively. In general, 26 chemicals were detected: Na, Mg, Al, Si, P, S, Cl, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Se, Br, Rb, Sr, Zr, Nb and Mo. The previous study with inorganic elements of Amapá milk [9], were performed only to Ca, P and Mg, using atomic absorption, and only to the species *B. parinarioides subsp parinarioide Ducke*.

In the inorganic study of amazonian sucuuba latex (*Himatanthus sucuuba*), unlike amapá milk, elements Ba, Pb and Th were observed [16]. The difference in composition may be related to differential accumulation of specific elements for each species [29]. The technique used by Silva et al. in the analysis of sucuuba latex, atomic absorption, only allows the observation of previously selected elements. Thus, elements present in *Brosimum* samples from this study that were not observed in sucuuba, such as Si, P, S, Cl, Sc, Se, Br, Rb, Nb and Mo may be present, since they not have been the object of that study.

Macroelements such as Na, Ca, K, Mg, P and Cl found in the three *Brosimun* species are observed in cow's milk [21,30-32], as well as most of microelements. The comparison with cow's milk becomes important in this case since the latex is used as a substitute of this food in some areas of the Amazon.

The presence of Ca and Mg has been studied in the latex of *Hevea brasiliensis*, the rubber tree, because they cause destabilization of this material. Moreover, Cu, Mn and Fe have also industrial importance, because they have a pro-oxidative and catalytic action, promoting oxidative deterioration of rubber [33]. All these elements were observed in the Amapá latex. Just as for sucuuba when comparing studies of *Hevea* with our data, we cannot say that the unreported elements for the *H. brasiliensis* are not present, once again, because of the different methodology applied in our work.

Of all the elements detected, only nine were quantified for comparison with previous studies, the availability of calibration curves and also since they are macroelements present in the latex: Na, Mg, Al, P, Cl, K, Ca, Se and Rb. Some trace elements, such as Si, Fe, Mo, among others, were detected at very low concentrations, below our current limits of quantification.

Qualitative analysis

Qualitative analysis of the detected elements allows the observation of inorganic composition fingerprints for latex of all three species studied, the presence of iron and chlorine in all samples. Various elements may be highlighted, they occur in at least 75% (minimum) of the samples: S, K, Ca, Cr, Mn, Co, Ni, Cu, Zn, Se, Br, Rb, Sr, and Zr (Table 1A).

On the other hand, some elements are most commonly observed in the samples of some species; rarer in other species, such as Si, P, Sc, Nb and Mo (Table 1B). This pattern could be used for the definition of biomarkers for different species of this genus.

Another possible interpretation can arise from the observation of elements that were detected in a uniform percentage of samples, but less than 75% in at least one of the species, such as Na, Mg, Al, Ti and V (Table 1C). For the Na, for example, the observed percentages were 66.7; 75.5 and 78.3% for BUT, BPO and BPA, respectively. For Al, the detection occurred in 33.3; 25.0 and 26.1% of the samples, respectively.

All elements detected in BPA were also observed in BPO. In BUT, only in Sc and Nb were not detected. BUT was the species with fewer samples collected, but, even so, the absence of these elements was confirmed in the duplicate analysis.

Smaller amounts were observed for Al, Sc and Nb. These elements can be found replacing Si, Ca and Zr in the structures of minerals in the Amazon soil [34,35]. Thus, the plant can absorb a small amount or even don't absorb these elements on the availability of the original soil [29], justifying their low percentage in the samples.

Quantitative analysis

Na was the element with the highest concentration in all samples (between 3 and 4% (w/w)), followed by Mg (just over 1%). The lower concentrations were obtained to Rb (0.002% (w/w)) and Cl (0.04% (w/w)). Interestingly, Cl was detected in all samples, but has extremely low concentration. In Table 2, the average of percentage for each element quantified can be observed.

Considering the elemental composition of each species, it is found that BPO and BUT have similar mean concentrations to most quantified elements, except only for the percentage of Se, and a subtle difference observed in Rb. BPA species has different average concentrations for Na, Mg, Al, P, K and Ca. It is worth noting the big difference for K, with 88% less of this element in BPA in relation to other species.

According to Franco [32], the World Health Organization (WHO) recommends that daily values in the diet of an adult are to: Na = 2200 mg; Mg = 350 mg; P = 800 mg; Cl = 3400 mg; K = 3750 mg; Ca = 800 mg; Se = 0.125 mg. These elements will present some toxicity only when the usual supply would constantly exceed. Hence, if ingested in moderation, either latex called Amapá milk studied here will not cause such undesired effect.

Thus, according to the results described in Table 1, the present study confirms the popular use of amapá milk as strengthening tonic property described in the working Galuppo [9], with substantial amounts of essential macroelements. Indeed, different amounts were identified by that author, as their samples were collected in the state of

A Elements		S	Cl	K	Ca	Cr	Mn	Fe	Co	Ni	Cu	Zn	Se	Br	Rb	Sr	Zr
% of samples with the element	BPA	91.3	100.0	95.7	100.0	91.3	91.3	100.0	95.7	82.6	95.7	95.7	78.3	91.3	82.6	82.6	87.0
	BPO	75.0	100.0	100.0	75.0	75.0	87.5	100.0	87.5	87.5	100.0	75.0	100.0	87.5	100.0	87.5	75.0
	BUT	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
B Elements		**Si**	**P**	**Sc**	**Nb**	**Mo**											
% of samples with the element	BPA	39.1	39.1	21.7	13.0	78.3											
	BPO	62.5	37.5	25.0	37.5	100.0											
	BUT	100.0	66.7	0.0	0.0	33.3											
C Elements		**Na**	**Mg**	**Al**	**Ti**	**V**											
% of samples with the element	BPA	78.3	73.9	26.1	87.0	82.6											
	BPO	75.0	62.5	25.0	87.5	75.0											
	BUT	66.7	100.0	33.3	66.7	66.7											

Table 1: Qualitative analysis of the observed elements (A: more than 75% of samples per species, B: very oscillating percentages, C: uniformly detected).

Species		Concentration (mg/g)								
		Na	Mg	Al	P	Cl	K	Ca	Se	Rb
BPO	Average	5.1247	1.9699	1.60870	0.9807	0.0456	0.7883	0.0550	0.1796	0.0021
	Standard Deviation	2.7063	0.1499	0.10074	0.7701	0.0003	0.3334	0.0041	0.0002	0.0004
BUT	Average	6.5499	1.9519	2.46665	0.7389	0.0452	0.8534	0.0599	0.1795	0.0019
	Standard Deviation	0.3058	0.1579	0.24515	0.0936	0.0027	0.3758	0.0104	0.0001	0.0001
BPA	Average	5.5852	1.8589	1.91889	1.1613	0.0452	0.1201	0.0584	0.1794	0.0019
	Standard Deviation	1.9512	0.9385	0.84724	0.7066	0.0014	0.0197	0.0076	0.0002	0.0003

Table 2: Average concentration of elements by species (mg/g).

Pará (Eastern Amazonia), with very different environmental conditions of the local collection of these samples, with distance of about 3000 km to each other.

Cow's milk is a food rich in minerals such as Na, Mg, P, Se and especially Ca and K [30,31,36] that may vary due to a number of factors such as lactation period the animal race, climate, season, animal diet composition and place of pasture [37]. In some Amazonian rural communities, *Brosimun* latex are used as temporary replacement for cow's milk, hence their popular name (Amapá milk). Using the average values, Franco [32] indicates that the values of certain minerals to cow's milk (Figure 1) can be compared to values observed in this study and in the Galuppo [9].

It can be seen that Amapá milks have levels of Na and Mg higher than cow's milk, but lower values of P, K and Ca, suggesting that the use as a substituent cow's milk should not be done by very long periods. Comparing *Brosimum* and *Himatanthus* latex [16], it is observed that Amapá has lower concentrations for all detected element. This is due in part to methodological differences applied in the two studies. The samples from Silva study [16], were lyophilized and *Himatanthus* elements were quantified in the digested samples. At the present study, samples were analyzed fresh, and the elements have their concentrations given in percent by weight of latex, without any external interference. Also according to Silva et al. [16], the sucuuba latex is popularly quoted as having anti-inflammatory and antitumor properties. These properties may be associated with the presence of Ca and Mg, as mentioned in the same work. The same properties are reported in work by popular Galuppo [9] to *B. parinarioide*, although the amounts of Ca and Mg are lower in Amapá milk. In a way, this type of property can be related to the presence of these elements, even in low concentrations.

In the work of Ng and Lai [33], *Hevea brasiliensis* latex samples were studied and the concentrations of Cu, Mn and Fe were observed to be below 3 mg/kg; and Ca were detected with mean value of 23.8 mg/kg, below Amapá values. According to Ng et al. [38], calcium helps the latex coagulation during production of rubber. Concentration above 30 mg/kg makes this procedure impracticable. Thus it is evident that the amapá latex, whose Ca concentration is about 500 mg/kg, is not a good source for rubber production.

Using EDXRF techniques it was possible to detect and quantify the elements in *Brosimum* latex samples, with interesting results. It was possible to conclude that the Amapá milk can be consumed both as a tonic, and as chewing gum, since no elements have concentrations above recommended by WHO and fits in Brazilian Sanitary

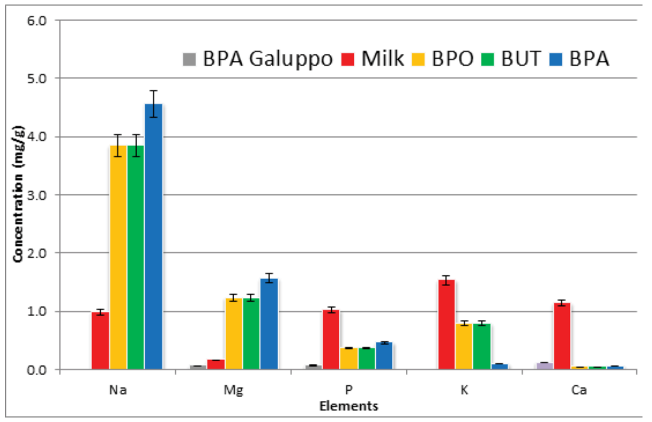

Figure 1: Elemental composition comparison of three species of amapá milk and cow's milk, were: BPA Gallupo and Milk are data of other researchers.

Authorities' values. The composition can help in the differentiation between species, and different types of latex, such as the *H. sucuuba* and *Hevea brasiliensis*. Since the latex species assessed in this study have chemical elements essential to the human diet, its use as chewing gum is appreciable, in addition to pharmacological and nutritional applications.

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