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Extracts and gel formulations of *Spondias mombin* L. stem and its main compound ellagic acid reduce cutaneous inflammation in experimental models

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***Spondias mombin* L. is a medicinal plant traditionally used for the treatment of vomiting, diarrhea, hemorrhoids, wounds and inflammation. In this study, the topical anti-inflammatory activity of *S. mombin* stem and ellagic acid was investigated. From the dried stem, powdered bark and wood were separately subjected to ethanol extraction by static maceration to obtain the EESB (bark) and EESW (wood) extracts. These extracts were analyzed by TLC and HPLC-DAD-UV. Using EESB and EESW (0.1, 0.5 and 1 mg/ear), GEESB and GEESW gel formulations (1, 3 and 5%) and ellagic acid (EA - 1 mg/ear), the topical anti-inflammatory activity was carried out by Croton oil-induced ear edema. Histopathological analysis, myeloperoxidase (MPO), N-acetyl-β-D-glucosaminidase (NAG) and nitric oxide (NO) were assessed. EA was identified in EESB and EESW as the main compound. After 6 h of treatment, EESB (53.83 to 63.06%), GEESB (41.45 to 43.48%), EESW (37.51 to 49.30%), GEESW (45.02 to 50.70%), and EA (58.59%) inhibited the ear thickness. In 24 h, the ear thickness was reduced by EESB (56.22 to 58.01%), GEESB (44.79 to 60.67%), EESW (44.11 to 62.69%), GEESW (49.38 to 62.88%) and EA (62.90%). In this time, ear weight, MPO, NAG, NO, leukocyte migration and vasodilation were decreased. These results suggest that *S. mombin* stem has topical anti-inflammatory activity, which supports the traditional uses and may represent an alternative for the treatment of cutaneous inflammation.**

Key words: *Spondias mombin*, ellagic acid, topical formulation, inflammation, anti-inflammatory agents.

INTRODUCTION

The treatment of skin inflammation is still a challenge for researchers and health professionals, since the skin is exposed to the external environment and undergoes metabolic modifications of the body itself (Eyerich et al.,

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2018). Clinically, skin problems are the most common and they present in many ways from the simplest and occasional eruptions and rednesses to chronic conditions like psoriasis, rosacea, dermatitis and acne (Brenner and Long, 2019). The inflammatory process, in turn, involves the release of chemical mediators that increase vascular permeability of leukocytes to migrate to tissues and painful mechanisms and the production of cardinal signals (heat, blush, pain and edema) (Pasparakis et al., 2014). Among the released mediators, histamine, serotonin, nitric oxide (NO), adhesion molecules (ICAM-1, others), vascular adhesion molecules (VCAM-1, Selectin-E, others), prostaglandins (PGE2, PGI2, others), leukotrienes (LTB4, LTC4, others), cytokines [tumor necrosis factor (TNF-α), interleukins (IL-1β, IL-6 and IL-10)], chemokines and others coordinate the events of inflammatory vascular changes and cell recruitment (Eyerich et al., 2018; Pasparakis et al., 2014). In addition, reactive oxygen species (ROS), mainly produced by neutrophils (polymorphonuclear), are capable of destroying host microorganisms and substances by the generation of superoxide which is converted to H_2O_2 and transformed into hypochlorite by the myeloperoxidase enzyme (MPO) (Forrester et al., 2018). To combat this disorder, anti-inflammatory drugs are used in clinical practice; however, they can cause adverse effects such as gastric irritation, ulceration, bleeding, renal failure, interstitial nephritis, headache, thrombocytopenia, hemolytic anemia, asthma, exacerbation, rash, angioedema and pruritus, which limit their prolonged use (Wongrakpanich et al., 2018). Faced with these harmful effects, one of the alternatives is the use of natural products and/or medicinal plants which has been a strategy for the search of new anti-inflammatory agents (Lordani et al., 2018).

With regards to relevant global biodiversity, we can find the *Spondias mombin* L. (Anacardiaceae family), popularly known as "cajá", "cajazeira", "cajá verdadeiro", "cajá-mirim" or "taperebá", a fruit species dispersed from the South of Mexico to Brazil, being cultivated in Africa and other continents (Silva et al., 2017; Duvall, 2006). In traditional medicine, this plant is used for its healing, anti-inflammatory, anti-thermal, antidiarrheal, antiblenorrhagic and anti-hemorrhoidal (Amaechi, 2015; Ayoka et al., 2008; Olugbuyiro et al., 2013; Nworu et al., 2011) properties. In particular, the bark obtained from the stem aids in labor and inflammation, fever, stomach pain and malaria (Adedokun et al., 2010), and for the treatment of wounds (Villegas et al., 1997). Experimental studies have proven these ethnomedicinal applications, since its leaf methanol extract has anti-inflammatory activity by reducing TNF-α and NO production (Nworu et al., 2011), as well as peritoneal leukocytes (Cabral et al., 2016). *S. mombin* exhibits antioxidant (Cabral et al., 2016; Brito et al., 2018; Ojo et al., 2018), anxiolytic and antidepressant (Sampaio et al., 2018), sedative, antiepileptic and antipsychotic (Ayoka et al., 2006), antiulcer (Brito et al., 2018; Sabiu et al., 2015), antidiabetic (Ojo et al., 2018) and antimicrobial (Olugbuyiro et al., 2009; Oladunmoye, 2007) activities. These activities may be related to the presence of compounds such as quercetin, chlorogenic acid, isoquercetrin, rutin and ellagic acid (EA), which may be directly correlated with wound healing, anti-inflammatory and antioxidant properties (Cabral et al., 2016; Silva et al., 2012).

From the phytochemical view-point, tannins, saponins, glycosylated anthraquinones, alkaloids (Corthout et al., 1991; Silva et al., 2011), triterpenes (Fred-Jaiyesimi et al., 2009), flavonoids (Silva et al., 2012) and glycosides (Ayoka et al., 2006) have been detected in *S. mombin* leaves. Saponins, alkaloids, tannins, flavonoids and phenolic compounds were quantified in its leaf, stem and root (Alobi et al., 2017). Ellagitannins (Corthout et al., 1991), geranine and galoylgeranine, chlorogenic acid, chlorogenic acid butyl ester and 2-*0*-caffeoyl-(+)-allohydroxycitric acid (Corthout et al., 1992) were identified in its leaves and stems. Quercetin, EA, kaempferol, isoquercetin and rutin (Silva et al., 2012), and lupeol (Lima et al., 2016) were found in leaves, while rhamnetin 3-*O*-rutinoside and quercetin 3-*O*-rutinoside were identified in leaves and/or stems (Pereira et al., 2015). In addition, stigmasta-9-en-3,6,7-triol and 3-hydroxy-22-epoxystigmastane were isolated from the methanol extract of *S. mombin* bark (Olugbuyiro et al., 2013), and the major components found in the essential oil were octadecane, heptacosane, hexatriacontane and β-caryophyllene (Lima et al., 2016). The presence of gallic, ellagic, and chlorogenic acids and isoquercetrin in *S. mombin* leaves was also identified by HPLC analysis (Cabral et al., 2016; Brito et al., 2018).

Considering its ethnomedicinal attributions, richness of chemical compounds and pharmacological evidences, this study aimed to investigate the active constituents and topical anti-inflammatory activity of extracts and gel formulations of *S. mombin* L. stem in order to contribute to the dissemination of their therapeutic properties and their possible application as herbal medicines.

MATERIALS AND METHODS

Plant material

Spondias mombin L. was collected at the Federal University of Bahia (UFBA), Salvador city, Bahia State, Brazil. A voucher specimen, identified by Dr. Lázaro Benedito da Silva, was deposited in the Herbarium Alexandre Leal Costa under nº 40/2016. Stems were collected on December 1, 2016 and subjected to drying at 50 °C, with forced ventilation until loss of 90 to 96% humidity.

Extract preparation

After stem collection and drying, the bark was removed and both materials were crushed separately in an electric mill with a defined granulation sieve (tamise n° 20) to extract preparation. These botanical materials (287.88 and 464.93 g, for bark and wood,

respectively) were extracted with ethanol (95%) (5.0 L) by static maceration with solvent renovation at room temperature until exhaustion. EESB (from bark) and EESW (from wood) extracts were obtained by removing the ethanol using rotary evaporator (Rotavapor RII®, Büchi, Flawil, Switzerland) and controlled temperature (55 ± 1°C). EESB and EESW were placed into a desiccator with silica for removal of water and residual solvent with a yield of 10.80 g (3.78%) and 14.36 g (3.08%) of dried extracts, in this order.

Chemicals

Drugs and reagents used in this study are as follows: Croton oil (≈100%), ellagic acid (≥ 95%), dexamethasone (≥ 97%), phosphoric acid (85%), rutin hydrate (≥ 94%), quercetin (≥ 95%), chlorogenic acid (≥ 95%), hexadecyltrimethylammonium bromide (≥ 99%), 3,3',5,5'-tetramethylbenzidine dihydrochloride hydrate (≥ 98%), *p*-nitrophenyl-acetamide-µ-*D*-glucopyranoside (≥ 98%), sodium phosphate (96%), glycine (≥ 98.5%), and sodium citrate dehydrate (≥ 99%) (Sigma-Aldrich® Co.), dimethylsulfoxide (DMSO, ≥ 99.7%), ethanol (95%), ethyl acetate (≥ 99%), toluene (≥ 99.5%), formic acid (≥ 98%), methanol HPLC grade (≥ 99.9%), and acetone (≥ 99%) (Vetec Química Farm Ltda), and ketamine chloride (10%) and xylazine chloride (2%) (Syntec).

Thin layer chromatography (TLC) analysis

To detect EA in *S. mombin*, standard EA (Sigma Aldrich®), EESB and EESW were solubilized in pyridine and analyzed by TLC in an aluminum plate (Alugram, Silica Gel 60, UV254 indicator, 20 x 20 cm dimensions and 0.20 mm thickness) using toluene: ethyl acetate: formic acid (5:5:1, v/v/v) as mobile phase and UV light 365 nm as revealer (Syed and Khan, 2016).

High performance liquid chromatography (HPLC) analysis

EESB and EESW were analyzed by Liquid Chromatograph (Waters® model) equipped with a diode array detector (DAD-UV) (Waters® 2998) at 190 - 400 nm, binary pump (Waters® 1525) and automatic injector (Waters® 2707) using a C18 reverse phase column (Sun Fire 4.6 × 250 mm, 5 µm). The flow rate was 1.0 ml/min with an injection volume of 30 µl and the column temperature was maintained at 40 °C. Extracts (2 mg) were prepared, in triplicate, homogenized in ultrasound (Sanders Medical mark, Soniclean 2 model) heated at 35°C for 15 min. Then, the samples were centrifuged (Kasvi microcentrifuge) for 5 min at 10,000 rpm and filtered through a filter membrane (0.45 µm pore PALL GHP mark). After this procedure, extract solutions were injected, in triplicate (n = 3), using an elution gradient composed of acidified water (pH 3 in 0.1% acetic acid) and linear gradient of methanol (from 20 to 100% in ultrapurified water) for 60 min. An isocratic system with 100% methanol (HPLC grade) until 70 min was also applied. Quercetin, rutin, chlorogenic acid and EA (1 mg/ml) were used as markers. The peaks and retention time of chromatogram and UV spectra were analyzed from the Empower 3 Software.

Gel preparation

EESB and EESW were used in the development of gel formulations according to the principles described by Ahmed (2015). Methylparaben (NIPAGIN, 0.2 g) was dissolved in water at 70°C followed by addition of hydroxyethylcellulose (2.2 g), under slow and constant stirring, until complete dissolution. Then, the preparation was cooled to 40°C, intercalating the stirring with periods of rest. Imidazolidinyl urea (0.1%), previously solubilized in water, was added to reach 100 ml with distilled water. To finalize the formulation, natrosol (2%), EESB (1, 3 and 5%) and EESW (1, 3 and 5%) were incorporated into the gel using propylene glycol as humectant, producing GEESB (1, 3 and 5%) and GEESW (1, 3 and 5%).

Animals

Male mice (25 - 30 g), 45 - 50 days old, were provided from the Center for Reproductive Biology of the Federal University of Juiz de Fora (UFJF). The animals were randomly separated into groups (n = 8) and housed into polypropylene cages (47 × 34 × 18 cm) in ventilated cabinet system for rodents with free access to food (Nuvilab® rodents) and water *ad libidum* at the Laboratory of Biomedicinal Chemistry and Applied Pharmacology of the Faculty of Pharmacy/UFJF. Mice were kept at room temperature (22 ± 2°C) and photoperiod controlled in cycles of 12 h light/dark. The experimental protocols (005/2016 and 001/2017) for the animal care and treatment were approved by the Ethical Committee for Animal Research/UFJF, which followed the recommendations of the Brazilian College of Animal Experimentation (COBEA).

Topical anti-inflammatory activity

Croton oil-induced ear edema in mice was made as described by Schiantarelli et al. (1982) with minor modifications. Induction was done in groups with eight mice (n = 8) applying 20 µl of 2.5% Croton oil (v/v, diluted in acetone) on the inner surface of the right ear, while the left one received 20 µl of acetone (vehicle). After 60 min, the treatment was performed on the right ear with extracts (EESB and EESW - 0.1, 0.5 and 1.0 mg/ear), gel formulations (GEESB and GEESW - 30 µl of 1, 3 and 5%), EA (1 mg/ear), dexamethasone (0.1 mg/ear, positive control) and negative control (20 µl of 0.9% saline). A basal group (untreated) was also inserted in the study. The ear thickness (mm) was measured after 6 and 24 h to follow the evolution of edema, while the weight (mg) was quantified after euthanasia with ear fragments (6 mm) by means of the difference between the right and left ears. Three fragments were preserved in 10% formaldehyde for histopathological analysis and three of them were used to determine myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) activities, and nitric oxide (NO) levels.

Obtaining the supernatant

Ear fragments of mice were treated with 1 ml of 80 mM sodium phosphate buffer (pH 5.4) containing 0.5% hexadecyltrimethylammonium bromide (HTBA). These fragments were triturated for 60 s in porcelain grail to yield the homogenates that were transferred into test tubes with addition of 1 ml of 80 mM sodium phosphate buffer (pH 5.4). From these homogenates, 2 ml were placed in an ice bath at 4°C, homogenized in ultrasound (Unique Ultral, Cleaner 1600A) for 10 min and centrifuged at 3,000 rpm at 4°C for 10 min. The supernatant was collected and used in the total protein, MPO, NAG, and NO assays (Young et al., 1989; Sánchez and Moreno, 1999).

Total protein dosage

The method used for protein dosing was described by Lowry et al. (1951) and modified by Sargent (1987). In triplicate, the supernatant obtained as described above (20 µl) was placed in test tubes with addition of distilled water (380 µl), reactive mixture (2 ml,

Figure 1. Identification of ellagic acid in the extracts of *S. mombin* by thin layer chromatography. EA = Ellagic acid. EESB = ethanol extract of *S. mombin* stem bark. EESW = ethanol extract of *S. mombin* stem wood. Mobile phase: toluene: ethyl acetate: formic acid (5:5:1, v/v/v). Revealer: 365 nm UV light.

1 g NaOH plus 5 g Na_2CO_3 to 250 ml distilled water), copper tartrate and copper sulfate. After 10 min, Folin solution (200 µl, 1:5) was added and kept for 30 min. The calibration curve was performed with ovalbumin. The absorbance was measured in a spectrophotometer (UV-VIS 1800, Shimadzu) at 660 nm and distilled water was used as blank.

Myeloperoxidase assay

The MPO activity was used as an indication of the presence of polymorphonuclear in the tissue by means of the methodology of Bradley et al. (1982) modified by Young et al. (1989). Supernatant (25 µl), in triplicate, was placed in 96-well microplate and 3,3',5,5'-tetramethylbenzidine (25 µl, 1.6 mM) in DMSO, hydrogen peroxide (100 µl, 0.003%, v/v) diluted in sodium phosphate buffer (0.08 M, pH 5.4) were added to start the reaction. The microplates were incubated at 37 °C for 5 min with sulfuric acid (100 µl, 4 M) at 4°C in each well to stop the reaction. The assay was colorimetrically determined using the Thermoplate TR-Reader® plate reader and the absorbance was measured at 450 nm. The MPO activity was expressed as optical density/mg tissue (OD/mg tissue) and distilled water was used as blank.

N-Acetyl-β-D-glucosaminidase assay

The NAG activity was used as indicative of the presence of mononuclear leukocytes applying the methodology of Sanchez and Moreno (1999). Supernatant (100 µl), in triplicate, was plated in 96-well microplate containing p-nitrophenyl-N-acetyl-β-D-glucosamine (100 µl, 2.24 mM) in sodium citrate/phosphate buffer (0.1 M, pH 4.5) to start the reaction. Then, the microplates were incubated at 37 °C for 10 min and the reaction was stopped with glycine buffer (100 µl, 0.2 M, pH 10.6) in each well. The assay was colorimetrically determined using a Thermoplate TR-Reader® plate reader and the absorbance was measured at 405 nm and expressed as optical density/mg tissue (OD/mg tissue). Distilled water was used as blank.

Nitric oxide assay

As recommended by Green et al. (1982), using Griess reagent (1.0% sulfanylamid and 0.1% α-naphthylethylenediamine solubilised in 5% phosphoric acid), NO was indirectly determined through the nitrite (NO_2^-) measurement by Griess's colorimetric method. The homogenate was obtained from Croton oil-induced ear edema using buffered saline (PBS, 3,000 µl, pH 7.2) and triturated about 60 s. Then, this homogenate (100 µl) and Griess reagent (100 µl) were added to 96-well microplates in triplicate and incubated for 20 min at room temperature. Absorbance measurements were performed on a microplate reader (Thermoplate®, TP-Reader®) at 540 nm. Sodium nitrite solutions (3.12 - 200 µM) were used to elaborate a standard curve and NO concentration was determined in µM.

Histopathological analysis

Fragments of excised ears were used to prepare histological sections (Chibli et al., 2014). The tissues were fixed in ALFAC solution (80% alcohol, 40% formalin and glacial acetic acid) over a period of 16 h and, after this time, maintained in alcohol (70%) until dehydration. Then, the tissues were dehydrated, embedded in paraffin, sectioned into 5 µm sections using a microtome and stained with hematoxylin and eosin. The edema (thickness of the histological section), leukocyte infiltration, vasodilation and thickness of the epidermis and dermis were evaluated in areas with 10× and 20× magnification. Representative sections were photographed using a microscope (Olympus BX41) coupled to the digital camera using Image Pro-Plus 4.5 software (Media Cybernetics).

Statistical analysis

The results were demonstrated by mean ± standard error of the mean (S.E.M.). Analysis of variance (ANOVA) followed by the Student Newman-Keuls test was used to measure the degree of significance ($p < 0.05$, $p < 0.01$ or $p < 0.001$). The statistical program GraphPad Prism version 5.01 was used to evaluate the data.

RESULTS AND DISCUSSION

Compound Identification

EA, a chemical marker of *S. mombin*, was detected in EESB and EESW by TLC using the commercially acquired standard from Sigma® (Figure 1). The chromatographic profile showed that the samples produced similar bands to EA as equivalent flow ratio\(FR) values (Figure 1). To confirm the presence of EA, analysis of EESB and EESW was performed by

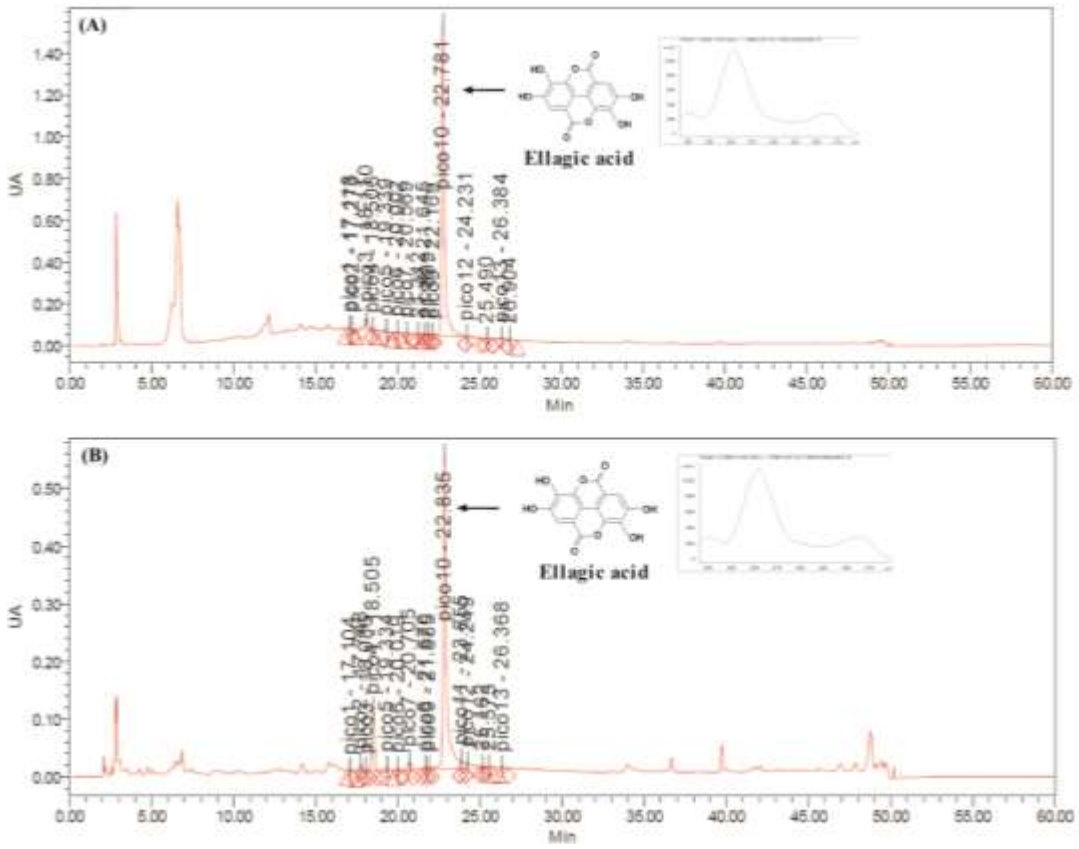

Figure 2. Chromatogram of EESB and EESW obtained by HPLC-UV-DAD at 254 nm indicating the presence of ellagic acid. (A) EESB = ethanol extract of *S. mombin* stem bark; (B) EESW = ethanol extract of *S. mombin* stem wood.

254 nm where it was possible to obtain a chromatogram with different peaks that are related to retention times and UV spectra (Figures 2A and 2B). In Figure 2A, it is shown that EESB peak 10, with mean retention time of 22.79 min, maximum absorption at wavelengths (199.3, 253.5 and 364.0 nm) and area of 87.95%, indicating be EA, since it presented UV spectrum and retention time similar to the standard compound. In addition, peak 10 of EESW (Figure 2B) produced a mean retention time of 22.84 min with maximum absorption at wavelengths (200.5, 253.5 and 364.0 nm) and area of 77.20%. These data are also suggestive of EA in the analyzed sample. Considering the constituents in both EESB and EESW, spectra profiles and retention times suggest differences in their chemical composition. However, the markers, chlorogenic acid, rutin and quercetin were not detected in these extracts.

In a previous study, EA has also been found in the leaves of *S. mombin* (Silva et al., 2012); however, our data showed that the stem bark and wood contain an expressive amount of this substance, since few bands were detected in TLC using EA standard compound (Figure 1). This TLC chemical profile was confirmed by HPLC analysis (Figure 2), which indicated EA as the major component with areas of 87.95% (EESB) and 77.20% (EESW) as shown by Bansal et al. (2014). This compound is described as a natural phenolic lactone that is a degraded product of hydrolysable tannins in plants (Khodadadi and Nasri, 2017). EA and its complex derivatives, such as ellagitannins, play an important role in human nutrition and are endowed with innumerable biological properties, including antibacterial, antioxidant, anti-hepatotoxic, anti-atherosclerotic, anti-inflammatory and anti-HIV replication activities. The interest about EA has increased mainly because of its anti-mutagenic and anticancer actions (Khodadadi and Nasri, 2017; Landete, 2011). Moreover, it was the qualitatively major component found in the ethanol extracts of *S. mombin*, and it is possible that the anti-inflammatory activities described in our results are due to the action of this important acid.

Topical anti-inflammatory effect

After 6 h of treatment, negative control group presented an expressive increase in ear thickness (Figure 3A). Doses of 0.1, 0.5 and 1.0 mg/ear of EESB significantly

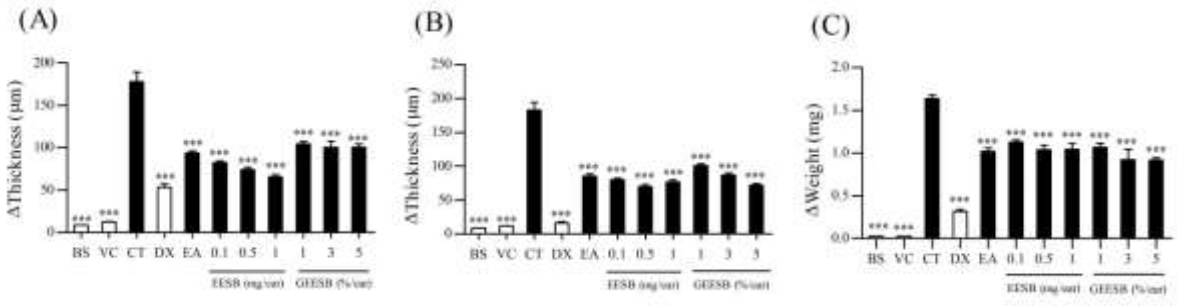

Figure 3. Anti-inflammatory effect of the ethanol extract and gel formulation of *S. mombin* stem bark on the Croton oil-induced ear edema. Each bar represents the mean ± SEM (n = 8).
***$p < 0.001$, different from the control group after analysis of variance followed by the Student-Newman-Keuls test. (A) Thickness measurements after 6 h. (B) Thickness measurements after 24 h. (C) Weight measurements after 24 h. BS = Basal; VC: Vehicle; CT: Negative control; DX: Dexamethasone; EA: Ellagic acid (1 mg/ear); EESB = ethanol extract of *S. mombin* stem bark; GEESB = gel formulation of *S. mombin* L. stem bark.

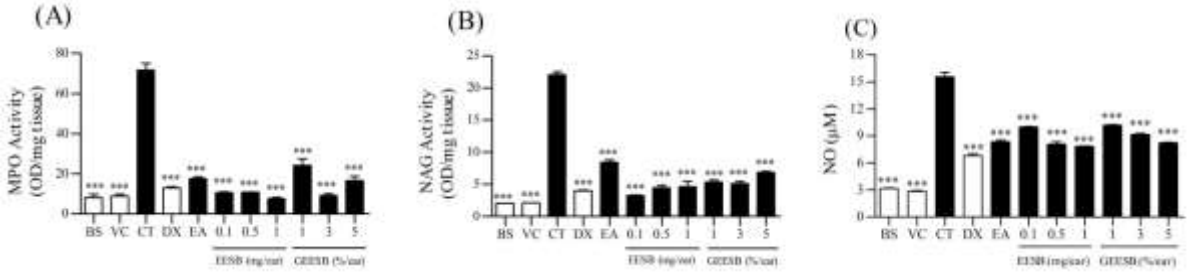

Figure 4. Effect of the extract and gels of *S. mombin* stem bark on the myeloperoxidase, N-acetyl-β-D-glucosaminidase and nitric oxide. Each bar represents the mean ± SEM (n = 3). (A) Myeloperoxidase (MPO); (B) N-acetyl-β-D-glucosaminidase (NAG); (C) Nitric oxide (NO).
***$p < 0.001$, different from the control group after analysis of variance followed by the Student-Newman-Keuls test. BS = Basal; VC = Vehicle; CT = Negative control; DX = Dexamethasone; EA: Ellagic acid (1 mg/ear); EESB = ethanol extract of *S. mombin* stem bark; GEESB = gel formulation of *S. mombin* L. stem bark.

inhibited the ear thickness (μm) (53.83 to 63.06%, $p < 0.001$), respectively, when compared to the negative control group. GEESB (1, 3 and 5%) application also reduced the ear thickness (41.45 to 43.48%) at the respective concentrations. EA caused 47.34% of inhibition, while dexamethasone, positive control, was effective in 69.91%.

In order to monitor the effect of EESB, GEESB and EA on the inflammatory process, the ear thickness was also evaluated after 24 h of Croton oil application. EESB (0.1 mg/ear = 56.22%, 0.5 mg/ear = 61.18% and 1 mg/ear = 58.01%), GEESB (1% = 44.79%, 3% = 52.61% and 5% = 60.67%) and EA (1 mg/ear = 53.45%) inhibited the edema formation compared to the negative control group (Figure 3B). At this time, EESB, GEESB and EA significantly decreased ($p < 0.001$) the ear weight (Figure 3C). As expected, dexamethasone reduced the thickness (91.19%) and ear weight (80.62%) after 24 h of treatment.

The Croton oil effect on the ear edema was also observed by the determination of MPO activity, which was increased in the negative control group when compared to the basal and vehicle groups (Figure 4A). In the groups submitted to treatment with EESB (0.1, 0.5 and 1.0 mg/ear) reduced MPO in 85.17, 84.87 and 89.54%, respectively ($p < 0.001$), in relation to the negative control group. GEESB also inhibited MPO by 66.29, 87.20 and 76.74% at concentrations of 1, 3 and 5%, in this order. EA reduced MPO in 75.54%.

Croton oil significantly raised the presence of NAG in the negative control group when compared to the basal and vehicle groups (Figure 4B). The treatment with EESB (0.1, 0.5 and 1.0 mg/ear) reduced NAG activity by 85.31, 79.86 and 79.23%, respectively, when compared to the negative control group. When treated with GEESB, NAG activity was decreased in 75.81, 76.86 and 77.95% at concentrations of 1, 3 and 5%, respectively, while EA caused 61.90% of inhibition. In addition, Figure 4C shows that EESB (35.93, 48.03 and 49.59%) and GEESB (34.51, 41.12 and 47.02%) reduced NO levels in dose dependent form. Dexamethasone was effective in inhibiting MPO, NAG and NO.

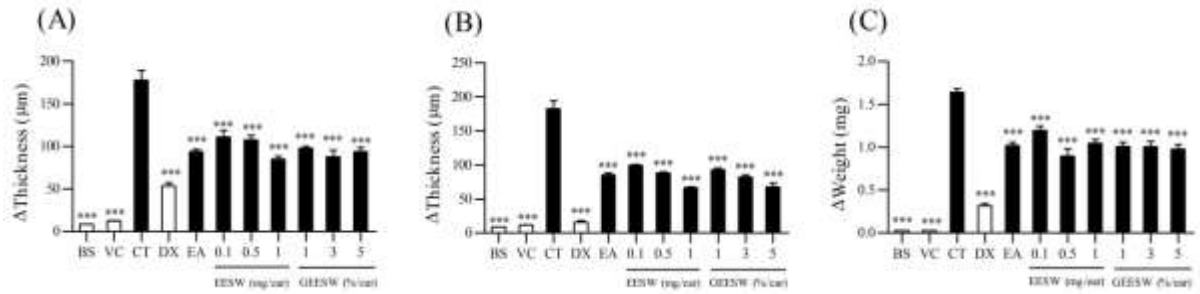

Figure 5. Anti-inflammatory effect of the ethanol extract and gels of *Spondias mombin* stem wood on the Croton oil-induced ear edema. Each bar represents the mean ± SEM (n = 8).
***$p < 0.001$, different from the control group after analysis of variance followed by the Student-Newman-Keuls test. (A) Thickness measurements after 6 h of treatment. (B) Thickness measurements after 24 h of treatment. (C) Weight measurements after 24 h of treatment. BS = Basal; VC: Vehicle; CT: Negative control; DX: Dexamethasone; EA: Ellagic acid (1 mg/ear); EESW = ethanol extract of *S. mombin* stem wood; GEESW = gel formulation of *S. mombin* L. stem wood.

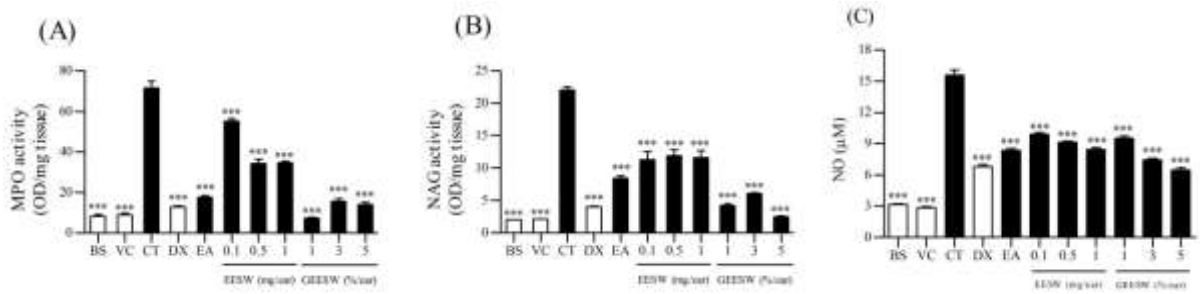

Figure 6. Effect of the extract and gels of *Spondias mombin* stem wood on the myeloperoxidase, N-acetyl-β-D-glucosaminidase and nitric oxide. Each bar represents the mean ± SEM (n = 3). (A) Myeloperoxidase (MPO); (B) N-acetyl-β-D-glucosaminidase (NAG); (C) Nitric oxide (NO).
***$p < 0.001$, different from the control group after analysis of variance followed by the Student-Newman-Keuls test. BS = Basal; VC = Vehicle; CT = Negative control; DX = Dexamethasone; EA: Ellagic acid (1 mg/ear); EESW = ethanol extract of *S. mombin* stem wood; GEESW = gel formulation of *S. mombin* L. stem wood.

Considering the Figure 5A, after 6 h of treatment, doses of EESW (0.1, 0.5 and 1.0 mg/ear) inhibited the ear thickness in 37.51, 39.48 and 52.23%, respectively, while GEESW (1, 3 and 5%) reduced in 45.02, 50.70, 47.33%, in this order, when compared to the negative control group. This effect was also observed after 24 h of treatment with EESW (0.1, 0.5 and 1.0 mg/ear) that reduced the ear thickness in 45.58, 51.93 and 63.67%, respectively, and 1, 3 and 5% of GEESW (reduction of 49.38, 55.06 and 62.88%, in this order) (Figure 5B). At this time, there was inhibition of ear weight (mg) in 27.09 (0.1 mg/ear), 45.35 (0.5 mg/ear) and 36.24% (1.0 mg/ear) by EESW, while 1, 3 and 5% GEESW decreased in 38.50, 38.74 and 40.27%, respectively (Figure 5C).

MPO activity was significantly reduced ($p < 0.001$) in 9.09, 52.15 and 51.62% after treatment with EESW (doses of 0.1, 0.5 and 1.0 mg/ear, respectively), when compared to the control group (Figure 6A). In addition, concentrations of 1, 3 and 5% GEESW inhibited MPO activity in 89.73, 78.15 and 80.43%, in this order. EESW (0.1, 0.5 and 1.0 mg/ear) was able to inhibit NAG activity in 48.84, 45.99 and 47.39%, respectively, relative to the negative control group. Treatment with 1, 3 and 5% GEESW produced an inhibitory response on NAG activity of 80.78, 72.69 and 88.79% at respective concentrations (Figure 6B). The data from Figure 6C show that, when compared to the negative control, EESW (36.44, 41.30 and 45.78%) and GEESW (38.93, 52.25 and 58.50%) decreased the NO levels after the treatment with 0.1, 0.5 and 1.0 mg/ear, respectively ($p < 0.001$). EA and dexamethasone were effective in inhibiting the inflammatory parameters of thickness, weight, MPO, NAG and NO.

To corroborate the results of thickness and weight ear and MPO and NAG activities, ear fragments were histopathologically analyzed and representative sections are shown in Figure 7 after treatment with EESB, GEESB, EESW, GEESW, EA, dexamethasone and

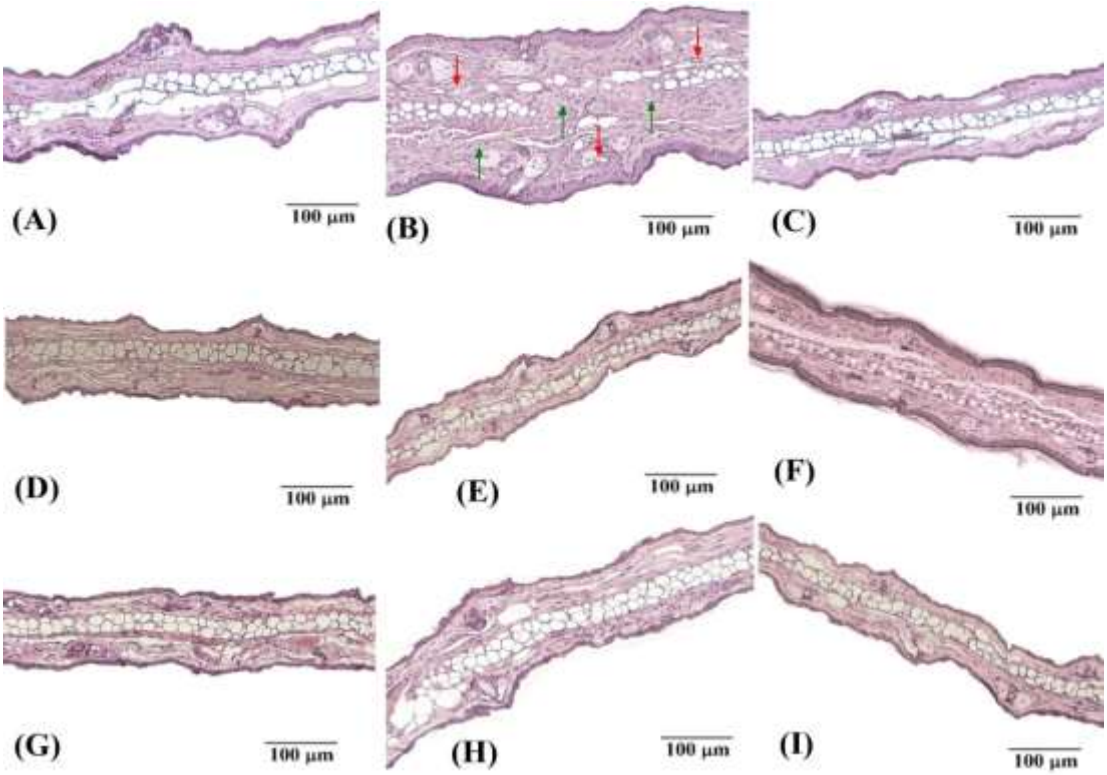

Figure 7. Representative histological sections of ear fragments with Croton oil-induced edema after treatment with extracts and gel formulations. Histological sections of mice ear stained with hematoxylin-eosin (10×, 100 µm scale).
(A) Basal (without inflammation); (B) Negative control (untreated); (C) Dexamethasone (positive control); (D) EESB 0.5 mg/ear; (E) EESW 0.5 mg/ear; (F) EA 1 mg/ear; (G) Vehicle; (H) GEESB 3%; (I) GEESW 3%. Green arrow up = Polymorphonuclear cells; Red arrow down = Vasodilation.

vehicle. The basal group (Figure 7A) and vehicle (Figure 7G) (without edema induction) showed no edema and no inflammatory process, whereas the negative control group (edema induction, untreated) showed increased ear thickness, polymorphonuclear and vasodilation (Figure 7B). Doses of 0.1, 0.5 and 1.0 mg/ear of EESB and EESW reduced ear thickness, polymorphonuclear, and vasodilation (representative sections by Figures 7D and E). At the concentrations 1, 3 and 5%, GEESB and GEESW were also active against edema formation, inhibiting the migration of polymorphonuclear cells and vasodilation (representative sections by Figure 7H and I). EA (Figure 7F) and dexamethasone (Figure 7C), positive control, were effective in reducing inflammatory parameters. The results indicate that extracts and gel formulations from *S. mombin* stem bark and wood presented a topical anti-inflammatory effect when evaluated by the ear edema model in mice after induction with Croton oil (Figures 3 and 5). Although there are no studies on the investigation of this activity using bark and wood, there is evidence that stem bark has anti-inflammatory property, being used in the treatment of hemorrhoids and as healing (Ayoka et al., 2008; Nworu et al., 2011). In addition, the anti-inflammatory potential of *S. mombin* leaves was confirmed using *in vivo* inflammation models, as well as *in vitro* protocols (Nworu et al., 2011; Cabral et al., 2016). The methanol extract of leaves (100, 200 and 400 mg/kg), for example, caused significant inhibition of carrageenin-induced paw edema in rats, reduced LPS-induced (systemic) levels of TNF-α in mice and decreased TNF-α induced by LPS and NO by BM-MØ by *in vitro* tests (Nworu et al., 2011). Cabral et al. (2016) showed that EA (2.5, 5 and 10 mg/kg) inhibited leukocyte migration to the site of inflammation, showing that it is one of the responsible for the anti-inflammatory action of *S. mombin* leaves, described in this study.

The ability of *S. mombin* extracts and gel formulations to reduce the effect of Croton oil shows an inhibitory potential on oxidative stress that prevents the generation of ROS and allows the suppression of inflammation of the skin and the proliferation of epidermal tissue (Calixto et al., 2004; Rahman et al., 2008). In addition to the inflammatory reaction with oxidative stress, the Croton oil application on the skin, 12-*O*-tetradecanoiforbol 13-acetate (TPA), as major component, promotes a vaso-dilation response, leukocyte infiltration and edema and

proliferation, and activation of nuclear oncogenes (Garg et al., 2008; Saraiva et al., 2010). TPA activates protein kinase C (PKC) which stimulates the pathway of mitogen-activated protein kinases (MAP kinase) and phospholipase A2 (PLA2) leading to increased gene expression of cyclooxygenase (COX) and translocation/ lipoxygenase (LOX) activation. Thus, the synthesis and release of proinflammatory mediators are associated with edema formation, cell hyperproliferation and leukocyte migration (Murakawa et al., 2006; Bernardis et al., 1994). As a consequence, activation of the MAP kinase pathway induces the expression of some nuclear transcription factors, such as NF-κB and AP-1, which are related to the regulation of the production of several proinflammatory proteins (IL-1, IL-8, TNF-α), proinflammatory enzymes (COX-2, iNOS and metalloprotease) and adhesion molecules (Pascual and Glass, 2006; Garcia-Piñeres et al., 2001). Furthermore, PKC phosphorylates PLA2, which removes arachidonic acid (AA) from membrane phospholipids to produce prostaglandins and leukotrienes by COX and LOX enzymes, respectively (Young et al., 1984). It is possible that EA and other components of EESB and EESW are capable of inhibiting one of these inflammatory process pathways as already described by Nworu et al. (2011) and Cabral et al. (2016).

The results in the Croton oil-induced ear edema model demonstrated that the extracts and gel formulations were able to inhibit edema after 6 and 24 h of treatment (Figures 3 and 5), promoting a similar response to dexamethasone. This anti-edematogenic action is related to the reduction of vascular permeability, since histopathological analysis (Figure 7) revealed a reduction in vasodilation and leukocyte migration (Christy et al., 2013; Geering et al., 2013). In addition, the reduction of MPO and NAG activities (Figures 4 and 6) also corroborated these results because MPO is often associated with the presence of polymorphonuclear cells (Bradley et al., 1982), whereas NAG is indicative of mononuclear occurrence in the site of inflammation, mainly in chronic inflammatory processes (Sánchez and Moreno, 1999).

The decrease in vasodilation by extracts, gel formulations and EA was also confirmed by NO assay (Figures 4 and 6), which is in accordance with the results of Nworu et al. (2011) and Cabral et al. (2016). NO, generated by the action of the enzyme induced nitric oxide synthase, contributes to the endothelial permeability, which results in cell infiltration and plasma and proteins leakage, favoring the edema formation and inflammatory process. In addition, leukocyte exposure to NO promotes increased interleukin-8 (IL-8) and tumor necrosis factor alpha (TNF-α) production, reinforcing the chemotactic gradient in damaged tissue (Corriveau et al., 1998; Muhl et al., 2000; Ma et al., 2004). It is likely that EA and extract constituents are capable of inhibiting NO pathway directly or indirectly through the production of prostanoids with reduced vasodilation and edema.

Conclusion

Ethanol extracts and gel formulations of *S. mombin* L. stem (bark and wood) and EA have topical anti-inflammatory activity by reducing the thickness and weight of Croton oil-induced ear edema, as well as inhibiting MPO and NAG activities, NO levels, vasodilation and leukocyte migration. EESB and EESW gel formulations can constitute phytotherapeutic options and represent an alternative for the treatment of inflammatory skin disorders.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests

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REFERENCES

Adedokun MO, Oladoye AO, Oluwalana SA, Mendie II (2010). Socio-economic importance and utilization of *Spondias mombin* in Nigeria. Asian Pacific Journal of Tropical Medicine 3(3):232-234.

Ahmed EM (2015). Hydrogel: Preparation, characterization, and applications: A review. Journal of Advanced Research 6(2):105-121.

Alobi NO, Bassey GA, Kalu RE, Enyi-Idoh KH (2017). Comparison of the phytochemical and nutritional properties of leaf, stem and root of *Spondias mombin* L. International Journal of Current Microbiology and Applied Sciences 6(6):804-810.

Amaechi OL (2015). Evaluation of uses and marketing potential of *Spondias mombin* Linn. (hog plum) in Ibadan metropolis. International Journal of Agriculture, Forestry and Fisheries 3(1):1-6.

Ayoka AO, Akomolafe RO, Iwalewa EO, Akanmu MA, Ukponmwan OE (2006). Sedative, antiepileptic and antipsychotic effects of *Spondias mombin* L. (Anacardiaceae) in mice and rats. Journal of Ethnopharmacology 103(2):166-175.

Ayoka AO, Akomolafe RO, Akinsomisoye OS, Ukponmwan OE (2008). Medicinal and economic value of *Spondias mombin*. African Journal of Biomedical Research 11:129-136.

Bansal V, Sharma A, Ghanshyam C, Singla ML (2014). Coupling of chromatographic analyses with pretreatment for the determination of bioactive compounds in *Emblica officinalis* juice. Analytical Methods 6(2):410-418.

Bernardis L, Leonardi G, Caruso A, Cutuli VM, Arnico-Roxas M (1994). Protective effects of papavarine salicylate in mouse ear dermatitis and PAF-induced rat paw oedema. Agents Actions 42(1-2):29-33.

Bradley PP, Priebat DA, Christensen RD, Rothstein G (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Journal of Investigative Dermatology

78(3):206-209.
Brenner EJ, Long MD (2019). Diagnosis and treatment of dermatologic diseases in inflammatory bowel disease. Current Opinion in Gastroenterology 35(4):330-336.
Brito SA, Almeida CLF, Santana TI, Oliveira ARS, Figueiredo JCBN, Souza IT, Almeida LL, Silva MV, Borges AS, Medeiros JW, Silva Neto JC, Gonçalves RCR, Kitagawa RR, Sant'Ana AEG, Rolim LA, Menezes IRA, Silva TG, Caldas GFR, Wanderley AG (2018). Antiulcer activity and potential mechanism of action of the leaves of *Spondias mombin* L. Oxidative Medicine and Cellular Longevity 2018:1-20.
Cabral B, Siqueira EMS, Bitencourt MAO, Lima MCJS, Lima AK, Ortmann CF, Chaves VC, Fernandes-Pedrosa MF, Rocha HAO, Scortecci KC, Reginatto FH, Giordani RB, Zucolotto SM (2016). Phytochemical study and anti-inflammatory and antioxidant potential of *Spondias mombin* leaves. Brazilian Journal of Pharmacognosy 26(3):304-311.
Calixto JB, Campos MM, Otuki MF, Santos ARS (2004). Anti-inflammatory compounds of plant origin: II. Modulation of pro-inflammatory cytokines, chemokines and adhesion molecules. Planta Medica 70(2):93-103.
Chibli LA, Rodrigues KCM, Gasparetto CM, Pinto NCC, Fabri RL, Scio E, Alves MS, Del-Vechio-Vieira G, Sousa OV (2014). Anti-inflammatory effects of *Bryophyllum pinnatum* (Lam.) Oken ethanol extract in acute and chronic cutaneous inflammation. Journal of Ethnopharmacology 154(2):330-338.
Christy AL, Walker ME, Hessner MJ, Brown MA (2013). Mast cell activation and neutrophil recruitment promotes early and robust inflammation in the meninges in EAE. Journal of Autoimmunity 42:50-61
Corriveau CC, Madara PJ, Van Dervort AL, Tropea MM, Wesley RA, Danner RL (1998). Effects of nitric oxide on chemotaxis and endotoxin-induced interleukin-8 production in human neutrophilis. The Journal of Infectious Diseases 177(1):116-126.
Corthout J, Pieters L, Claeys M, Geerts S, Berghe DV, Vlietinck AJ (1991). Antiviral ellagitannins from *Spondias mombin*. Phytochemistry 30(4):1129-1130.
Corthout J, Pieters LA, Claeys M, Berghe DV, Viletinck AJ (1992). Antiviral caffeoyl: esters from *Spondias mombin*. Phytochemistry 31(6):1979-1981.
Duvall CS (2006). On the origin of the tree *Spondias mombin* in Africa. Journal of Historical Geography 32(2):249-266.
Eyerich S, Eyerich K, Traidl-Hoffmann C, Biedermann T (2018). Cutaneous barriers and skin immunity: Differentiating a connected network. Trends in Immunology 39(4):315-327.
Fred-Jaiyesimi A, Kio A, Richard W (2009). α-Amylase inhibitory effect of 3β-urs-12-en3-il (9z)-hexadec-9-enoato isolated from *Spondias mombin* leaf. Food Chemistry 116(1):285-288.
Forrester SJ, Kikuchi DS, Hernandes MS, Xu Q, Griendling KK (2018). Reactive oxygen species in metabolic and inflammatory signaling. Circulation Research 122(6):877-902.
Garcia-Piñeres AJ, Castro V, Moraes G, Schmidt TJ, Strunck E, Pahl HL, Merfort I (2001). Cysteine 38 in p65/NF-KB plays a crucial role in DNA binding inhibition by sesquiterpene lactones. Journal of Biological Chemistry 276(43):39713-39720.
Garg R, Ramchandani AG, Maru GB (2008). Curcumin decreases 12-*O*-tetradecanoylphorbol-13-acetate induced protein kinase C translocation to modulate downstream targets in mouse skin. Carcinogenesis 29(6):1249-1257.
Geering B, Stoeckle C, Conus S, Simon H (2013). Living and dying for inflammation: neutrophils, eosinophils, basophils. Trends in Immunology 34(8):398-409.
Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR (1982). Analysis of nitrate, nitrite, and [15n]nitrate in biological fluids. Analytical Biochemistry 126(1):131-138.
Khodadadi S, Nasri H (2017). An update on ellagic acid as a natural powerful flavonoid. Annals of Research in Antioxidants 2(2):1-5.
Landete JM (2011). Ellagitannins, ellagic acid and their derived metabolites: a review about source, metabolism, functions and health. Food Research International 44(5):1150-1160.
Lima EQ, Oliveira E, Brito HR (2016). Extraction and characterization of the essential oils from *Spondias mombin* L. (Cajá), *Spondias purpurea* L. (Ciriguela) and *Spondia* ssp (Cajarana do sertão). African Journal of Agricultural Research 11(2):105-116.
Lordani TVA, Lara CE, Ferreira FBP, Monich MST, Silva CM, Lordani CRF, Bueno FG, Teixeira JJV, Lonardoni MVC (2018). Therapeutic effects of medicinal plants on cutaneous wound healing in humans: A systematic review. Mediators Inflammation 2018:1-12.
Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. Journal of Biological Chemistry 193(1):265-275.
Ma P, Cui X, Wang S, Zhang J, Nishanian EV, Wang W, Wesley RA, Danner RL (2004). Nitric oxide post-transcriptionally up-regulates LPS-induced IL-8 expression through p38 MAPK activation. Journal of Leukocyte Biology 76(1):278-287.
Muhl H, Chang JH, Huwiler A, Bosmann M, Paulukat J, Ninic R, Nold M, Hellmuth M, Pfeilschifter J (2000). Nitric oxide augments release of chemokines from monocytic U937 cells: modulation by anti-inflammatory pathways. Free Radical Biology and Medicine 29(10):969-980.
Murakawa M, Yamaoka K, Tanaka Y, Fukuda Y (2006). Involvement of tumor necrosis factor (TNF)-α in phorbol ester 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced skin edema in mice. Biochemical Pharmacology 71(9):1331-1336.
Nworu CS, Akah PA, Okoye FB, Toukam DK, Udeh J, Esimone CO (2011). The leaf extract of *Spondias mombin* L. displays an anti-inflammatory effect and suppresses inducible formation of tumor necrosis factor-α and nitric oxide (NO). Journal of Immunotoxicology 8(1):10-16.
Ojo AO, Afon AA, Ojo AB, Ajiboye BO, Oyinloye BE, Kappo AP (2018). Inhibitory effects of solvent-partitioned fractions of two Nigerian herbs (*Spondias mombin* Linn. and *Mangifera indica* L.) on α-amylase and α-glucosidase. Antioxidants (Basel) 7(6):1-11.
Oladunmoye MK (2007). Comparative evaluation of the effects of leaf extract from *Spondias mombin* on rats with induced infections from *Bacillus cereus* and *Clostridium sporogenes*. Research Journal of Phytochemistry 1(2):68-73.
Olugbuyiro JÁ, Moody JO, Hamann MT (2013). Phytosterols from *Spondias mombin* Linn with antimycobacterial activities. African Journal of Biomedical Research 16(1):19-24.
Olugbuyiro JAO, Moody JO, Hamann MT (2009). AntiMtb activity of triterpenoid-rich fractions from *Spondias mombin* L. African Journal of Biotechnology 8(9):1807-1809.
Pascual G, Glass CK (2006). Nuclear receptors versus inflammation: mechanisms of transrepression. Trends in Endocrinology and Metabolism 17(8):321-328.
Pasparakis M, Haase I, Nestle FO (2014). Mechanisms regulating skin immunity and inflammation. Nature Reviewa Immunology 14(5):289-301.
Pereira C, Oliveira LL, Gonçalves R, Amaral ACF, Kuster RM, Sakuragui CM (2015). Phytochemical and phylogenetic analysis of *Spondias* (Anacardiaceae). Química Nova 38(6):813-816.
Rahman S, Bhatia K, Khan AK, Kaur M, Ahmad F, Rashid H, Athar M, Islam F, Raisuddin S (2008). Topically applied vitamin E prevents massive cutaneous inflammatory and oxidative stress responses induced by double application of 12-O-tetradecanoylphorbol-13-acetate (TPA) in mice. Chemico-Biological Interactions 172(3):195-205.
Sabiu S, Garuba T, Sunmonu T, Ajani E, Sulyman A, Nurain I, Balogun A (2015). Indomethacin-induced gastric ulceration in rats: Protective roles of *Spondias mombin* and *Ficus exasperata*. Toxicology Reports 2:261-267.
Sampaio TIS, Melo NC, Paiva BTF, Aleluia GAS, Silva Neto FLP, Silva HR, Keita H, Cruz RAS, Sánchez-Ortiz BL, Pineda-Peña EA, Balderas JL, Navarrete A, Carvalho JCT (2018). Leaves of *Spondias mombin* L. a traditional anxiolytic and antidepressant: Pharmacological evaluation on zebrafish (Daniorerio). Journal of Ethnopharmacology 224:563-578.
Sánchez T, Moreno JJ (1999). Role of leukocyte influx in tissue prostaglandin H synthase-2 overexpression induced by phorbol ester and arachidonic acid in skin. Biochemical Pharmacology 58(5):877-879.
Saraiva RA, Araruna MKA, Oliveira RC, Menezes KDP, Leite GO, Kerntopf MF, Costa JGM, Rocha JBT, Tome AR, Campos AR,

Menezes IRA (2010). Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. Journal of Ethnopharmacology 136(3):504-510.
Sargent MG (1987). Fiftyfold amplification of the Lowry protein assay. Analytical Biochemistry 163(2):476-481.
Schiantarelli P, Cadel S, Acerbi D, Pavesi L (1982). Antiinflammatory activity and bioavailability of percutaneous piroxicam. Arzneimittelforschung/Drug Research 32(3):230-235.
Silva BM, Rossi AAB, Tiago AV, Schmitt KFM, Dardengo JFE, Souza SAM (2017). Genetic diversity of Cajazeira (*Spondias mombin* L.) in three geographic regions. Genetic and Molecular Research 16(1):1-11.
Silva ARA, Morais SM, Marques MMM, Lima DM, Santos SCC, Almeida RR, Vieira IGP, Guedes MIF (2011). Antiviral activities of extracts and phenolic components of two *Spondias* species against dengue virus. Journal of Venomous Animals and Toxins Including Tropical Diseases 17(4):406-413.
Silva ARA, Morais SMM, Marques MMM, Oliveira DF, Barros CC, Almeida RR, Vieira IGP, Guedes MIF (2012). Chemical composition, antioxidant and antibacterial activities of two *Spondias* species from Notheastern Brazil. Pharmaceutical Biology 50(6):740-746.
Syed YH, Khan M (2016). Chromatographic profiling of ellagic acid in *Woodfordia fruticosa* flowers and their gastroprotective potential in ethanol-induced ulcers in rats. Pharmacognosy Research 8(Suppl 1):5-11.
Villegas LF, Fernandez ID, Maldonado H, Torres R, Zavaleta A, Vaisberg AJ, Hammond GB (1997). Evaluation of the wound-healing activity of selected traditional medicinal plants from Peru. Journal of Ethnopharmacology 55(3):193-200.
Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J (2018). A comprehensive review of non-steroidal anti-inflammatory drug use in the elderly. Aging and Disease 9(1):143-150.
Young LM, Kheifets JB, Ballaron SJ, Young JM (1989). Edema and cell infiltration in the phorbol ester-treated mouse ear are temporally separate and can be differentially modulated by pharmacologic agents. Agents Actions 26(3-4):335-341.
Young JM, Spires DA, Bedord CJ, Wagner B, Ballron SJ, Young LM (1984). The mouse ear inflammatory response to topical arachidonic acid. Journal of Investigative Dermatology 82(4):367-371.