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Original Paper

The Relationship between Elementary Chemical Composition and Extraction Method for Protium Strumosum Daly Species Resin

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Abstract

The genus Protium Burm f., the most abundant of the Burseraceae family, has its primary center of diversity in the Amazon. Its main characteristic is the exudation of "Breu", an aromatic resin collected directly from the trunk of the trees.

Stimulating pastes have been used to increase resin flow, such as 2-chloroethylphosphonic (Ethephon). It is important to evaluate variations in the composition of the resin extracted naturally and with the use of the stimulant, to guarantee the product quality. The inorganic analysis is also fundamental to verify the presence of compounds harmful to health.

A quali-quantitative elemental variation of the resin between the two extraction modes was proved by WD-XRF analysis. In 15 samples, Mg and F were only observed in those using Ethephon. In samples from the same tree, the qualitative difference is 54%, proving a discrepancy in the chemical composition of Breu, which can alter its properties.

Keywords

Protium strumosum Daly, ethephon, Elementary Chemical Composition, WDXRF

1. Introduction

Burseraceae family's origin is probably from North America reaching the South by migration. Consequently, it expanded to other areas of the world, mainly subtropical and tropical areas, reaching Europe, Africa, Asia and Oceania (Weeks, Douglas, & Beryl, 2005). There are about 700 species divided into 19 genera. The *Protium Burm f.* is the most abundant genus with approximately 150 species (Murthy, Reddy, Rani, & Pullaiah, 2016). Its primary center of diversity lies in Amazon with 73 species of which 42 are endemic (Aguilar-Sierra, 1998).

As well as most species of the family Burseraceae, the main attribute of the genus *Protium* is the exudation of an aromatic oleoresin, popularly known as Breu. It is widely found and commercialized in the Amazon region with economic, medicinal and cultural importance (Zoghbi, Andrade, & Maia, 2002). Generally, it is collected directly from the trunk of the trees being exuded by cracks, splinters or insect action (Daly, 1992).

Among the well-known applications of Breu stands out: fire lighter, boat caulking and insect repellent action. On traditional medicine, natives commonly use its properties as anti-inflammatory, analgesic, expectorant and cicatrizing (Daly, 1989; Siani et al., 1999; Duwiejua, Zeitlin, Waterman, Chapman, Mhango, & Provan, 1993; Rüdiger, Siani, & Veiga Junior, 2007). On the industrial area, breu resin has been used in the cosmetics industry due to its aromatic composition in soaps, perfumes and varnish (Tourneau & Greissing, 2010).

The induced extraction of oleoresins, gums and latex through cuts or holes in the trunk of the tree is done commercially for domesticated and native species found in forests. Sometimes the plant does not provide a satisfactory amount of resin or don’t even release it. Based on this, stimulating pastes have been widely used, aiming to increase resin flow for a longer time (Abeles, Page, & Mikal, 2012; Zuñiga, Paulo, & Valdir, 2017; SCHALLER, 2012).

The most common stimulant paste is 2-chloroethylphosphonic, better known as Ethephon. In contact with pH above 3.5, this paste decomposes into ethylene (C_2H_4), a plant hormone that acts at the cellular level, triggering a series of processes within the plant. The stimulation improve resin production and endurance in wound healing, rising the resin production flow to remain active for a longer period (Miller, 1938).

Some studies have already verified the efficacy of ethephon increasing of the resin production by the trees (Zuñiga, Paulo, & Valdir, 2017). However, it is important to evaluate if there was any variation in the chemical composition of the resin extracted with the stimulant use, to guarantee the chemical quality of the product offered in the market. Furthermore, inorganic chemical analysis is essential to verify the presence of compounds that may be harmful to human health if absorbed from a certain concentration, such as Chromium, Bromine, Manganese and Chlorine (Malavolta, 1980).

Among the inorganic elements absorbed by plants, a special group called essential elements stands out. They are part of some important compound or reaction that maintain the plant health (Epstein, 1975). In addition to C, O and H (organic), sixteen mineral elements are classified essential, divided by the concentration required by the plant in two groups: macronutrients (N, P, K, Ca, Mg, S and Si) and micronutrients (B, Cl, Cu, Fe, Mn, Mo, Zn, Ni and Na). Macronutrients come into concentrations of 10 to 5000 times higher than micronutrients (Taiz & Zeiger, 2004).

This work aimed to differentiate the inorganic chemical composition of an oleoresin, collected from

Protium strumosum Daly, with two different extraction methods, natural and with ethephon. Then run an analysis with wave dispersion X-ray fluorescence (WD-XRF), showing how much qualitatively and quantitatively samples differs from one another.

2. Experimental

2.1 Samples

The samples were collected at Adolpho Ducke Forest Reserve, located at the northeast of of Manaus city, at coordinates 02º55 ‘S and 59º59’ W. Ten trees of the species *Protium strumosum* Daly were identified by the Herbarium of the Institute National Research Center of the Amazon - INPA. For ethephon application, cuts were made on the trunk of the trees with a syringe knife. The ethephon used was the commercial 10% concentration, which was diluted in equal parts (500 ml of water with 500 ml ethephon 10%, totalizing 1 L of 5% ethephon) then it was applied using a brush. The resin was collected after 60 days (Rüdiger, Cláudia, & Valdir, 2009). The resin exuded naturally was also collected in the trunk in five of these trees, totaling fifteen samples.

The samples were stored in glass containers protected from light and kept under refrigeration until analysis. Different textures, stains and amounts of resin were observed in each sample collected.

2.2 Samples Analysis by WD-XRF

Due to its different condition, liquid and solid specimens were prepared in different ways. Those that still had a certain viscosity (liquid) were impregnated in suitable filter paper for analysis. On the other hand, samples too dry (solid) were sprayed with the aid of a mortar and a pestle until they reached the appropriate homogeneous granulometry then poured inside a plastic sample holder and finally capped with a polypropylene film to isolate the material. The analyzes were performed on WD-XRF of Rigaku brand, model SuperMini, palladium tube, 200 sec of exposure time, with power of 200 W. The conditions were set taking into account the sample matrix and the sample holder. The analyzing crystals LIF 200, PET and RX25 were used. In this equipment the SC and PC detectors are available, both used in the analysis of these samples. A sample of the blank filter paper had its data collected, to serve as reference during the analysis process, as well as the polypropylene film. The samples had their data collected under the same conditions as the filter paper / polypropylene film. Once the parameters have been adjusted for analysis, the results obtained by different methods of sample preparation can be compared.

Qualitative analysis were performed by observing the characteristic energy peaks of each sample. It was possible to identify the elements by their Kα and / or Kβ energies (Rene & Andrzej, 2001; Janssens, Proost, & Falkenberg, 2004). The quantification analysis were done through mathematical relations, the emission peaks are related to the respective concentrations of a certain element. To simplify the comparison between the two methods, average concentration values were obtained from the data and divided and two groups of results.

3. Results and Discussion

3.1 Qualitative Aspects

The composition of the oleoresins from the two extraction methods are presented in Table 1. In qualitative terms, the Mg and F elements were observed only in the samples extracted with ethephon, indicating the existence of the difference in the result between the extraction methods. Macronutrients were detected in all specimens. The micronutrients observed in the samples were Cl, Fe, Zn and Na.

Table 1. Elemental Chemical Composition of Breu Extracted with the Two Methods of Analysis

Samples	Elements
Ethephon	K, Zn, Cu, Ag, Cd, Fe, Si, Cl, S, Na, P, **Mg**, I, Al, **F**, Ca.
Natural	K, Zn, Ag, Cd, Fe, Si, Cl, S, Na, I, Al, Ca.

In a previous comparative study among 5 species of the family Burseraceae (*Protium cf. apiculatum, Protium cf. hebetatum, Protium cf. heptaphyllum, Protium tenuifolium* and *Trattinnikia peruviana*) (Janssens, Proost, & Falkenberg, 2004), the following elements were detected: S, Cl, Ca, Fe, Ti, V, Si, Na, P, Al, Mg, K, Cr and Mn. In the composition of resin, oils exuded by these species without the use of ethephon by the dispersive energy X-ray fluorescence method. With the exception of the elements Ti, V, P, Cr and Mn, all the others were found in this study with the pitch of the species *Protium strumosum* Daly.

Some oxides, such as SiO_2, Al_2O_3, Fe_2O_3, MgO, CaO, Na_2O, K_2O, TiO_2, are found in several rivers forming the Amazon Basin: Sampaio, Marmelos, Antônio, Puruzinho, Jamari, Madeira, Aripuanã, Manicoré and Machado (Queiroz, Adriana, & Candido, 2011). These oxides come from some minerals such as Illita, Muscovite, Montmorillonite, Quartz and Tourmaline, being probable sources of the elements Si, Al, Fe, Mg, Ca, Na and K. The last four are the main cations found in the soil (Pavinato & Rosolem, 2008).

The essential elements observed in the Burseraceae oil-resin in this work are commonly observed in other plants, with varying concentrations depending on the plant species, region where the sample was collected and the part of the plant studied. They are elements common to all higher plants, being present in all their tissues (Janssens, Proost, & Falkenberg, 2004).

Among the non-essential elements found, we have Ag, I, Al, F and Cd. Compared to other studies in the literature (Marques, Sartori, Lemos, Machado, Souza, & Monte, 2010; Silva, Milena, Julie, Gil, & Valdir, 2016), silver is not commonly found in pitch samples, not even in resins of other species. Its presence in all samples can be associated with the substitution of another essential element in low concentration in the soil. In higher plants and fungi, silver accumulation is expected only in areas contaminated with silver, such as silver mine tailings and modified soils. The use of industrial and household waste as organic fertilizer is a possible source. The needs of heavy fertilizers of mineral origin, as well as agrochemicals and herbicides, also add to the soil large quantities of heavy metal, which may explain the presence of silver (Zámbò, Tétényа, & Bernáth, 1989). Since the samples come from native forest, there is no obvious explanation for the presence of silver in the samples.

Cadmium is a element highly damaging to life health, from 0.5 ppm is already considered toxic to any plant material. Mineral fertilizers, mainly phosphates, tend to add Cd to the soil (Fernandes, Flávio, Lidia, & Maria, 2016).

Aluminum is one of the most abundant cations in the earth's crust. The release of Al^{3+} in the soil is directly related to the soil acidification process (Marschner, Horst., 2011). It is involved in reducing the availability and uptake of soil P by the plant, and in the competitive inhibition of the absorption of cationic nutrients, such as Ca^{2+}, Mg^{2+}, K^{+}, and micronutrients. It also presents a damaging action on the cell membranes of plants, binding to its components, drastically reducing its permeability (Mengel & Kirkby, 1987).

Iodine is an essential element to humans and animals. Naturally, iodine poisoning in plants is difficult (Volkweiss, 1986). The levels of the element in the water reflect the content of the element in the rocks and soils of a region and, consequently, its contents in the plants.

Plants absorb fluoride as F- from the soil and increase pH by liming decreases their availability and absorption by the roots (Lopes, Almeida, Nogueira, Magalhães, & Morais, 2002). The low contents in most soils, the small availability and the small absorption, help to explain the low frequency of F-intoxication found in plants (Freire, 2005).

3.2 Quantitative Aspects

Table 2 shows the results about the concentration averages of all samples. For the purpose of direct comparison, these results focus on two samples extracted from the same tree. In this case, of all eleven elements observed in the sample with ethephon, only the elements Cd, Ag, Fe, K, Cl and Si were in common with the natural sample. However, Na, P, S, Cu and Zn were only observed in the sample extracted with ethephon. Reinforcing the conclusion that the chemical composition of the resin, when extracted with ethephon, becomes different.

Table 2. Comparison between Two Samples Extracted from the Same Tree

Elements	Natural Extraction (mg/g)	Ethephon (mg/g)
Si	0.0135	0.0162
Cl	0.0118	0.0868
K	0.0439	0.0366
Fe	0.0137	0.0100
Ag	0.1122	0.1866
Cd	0.0722	0.0701
Na	NQ**	0.1032
P	NO*	0.0069
S	NQ**	0.0137
Cu	NO*	0.0034
Zn	NQ**	0.0060

*Not observed; ** Observed, but not quantified (Below the limit of quantification)

Figure 1 indicates that the values are higher in the samples with ethephon. The stimulant acts to potentiate the production flow of the resin, resulting in this arrangement (Fusatto, 2013). With the exception of Ca, Na and Cd, all other elements appeared in greater quantity in the samples extracted with ethephon.

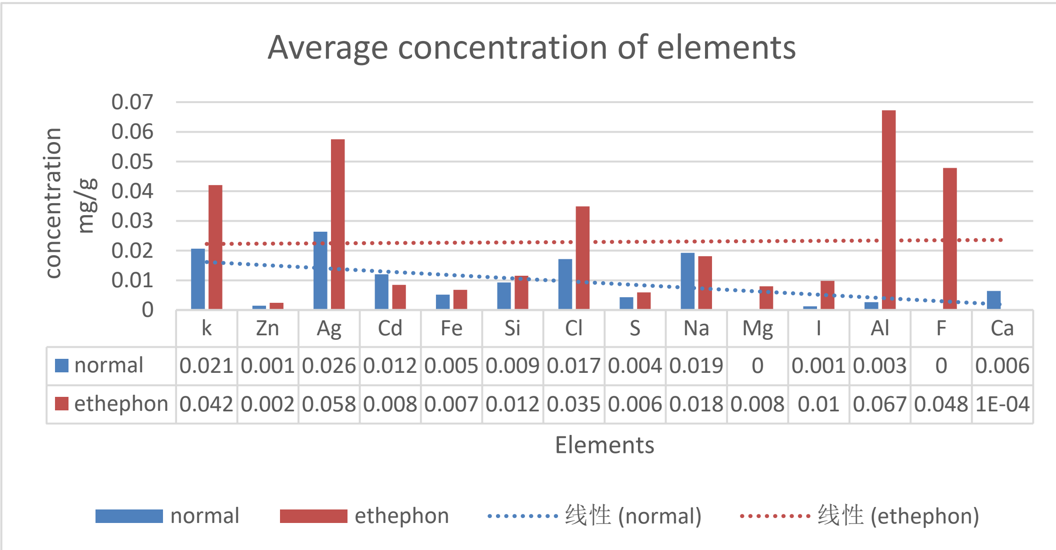

Figure 1. Average Concentration of the Elements Presents in the Samples

Study on the use of ethephon in resin production in *Pinus elliottii* var. *elliottii* (Lopes, Almeida, Nogueira, Magalhães, & Morais, 2002), found the increase in resin exudation at about 70% more than normal. Another study, with the species *Pinus caribaea var. Hondurensis*, indicated that ethephon increases by more than 100% the production of resin by the tree (Capitani, 1982).

The majority of micronutrients are predominantly constituents of enzyme molecules, therefore essential only in small concentrations throughout plant levels. In contrast, macronutrients are constituents of organic molecules, such as proteins and nucleic acids, or acting on osmosis, appearing in higher concentrations (Ratte, 1999). The average of macronutrients found in natural samples (K, Mg, Ca, S and Si) was 0.0091 mg/g. On the other hand, samples extracted with ethephon (k, Mg, Ca, S and Si) presented 0.0135 mg/g, while the micronutrients (Zn, Fe, Cl and Na) in the natural samples had an average value of 0.0108 mg/g, whereas in the samples with ethephon use it was 0.0155 mg/g.

In both situations, it was observed that in the samples obtained with ethephon the number was higher, indicating the influence of ethephon on the chemical composition of the sample. Contrary to literature (Meyer, Anderson, Bohning, & Fratianne, 1983), the average in both natural and ethephon samples, of macronutrients was lower than micronutrients.

Chlorine and Phosphorus are part of the chemical composition of ethephon structure (Carvalho, 2003). The higher frequency of chlorine in samples with ethephon support extraction may indicate the low retention of this element by the plant, therefore its prompt exudation by the vegetal system.

A previous study about amapa milk, presented potassium in almost 100% of the samples, its quantitative average was contacted around 0.1201mg/g in the species studied. Its presence can be observed in large scale in natural resins due to its regulation enzymatic functions, electrochemical balance and synthesis of proteins (Alva, 2006).

Cadmium found in concentrations from 0.0005 mg/g on any vegetable material is already considered toxic (Faquin & Vale, 1991). In the natural samples, its average was 0.012 mg/g and in the samples with ethephon was 0.0085 mg/g, indicating cadmium intoxication in the vegetal material of the plant.

The metal ions of Ca and Mg are considered destabilizing in the latex of fresh rubber in the species *Hevea brasiliensis* when they are in abnormal large concentrations (Ng, Hsia, & Lai, 1970). Results showed concentrations below 0.01 mg/g, suggesting normality in Breu.

4. Conclusions

The chemical analysis of the Breu elemental composition is vital to elucidate the differences between two modes of extraction. Qualitatively, it was observed that on the average compositions of all 15 samples, Mg and F were only observed in the samples extracted with ethephon. Quantitatively, the average elemental compositions were higher in the ethephon samples, following the literature. In two samples of the same tree, it is noticed that the qualitative difference shows even greater, being 54% the difference, proving the difference caused in the chemical composition of the oleoresin. These differences may cause changes in Breu’s properties, showing the need for a more in-depth study of the composition of the industry, since the material is used in the production of cosmetics.

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