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Characterization of Nutrients in the Leaves and Fruits of Embaúba (Cecropia Pachystachya) Trécul

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Abstract

The fruits and leaves of the embaúba tree (*Cecropia Pachystachya* Trécul) harvested in the region of the state of Rio de Janeiro were analyzed for moisture, protein, fat, ash, soluble fiber, insoluble fiber, carbohydrates, pH, acidity and minerals. The fresh fruit and leaves had 71.8 and 62.4% moisture, 0.54 and 1.13% protein, 0.68 and 0.46% total fat, 0.50 and 0.96% ash, 0.11 and 0.19% soluble fiber, 2.60 and 2.19% insoluble fiber, 23.8 and 32.7% total carbohydrates, 0.04 and 0.06% acidity and pH values of 5.98 and 5.1, respectively. The fruit and leaves are also good sources of magnesium (Mg), potassium (K), manganese (Mn) and iron (Fe). They provide >100% of the dietary reference intake (DRI) for adults. Lyophilized fruits and leaves were also extracted by hot, pressurized, dry methanol (100°C, 10 MPa). This solubilized 27% of the fruit and 15% of the leaves. The extract was partitioned between water and methylene chloride (CH_2Cl_2). The amphiphilic compounds went into the CH_2Cl_2 phase. They accounted for 2.13% and 5.15% of the lyophilized fruit and leaves, respectively. The amino acid concentrations were also measured. NMR analysis showed that the methanolic extract contained primarily fatty acid glycosides, with smaller amounts of aromatic compounds. The NMR spectra of the amphiphilic compounds showed the presence of triglycerides in the fruit, but not the leaves. This will give regulators several ways of determining whether or not food products labeled as containing embauba are genuine. In addition, it will help regulators decide if embaúba should be classified as generally regarded as safe (GRAS). It will also help regulators decide what should be on the label for food products made from embauba.

Keywords: Embaúba tree, *Cecropia Pachystachya*, Fruits, Leaves, Chemical composition

1. Introduction

Cecropia Pachystachya Trécul is a tree that is known as embaúbeira, embaúba, embaúva, umbaúba, ambaíba and "lazy tree". A photo of it is shown in Figure 1. It is native to the Americas. It can grow 4-7 m tall, with a 15-25 cm diameter trunk and unisexual flowers. It is useful in reforestation, due to its rapid growth [1]. Their simple leaves with long, rough, white petioles are eagerly consumed by the pale-throated sloth (Bradypus tridactylus) and by birds. The word embaúba originated from the term "ambaíba", from the Tupi language. It means "tree with holes" or "tree that is not good for construction". As a result, about 80% of the *Cecropia* species are mirmecophytes that live in a mutualistic relationship with ants that live inside the hollow stems [2]. These plants provide corpúsculos mülleriano corpuscles that contain glycogen and proteins as well as shelter for ants [3].

Embaúba fruits are produced in abundance every year. They have small seeds surrounded by a sweet, meaty pulp that birds and monkeys eat. These fruits are similar to figs, with a soft pulp filled with many small seeds [4–6]. The *Cecropia* (Cecropiaceae) are used widely in Brazil [5]. The leaves of *C. pachystachya* Trécul (also known as *C. pachystachya* Mart. and simply *C. pachystachya*) are used traditionally as an expectorant, antiasthmatic, and hypoglycemic agent [5, 7]. In addition, the leaves are used in several Latin American countries to treat hypertension, as well as pulmonary and cardiac diseases [8, 9]. Other names for *C. pachystachya* include embaúba, umbaúba, imbaúba and embaúva in Brazil and as ambay, ambaí, amba-hu, ambaiba or ambay-guazú in Argentina [9]. The

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Figure 1: The embaúba tree

leaves are also used to treat coughs and asthma [9] and diabetes [10]. There are about 60 different species of *Cecropia* in Latin America, including Brazil [11]. *C. glaziovii* Sneth. and *C. pachystachya* are the two most common species in the Southeast and South of Brazil. These species are both popularly known as embaúba. They have different heights and colors of their leaves. Both of them are widely used in to treat cough, asthma, high blood pressure, inflammation, and as a diuretic. The main pharmacological activities described in the literature for these species are hypotensive activity and effects on the central nervous system, including anxiolytic and antidepressant-like activities. There are some phytopharmaceutical preparations made from both species in some countries, such as Brazil and France. The two species can be distinguished by the differences in their phenolic compounds. Chlorogenic acid was found in both, but only *C. pachystachya* had orientin [11].

More recent studies have shown that the leaves of *C. pachystachya* acted as an antidepressant in a mouse model of chronic, unpredictable stress [12]. An extract of the leaves also had topical anti-inflammatory and *in vitro* antioxidant effects [13]. A similar extract of *C. pachystachya* showed leishmanicidal activity and was able to inhibit the enzyme arginase, the concentration of which is elevated in hypertension, asthma, and diabetes [10]. Some of the phenolic compounds in an extract of the leaves were shown to be quorum sensing (QS) inhibitors [14]. So, the extract and the phenolic compounds in it may be useful as antipathogenic drugs or antifoulants (same ref). Finally, an extract of the leaves prevented ketamine-induced manic behavior and oxidative stress in rats [12]. This suggests that *C. pachystachya* leaves might be useful in preventing bipolar disorder, reducing the episode relapse and the oxidative damage associated with the manic phase of this disorder [12].

Some of the compounds that have been found in *C. pachystachya* leaves using high performance liquid chromatography (HPLC) with UV detection include chlorogenic acid, isoorientin, orientin, catechin, epicatechin, isoquercitin, isovitexin, vitexin, rutin, procyanidin B2, sitosterol and α-amyrin, as well as ursolic, pomolic and oleanolic acids [11, 13–15]. However, the chemical composition and health effects were determined using methanolic extracts obtained at room temperature and/or pressure. In contrast, pressurized liquid extraction (PLE) using methanol at 100°C and 10 MPa (100 atm) pressure can solubilize much more material from a variety of lyophilized fruit pulps, stems, seeds and leaves [16]. This includes an underappreciated class of bioactive compounds called fatty acid glycosides, which are esters of fatty acids and one or more sugars, usually glucose [16, 17].

To the best of our knowledge, the chemical composition of the fruits have never been described. Neither the fruits nor the leaves have been extracted with hot, dry methanol in a sealed contained (Accelerated Solvent Extractor) nor anlyzed by NMR. So, the objectives of this study were to determine how much of the dried leaves and fruits of *C. Pachystachya* can be solubilized by hot, dry pressurized methanol, analyze the extracts by NMR, as well as measure the moisture content, protein, lipids, ash, soluble fiber, insoluble fiber, carbohydrates, pH, acidity, amino acids and minerals of the dried leaves and fruits. This will give regulators several ways of determining whether or not food products labeled as containing embauba are genuine. In addition, it will help regulators decide if embaúba should be classified as generally regarded as safe (GRAS). It will also help regulators decide what should be on the label for food products

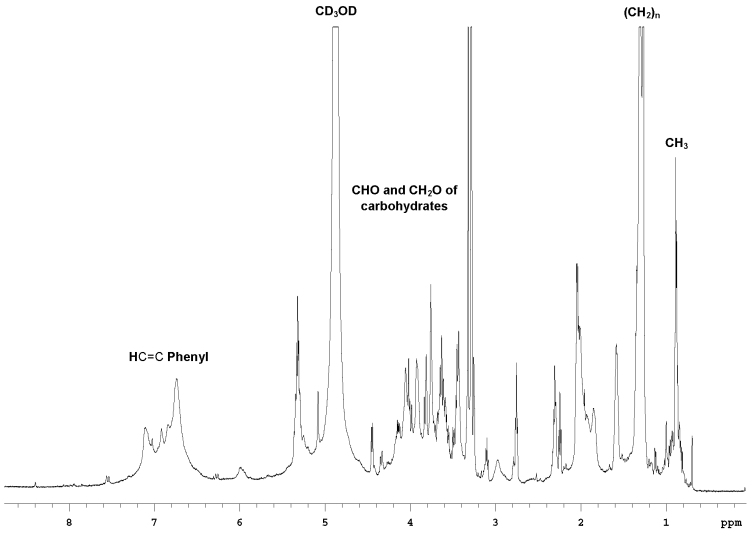

Figure 2: ^{1}H-NMR of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba fruit, in CD_3OD

made from embauba.

2. Materials and Methods

2.1. Materials

The fruits and leaves of the embauba tree (*C. Pachystachya*) were harvested in the Mazomba region in the eastern part of the state of Rio de Janeiro, where these trees are thick. Only fresh, healthy leaves with rough, white surfaces were used. They were lyophilized. Portions were analyzed in Brazil, while other portions were sent to the U.S. FDA for analysis by NMR. Methanol (CH_3OH), methylene chloride (CH_2Cl_2), deuterated chloroform ($CDCl_3$) and deuterated methanol (CD_3OD) were from SigmaAldrich, St. Louis, MO.

2.2. ASE extraction and separation of amphiphilic compounds

About 10 g each of lyophilized fruit and leaves of *C. pachystachya* were mixed with enough HydroMatrix™(SigmaAldrich, St. Louis, MO) to fill the 100 mL stainless steel sample cell used in an Accelerated Solvent Extractor (ASE, ThermoFisher Scientific, Sunnyvale, CA). Then, CH_3OH was added while the temperature and pressure were increased to 100°C and 10.3 MPa (1500 psi, 100 atm) over a 3 min time (static time). Next, the solvent was purged into a collection vessel. A total of four cycles were run to statically extract the sample, resulting in a total volume of about 160 mL. The solvent was evaporated off and the oily residues remaining were weighed. A portion of each residue was redissolved in CD_3OD for NMR analysis while another portion was partitioned between CH_2Cl_2 and water. The CH_2Cl_2 phase was collected to obtain the amphiphilic portion of each methanolic extract. The CH_2Cl_2 was evaporated off and the residues were redissolved in $CDCl_3$ for NMR analysis.

2.3. NMR analyses

NMR analyses were done using an Agilent DD2 600 MHz NMR (Santa Clara, CA). A 30° pulse width and 1 sec pulse delay were used for the ^{1}H NMR, while a 30° pulse width and 2 sec pulse delay were used for the ^{1}H-coupled ^{13}C-NMR spectra, also known as $^{13}C\{^{1}H\}$-NMR. Chemical shifts were referenced to the CD_3OD signals at 3.35 and 4.78 ppm (for ^{1}H) and 49.3 ppm (for ^{13}C) for the spectra of the methanolic extracts and to the $CDCl_3$ signals at 7.27 and 77.23 ppm, for ^{1}H and $^{13}C\{^{1}H\}$-NMR, respectively for the analysis of the amphiphilic compounds.

2.4. Analysis of peroxidase activity

To evaluate the effect of temperature on peroxidase activity in the leaves and fruit pulp, portions of each were briefly immersed in water at 100°C. After 30 secs, the leaves were removed and washed with 50 mL of distillied water. They were transferred to sample tubes to which three or four drops each of an alcoholic solution of guaiacol and hydrogen peroxide, both 0.5% (v/v). After thorough mixing, the formation of color indicated the presence of active peroxidase.

2.5. Physical and physical-chemical analyses

The following analyses were done in triplicate: moisture, total protein, carbohydrates, lipids (fat), soluble fiber, insoluble fiber and pH. They were done as described by the Adolfo Lutz Institite [18]. Minerals were quantified by inductively coupled plasma spectrometry (ICP), using a Perkin Elmer-Sciex ELAN 6000 [19].

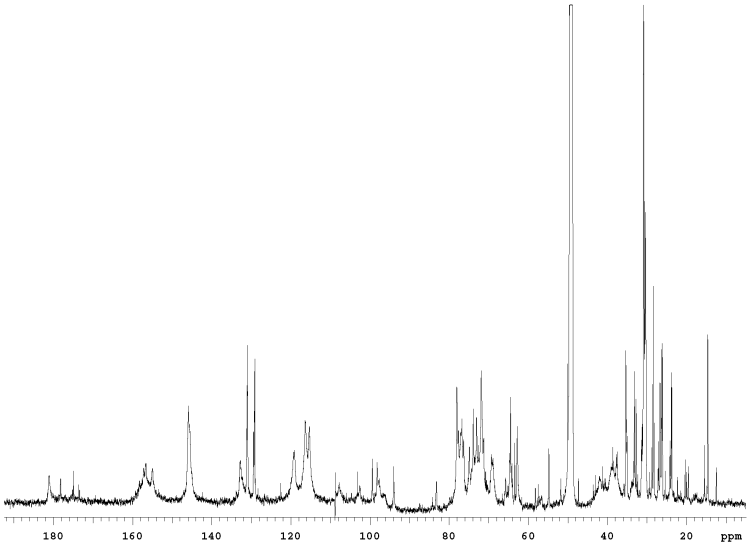

Figure 3: $^{13}C\{^{1}H\}$-NMR of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba fruit, in CD_3OD

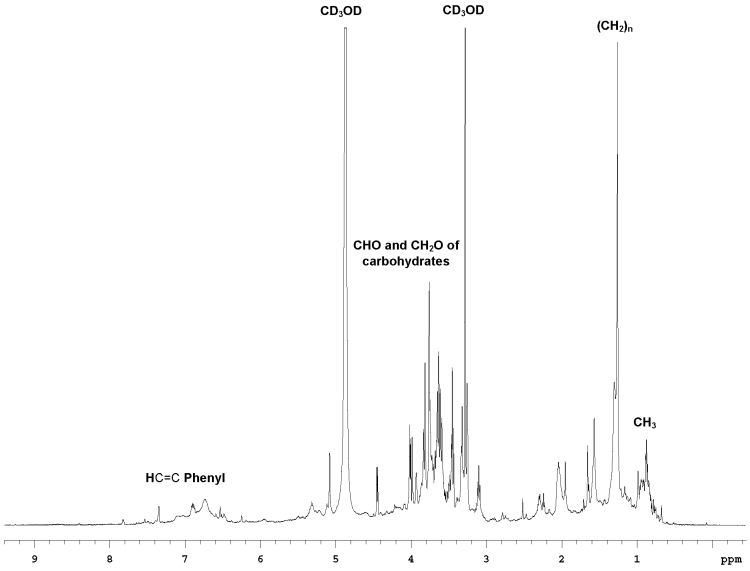

Figure 4: ^{1}H-NMR of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba leaves, in CD_3OD

3. Results and Discussion

The peroxidase activities of the fruits and leaves fell to zero after 90 secs of being immersed in water at 100°C. Controlling peroxidase activity is important, because it can cause undesirable effects by degrading healthy phenolic compounds and causing the fruits and leaves to darken [20, 21]. The purpose for peroxidase activity analysis is to identify a time point that healthy phenolic compounds will not be degraded by the enzyme. The ideal time point (90 secs) can be used in the product analysis to prevent such damage to the samples. The chemical composition and physical-chemical properties of the fruit and leaves are listed in Table 1. The amino acid profiles are in Table 2 and metal content is in Table 3. The fresh fruit and leaves had 71.8 and 62.4% moisture, 0.54 and 1.13% protein, 0.68 and 0.46% total fat, 0.50 and 0.96% ash, 0.11 and 0.19% soluble fiber, 2.60 and 2.19% insoluble fiber, 23.8 and 32.7% total carbohydrates, 0.04 and 0.06% acidity and pH values of 5.98 and 5.1, respectively. The fruit and leaves are also good sources of magnesium (Mg), potassium (K), manganese (Mn) and iron (Fe). They provide >100% of the dietary reference intake (DRI) for adults [22].

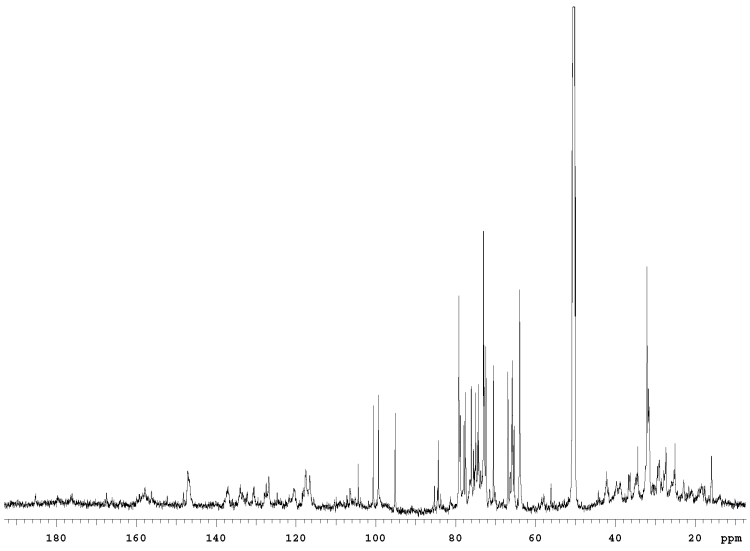

Figure 5: $^{13}C\{^{1}H\}$-NMR of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba leaves, in CD_3OD

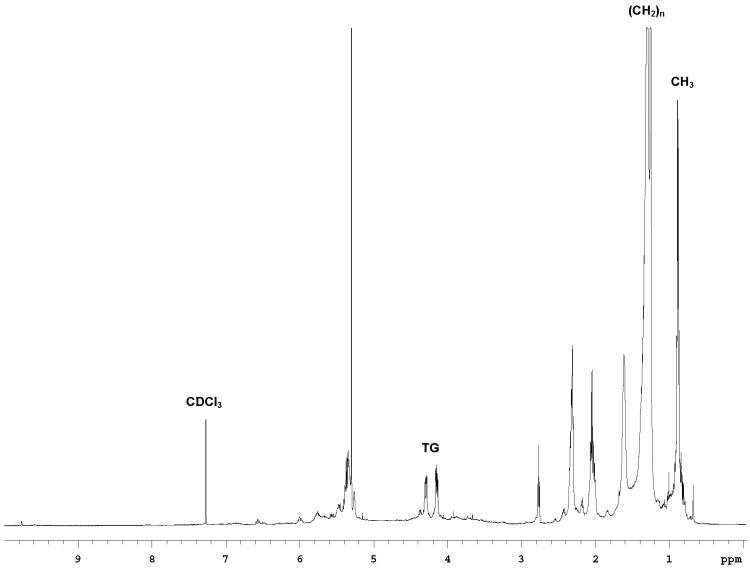

Figure 6: ^{1}H-NMR of the amphiphilic portion of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba fruit, in $CDCl_3$. The peaks due to the $-CH_3$ and $(CH_2)_n$ of fatty acyls, the $-CH_2O-$ of triacylglycerides and $CDCl_3$ solvent are labeled

Dry methanol solubilized 27% of the fruit and 15% of the leaves. The ^{1}H and $^{13}C\{^{1}H\}$-NMR spectra of the residues remaining after evaporating off the methanol are shown in Figures. 2 – 5. Like the spectra of other fruits and leaves that have been extracted and analyzed this way, the NMR spectra are dominated by signals in the carbohydrate region (38.6 and 46.6% of the total peak areas in fruits and leaves, respectively), with smaller peaks from about 0.6 – 1.0 ppm due to CH_3 groups that are attached to $(CH_2)_n$ groups that produced signals from about 1.2 – 1.4 ppm. These form fatty acyls that can be part of either fatty acids or esters of fatty acids and sugars (fatty acid glycosides). However, they are not part of triacylglycerides that may have been present in the fruit and/or leaves, but are not soluble in methanol. There are also signals from about 6.5 – 7.6 ppm due to HC=C in phenyl groups and/or phenolic compounds that were 13.0 and 7.47% of the total peak areas in fruits and leaves, respectively. These are unusually high values, suggesting that both the fruit and leaves could be good sources of phenolic compounds that may act as antioxidants. In comparison, signals due to HC=C in phenyl groups and/or phenolic

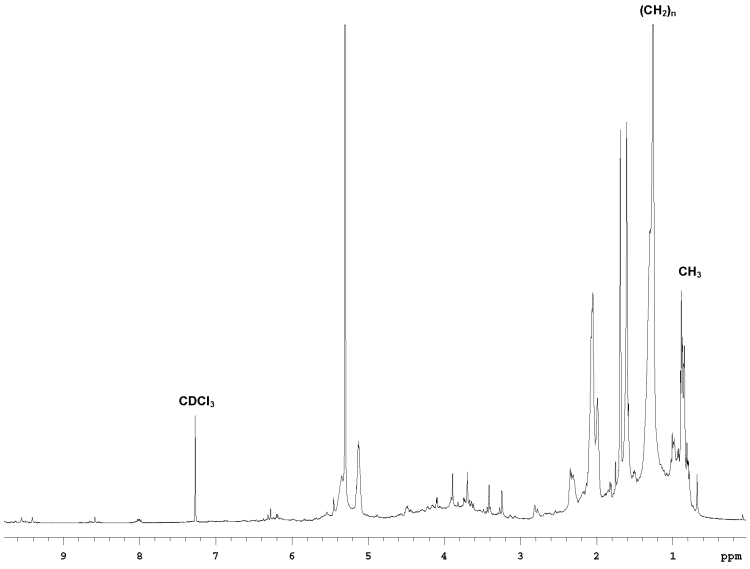

Figure 7: ^{1}H-NMR of the amphiphilic portion of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba leaves, in $CDCl_3$. The peaks due to the $-CH_3$ and $(CH_2)_n$ groups, as well as the $CDCl_3$ solvent are labeled

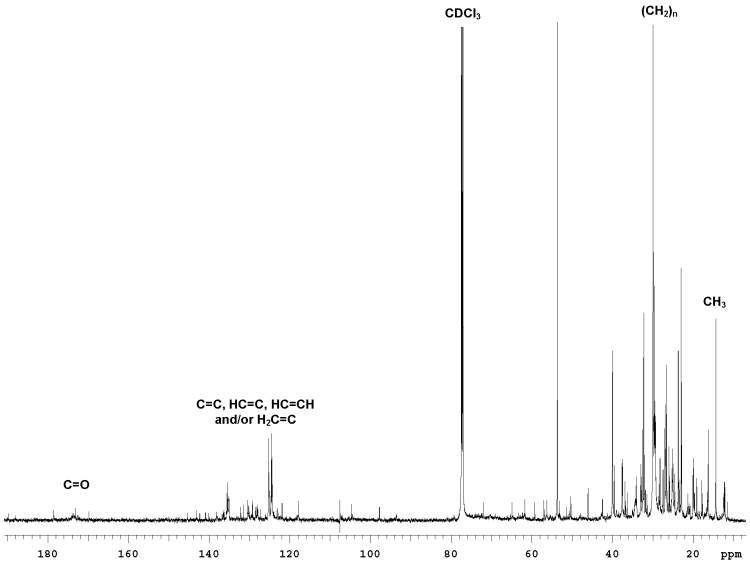

Figure 8: $^{13}C\{^{1}H\}$-NMR of the amphiphilic portion of the hot, pressurized (100°C, 10 MPa) methanolic extract of lyophilized embaúba leaves, in CD_3OD

compounds in açaí (*Euterpe oleracea*), elderberries (*Sambucus nigra*) and graviola (*Annona muricata*) were only 2.52, 0.97 and 0.80%, respectively [16].

About 2.13 and 5.15% of the methanolic extracts of the lyophilized fruits and leaves, respectively partitioned into CH_2Cl_2 instead of water. The ^{1}H-NMR spectra of these amphiphilic compounds are shown in Figures 6 & 7. There were signals in the ^{1}H spectra that due to $-CH_3$ (0.6 – 1.0 ppm) and $(CH_2)_n$ (1.0 – 1.6 ppm) in both the fruits and leaves. There were also signals due to the $-CH_2O-$ groups in triglycerides (4.14 and 4.28 ppm) in the fruits, but not the leaves. The signal due to the –CHO– in triglycerides (5.26 ppm) overlapped with larger signals from 5.1 – 5.5 ppm that are probably due to carbohydrates in the fruit. However, the carbohydrates must be covalently attached to hydrophobic groups, like the long chain fatty acyls in fatty acyl glycerides that have been seen in many other fruits [16]. There wasn't enough amphiphilic material in the extract of the fruits to obtain a good $^{13}C\{^{1}H\}$-NMR spectrum, but there was in the leaves. It is shown in Figure 8. Signals due to the $-CH_3$ (14.34 ppm) and $(CH_2)_n$ (21.69 – 35.07 ppm) groups, as

Table 1. Chemical and physical-chemical properties of C. pachystachya (embaúba) fruit (pulp and seeds) and leaves.

Properties	Fruits	Leaves
Moisture (%)	71.8	62.4
Protein (%)	0.54	1.13
Lipids (%)	0.68	0.46
Ash (%)	0.50	0.96
Soluble fiber (%)	0.11	0.19
Insoluble fiber (%)	2.60	2.19
Carbohydrates (%)	23.8	32.7
pH	5.98	5.91
Acidity (%)	0.04	0.06

Table 2. Concentrations of amino acids in *C. pachystachya* (embaúba) (g/100 g).

Amino acid (g/100 g)	Fruit	Leaves
Leucine	0.53	1.03
Lysine	0.52	1.16
Phenylalanine	0.41	0.81
Tyrosine	0.31	0.53
Valine	0.50	0.89
Histidine	0.26	0.32
Isoleucine	0.34	0.67
Methionine	0.03	0.13
Methionine + Cysteine	0.06	0.16
Glutamic acid + Glutamine	1.45	1.84
Arginine	0.85	0.92
Aspartic acid + Asparagine	1.02	1.01
Glycine	0.46	0.82
Serine	0.50	0.49
Proline	0.39	0.68
Alanine	0.46	0.83

well as a mixture of C=C, HC=C, HC=CH and H2C=C carbons (118 – 145 ppm) as well as C=O carbons in esters (172 – 174 ppm) were seen.

4. Conclusions

The fresh fruit and leaves had 71.8 and 62.4% moisture, 0.54 and 1.13% protein, 0.68 and 0.46% total fat, 0.50 and 0.96% ash, 0.11 and 0.19% soluble fiber, 2.60 and 2.19% insol-

Table 3. Concentrations of minerals in the leaves and fruits of *C. pachystachya* (embaúba) as mg per 100 g dry weight (mg/100g-dw) for macronutrients and µg/g-dw for micronutrients, as well as comparisons to the dietary reference intake (DRI) a Ingestão Recomendada Diária (2004)

Minerals	Leaves	DRI (mg/day)	Fruits
B (mg/100g-dw)	7.89	3 - 20	4.95
Na (mg/100g-dw)	37.3	0.12 – 5.0	6.84
Mg (mg/100g-dw)	634	30 - 420	460
K (mg/100g-dw)	1273	400 - 5100	1995
Ca (mg/100g-dw)	3248	210 - 1300	1970
V (mg/100g-dw)	0.13	1.8	0.018
Cr (µg/100g-dw)	0.46	0.2 - 45	0.15
Mn (mg/100g-dw)	9.51	0.003 – 2.6	5.87
Fe (mg/100g-dw)	80.2	0.27 - 27	18.6
Ni (mg/100g-dw)	0.21	0.20 – 1.0	0.16
Cu (µg/100g-dw)	1.72	200 - 1300	2.4
Zn (mg/100g-dw)	6.59	2.5 – 10.9	7.02
Se (µg/100g-dw)	0.02	17 - 70	0.04
Mo (µg/100g-dw)	0.18	13 - 40	0.21
I (µg/100g-dw)	0.52	90 - 290	0.13

DRI Dietary Reference Intakes. Otten, J.J.; Hellwig, J.P.; Meyers, L.D. Dietary Reference Intakes. The National Academies Press, Washington, D.C. 2006

uble fiber, 23.8 and 32.7% total carbohydrates, 0.04 and 0.06% acidity and pH values of 5.98 and 5.1, respectively. The fruit and leaves are also good sources of magnesium (Mg), potassium (K), manganese (Mn) and iron (Fe). They provide >100% of the dietary reference intake (DRI) for adults. Lyophilized fruits and leaves were also extracted by hot, pressurized, dry methanol (100°C, 10 MPa). This solubilized 27% of the fruit and 15% of the leaves. The extract was partitioned between water and methylene chloride (CH_2Cl_2). The amphiphilic compounds went into the CH_2Cl_2 phase. They accounted for 2.13% and 5.15% of the lyophilized fruit and leaves, respectively. The amino acid concentrations were also measured. NMR analysis showed that the methanolic extract contained primarily fatty acid glycosides, with smaller amounts of aromatic compounds. The NMR spectra of the amphiphilic compounds showed the presence of triglycerides in the fruit, but not the leaves.

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6. Article Information

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