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HPLC profile and antiedematogenic activity of *Ximenia americana* L. (Olacaceae) in mice models of skin inflammation

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ABSTRACT

The aim of this study was to evaluate the anti-edematogenic activity of X. americana L. (HEXA) hydroethanolic extract in ear edema models (acute and chronic) induced by croton oil and by different phlogistic agents (arachidonic acid, capsaicin, phenol and histamine), identifying the possible anti-edematogenic mechanism. HEXA demonstrated a significant anti-edematogenic effect at concentrations of 100–500 μg/ear in ear edema induced by croton oil with higher inhibition of edema of 39.37. However, the concentrations of 100 and 200 μg/ear were taken as a standard, demonstrating the effect in the chronic model induced by croton oil with inhibition of 61.62% and 48.74%. In the AA-induced ear edema model, HEXA showed inhibition of: 24.45% and 32.31%; capsaicin inhibition of 72.72% and 47.57%; phenol inhibition of 34% and 20.1%; and histamine inhibition of 31.8% and 21.62%. Then, the results were showed that HEXA demonstrated an anti-edematogenic effect in acute and chronic inflammation models, demonstrating a probable mechanism of action by the inhibition or modulation of key mediators of the inflammatory process. The chemical profile and presence of flavonoids guaranteeing a profile of activity similar to natural drugs that act or modulate the production of mediators of inflammations.

1. Introduction

The skin is an important barrier between the body and the external environment, playing a key role in protection and regulation of the body homeostasis. However, this protective barrier is constantly exposed to different noxious stimuli, such as: pathogens, ultraviolet rays, oxidative stress (and reactive oxygen species (ROS), and various mechanical, physical or chemical stimuli; and therefore, it works as an interface for triggering local inflammatory responses (Elias, 2007).

Exposure to these noxious stimuli, as well as mounting inadequate or misdirected immune responses that lead to the production of specific cytokines, may be critical for the pathogenesis of skin inflammatory conditions, such as psoriasis and dermatitis (Kupper and Fuhlbrigge, 2004; Song et al., 2008; Emre et al., 2012). These events result in the production and release of several inflammatory mediators, including the vasoactive amines (histamine and serotonin), metabolites of the arachidonic acid (prostaglandins, thromboxanes, leukotrienes and platelet activating factor (PAF)), bradykinin, nitric oxide, neuropeptides and cytokines (Coutinho et al., 2009).

Corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) and histamine receptor antagonists are widely used to treat inflammatory conditions because these drugs modulate essential steps in the cascade of production or action of inflammatory mediators. Nevertheless, there are some conditions under which these drugs are not effective, and in addition, they cause significant side effects, justifying the search for new drugs to treat inflammatory and allergic

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diseases (Davies et al., 2006 . Schoepe et al., 2006).

In this context, the floristic richness of the Brazilian territory, in the case of plants popularly used for the treatment of inflammatory processes, contribute to the elucidation of new bioactive molecules and to the production of new anti-inflammatory drugs (Coutinho et al., 2009) in order to reduce the undesirable effects of current drugs, in the search for more effective and safe substances. Others species of plants present significative effects in preclinical and clinical studies such, as *Matricaria recutita*, *Hamamelis virginiana, Arnica montana*, and *Calendula officinalis* (Bedi and Shenefelt, 2002), *Vanillosmopsis arborea* (Leite et al., 2011), *Caryocar coriaceum* (Saraiva et al., 2011) and *Lippia sidoides* (Veras et al., 2013), *Astronium fraxinifolium* (Brito et al., 2018) were evidenced.

The species *Ximenia americana* L. (Oleaceae), popularly known as "ameixa brava", is a tropical plant species broadly found in northeastern Brazil, but also found in Africa, India, New Zealand, Central America and South America, where it is used in folk medicine to treat stomach pain, syphilis, rheumatism, cancer and mouth infections (Brasileiro et al., 2008). In Brazil, their shells are popularly used to treat leprosy, malaria, skin infections, hemorrhoids, inflammation, headache, dermatitis, and also as antipyretic and cicatrizing (Souza et al., 2014).

Previous phytochemical studies with this species demonstrated the presence of saponins, flavonoids, glycosides, tannins, phenolic compounds, alkaloids, quinones and terpenoids, whose biological activities have been elucidated and include: antimicrobial, antifungal, anticancer, anti-trypanosome, anti-rheumatic, antioxidant, analgesic, molluscicide, pesticide, and modulator of hepatic and blood disorders. However, the anti-inflammatory topic effect of *Ximenia americana* L. remain to be elucidated. Thus, considering the ethnopharmacological data regarding the use of *X. americana* to treat inflammatory conditions, this study aims to characterize the topic antiedematogenic effect of the hydroethanolic extract of *X. americana* (HEXA) using mice models of skin inflammation.

2. Materials and methods

2.1. Botanical material

The shells of *Ximenia americana* L. were collected in a Caatinga area, the "Sítio lambedor" located in the municipality of Farias Brito, Ceara State, Brazil, in June 2014, following the geographical coordinates: SAD 69 - Latitude 06° 57 ′2630' 'and Longitude 39° 32 ′1740' '- the southern hemisphere. A sample containing leaves, flowers and fruits was used for identification, and then it was deposited in the Dárdano Andrade-Lima caririense herbarium (HCDAL) under number 10976.

2.2. Chemicals

Analytical grade chemicals were used in all experiments. Acetone and ethanol were acquired from Dynamics (Brazil). Methanol, acetonitrile, phosphoric acid, gallic, caffeic acid, ellagic acid and chlorogenic acid were purchased from Merck (Darmstadt, Germany); the croton oil, the arachidonic acid, phenol, histamine, indomethacin, quercetin, rutin, catechin, quercitrin, gallic acid, chlorogenic acid and L-ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA); dexamethasone (Decadron) was purchased from Aché (Brazil) and Ketamine hydrochloride and Xylazine chloride were purchased from Syntec (Brazil).

2.3. Animals

Female Swiss mice (*Mus musculus*), weighting 20–30 g were obtained from the Animal Containment Unit of the Regional University of Cariri (URCA). The animals were maintained with food and water *ad libitum* (Labina, Purina, Brazil) in a room with a temperature ranging from 22 to 24 °C and a 12 h light/dark cycle. Before starting the experiments the animals were kept in the laboratory of Molecular Pharmacology and Chemistry of the Regional University of Cariri - URCA by a period of 24 h for adaptation. This study was carried out in accordance with the recommendations of the Brazilian National Council for the Control of Animal Experimentation (CONCEA). The protocols were approved by the Experimentation and Animal Use Committee of the Regional University of Cariri (CEUA Nº 82/2015, URCA).

2.4. Extract preparation

The plant bark was dried under the sunlight for two consecutive days from 10:00 a.m. to 3:00 p.m. Then, they were weighted, separated, triturated and transferred to a transparent glass container, where they were soaked in a mixture of distilled water and absolute ethanol (1:1) for a period of 72 h. The hydroethanolic extract obtained from *Ximenia americana* shells (HEXA) was filtered to retaining the solids and then transferred to a rotary evaporator (27–35 rpm; 45 °C) to remove ethanol. After removing the solvent, the extract was kept under water bath for ethanol evaporation and 24 h later it was frozen to finally be lyophilized. The identification and quantification of the chemical compounds present in the HEXA was performed by HPLC-DAD.

2.5. Ear edema induced by single application of the croton oil

The ear edema induced by single application of croton oil was performed as described by Tubaro et al. (1986). Briefly, the mice (n = 6) had their right ears pretreated topically with distilled water (negative control), dexamethasone (4 mg/mL -positive control) or HEXA at 100, 200 or 500 mg/ear, in a volume of 20 μL (10 μL in each side of the ear). One hour later 20 μL of the croton oil (5% v/v in acetone) was administered in the right ear and 20 μL of the vehicle (acetone) was administered in the left ear. After 4 h, the animals were euthanized, the ears were removed, cut into 6 mm diameter disks and weighed on an analytical balance.

2.6. Ear edema induced multiple applications of the croton oil

Mice (n = 6) were topically administered with 20 μL of croton oil (5% v/v in acetone) in the right ear and 20 μL of the vehicle (acetone) in the left ear daily, for 9 days. From day 5–9 of the experimental protocol, the animals were topically treated with 20 μL of distilled water, dexamethasone (4 mg/mL) or HEXA (100, 200 or 500 mg/ear). The animals had both ears measured daily using a digital caliper. One hour after the last administration of the croton oil, the animals were euthanized, the ears were removed, cut into 6 mm diameter disks and weighed on an analytical balance (Stanley et al., 1991).

2.7. Ear edema induced by arachidonic acid, capsaicin or phenol

To perform these protocols, the animals (n = 6) had their ears injected with different inflammatory agents: arachidonic acid (AA) 0.1 mg/μL diluted in acetone (Young et al., 1984), capsaicin 0.01 mg/μL diluted in 90% ethanol (Gábor and Rázga, 1992) and phenol 10% (v/v) diluted in acetone. One hour before the administration of each agent, the animals were topically treated in the right ears with distilled water, dexamethasone (4 mg/mL), indomethacin (25 mg/mL) or HEXA at 100 or 200 mg/ear, in a volume of 20 μL (10 μL on each side of the ear). One hour after AA or phenol administration and 30 min after capsaicin injection the animals were euthanized, the ears were removed, cut into 6 mm diameter disks and weighed on an analytical balance.

2.8. Ear edema induced by histamine

Groups of 6 mice anesthetized with ketamine hydrochloride (20 mg/Kg, i.p.), and xylazine hydrochloride (10 mg/Kg, i.p.) were