Morphoanatomic aspects and phytochemical screening of Plinia edulis (Vell.) Sobral (Myrtaceae)
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Plinia edulis (Myrtaceae), popularly known as “cambucá”, is a Brazilian medicinal plant employed in the treatment of stomach problems and throat affections by the “caiçaras”, fishermen of coastal localities. Aiming to contribute with the species knowledge the leaves of P. edulis were analyzed macro and microscopically and the chemical composition of the volatile oil was determined using a combination of GC/MS and retention indices. The antimicrobial assay and the phytochemical screening of the aqueous ethanol extract of the leaves have been performed to correlate the secondary metabolites and the traditional use. Leaves present morphological characteristics of others Myrtaceae species and some particularities, such as the circular idioblasts in number of 2 to 4, scattered perpendicularly at the adaxial surface, with druses or prismatic crystals. In the volatile oil fifteen components have been identified, of which epi-α-cadinol (21.7%), α-cadinol (20.2%) and trans-caryophyllene (14.2%) were major. The phytochemical screening of the aqueous ethanol extract showed the presence of substances with pharmacological interest, such as flavonoids, tannins, saponins and terpenoids but, despite of the presence of these classes, the extract did not inhibit the growth of Aspergillus niger, Candida albicans, Escherichia coli and Staphylococcus aureus in a concentration of 1,000 mg/mL.

INTRODUCTION

The family Myrtaceae native in tropical and subtropical regions comprises about 3,5 thousand species (Barroso, 1984). Some Myrtaceae had been proven to be effective to inhibit the growth of microorganisms (Chaimb et al., 2007; Ndounga et al., 1994; Anesini, Perez, 1993). The genus Plinia L. comprises about 30 species from Costa Rica to southern South America and in the West Indies (Landrum, Kawasaki, 1997). These species have been subject of chemical and biological studies. Some species accumulate flavonoids (Mendez et al., 1997; 1994) which have been evaluated as inhibitors of the enzyme xanthine-oxidase (Theoduluz et al., 1988). Apel et al. (2006) analyzing the volatile oil of four Plinia species verified predominance of sesquiterpenes in their.
**Plinia edulis** (Vell.) Sobral is a tree, which attains 5-10 m high, growing in Brazil from Rio de Janeiro to Santa Catarina States (Lorenzi et al., 2006). It is endemic to the Atlantic rainforest and popularly designated as “cambucá” (Lorenzi et al., 2006). It is well known due their juicy fruits and uses in folk medicine against stomach problems and throat affection (Nascente, 2008; Maciel, Cardoso, 2003). Although the species has been commonly used in traditional medicine, scientific reports on it are limited. This plant has been shown to exhibited gastroprotective activity (Ishikawa et al., 2003).

Despite its therapeutic activity there are not studies concerning morphoanatomical and phytochemical characterization to distinguish this starting plant material from adulterants. So, the leaves of *P. edulis* were described macro and microscopically. In order to rationalize relationship between chemical constituents and traditional use of the medicinal plant, the composition of the volatile oil from leaves was determined, as well as the phytochemical analysis and evaluation for antimicrobial effect of leaves aqueous ethanol extract.

### MATERIAL AND METHODS

#### Plant material

The leaves of *P. edulis* were collected during flowering at morning period in Trindade, Paraty City, Rio de Janeiro State, Brazil. Flowering material was identified and a voucher specimen has been deposited in the Instituto de Botânica de São Paulo, under number SP 356.472.

#### Macro and microscopical analysis

The crude drug consisting of dried leaves of *P. edulis* was analyzed for its dimension, form, color, surface and organoleptic characteristics. The material was observed by aid of a stereomicroscope Willd. Fresh vegetable material or fixed in FAA 70 (formalin, acetic acid and 70% ethanol) and maintained in 70% ethanol solution (Berlyn, Miksche, 1976) was used for anatomical analysis. Transverse sections were made at the middle part of the midrib of leaves and at the mid-region of the petiole. Sections were then cleared in bleach, washed and stained in 0.1% safranin in 50% ethanol and astra blue (Roeser, 1962). Cuticular macerations and dissociation of the stem were made. The material used was left overnight in equal volumes of 20% hydrogen peroxide and glacial acetic acid at 60 °C (Franklin, 1945). The material obtained was stained in safranin/astra blue solution.

The results were registered with aid of photomicroscope Nikon, Optiphot. Scales were obtained in the same optical conditions.

#### Volatile oil

Fresh leaves (700 g) were submitted to hydrodistillation during 4 h in a Clevenger-type apparatus.

#### Volatile oil analysis

The leaf oil was analyzed by GC/MS in a Shimadzu, QP-5000 GC/MS instrument under the following conditions: dimethylpolysiloxane B-5 fused silica capillary column (30m x 0.25mm; film thickness 0.25mm); carrier gas He (1 mL/min); injector temperature 240 °C; detector temperature 230 °C; column temperature 60-240 °C at 3 °C/min; mass spectra electron impact 70 eV. The identification of the constituents was performed by computer library search (Nist 62 library), retention indices (Adams, 1995) and interpretation of the mass spectra (McLafferty and Stauffer, 1989).

#### Preparation of the freeze-dried extract

The plant material was dried at 40-45 °C in oven with air circulation. The dried leaves were grounded and percolated with 70% ethanol at room temperature (Farmacopéia, 1988). The solution was concentrated at 40 °C under low pressure and the residue was freeze-dried.

#### Phytochemical screening

The freeze-dried extract was assayed for the occurrence of flavonoids, saponins and tannins using TLC methods (Wagner, Bladt, 1996).

#### Antimicrobial activity

Antimicrobial activity was evaluated against Gram positive bacteria *Staphylococcus aureus* (ATCC 6538); Gram negative bacteria *Escherichia coli* (ATCC 8739) and the fungi *Candida albicans* (ATCC 10231) and *Aspergillus niger* (ATCC 16404). The minimum inhibitory concentration (MIC) was determined by the macrobroth dilution method (Wadt et al., 1996). The microorganisms suspensions were adjusted to the standard concentration of approximately 1.0 x 10^2 CFU/mL. The test bacterial strains were inoculated into Difco™ Tryptic Soy Broth and the fungi into Difco™ Sabouraud Dextrose Broth. Each sample concentration was added with test microorganisms, in triplicates. At the same conditions, a solution of chloranfenicol (5 µg/mL) was added with test
bacteria and anfotericin B (1 µg/mL) with test fungi (positive controls), while the solvent was used as negative control. Tubes were incubated at 35-37 ºC for 24 h (bacteria) and 20-25 ºC for 48 h (C. albicans) or 5 days (A. niger). The MIC was as taken as the lowest concentration showing no noticeable growth (turbidity).

RESULTS AND DISCUSSION

In general, the macro and microscopical analysis of P. edulis evidenced typical features of Myrtaceae (Metcalfe, Chalk, 1950; Landrum, Kawasaki, 1997), but some anatomical features are peculiars in this species. Secretory cavities, bicollateral vascular bundles and crystalliferous cells have been mentioned as being widespread in Myrtaceae (Metcalfe, Chalk, 1950). There are few comparable studies on the botanical aspects of Plinia (Landrum, Kawasaki, 1997; Kawasaki, Holst, 2002).

The macroscopical analysis showed that the leaves of P. edulis are simple, lanceolate to oblong-lanceolate with entire margins (Figure 1A). The apex is acute to acuminate and the base is cuneate to obtuse. The foliar lamina has from 14 to 17 cm in length, from 4 to 6 cm in width and translucent glands. The venation is pinnate and brochidodromous. The lateral veins anastomosing about 1-3 mm from the margin form an inframarginal vein. Leaf venation pattern seems to combine characteristics of other Plinia species (Kawasaki, Holst, 1994; 2002). Non-glandular trichomes cover the whole leaf surface but they are more abundant on the abaxial surface and along the veins. The lamina is chartaceous, brownish-green and brittle. The cylindrical petiole has from 1.5 to 2.5 cm in length. The leaves have aromatic odor and astringent taste.

In frontal view, the epidermis is composed of polygonal cells with slightly undulate anticlinal walls. In transverse section, the unilayered epidermis presents quadrangular cells (Figures 1B and 1C). Secretory cavities are more frequent beneath the epidermis (figure 1B), although they are distributed throughout the lamina. The pattern of development of these cavities was not studied here. In surface view, distinct cells in pairs can be seen over the secretory cavities. These cells differ from the other epidermal cells in form, size and the dividing wall is sinuous. The mesophyll is dorsiventral with one layer of palisade parenchyma that comprises about 1/5 of the mesophyll width. The spongy parenchyma has conspicuous air spaces. Large idioblasts, in number of two to four, with druses and prismatic crystals of calcium oxalate are scattered perpendicularly at the adaxial face (Figure 1C). The leaves are hypostomatic, with anomocytic stomata predominantly (Figure 1D). The adaxial surface cells are comparatively bigger than the cells of the other face. The outer wall of adaxial and abaxial cells are thickly cutinized. Lignified, curved, non-glandular trichomes are present on both lamina surfaces, especially at the abaxial face of the midrib, where they are longer. These trichomes are unicellular, with acute apical cells (Figure 1E). This finding is in agreement with some authors (Landrum, Kawasaki, 1997; Kawasaki, Holst, 2002).

The transverse section of the midrib is nearly plane-convex. The vascular system forms an incomplete cylinder with incurved ends. The vascular bundles are bicollateral and fibers form a cylinder around the outer phloem (Figure 1F).

The petiole in transection is plane-convex or slightly concave-convex. The epidermal cells are anticlinally elongated and thickly cutinized. The vascular system is arranged in a broad crescent shape with deeply incurved

FIGURE 1 - Macro and microscopical analysis of leaves of Plinia edulis (Vell.) Sobral. A: crude drug. Transverse sections (B, C, F) and frontal view (D, E). B: secretory cavity (sc); C: idioblasts (arrow); D: stomata (st) and basis of trichomes (arrow); E: non-glandular trichome (arrow); F: midrib. Bars: 10 µm (B, E); 20 µm (D); 40 µm (C); 100 µm (F).
adaxial ends (Figure 2A). The indumentum is composed of the same type of non-glandular trichome, but compared to lamina, they are more abundant (Figure 2B). Druses and some prismatic crystals are present in the cortex (Figure 2C) but they are more numerous in the medullar region. Secretory cavities are frequent (Figure 2D). Phloem contains numerous prismatic crystals arranged in crystal series which are observed in the dissociated tissue (Figure 2E).

Various plant morphological features may affect the foraging efficacy of predatory insects or parasitoids. The effectiveness of the defense mechanisms in response to environmental conditions and predatory insects or parasitoids may include physical and chemical factors (Horgan et al., 2007). Non-glandular trichomes and calcium oxalate crystals may form physical obstacles to herbivores, whereas the secretory cavities and tanniferous cells may release chemical repellents. The lipophytic content of secretory cavities and phenolics have been demonstrated antimicrobial activity, repellent properties and sometimes represent a feeding barrier (Omolo et al., 2004; Harborne, 2001)

The morphoanatomical analysis has been proven to be a rapid and reliable method for quality control of the plant material used for medicinal purposes. Despite the overall similarity of structures with other Myrtaceae, the combination of lamina and midvein structure, together with series of two to four idioblasts scattered perpendicularly at the adaxial face may be a criterion for the analysis of this crude drug.

On the other hand, the presence of lipophilic content observed in the secretory cavities leads to the phytochemical analysis of the composition of the oil, which is summarized in Table I. The hydrodistillation yielded 0.02% of oil, which contains as major components the epi-α-cadinol (21.7%), the α-cadinol (20.2%) and the trans-caryophyllene (14.2%). In this analysis, the oil composition was quite different from that described to specimen collected in Porto Alegre (South Brazil) (Apel et al., 2006), that showed as main components the β-caryophyllene and its oxide (39.3%). Although the difference in composition could be due to distinct preparation procedure, geographical or seasonal variations, this finding could suggest alternatively the occurrence of chemotypes.

The traditional use of P. edulis and the presence of flavonoids, tannins, and terpenoids evidenced by the preliminary phytochemical analysis lead us to search for antimicrobial activity of the extract. The antibacterial and antifungal activity of several plants have been attributed to terpenoids (Wyatt et al., 2005; Filipowics et al., 2003) and

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<th>Constituents</th>
<th>Retention Indices</th>
<th>Percentage</th>
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<tr>
<td>1,8-cinole</td>
<td>1030</td>
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<tr>
<td>trans-caryophyllene</td>
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<td>α-humulene</td>
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<td>allo-aromadendrene</td>
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<td>α-selinene</td>
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<td>α-muurolene</td>
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<tr>
<td>γ-cadinene</td>
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<tr>
<td>α-cadinol</td>
<td>1654</td>
<td>20.2</td>
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| FIGURE 2 - Microscopical analysis of the petiole of *Plinia edulis* (Vell.) Sobral. Transverse sections (A, C, D) and dissociated tissue (B, E). A: vascular system; B: non-glandular trichomes; C: druses (arrow); D: secretory cavity (sc); E: druses (dr) and prismatic crystals (pc). Bars = 20 µm (C, D, E); 40 µm (B); 100 µm (A).
phenolics (Heinonen, 2007) such as tannin, responsible for the astringent taste of \textit{P. edulis} leaves. Despite of the fact that these classes of compounds had shown antibiotic activity against some types of strains (Heinonen, 2007; Kim, Shin, 2004; Kubo, Muroi, Kubo, 1993), the leaf extract did not inhibit neither the growth of \textit{A. niger} and \textit{C. albicans} in a concentration of 2.000 mg/mL nor the growth of \textit{E. coli} and \textit{S. aureus} in a concentration of 1.000 mg/mL. Further biological and phytochemical studies have been developing to verify the relationship of the pharmacological activity and chemical constituents of \textit{P. edulis}.

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REFERENCES


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