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Research paper

Spondias mombin: Quality control and anti-inflammatory activity in human neutrophils

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ABSTRACT

Spondias mombin L. (Anacardiaceae) is a tree known in Northeast Brazil as "cajazeira". Besides producing fruit that is widely consumed (yellow mombin or hog plum in English, *caja* in Portuguese, among others), its leaves are extensively used in traditional medicine for its anti-inflammatory, antimicrobial and antiviral properties. This study aimed to develop a standardized hydroalcoholic extract of *S. mombin* leaves and to investigate the anti-inflammatory activity of the extract and geraniin from *S. mombin* in human neutrophils. The authors used an integrative approach, including histological and histochemical examination, HPLC analysis and microbiological control. The anti-inflammatory bioactivity of the standardized extract of *S. mombin* and geraniin was evaluated in a degranulation assay in human neutrophils. The leaves of *S. mombin* exhibited distinguishable histological features compared with other *Spondias* species. Idioblasts with phenolic compounds were histochemically identified. Purity parameters were determined in the drug as well as the total polyphenol content in the plant extract. HPLC analysis allowed detection and quantification of two bioactive phenols, geraniin and chlorogenic acid. The hydroalcoholic extract of *S. mombin* leaves and geraniin exhibited significant anti-inflammatory activity in human neutrophils. This study establishes an interdisciplinary method along with unprecedented specifications for the quality control of *S. mombin* and demonstrates that both standardized plant extract and geraniin showed anti-inflammatory activity.

1. Introduction

In common with drugs, it is vital to ensure the quality, safety and efficacy of botanical preparations for human consumption (Schilter et al., 2010). To date, various macroscopic and microscopic techniques, molecular marker authentication methods and chromatographic analysis, including the use of HPLC, have been established as powerful tools for comparing the chemical profiles of herbal medicines, by identifying and measuring the content of chemical markers (Araruna et al., 2013; Costa et al., 2015; Lin et al., 2015).

Spondias mombin L. (Anacardiaceae) is a tree extensively used in herbal medicine programs in Brazil (Matos, 2000; Brasil, 2010). The juice and leaf powder are traditionally used topically for healing and anti-inflammatory activity. The home-made preparations (teas) of the trunk bark, leaves or flowers are utilized for treating diarrhea, emesis, hemorrhoids and throat inflammation. In addition, the leaves are also used as antiviral (herpes simplex 1) by the public phytotherapy program called "Farmácias Vivas" (Akubue et al., 1983; Villegas et al., 1997; Matos, 2000).

Chemical studies (Corthout et al., 1991, 1992; Silva et al., 2012) of *S. mombin* leaves showed the presence of various phenols including phenolic acids (chlorogenic and caffeic acids), flavonols (quercetin) and ellagitannins (geraniin and galoilgeraniin). Several pharmacological actions of organic or aqueous extracts of *S. mombin* (not standardized)

Abbreviations: ±, More or less; %, Percentage; °C, Degree Celsius; µg, Microgram; µL, Microliter; GAE, Gallic acid equivalent; HPLC, igh-performance liquid chromatography; PDA, hotodiode array detector; UFC, ederal University of Ceará; MTT, -(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide; DMSO, imethyl sulfoxide; HAE, ydroalcoholic extract; PMA, 12-phorbol 13-myristate acetate; HEMOCE, Hematology Center of Ceará; HBSS, Hank's balanced salt solution; FC, Folin-Ciocalteu reagent; SC, Sodium carbonate; CFU, Colony-forming unit; ICH, International Council for Harmonization; HAE, Hydroalcoholic extract.

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have been demonstrated, such as antibacterial and antifungal (Osuntokun et al., 2018), leishmanicidal (Accioly et al., 2012), gastroprotective (Sabiu et al., 2016), anti-inflammatory (Cabral et al., 2016) as well as antiviral against poliomyelitis, coxsackie B_4 and herpes simplex 1 viruses (Corthout et al., 1991, 1992).

For the development of phytomedicine, clear and systemic characterizations of the taxonomy, phytochemical components and the bioactivities of the candidate medicinal plant are needed. In the present study, an integrative approach was used, including histological, histochemical and physicochemical analysis, to authenticate *S. mombin* leaves. Also, the anti-inflammatory effects of this standardized plant extract were evaluated in human neutrophils as a basic and preliminary way to establish quality standards of pharmacological effect.

2. Materials and methods

2.1. Plant material

Leaves of *Spondias mombin* L. were collected during the winter in the Medicinal Plant Garden of Ceará Federal University (UFC), Fortaleza, Ceará, Brazil (3º 44 '50 ' S and 38º 34 ' 22' W). A voucher specimen was deposited in the Prisco Bezerra Herbarium of UFC, under EAC no. 38575.

2.2. Histological and histochemical analysis

The histological analysis used fully expanded leaves of three plants. Fragments of the middle region of the lamina were fixed in 4% paraformaldehyde, 1% glutaraldehyde and phosphate buffer pH 7.2 (Karnovsky, 1965), dehydrated in ascending ethanol series, and infiltrated and embedded in methacrylate resin (Historesin, Leica®). Transections of 5 μm, obtained with an auto-advance rotary microtome (Leica® RM 2245) were stained with Astra blue and safranin 9:1 (Bukatsch, 1972). Histochemical tests for total phenolic compounds were performed using ferrous sulfate (Gerlach, 1984). The reagent Sudan IV was used for cuticle evidence (Gerlach, 1984). The slides were analyzed, and photomicrographs obtained with a Leica DM4000 B LED optical microscope coupled to an image capture system.

2.3. Preparation and characterization of the plant drug

Leaves of *S. mombin* were dried in a forced-air oven with continuous renewal of air for 2-120 h at 60 ± 5 °C and monitored for moisture. The moisture was determined using an infrared balance, whereas the mean size of the particles, total ash content, acid insoluble ash, and microbiological parameters were determined as previously described (Brazilian Pharmacopoeia, 2010). The phytochemical profile was ascertained as described by Matos (2009), by identification reactions based on the chemical group to be investigated.

2.4. Preparation of the S. mombin extract and characterization

The biological activities of *S. mombin* extract have been related at least in part to the presence of phenols, as determined in hydroethanolic extracts. Based on these chemical characteristics of the plant and in its traditional use, the present study prepared a hydroethanolic extract from the leaves of *S. mombin* by dynamic maceration at room temperature. The plant drug from the leaves (3 g) was macerated in 70% ethanol (10 mL) in water during 2.5 h. The extract was submitted to vacuum filtration and the ethanol was evaporated with the aid of rotary evaporator (60 °C) and the volume was brought to the desired concentration before biological assays. The yield was 6.6 ± 0.53 % (w:v) based on solid residues. The extract was maintained under refrigeration at 4 °C until the assays were carried out.

2.4.1. Total phenol content of the hydroalcoholic extract (HAE) of S. mombin leaves by spectrophotometry

The total polyphenol content in extracts was determined by the Folin-Ciocalteu colorimetric method (Fogliano et al., 1999). An aliquot (100 μL) of sample was mixed with 250 μL of Folin-Ciocalteu reagent, 400 μL of water, and 500 μL of Na_2CO_3 (10 %). After one hour standing in the dark, the color was measured in a spectrophotometer (Genesys 10S UV-Vis, Thermo Scientific, USA) at 715 nm. The content of total polyphenols was calculated from the regression equation of the calibration curve of the gallic acid standard (dissolved in Milli-Q water), at concentrations ranging from 2 to 8 μg/mL. The analyses were performed in triplicate and the data were expressed as mg of gallic acid equivalents (GAE) per mL of extract. The method was validated according to the ICH guidelines and Brazilian regulations, including specificity, linearity, precision, accuracy and robustness (ICH, 2014;Brasil, Ministério da Saúde. Agência Nacional de Vigilância Sanitária, 2017).

2.4.2. Chromatographic analysis of the HAE of S. mombin leaves by HPLC-PDA

This analysis was performed to identify and quantify two bioactive markers (geraniin and chlorogenic acid) in the HAE of *S. mombin* leaves. HPLC analysis was carried out with an Alliance HPLC-PDA system (Waters, USA) using a 5 μm Phenomenex Kinetex Evo C18 column (4.6 mm × 250 mm). The gradient elution was performed, while the detection of geraniin and chlorogenic acid were at 276 and 325 nm, respectively.

2.5. Anti-inflammatory activity: effect on neutrophil degranulation

Human blood was obtained from the Hematology and Hemotherapy Center of the Ceará State. The cells suspension containg predominantly neutrophils (80-90%) with viability of 97.7 ± 0.94 % (Trypan blue test) were isolated according to a previously described method (Lucisano and Mantovani, 1984). The study was approved by the Human Research Ethics Committee of Federal University of Ceará.

2.5.1. Degranulation assay

The cells (5×10^6 cells/mL) were incubated with HAE of *S. mombin* leaves (1 - 200 μg/mL), geraniin (1 - 100 μg/mL), indomethacin (36 μg/mL, standard drug), DMSO (1% v/v, vehicle-control group) or HBSS (untreated cells) for 15 min at 37 °C. Human neutrophils were stimulated by the addition of 12-phorbol 13-myristate acetate (PMA) (0.1 g/mL) for 15 min at 37 °C. The reaction was stopped by cooling, the cell suspension was centrifuged (2000 g, 10 min, 4 °C) and the supernatant obtained was used to determine the concentration of myeloperoxidase (MPO) was measured at 620 nm according to the methodology described by De Young et al. (1989). The results are expressed as percentage of the release of MPO.

2.5.2. Cytotoxicity assay: MTT test

Neutrophils (2.5×10^6 cells/mL) were incubated at 37 °C with HAE of *S. mombin* leaves (1- 200 μg/mL), geraniin (1 - 100 μg/mL), HBSS (untreated cells), DMSO (1% v/v, vehicle-control group) or Triton X-100 0.2% (cytotoxic standard) for 30 min at 37 °C. After this period, the plate was centrifuged, and the supernatant discarded and a new incubated solution (200 μL) containing 10% of MTT (10 mg/mL). Finally, the plate was centrifuged again, the supernatant was discarded and then added to 150 μμL of pure DMSO for cell lysis and solubilization of the formazan. At this time, the plates were shaken for 15 minutes. The absorbance was measured at 540 nm (Mosmann, 1983). Cell viability was expressed by percentage.

2.6. Statistical analysis

The results were submitted to analysis of variance (ANOVA), followed by the Tukey post hoc test (GraphPad Prism, USA), used for

multiple comparisons. Whenever needed, the unpaired Student t-test was used for comparisons between two means. Differences were considered significant at $p < 0.05$.

3. Results

3.1. Histological and histochemical characterization

Transections showed that the leaf blade epidermis is uniseriate on the adaxial and abaxial faces (Fig. 1A-C). Thickened cuticle is observed covering the epidermal cells (Fig. 1E). Short or long non-glandular trichomes occur on both leaf blade faces (Fig. 1E).

The mesophyll is dorsiventral with a one-layered palisade parenchyma on the adaxial face and five-layered spongy parenchyma on the abaxial face (Fig.1A, B). Collateral vascular bundles are encircled by a parenchymatous sheath, with sheath extension on the adaxial face (Fig. 1C). Idioblasts with druses are observed in both palisade and spongy parenchyma (Fig. 1A-C).

The midrib has a biconvex transection plane. Collateral vascular bundles in a U-arrangement pattern occur centrally. Three groups of accessory collateral vascular bundles are also observed (Fig. 1D).

Reddish-colored idioblasts with acidophilic content are observed in the mesophyll and midrib (Fig. 1C, D). In the mesophyll, they occur among the parenchyma cells of the vascular bundles, as well as along the vascular bundle sheath (Fig. 1C). In the midrib, they are mainly present surrounding the vascular system and also occur among the parenchyma cells of the vascular bundles (Fig. 1D). The histochemical tests indicated these idioblasts are rich in total phenolic compounds (Fig. 1F-H). Some palisade cells of the mesophyll rendered positive results for total phenolic compounds (Fig. 1F).

3.2. Preparation and characterization of the plant drug

The preparation of the plant drug from *S. mombin* was carried out in a forced-air oven with continuous renewal of air. The moisture content of the fresh plant material (75.13 ± 1.3 %) declined significantly (4.39 ± 0.14 %). The moisture content of the plant drug decreased in the first 4 hours and remained almost constant thereafter (Table. 1).

After drying, the material was ground to an average particle diameter of 0.369 mm (Table 2), characterized as a moderately coarse powder. Other parameters, such as total and acid-insoluble ash were also determined (Table 2).

The results of the analysis of dried *S. mombin* material showed there was no microbial growth of *Salmonella* spp or *Escherichia coli*, both considered pathogenic microorganisms. In the total count of bacteria and fungi/yeasts, the contamination level found was $< 10^1$ CFU/g (Table 2), lower results than the pharmacopoeia specifications (Brazilian Pharmacopoeia, 2010), both for the hot extraction process (10^5 CFU/g) and cold extraction process (10^3 CFU/g).

3.3. Phytochemical profile

A preliminary chemical analysis of the leaves of *S. mombin* was also performed which confirmed presence of phenols in the plant extract, particularly tannins, besides other chemical constituents, such as alkaloids.

3.4. Total phenol content of the HAE of S. mombin leaves by spectrophotometry

To determine the content of polyphenols in the *S. mombin* extract, a spectrophotometric method was developed and validated. Analysis of the results of the specificity test indicated that the conditions were satisfactory. The method was linear ($y = 0.1063x + 0.0898$, $r > 0.99$). The limits of detection and quantification were 0.28 µg/mL and 0.86 µg/mL, respectively. The repeatability and the intermediate precision were 17.63 ± 0.2 (1.15%) and 17.42 ± 0.27 (1.58%) GAE mg/mL of extract, respectively, with no significant difference between them ($p = 0.0946$). The accuracy, determined by a recovery study, presented an average recovery in about 100% (100.4 ± 3.5%). Alterations in volumes of Folin-Ciocalteu reagent and Na_2CO_3, as well as the change of the wavelength used (715 to 785 nm), did not result in significant variation in terms of quantification according to the p-values obtained in the statistical analysis by the T-test ($p = 0.3441$) and F-test ($p = 0.2480$), showing that the method was robust for the parameters evaluated. The results indicated 17.5 mg/mL of total phenols in the HAE of *S. mombin* leaves.

3.5. Chromatographic analysis of the HAE of S. mombin leaves by HPLC-PDA

The chromatographic analysis allowed simultaneously detecting geraniin and chlorogenic acid (retention time of peaks: 22.93 min and 9.17 min, respectively) (Fig. 2), which presented satisfactory peak purity since the purity angles were lower than the threshold angles and the threshold curves did not intersect the purity curves. The chromatographic method was linear, and the correlation coefficients were above 0.99. The concentrations of geraniin and chlorogenic acid in the *S. mombin* extract were 7.3 ± 1.9 mg/mL and 0.8 ± 1.8 mg/mL, respectively.

3.6. Anti-inflammatory activity: degranulation and citotoxic assays

The effect of HAE of *S. mombin* or geraniin on neutrophil degranulation was measured by MPO release after exposure to PMA. The addition of PMA to the cells suspension caused a significant increase in the MPO release (control group: 100 ± 8.4%) compared with basal condition (HBSS group: 19.7 ± 1.2%). The addition of HAE from *S. mombin* (1, 10, 50, 100 and 200 µg/mL) to the cell before PMA exposure partially reversed the increase in MPO release compared to that produced by PMA (control group) showing maximal inhibitions at concentrations of 100 and 200 µg/mL (20.3 and 45.2% inhibition, respectively). The addition of geraniin (1 – 100 µg/mL) in the cell suspension induced a dual effect, increasing the MPO release at lower concentrations (1 to 25 µg/mL) and reducing it by 37.7 and 83.9% at the highest concentrations (50 and 100 µg/mL, respectively). The anti-inflammatory effect of geraniin at 100 µg/mL was better than that of indomethacin (36 µg/mL), a non-selective inhibitor of cyclooxygenase (73.9% inhibition) (Fig. 3).

The addition of HAE of *S. mombin* or geraniin to the cell culture at concentrations ranging from 1 to 100 µg/ml produced no effect on cell viability evaluated by MTT test. However, the MTT absorbance at 200 µg/mL HAE concentration was lower as compared to control group which is indicative of a reduction in the number of viable cells (HAE 200: 80.9 ± 1.9%; control: 94.4 ± 5.1%) (Fig. 3). So, the anti-inflammatory effect of HAE in the highest concentration is partially related to its toxicity.

4. Discussion

The initial analysis of medicinal plants consists of confirming their identity, based on macro and microscopic descriptions associated with chemical analysis of the vegetal organs, and in some cases, molecular analysis (Lin et al., 2015). Such information is important in the pharmacobotanical quality control of plant raw materials, providing conditions for the identification of adulterated lots (Sanches et al., 2007).

The leaf anatomical features of cultivated *S. mombin* are described for the first time here. Mesophyll organization, vascular system pattern of the midrib, and idioblasts as accumulation places of phenolic compounds are notable histological and histochemical differences in comparison with *S. mombin* harvested in the wild (Chisom et al., 2014; de Vasconcelos et al., 2016) and with others species of the *Spondias* genus, such as *S. purpurea* L., *S. tuberosa* Arruda, and *S. dulcis* Forst. F. (Barrios and Hernandez, 2003; Silva and Paiva, 2007; Santos et al., 2012).

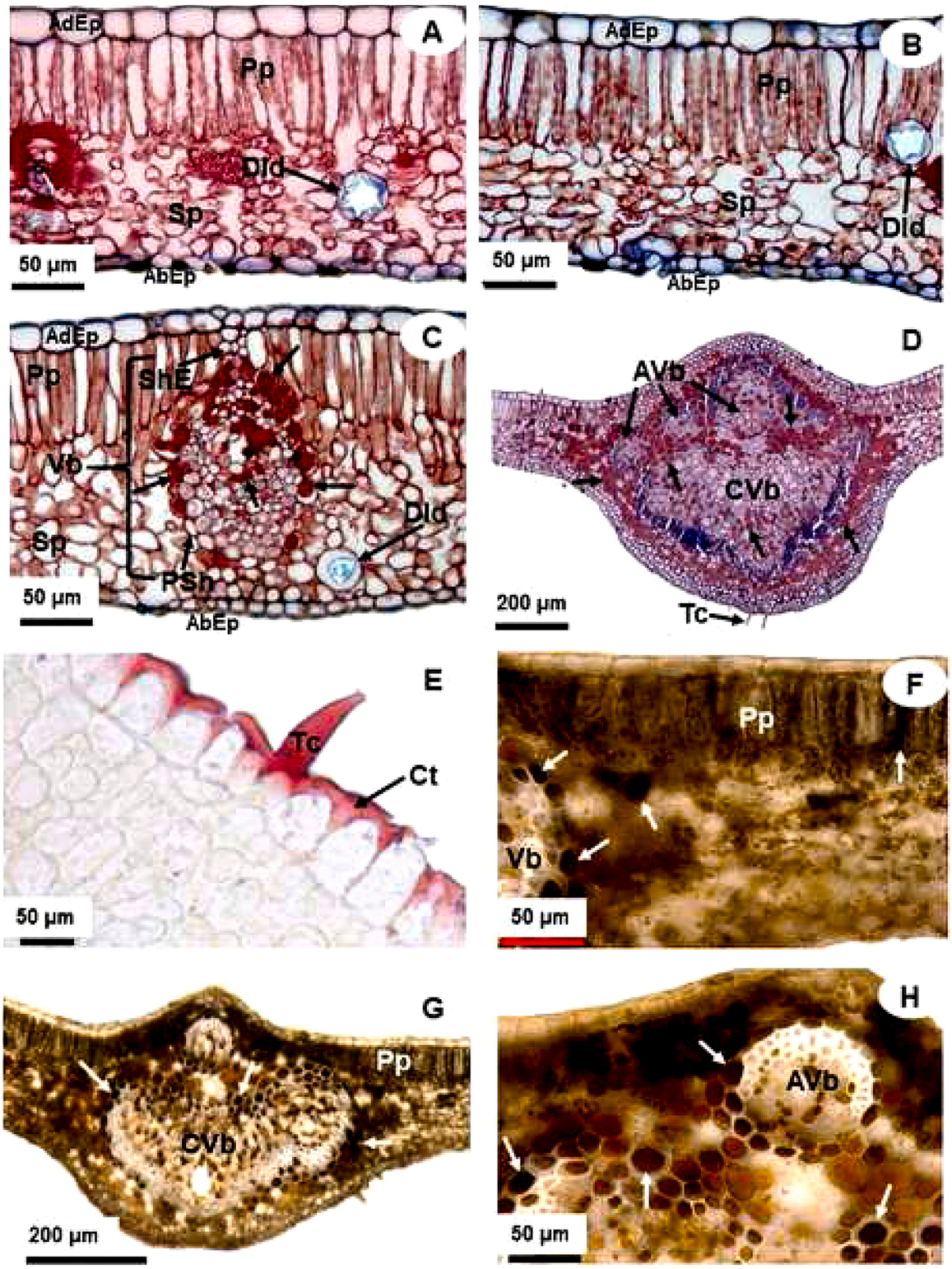

Fig. 1. Photomicrographs of transections of the leaf blade of *Spondias mombin* L. (Anacardiaceae). **A**; **B**; **C** and **F**. Mesophyll. **D**; **E**; **G** and **H**. Midrib. **A-E**. Structural characterization. **A; B; C** and **D**. An overview of the histological organization. Note reddish-colored idioblasts with acidophilic content (arrows). **E**. Details of the non-glandular trichome and cuticle. **F-H**. Positive results from histochemical tests for total phenolic compounds. Arrows indicate some idioblasts with intensive positive reaction. Adaxial epidermis (AdEp). Abaxial epidermis (AbEp). Palisade parenchyma (Pp). Spongy parenchyma (Sp). Idioblasts with druse (DId). Vascular bundle (Vb). Accessory vascular bundle (AVb). Central vascular bundle (CVb). Parenchymatic sheath (PSh). Bundle sheath extension (ShE). Non-glandular trichome (Tc). Cuticle (Ct).

Table 1
The moisture content of the plant drug from *S. mombin* determined by the method of drying on an infrared balance and prepared in an oven with circulation and continuous renewal of air.

Oven time (h)	Moisture content (%)
0	75.13 ± 1.3 (1.74)
2	36.6 ± 1.16 (3.16)
4	7.45 ± 0.06 (0.79)
6	5.12 ± 0.19 (3.63)
8	4.83 ± 0.13 (2.64)
24	4.87 ± 0.21 (4.27)
48	4.72 ± 0.10 (2.12)
72	4.52 ± 0.18 (4.03)
96	4.43 ± 0.15 (3.42)
120	4.39 ± 0.14 (3.13)

The results were expressed as mean ± standard variation (coefficient of variation). Analyses were carried out in triplicate.

Table 2
Pharmacognostical and microbiological parameters of the plant drug of *S. mombin*.

Parameters	Results
Mean diameter of particles	0,369 ± 0,007 (2,01)
Total ashes	5,79 ± 0,180 (3,10)
Ashes insoluble acid	0,629 ± 0,039 (6,25)
Microorganism aerobic bacteria	$< 10^1$ CFU/g
Fungi/Yeats	$< 10^1$ CFU/g
Escherichia coli	Absent
Salmonella spp	Absent

The results were expressed as mean ± standard variation (coefficient of variation). Analyses were carried out in triplicate.

Therefore, differences between wild and cultivated *S. mombin* must be considered for botanical authentication purposes. Furthermore, the data that was obtained will be useful to distinguish *S. mombin* from closely related *Spondias* species.

In order to guarantee the quality of raw material, the results presented here enable the standardization of a drying method to attain moisture content close to the minimum level (8-14%) established in different pharmacopoeias (Simões et al., 1999). They also support evaluation of the purity of the plant material (Brasil, 2014) determined through tests, such as ash content, acid insoluble ash content and determination of microbiological contaminants. In this context, the ash content of *S. mombin* determined in this study is important for the standardization of the raw material, by indicating previously unpublished specifications for quality control, since there is no pharmacopeia monograph of the species or data of these parameters in the literature.

Further studies allowed the determination of the phytochemical profile of the *S. mombin* hydroalcoholic extract, corroborating the results of previous studies that also observed the presence of tannins, flavonoids, and saponins in Anacardiaceae (Silva et al., 2012) as well as phenolic, ellagic and chlorogenic acids (Cabral et al., 2016). The chromatographic profile of HAE of *S. mombin* leaves was also determined, where it was possible to identify and quantify for the first time the bioactive marker geraniin. This substance was not identified in chromatographic studies (Cabral et al., 2016) and other species of the *Spondias* genus like *S. tuberosa* (Silva et al., 2011), *S. purpurea* (de Almeida et al., 2017) and *S. venulosa* (Pereira et al., 2015). Therefore, the botanical description, the purity parameters, and the chromatographic analysis performed in the present study produced a group of results that together will certainly differentiate *S. mombin* from other species.

After being fully characterized, the *in vitro* anti-inflammatory activity of HAE *S. mombin* leaves was evaluated. The results showed that the extract was able to modulate neutrophil activation, determined by reduction of the MPO release by neutrophils, which may play an important role in the *in vivo* anti-inflammatory activity of the species described by Cabral et al. (2016). Part of the observed effect is related to geraniin (in the highest concentrations), as well as the possible presence of other compounds, such as chlorogenic acid (Hwang et al., 2014; Ohkawara et al., 2017). In addition, these results corroborate previous

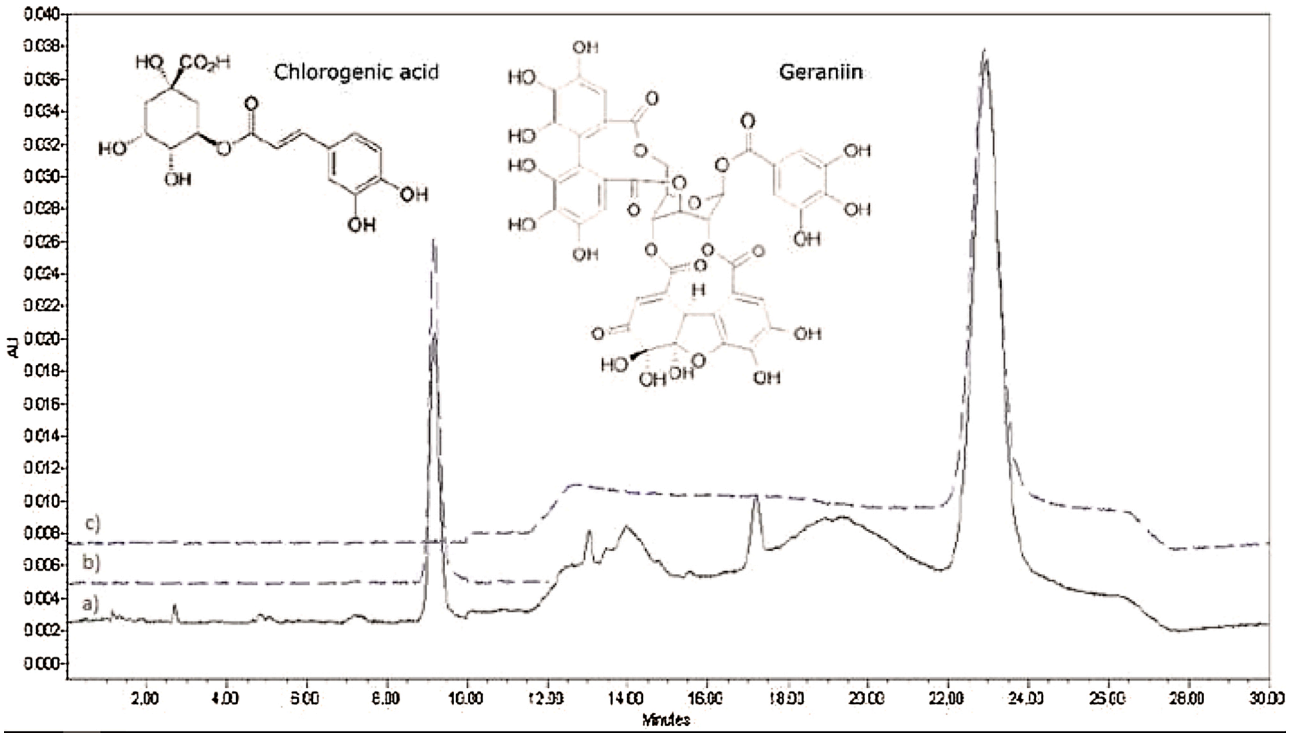

Fig. 2. Chromatograms in Timed Wavelength mode (0-10 min in λ = 325 nm and 10-30 min in λ = 276 nm) a) HAE of *S. mombin* leaves; b) Chlorogenic acid 7,35 μg/mL; c) Geraniin 64,08 μg/mL.

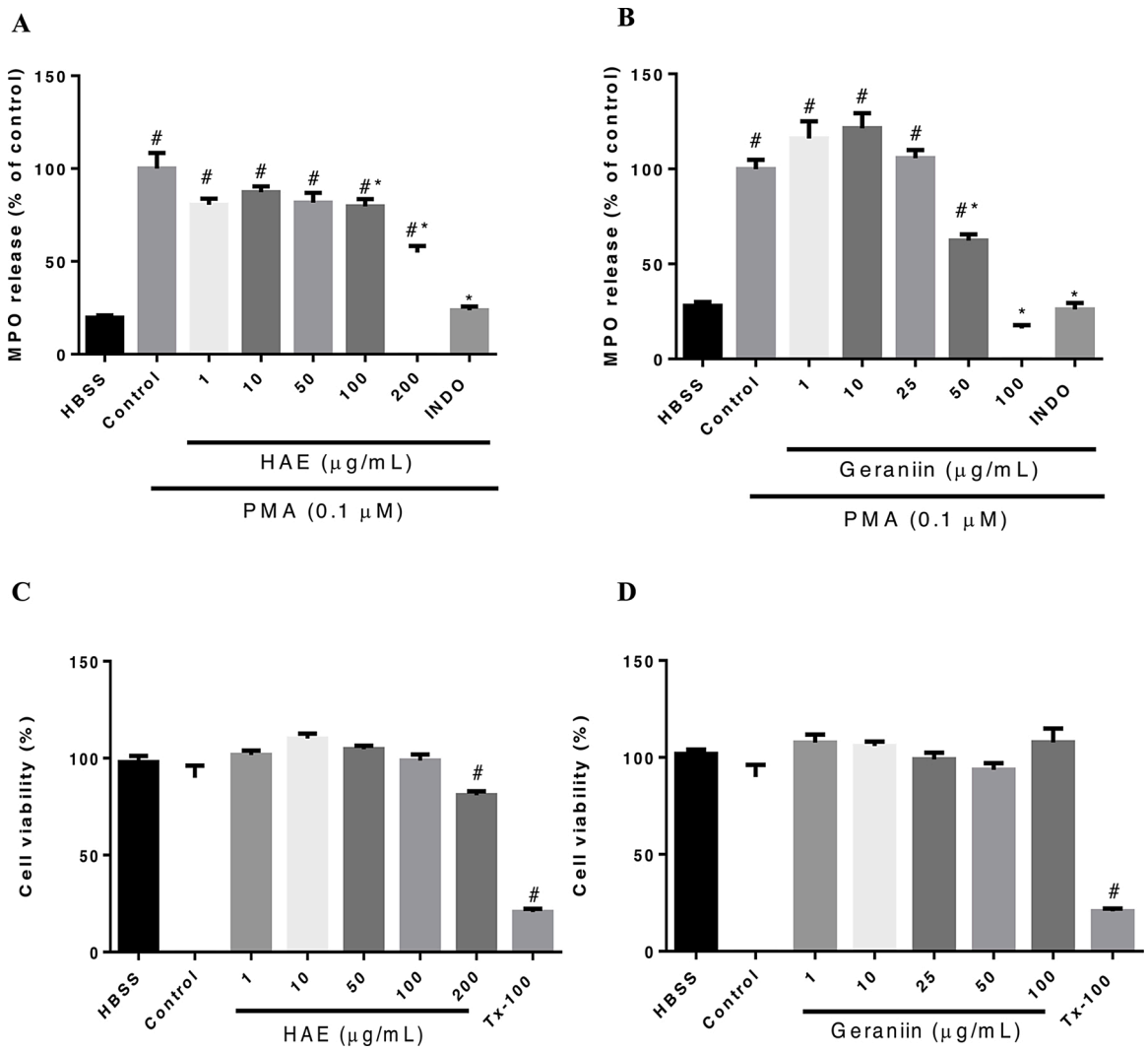

Fig. 3. Effects of HAE of *S. mombin* leaves (A) and geraniin (B) on the human neutrophil degranulation assayed using release MPO as marker and effects of HAE of *S. mombin* leaves (C) and geraniin (D) on the cytotoxicity of neutrophils determined by the MTT assay. Results represent means ± S.E.M. ($p < 0.05$; ANOVA and Tukey as the *post hoc* test). # vs HBSS; *drug effect vs Control; HBSS: Hanks' buffered saline solution (untreated group); Control: 1% DMSO (vehicle); INDO: indomethacin (36 µg/mL); Triton X-100 (0.2% v/v) was used as cytotoxic standard.

studies (Krakauer, 2002; Wang et al., 2015) that showed the potential of other plant extracts.

5. Conclusions

This study establishes an interdisciplinary method and specifications for quality control of the traditional medicinal plant, *S. mombin*, which has anti-inflammatory activity. The histological and histochemical characteristics of the leaves of *S. mombin* described in the present study are certainly useful to support this quality control. Both the standardized HAE of *S. mombin* leaves and geraniin inhibited the human neutrophil pro-inflammatory response.

Declaration of Competing Interest

The authors declare no conflict of interest.

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References

Accioly, M.P., Bevilaqua, C.M., Rondon, F.C., de Morais, S.M., Machado, L.K., Almeida, C.A., de Andrade Jr., H.F., Cardoso, R.P., 2012. Leishmanicidal activity in vitro of *Musa paradisiaca* L. and *Spondias mombin* L. fractions. Vet. Parasitol. 187, 79–84.

Akubue, P.I., Mittaland, G.C., Aguna, C.N., 1983. Preliminary pharmacological study of some Nigerian Medical plants. J. Ethnopharmacol. 8, 53–63.

Araruna, S.M., Silva, A.H., Canuto, K.M., Silveira, E.R., Leal, L.K.A.M., 2013. Influence of process conditions on the physicochemical characteristics of cumaru (*Amburana cearensis*) powder produced by spray drying. Revista Brasileira de Farmacognosia 23, 132–137.

Barrios, E.P., Hernandez, B.C.R., 2003. Phenology, growth, and response to light of Ciruela Mexicana (*Spondias purpurea* L., Anacardiaceae). Econ. Botany 57, 481–490.

Brasil, 2010. diário oficial da União. Portaria nº 886, 20 de abril de. institui a Farmácia Viva no âmbito do Sistema Único de Saúde (SUS).

Brasil, 2014. Agência Nacional de Vigilância Sanitária. Resolução da Diretoria Colegiada – RDC nº 26, de 13 de maio de 2014.

Brasil, Ministério da Saúde. Agência Nacional de Vigilância Sanitária, 2017. Resolução da Diretoria Colegiada – RDC nº 166 de 24 de julho de.

Brazilian Pharmacopoeia, 2010. 5th ed. ANVISA, Brasília.

Bukatsch, F., 1972. Bermerkungen zur Doppelfarbung Astrablau-Safranin. Mikrokosmos 61, 255.

Cabral, B., Siqueira, E.M.S., Bitencourt, M.A.O., Lima, M.C.J.S., Lima, A.K., Ortmannd, C. F., Chaves, V.C., Fernandes-Pedrosa, M.F., Rochac, H.A.O., Scortecci, K.C., Reginatto, F.H., Giordani, R.B., Zucolotto, S.M., 2016. Phytochemical study and anti-inflammatory and antioxidant potential of *Spondias mombin* leaves. Braz. J. Pharmacognosy 26, 304–311.

Chisom, I.F., Okereke, C.N., Okeke, C.U., 2014. A comparative foliar anatomical and morphological study on *Anacardium occidentale* L. and *Spondias mombin* L. International J. H. Medicine 2, 150–153.
Corthout, F.J., Pieters, L.A., Claeys, M., Vlietinck, A.J., 1991. Antiviral; Ellagitannins from *Spondias mombin*. Phytochemistry. 30, 1190.
Corthout, J., Pieters, L.A., Claeys, M., Vanden Berghe, D.A., Vlietinck, A.J., 1992. Antiviral Caffeoyl; Estersfrom *Spondias Mombin*. Phytochemistry. 31, 79.
Costa, M.F.B., Liborio, A.B., Miron, D., Silva, A.H., Leal, L.K.A.M., Martins, A.M.C., 2015. Red propolis ameliorates ischemic-reperfusion acute kidney injury. Phytomedicine 22, 787–795.
de Almeida, C.L.F., Brito, S.A., de Santana, T.I., Costa, H.B.A., Júnior, C.H.R.C., da Silva, M.V., de Almeida, L.L., Rolim, L.A., dos Santos, V.L., Wanderley, A.G., da Silva, T.G., 2017. *Spondias purpurea* L. (Anacardiaceae): Antioxidant and Antiulcer Activities of the Leaf Hexane Extract. Oxid. Med. Cell. Longevity 2017.
de Vasconcelos, A.L., de Vasconcelos, A.L., Randau, K.P., 2016. Pharmacognostic characterization of *Spondias mombin* L. (Anacardiaceae). Pharmacognosy J. 8, 513–519.
De Young, L.M., Kheifets, J.B., Young, J.M., 1989. Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separated and can be differentially modulated by pharmacologic agents. Agents Actions 26, 335–341.
Fogliano, V., Verde, V., Randazzo, G., Ritieni, A., 1999. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J. of Agricultural and Food Chemistry. 47, 1035–1040.
Gerlach, D., 1984. Botanische Mikrotechnik. Stuttgart, Georg Thieme Verlag.
Hwang, S.J., Kim, Y.-W., Park, Y., Lee, H.-J., Kim, K.-W., 2014. Anti-inflammatory effects of chlorogenic acid in lipopolysaccharide-stimulated RAW 264.7 cells. Inflammation Res. 63, 81–90.
ICH, 2014. Topic Q2 (R1). Validation of analytical procedures: text and methodology.
Karnovsky, M.J., 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. J. Cell Biol. 27, 137–138.
Krakauer, T.I., 2002. The polyphenol chlorogenic acid inhibits staphylococcal exotoxin-induced inflammatory cytokines and chemokines. Immunopharmacol. Immunotoxicol. 24, 113–119.
Lin, W.C., Wen, C.C., Chen, Y.H., Hsiao, P.W., Liao, J.-W., Peng, C., Yang, N., 2015. Integrative approach to analyze biodiversity and anti-inflammatory bioactivity of *Wedelia* medicinal plants. PLOS One 10, 1–24.
Lucisano, Y.M., Mantovani, B., 1984. Lysosomal enzyme release from polymorphonuclear leukocytes induced by immune complexes of IgM and IgG. J. Immunol. 132, 2015–2020.
Matos, F.J.A., 2000. Plantas Medicinais: guia de seleção e emprego de plantas usadas em fitoterapia no Nordeste do Brasil. 2ª Edição, Fortaleza, 2000.
Matos, F.J.A., 2009. Introdução à Fitoquímica Experimental. EUFC, Fortaleza.
Mosmann, T.J., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Immunol. Methods 65, 55–63.
Ohkawara, T., Takeda, H., Nishihira, J., 2017. Protective effect of chlorogenic acid on the inflammatory damage of pancreas and lung in mice with l -arginine-induced pancreatitis. Life Sci. 190, 91–96.
Osuntokun, O.T., Idowu, T.O., Cristina, G.M., 2018. Antibacterial and antifungal efficacy of partially partitioned fractions of *Spondias mombin* (Linn) extracts (root, leaf and stem bark) against clinical and environmental isolates. Méd. Chem. 8, 10–17.
Pereira, C., de Oliveira, Gonçalves, R., Amaral, A.C.F., Kustera, R.M., Sakuraguic, C.M., 2015. Phytochemical and phylogenetic analysis of Spondias (Anacardiaceae). Quim. Nova. 38, 813–816.
Sabiu, S., Garuba, T., Sunmonu, T.O., Sulyman, A.O., Ismail, N.O., 2016. Indomethacin-induced gastric ulceration in rats: ameliorative roles of *Spondias mombin* and *Ficus exasperata*. Pharm. Biol. 54, 180–186.
Sanches, A.C.C., Lopes, G.C., Toledo, C.E.M., Sacramento, L.V.S., Sakuragui, C.M., Mello, J.C.P., 2007. Estudo Morfológico Comparativo das Cascas e Folhas de *Stryphnodendron adstringens, S. polyphyllum e S. obovatum- Leguminosae*. Lat. Am. J. Pharm. 26, 362–368.
Santos, B.F.S., Azevedo, A.A., Silva, L.C., Oliva, M.A., 2012. Diagnostic and prognostic characteristics of phytotoxicity caused by fluoride on *Spondias dulcis* Forst. F. (Anacardiaceae). An. da Academia Brasileira Ciências. 84, 689–702.
Schilter, B., Andersson, C., Anton, R., Constable, A., Kleiner, J., O'Brien, J., Renwick, A. G., Korver, O., Smit, F., Walker, R., 2010. Guidance for the safety assessment of botanicals and botanical preparations for use in food and food supplements. Food Chem. Toxicol. 41, 1625–1649.
Silva, A.R.A., Morais, S.M., Marques, M.M.M., Lima, D.M., Santos, S.C.C., Almeida, R.R., Vieira, I.G.P., Guedes, M.I.F., 2011. Antiviral activities of extracts and phenolic components of two *Spondias* species against dengue virus. J. Venom. Anim. Toxins incl. Trop. Dis. 17, 406–413.
Silva, A.R.A., Morais, S.M., Marques, M.M.M., Oliveira, D.F., Barros, C.C., Almeida, R.R., Vieira, I.G.P., Guedes, M.I.F., 2012. Chemical composition, antioxidant and antibacterial activities of two *Spondias* species from Northeastern Brasil. Phamaceut. Biol. 50, 740–746.
Silva, O.N., Paiva, J.G.A., 2007. Estudos morfológicos e anatômicos em folhas adultas de *Spondias tuberosa* Arruda (Anacardiaceae Lindley). Bol. Latinoam. y del Caribe de Plantas Medicinales y Aromáticas. 6, 36–43.
Simões, C.M.O., Schenkel, E.P., Gosmann, G., 1999. Farmacognosia: da da planta ao medicamento. Porto Alegre/Florianópolis Ed.Universidade/UFRGS/Ed. Da UFSC.
Villegas, L.F., Fernandez, I.D., Maldonado, H., Torres, R., Zavaleta, A., Vaisberg, A.J., Hammond, G.B., 1997. Evaluation of the wound healing activity of selected traditional medicinal plants from Peru. J. Ethnopharmacol. 55 (3), 193–200.
Wang, P., Peng, X., Wei, Z.F., Wei, F.Y., Wang, W., Ma, W.D., Yao, L.P., Fu, Y.J., Zu, Y.G., 2015. Geraniin exerts cytoprotective effect against cellular oxidative stress by upregulation of Nrf2-mediated antioxidant enzyme expression via PI3K/AKT and ERK1/2 pathway. Biochimica et Biophysica Acta 1850, 1751–1761.