Pharmacognostic Characterization, Bioactive Compounds and Powder Antioxidant Action of Leaves of Araçás (Psidium cattleianum (Myrtaceae))

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Abstract

Araçás spp., have been used in traditional medicine for possessing several pharmacological properties such as antiseptic, digestive, anti-hemorrhagic, controlling blood pressure and diuretic. This study aimed to make a pharmacognostic characterization to establish control parameters of the plant drug quality (leaf), the determination of phenolic compounds, flavonoids, tannins, vitamin C and β-carotene, and to evaluate the powder antioxidant capacity of Psidium cattleianum leaf. From powder of P. cattleianum leaves, pharmacognostic analyzes were performed and quantification assays of bioactive compounds and antioxidant activity by DPPH capture using spectrophotometric methods. In the pharmacognostic characterization were determined the density (0.366 g. mL−1), the ash content (17.52% ± 1.002), moisture (9.32% ± 1.006) and the pH (5.4 ± 0.047). The quantification assays of phenolic compounds, flavonoids, tannins, vitamin C, carotenoids and showed that the plant drug presents significant amounts of these bioactive compounds. The antioxidant activity analysis indicates that the leaf of this plant species has potentially active compounds in combating free radicals. The findings open perspectives for the realization of other biological assays for evidence of possible biological activities presented by the plant species in question.

Keywords: Traditional medicine; Secondary metabolites; Drugs; Free radicals

Introduction

Since the dawn of civilization, the use of substances of vegetable origin, in the form of extracts, powders and essential oils, have played an important role in the treatment, cure and prevention of various diseases that afflict humanity [1]. The medicinal effects of plants are related to a group of different metabolites, which are termed secondary metabolites, such as flavonoids, alkaloids, triterpenes, sesquiterpenes, tannins, lignins and others.

The secondary metabolites are defined as bioactive compounds that whose function is to adapt the plant to the environment, performing important functions such as defense against infection, injury, pests, pathogens, ultraviolet rays and herbivores [2-4]. These classes of substances, frequently, are targets of interest of researchers who see them as a promising source for discovery of new drugs, cosmetics and agrochemicals [5-7].

Botanical species of the family Myrtaceae (such as Pimenta dioica, Pimenta racemosa, Eucalyptus globulus, Syzygium aromaticum, Psidium guajava, Eugenia uniflora and Melaleuca alternifolia). They have been studied in scientific research over the years because they are promising candidates for the discovery of novel compounds having therapeutic properties [7-9]. It is also worth mentioning that the great diversity of species of this family, by being used in popular means and be the subject of scientific research, is described in Monographs of Pharmacopoeia Brasileira to ensure the safe medical use of these plants.

Among the Myrtaceae family, has the Araçazeiro (Psidium cattleianum), small shrub, distributed in a large area of Brazil, from Bahia to Rio Grande do Sul. This plant species has a very similar fruit to guava, but a little acider, is widely consumed in natura, in juices, jams and ice cream and has good acceptance by consumers. This plant is widely grown in home gardens, especially in the southern region [10-15].

In traditional medicine, the species has been used for various purposes, such as antiseptic, digestive, anti-hemorrhagic, controlling blood pressure and diuretic. There are reports, for example the use of leaves of this species in decoctions for treating diarrhea. Referenced in the literature studies reinforce the pharmacological properties of fruits and leaves of Araçás spp., such as, for example, antioxidant activity, antimicrobial [4] and increased sleep induced by ketamine in mice.

Among the bioactive plant compounds that represent a significant pharmacological potential, especially the antioxidant activity in combating free radicals, highlights the phenolics, flavonoids, tannins, vitamin C and β-carotene. It is known that the excess of free radicals in the human body may cause rupture of the cell and cellular content damage (proteins, lipids, carbohydrates and DNA) causing oxidative processes to tissues. Various pathological states such as acceleration of...
aging, cancer, cardiovascular disease, cataracts, decline of the immune system, and brain dysfunctions such as Alzheimer’s are associated with uncontrolled production of free radicals in biological systems. Antioxidants are substances that react and neutralize the free radicals before they attack cells.

Although *P. cattleianum* spp., is been used in traditional culture, there is still no description of quality control parameters of the plant drug in the Brazilian Pharmacopoeia and studies on the bioactive compounds quantification and antioxidant activity of the leaves of this *spp.* Thus, this study was aimed to characterize pharmacognostic to establish quality control parameters of the plant drug (leaf), the determination of phenolic compounds, flavonoids, tannins, vitamin C and β-carotene, as well as evaluates the antioxidant capacity of *P. cattleianum* leaf powder [16-19].

### Material and Methods

The *P. cattleianum* leaves were collected in the municipal district of Pire do Rio, State of Goiás, Brazil, in March 2014. A voucher specimen, under record number 9203 was deposited in herbarium of the State University of Goiás, University Unit of Exact Sciences and Technology (UEG/UnUCET).

From the powdered dried leaves were determined in triplicate, the density of the plant drug powder, pH, total ash content and moisture content. The tests to determining those parameters were conducted at Laboratory of Physical-Chemical analysis of the Federal Institute Goiano - Câmpus Urutai, as described by the Pharmacopoeia Brazil [20].

Dosages assays were performed to phenolic compounds using Folin Ciocalteau method [21], flavonoids [22], tannins through the vanillin method [23] with three replicates for each sample. The absorbance readings were performed in the dark at room temperature in a spectrophotometer Thermo Fisher Scientific, Bio Mate Model 3. The results are expressed in milligrams per 100 g of sample. These tests were conducted at the Science and Food Technology Laboratory of Embrapa Cerrado (Planaltina - DF).

For evaluation of total carotenoid about 15 g of leaf powder were homogenized proceeding to extraction with pure acetone until no more observe of the characteristic color of the carotenoids in the residue. The extract was filtered through sintered funnel plate and transferred to a separatory funnel containing diethyl ether and petroleum ether (1:1). The extract was filtered with sodium sulfate to remove water. The extract was transferred to a 50 mL volumetric flask and measured with petroleum ether. Then there was the reading in spectrophotometer at 450 nm, resetting the equipment with petroleum ether [24].

Quantification was performed by Equation:

\[
TC (mg/100 g) = \frac{10^5 \times Abs \times Vol}{1\%E1 \ cm \times m}
\]

Where, 
- **TC**=Total carotenoid
- **Abs**=Absorbance at 450 nm
- **Vol**=balloon volume utilized in the dilution (mL)
- **m**=sample weight (g)
- **E1 cm 1%**=2592 (for β-Carotene in petroleum ether)

For evaluation of the vitamin C, about 2 g of the leaf powder was weighed and mixed with 20 mL of an acid solution of 6% HPO₃, containing 2 N acetic acid. It was centrifuged at 15,000 rpm for 20 min at 4°C, and filtered the supernatant. 1 mL of the solution was pipetted and added to 50 µL solution of 0.2% 2.6 dichlorophenolindophenol (DCPIP); it was stirred and incubated at room temperature for 1 hour. Add 1 mL of 2% thiourea and stir it well and then add 0.5 ml of 2% dinitrophenylhydrazine (DNPH) solution (except white). Mixed, capped and leave in water bath at 60°C for 3 h (except white). Placed in an ice bath and added carefully 2.5 mL of cold H₂SO₄ (including white) and stirred and then 0.5 mL of 2% DNPH white was added and stirred. Read the absorbance at 540 nm. A standard solution of ascorbic acid (AA) with ascorbic acid in 100 ml of acid solution 6% HPO₃, containing 2 N acetic acid was prepared for the calibration curve [25].

The antioxidant activity was determined according to the capture of free radical 2,2-diphenyl-1-picryl-hidrazil (DPPH) method, as described by Sánchez-Moreno et al. [26] with modifications. Briefly, in a 100 mL volumetric flask were added 1 g of leaf powder, 40 mL of 50% methanol and 40 mL of 70% acetone and completed the volume with distilled water. The reading was performed using aliquots of 100 µL extract, at four different concentrations and added to 3.9 ml of methanol solution of DPPH (2,2-diphenyl-1-picryl-hidrazil) 6 µM. The absorbance readings were performed after incubation for 30 min in the dark, in environment temperature in spectrophotometer (Thermo Fisher Scientific, Bio Mate Model 3) at 515 nm. The methyl alcohol was used as white, to calibrate the spectrophotometer.

To calculate the DPPH sequestration percentage was used the following equation: Radical DPPH sequestration (%) = [(White absorbance-absorbance sample)/(absorbance white)] × 100.

### Results and Discussion

#### Pharmacognostic characterization

The results of the pharmacognostic analysis in *P. cattleianum* leaves are shown in Table 1.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Average results ± Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>0.366 ± 0.015 g/mL⁻¹</td>
</tr>
<tr>
<td>pH</td>
<td>5.4 ± 0.047</td>
</tr>
<tr>
<td>Content of total ash</td>
<td>16.98 ± 1.002%</td>
</tr>
<tr>
<td>Moisture content</td>
<td>9.32 ± 1.006%</td>
</tr>
</tbody>
</table>

Table 1: Results obtained in the pharmacognostic aliases expressed as mean ± standard deviation.

The pharmacognostic analyzes aim to assess the quality and purity of certain plant drug when used as raw material for the development of phytotherapeutic and phytocosmetic products. In general, quality control parameters, according to the Pharmacopoeia Brazil (2010), include the absence of foreign elements in the drug; especially dirtiness of earthly origin (sand, stone and soil), insects and other organisms; the determination of moisture, non-compacted apparent density, determination of pH and the content of total ash. It is worth noting that this type of analysis is important, since the presence of impurities and poor quality of herbal drugs marketed in fair and official
establishments suggest that they may have been tampered with or that there was poor maintenance and negligence in preparing the same.

According to Nascimento et al. [27], the high content of impurities in plant drugs can result in health problems to those who use the products based on medicinal plants (whether for food or medicinal purposes), because do not cause the expected therapeutic effect and even the occurrence of adverse reactions such as poisoning and allergic reactions, resulting in a serious health problem.

In this herein study, the drug powder had an apparent density, seen as low density according to Pharmacopoeia Brazil (2010) [20]. This parameter can directly influence the extraction process and obtaining of extracts with different solvents once the low density of plant drug can indicate difficulties in compression of the particles and therefore the necessity of using a larger volume of solvent in the process maceration to avoid the fluctuation of powdered drug [28].

Regarding to pH, it was found solution of acid character, suggesting that the leaf of this plant should contain predominantly acids constituents. It is worth noting, as Hubinger [29], which in plants are various inorganic and organic acids present in general combined in the form of salts, esters, lactones, lipids, essential oils, resins, proteins, among others. For the author, one of these acids also lies in the free state, solubilized in the cytoplasm, which can characterize the basic character, neutral and acid of specific plant extract. In the case of herbal drugs, the acid nature of the chemicals contained in the plant material has great importance because, generally avoids the presence and attack of micro-organisms [30].

Another important point to be considered in identifying purity of herbal drugs relates to the determination of the content of total ash. The total ash, derived from plant tissue (physiological ash) and derived from foreign materials (non-physiological ash), allow verifying the presence of non-volatile inorganic impurities which may be present as contaminants in the plant material [31,32] after incineration process (taken to coal state). No data recommended by Pharmacopoeia Brazil, 2010 [20] to assess the total ash content obtained for the studied spp., so the results of this study can serve as a benchmark for this plant drug.

Another essential parameter to ensure the quality of vegetable drug is the residual water content found in the dry plant material. The determination of moisture of the plant drug had a percent within the specified limit as regarding by the current Pharmacopoeia, which may result in the loss of material. The moisture content in the leaves powder of the studied species, showed an amount considered safe and that ensures the stability of the material.

Quantification of bioactive compounds

The results of quantitation of phenolic compounds, flavonoids, tannins, vitamin C and β-carotene in P. cattleianum leaf powder (moisture range of 9.32 ± 1.006%) are shown in Table 2.

<table>
<thead>
<tr>
<th>Bioactive compounds</th>
<th>Plant species</th>
<th>Feedstock</th>
<th>Methodology used</th>
<th>Result (mg/100 g ± Standard Deviation)</th>
<th>Linear equation</th>
<th>Correlation coefficient (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic compounds</td>
<td>Uvaia</td>
<td>Pulp in natura</td>
<td>Folin and Ciocalteau [21]</td>
<td>127</td>
<td>Y=0.016 x + 0.000</td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Eugenia pyriformis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Jambofoá</td>
<td>Pulp in natura</td>
<td>Folin and Ciocalteau [21]</td>
<td>185</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Syzygium jambolanum</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goiaba</td>
<td>Frozen pulp</td>
<td>-</td>
<td>83.0</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Psidium guajava</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acerola</td>
<td>Frozen pulp</td>
<td>-</td>
<td>580.1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Acerola</td>
<td>Pulp in natura</td>
<td>Lees and Francis</td>
<td>9.31 ± 0.22</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2: Results obtained in the quantification of bioactive compounds in Psidium cattleianum leaf powder.

The plant drug presents important bioactive compounds amount, justifying some therapeutic activities of popular use of this species. The leaves have high levels of bioactive compounds in comparison with the results of other studies conducted with fruits of species, of the botanical family Myrtaceae, as shown in Table 3.
Table 3: The results obtained for bioactive compounds in fruit species, of the botanical family Myrtaceae.

<table>
<thead>
<tr>
<th>Fruit Name</th>
<th>Extract Type</th>
<th>Source</th>
<th>Reference</th>
<th>Tannins</th>
<th>Vitamin C</th>
<th>β-Carotene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cagaita</td>
<td>Pulp in natura</td>
<td>Lees and Francis</td>
<td>[22]</td>
<td>7.07</td>
<td>-</td>
<td>0.77</td>
</tr>
<tr>
<td>Eugenia dysenterica</td>
<td>Frozen pulp</td>
<td>Lees and Francis</td>
<td>[22]</td>
<td>4.86</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atomized pulp</td>
<td>Lees and Francis</td>
<td>[22]</td>
<td>42.93</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Cagaita</td>
<td>Pulp in natura</td>
<td>Waterman and Mole</td>
<td>16.62</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen pulp</td>
<td>Waterman and Mole</td>
<td>[23]</td>
<td>21.30</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atomized pulp</td>
<td>Waterman and Mole</td>
<td>[23]</td>
<td>67.00</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Cagaita</td>
<td>Pulp in natura</td>
<td>HPLC-DAD</td>
<td>34.11</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Frozen pulp</td>
<td>Benassi and Antunes</td>
<td>[41]</td>
<td>27.46</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Atomized pulp</td>
<td>Benassi and Antunes</td>
<td>[41]</td>
<td>26.97</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>β-Carotene</td>
<td>Cagaita</td>
<td>Pulp in natura</td>
<td>HPLC-DAD</td>
<td>0.77</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Goiaba</td>
<td>Fresh fruit</td>
<td>HPLC-DAD</td>
<td>13.8</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

The vitamin C is used in the prevention of numerous diseases, such as diabetes, cataracts, glaucoma, macular degeneration, atherosclerosis, stroke, heart disease and cancer, in addition to stimulating the immune system and has antioxidant [35]. Similarly, carotenoids are associated with many therapeutic properties such as antioxidant, epithelial protection and fetal development [55].

Determination of antioxidant activity

The linear equation obtained ($y=0.009 x + 0.015$) with a coefficient of determination ($R^2$) equal to 0.997 was used to determine the effective concentration ($EC_{50}$), in other words the amount of plant drug required to reduce the initial concentration of DPPH by 50%. The data show that the plant drug powder drug has scavenging activity of free radicals with a value of $EC_{50}=97.91 \pm 0.49 \mu M$. This means that 1 gram of plant drug powder has antioxidants capable of reducing 97.91 µmol of DPPH by 50%.

The Table 4 shows the results of the percentage of free radical DPPH capture by antioxidants present in the P. cattleianum leaf according to the concentrations tested.
The results show that the plant drug powder presents good antioxidant activity. The antioxidant activity has been described for some species of the Myrtaceae family, as *Psidium guajava* [56], *Eugenia uniflora* [57], *Myrciaria cauliflora* [58]. Some authors, such as Fetter et al. [59], Medina [14] Nora et al. [60] reported antioxidant action in fruit of *Aracá* spp., However, there are still no reports of antioxidant potential studies with the leaf of this species.

### Conclusions

The pharmacognostic point of view, the results found for the plant drug under study are in line with the requirements recommended by the Pharmacopoeia Brazil, 2010. Therefore, the conditions used in the different steps of obtaining plant drug (from the collection of leaves to the final product) contributed to the quality of raw material used in the study. The results showed that the plant drug has significant quantities of bioactive compounds as well as antioxidant activity. It is noteworthy that these findings open up prospects for the realization of other biological assays for evidence of possible biological activities presented by the plant species.

### References


