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ORIGINAL ARTICLE

Pharmacognostic Evaluation of *Carapa guianensis* Aubl. Leaves: A Medicinal Plant Native from Brazilian Amazon

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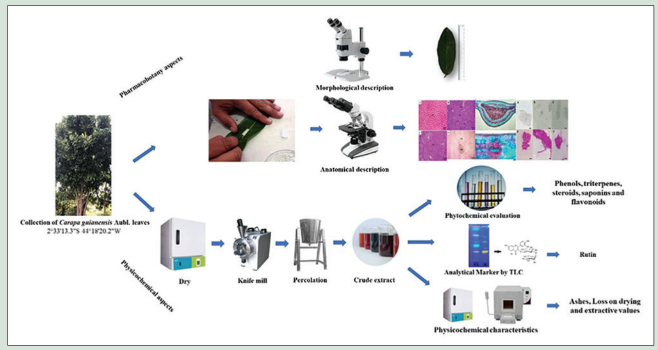
ABSTRACT

Background: *Carapa guianensis* Aubl., known as crabwood, has been used in folk medicine as anti-inflammatory, wound healing, and for the treatment of flu and colds. **Objective:** The present study aimed to establish the pharmacognostic features of *C. guianensis* leaves. **Materials and Methods:** The leaves were investigated according to the World Health Organization guideline on the pharmacognostic specification, which comprised macroscopic and microscopic assessment, phytochemical screening, and physicochemical characterization of the leaves, besides the microscopic analysis of the powder. **Results:** Leaves were characterized as a compound, coriaceous with elliptic shape, entire margin, acuminate apex, obtuse base, and opposite phyllotaxis. The epidermis has straight periclinal and anticlinal walls. Calcium oxalate crystals were observed in druses, anomocytic stomata just on a lower side (hypostomatic), and dorsiventral mesophyll. Phytochemical screening revealed the presence of flavonoids, saponins, triterpenes, and steroids in the crude extract. The values of the physicochemical parameters such as total ash, acid-insoluble ash, and loss on drying are 7.16%, 1.03% and 7.93%, respectively; the ethanol and water-soluble extractive values are 19.47% and 15.97%, respectively. **Conclusions:** The information obtained with botanical, physicochemical, and phytochemical studies could be used to identify *C. guianensis* and to certify the authenticity of commercial samples.

Key words: Andiroba, morphoanatomy, phytochemical screening, quality control, rutin

SUMMARY

- Rutin can be used as chemical marker for quality control of *C. guianensis*.
- The crude extract of the leaves has phenolic compounds, saponins, triterpenes and steroids.
- The leaves are characterized by the presence of cells with straight anticlinal walls and polyhedral shape, calcium oxalate crystals in the form of rosette in secondary ribs and anomocytic stomata.



Abbreviations Used: TLC: Thin-layer chromatography.

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INTRODUCTION

Medicinal plants are a rich source of secondary metabolites with interesting biological activities,[1] and since the beginning of civilization, plants have been used for man to heal, cure, or prevent diseases; they have great potential for producing new drugs of great benefit to humankind.[2,3]

Carapa guianensis Aubl. (*Meliaceae*), commonly known as andiroba and crabwood, is a tree distributed in the Amazon basin, Central America, and the Caribbean. In Brazil, it was included in a national list of medicinal plants with the potential to advance in the production chain and generate products of interest to the public health system.[4]

Ethnopharmacological studies have reported the therapeutic use of *C. guianensis*. Leaves and bark are used to prepare the tea for treating fever, worms, and skin illness.[5] The seed oil is largely utilized to treat diseases of the respiratory system, inflammation, and as an insect repellent.[6-9] Owing to its traditional use for therapeutic purposes for centuries, this species, mainly the oil has been investigated for its pharmacological properties. However, studies with the *C. guianensis* leaves are lacking. Therefore, it is required studies to validate the popular use of this plant species, in a real perspective of the development of products derived from *C. guianensis*.[10,11]

However, the legislation[12] emphasizes that for the development of herbal medicines, it is necessary to define parameters of efficacy, safety, and quality (authenticity, integrity, and purity) of both the raw material and the finished product, which also allows the inclusion in pharmacopoeias and official codes.

The integrity tests aimed at qualitative and quantitative evaluation of markers of plant material, assays based on characterization and chemical

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constituents of plant species, which can be started with the phytochemical analysis to identify the relevant secondary metabolites groups.[13,14]

As explained above and considering that despite the widespread popular therapeutic use of *C. guianensis* leaves, studies to define the parameters of quality, efficacy, and safety of that plant species are scarce. In this regard, the present work reports the pharmacobotany, phytochemical, and physicochemical parameters of *C. guianensis* leaves, with the aim to effectively contribute to quality control of that vegetable raw material.

MATERIALS AND METHODS

Plant material collection and identification

The leaves of *C. guianensis* were collected in São Luís, Maranhão, Brazil (2°33'13.3"S 44°18'20.2"W) in February 2017. The plant material was identified, and a voucher specimen deposited in the Ático Seabra Herbarium of the Federal University of Maranhão with registration code SLS-01253.

Pharmacobotany characterization

The pharmacobotanical study was conducted from the morphological and anatomical characterization of adult leaves of *C. guianensis*. To perform this study, the adult leaves were collected in three replicates. The morphological descriptions were evaluated using fresh leaves with naked eye and stereomicroscope as per observations described in the literature.[15] For the anatomical description of *C. guianensis*, transversal sections were cut using stainless steel blades. The sections were bleached in 10% sodium hypochlorite solution, washed in distilled water, and then stained in astra blue and basic fuchsin. Sections were then mounted between slide and coverslip using hydrated glycerin and analyzed by light microscopy.[16] The pictures were taken using a digital camera attached to the microscope. The stomata were classified as method followed by Metcalfe and Chalk.[17] The characterization of cell walls and mesophyll was performed based on Appezzato-da-Glória and Carmello-Guerreiro.[18]

Obtaining crude extract

The leaves of *C. guianensis* were dried at room temperature under the shade for 7 days, and then cut with knife mill using 2-mm diameter mesh to obtain a coarse powder. The leaf powder was subjected to a percolation extraction process with 70% ethanol, using drug solvent ratio 1:12 (w/v) under cover of light. It was then filtered and concentrated using rotary evaporator.

Physicochemical characteristics

Total ash, acid-insoluble ash, loss on drying, and extractive matter parameters of *C. guianensis* leaves were analyzed according to the World Health Organization guideline for quality control methods for medicinal plant materials.[19] All samples were analyzed in triplicate. Grand mean and the pooled standard deviation was calculated.

Phytochemical evaluation

The crude extract and the fractions were subjected to phytochemical screening tests to detect the secondary metabolites groups.[20] All tests were performed in triplicate.

Thin-layer chromatography of *Carapa guianensis*

The extract was dissolved in methanol and then plated on silica gel GF254 (Merck) analytical thin-layer chromatography (TLC) plate. For analysis, the AcOEt: H_2O: CH_2O_2 (80:10:10) mixture was used as the mobile phase, the plates were then nebulized with ethanolamine diphenylborate, followed by 5% (w/v) polyethylene glycol 4000 in methanol (reagent Natural A, NA) and examined under ultraviolet light (365 nm). As an analytical standard, the rutin (99%) was used for comparison with the compounds present in the extract.

RESULTS

Pharmacobotany aspects

Morphological description

The leaves of *C. guianensis* are paripinnate, elliptic shape with an entire margin, leathery texture, smooth surface, acuminate apex, obtuse base with opposite phyllotaxis, and veining pinnate camptodrome type, measuring 25–60 cm in length. Leaves are concolor dark green observed with the naked eye in both sides [Figure 1].

Anatomical description

The epidermis (in front view) has cells with straight anticlinal walls and polyhedral shape on both sides [Figure 2a]. The secondary ribs presented the elongated cells with a rectangular shape and showed the presence of calcium oxalate crystals in the form of drusen [Figure 2b]. The anomocytic stomata were found restricted to the abaxial surface [Figure 2b and c] of the leaf. Extrafloral nectary are observed in both sides of the leaves [Figure 2d].

Mesophyll

The leaf has dorsiventral mesophyll, having two layers of palisade and eight to ten layers of spongy and loosely packed parenchyma. The vascular bundles observed in leaf blades are the collateral type, with xylem facing the adaxial and phloem toward the abaxial epidermis [Figure 3].

Midrib

The midrib has a biconvex shape with quietly convexity in upper layers as observed in cross section image. The epidermal cells are like the rest of the blade, internally having a layer of cells with sclerified walls. There are three to four layers of chlorenchyma ring and common parenchyma. The central vascular bundle has a triangular shape and is surrounded by sclerenchyma fibers, xylem having the central region being surrounded by the phloem [Figure 4].

Microscopy of Carapa guianensis powders

The microscopic observation of *C. guianensis* powder demonstrates characteristics that identify the species such as cells with straight anticlinal walls and polyhedral shape [Figure 5a], elongated cells with rectangular shape and showed the presence of calcium oxalate crystals in the form of rosette [Figure 5b, d and e], in secondary ribs as well as anomocytic stomata [Figure 5c, d and f].

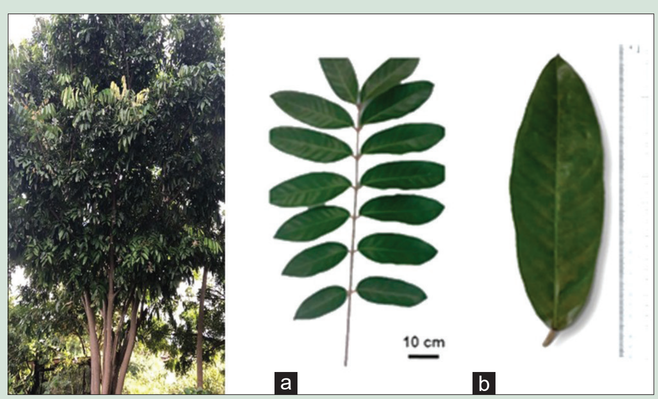

Figure 1: *Carapa guianensis* Aubl. (a) Leaf (b) Leaflet

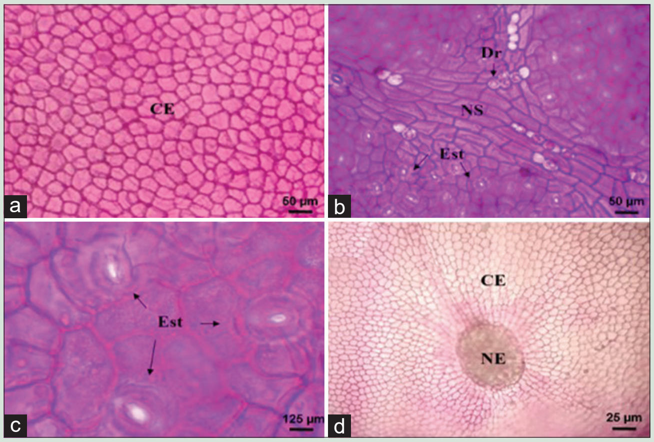

Figure 2: *Carapa guianensis* Aubl. vertical cross sections of epidermis. (a) Adaxial surface. (b) Abaxial epidermis. (c) Anomocytic stomata. (d) Abaxial epidermis, especially the nectaries. NS: Secondary vein; Dr: Druses; Est: Stomata; CE: Epidermal cells; NE: Extrafloral nectaries

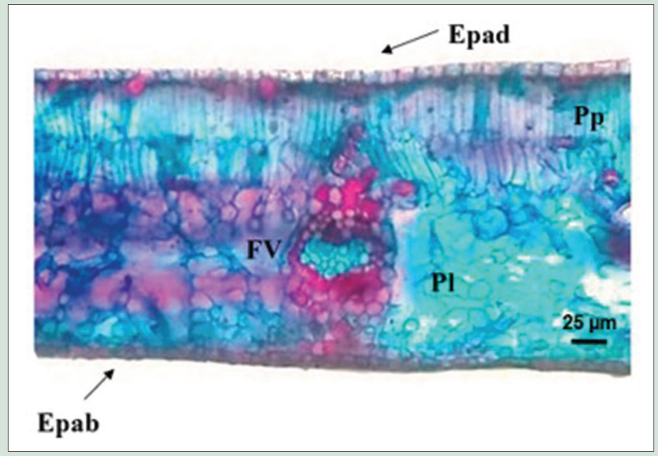

Figure 3: *Carapa guianensis* Aubl. cross-section of mesophyll. Epab: Lower epidermis; Epad: Upper epidermis; Pp: Palisade parenchyma; Pl: Spongy parenchyma; FV: Vascular bundles

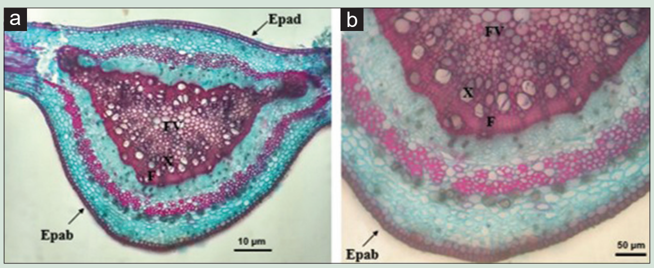

Figure 4: Cross-section showing midrib of *Carapa guianensis* Aubl. Epab: Lower epidermis; Epad: Upper epidermis; X: Xylem; F: Phloem; FV: Vascular strand (a) Midrib (b) Midrib - focus on epidermis details

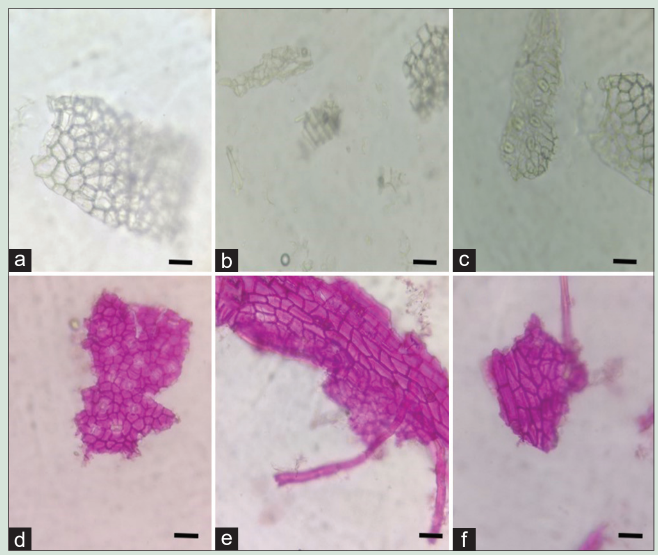

Figure 5: Microscopy of *Carapa guianensis* Aubl. leaf powder. (a and b) epidermis cells (c and d) anomocytic stomata (e) calcium oxalate crystals in the form of drusen (f) secondary ribs with elongated cells

Phytochemical profile

The yield of the crude extract was 19.47%, and the phytochemical studies of leaves of this species revealed the presence of phenols, flavonoids in prominent; in addition to saponins, triterpenes, and steroids. The results of the phytochemical screening are described in Table 1.

Chemical profile of thin-layer chromatography of *Carapa guianensis* Aubl. crude extract

TLC of the hydroethanolic extract shows a profile of five spots, the results are shown in Figure 6 and Table 2.

When comparing the analytical profile obtained by the application of the crude extract with the rutin standard in the TLC, it was possible to confirm the presence of this flavonoid, a comparison made through the retention factor. Rutin, a flavonoid known for its antioxidant activity, is widely used in the world.

Physicochemical characteristics

The physicochemical evaluation (% by weight) of *C. guianensis* leaves was demonstrated in Table 3.

DISCUSSION

The quality control of herbal medicines is important to ensure the efficiency and quality of these products. This analysis includes many botanical and chemical methods. Botanical analysis, which includes macroscopic and microscopic parameters, is the simplest and cheapest way of attesting the authenticity of the plant drug;[19] and because most herbals are commercialized in the powder form, the analysis of pulverized plant is essential to assess the quality.

The leaf morphology is consistent with results as described earlier.[21] However, it differs as reported by Pantoja[22] which describes phyllotaxis as an alternate.

The presence of anomocytic stomata in *C. guianensis* leaves and occurs in other species of the family *Meliaceae*.[23] The presence of extrafloral nectary in the leaflets of *C. guianensis* has also been reported by Ferraz *et al.*[24]

The characteristics found on mesophyll highlight the presence of bi-stratified palisade parenchyma as observed in trees that grow in places with high light intensity.[25] In general, the anatomical patterns described in this study for *C. guianensis* corroborate the attributes proposed by Metcalfe and Chalk;[26] for species of the family *Meliaceae*, with the exception of trichomes which are absent and mesophyll that not presents secretory cells.

Table 1: Phytochemical screening of *Carapa guianensis* Aubl. leaves crude extract

Secondary metabolites	*Carapa guianensis* leaf
Phenols	Present
Tannins	Negative
Coumarins	Negative
Triterpenes	Present
Steroids	Present
Saponins	Present
Alkaloids	Negative
Anthocyanidins and anthocyanins	Negative
Flavones, xanthones, and flavonols	Present
Flavones	Present
Catechins	Present
Flavones	Present

Table 2: Profile of thin-layer chromatography of *Carapa guianensis* Aubl.

Absorbent	Mobile phase	Spraying reagent	Number of spots	R_f values	Color
Silica gel GF254 (Merck)	AcOEt:H_2O:CH_2O_2 (80:10:10)	Reagent natural A	1	0.21	Yellow
			2	0.36	Blue
			3	0.63	Green
			4	0.84	Blue
			5	0.90	Red

Table 3: Physicochemical specification (percentage by weight) of *Carapa guianensis* Aubl.

Parameter	Content (percentage by weight)*
Loss on drying of fresh leaves	43.30±2.82
Loss on drying of powder	7.93±0.15
Total ash	7.16±0.25
Acid-insoluble ash	1.03±0.12
Ethanol extractive values	19.47±1.23
Water extractive values	15.97±1.54

*Values express in mean±SD. SD: Standard deviation

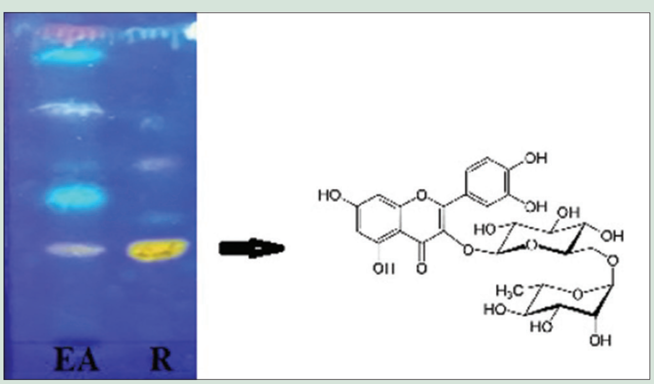

Figure 6: Thin-layer chromatography of *Carapa guianensis* Aubl. EA: Crude extract of *Carapa guianensis;* R: Rutin

Phytochemical screening indicates the groups of secondary metabolites present in plants, being of great relevance when there are no data regarding the chemical composition of the plant species of interest.[27,28]

Phenolic compounds stand out because of their reducing property and provide a structural variety, which can be simple or more complex. Phenols present hydroxyls act in neutralizing or scavenging-free radicals and[29,30] presenting itself as an alternative to prevent oxidative deterioration of food and minimize oxidative damage in living beings.[31]

Saponins are secondary metabolites produced by the plants,[28] and these present surface-active property, which may complex with proteins and cell membrane phospholipids, altering the permeability of membranes, assisting in the uptake of substances or possess toxic action in destroying the membrane.[32] Saponins have also been highlighted due to their biological activities such as wound healing[33] and anti-inflammatory.[34]

The triterpenes and steroids have anti-inflammatory activity, which exploited the traditional use of this plant species. The evidence of this activity in this plant species has been described by testing the isolates of *C. guianensis* triterpenes.[35]

In this way, the phytochemical prospection indicates an enormous potential of the vegetal species for diverse biological activities such as anti-inflammatory, antioxidant, and antimicrobial, mainly by the presence of compound of rutin, a natural compound belonging to the class of flavonoids, which have various biological activities described in the literature as antifungal,[36,37] anticancer,[38] hepatoprotective,[39] neuroprotective,[40] antioxidant;[41,42] and the ability to inhibit the activity of lipoxygenase, cyclooxygenase, and phospholipase A.[43-46]

In therapy, this compound is widely used because of its thrombolytic and antioxidant capacity, being able to bind to the platelet membrane and eliminate free radicals, by blocking the enzymatic protein disulfide isomerase found in all cells involved in blood coagulation.[47] It can also be used as an anticoagulant,[47,48] has been sold as an herbal supplement approved by the US Food and Drugs Administration.

The phytochemical analysis by Silva and Almeida[30] in the crude hydroethanolic extract of *C. guianensis* bark showed the presence of phenols, tannins, and anthraquinones as major metabolites; while the analysis of the ethanol extract of the leaves demonstrated the presence of alkaloids, tannins, saponins, and essential oils, and no flavonoids and triterpenes.[49] In the present study, it is noteworthy to mention that crude extract did not show the presence of alkaloids and tannins. This can be explained by the fact that metabolites vary in composition and proportion depending on several factors, mainly the place of collection and time of year when the plant material has been collected.[50]

The physicochemical evaluation of plants is important for detecting adulteration and testify the quality of the drug.[51-54] For instance, the total ash is used to indicate the presence of foreign inorganic matter and the purity of the drug. A high value can be due problems in manipulation or even addition of sand.

Although the quality standards are fundamental for the validation of the use of plant species in the therapeutics, few plant products have these parameters described in the literature, as is the case of *C. guianensis*. In this way, the present work contributes significantly, given the scarce information in the quality control of herbal products.

CONCLUSION

This study described the morpho-anatomical characteristics that can be used to identify the *C. guianensis* Aubl. and established physicochemical parameters that allow the evaluation of commercial samples of this vegetal raw material.

Acknowledgement

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Conflicts of interest

There are no conflicts of interest.

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