Journal of Ethnopharmacology 192 (2016) 178–182

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Ethnopharmacological communication

Evidence of anti-inflammatory and antinociceptive activities of *Plinia edulis* leaf infusion

Lara F. Azevedo [a], Simone Maria da Silva [b], Lucas B. Navarro [c], Lydia F. Yamaguchi [c], Carlos Giovani O. Nascimento [a], Roseli Soncini [a], Tati Ishikawa [b,*]

[a] Department of Physiological Sciences, Institute of Biomedical Sciences, Federal University of Alfenas, 37130-000 Alfenas, MG, Brazil
[b] Department of Food and Drugs, Faculty of Pharmaceutical Sciences, Federal University of Alfenas, 37130-000 Alfenas, MG, Brazil
[c] Department of Fundamental Chemistry, Institute of Chemistry, University of São Paulo, 05599-970 São Paulo, SP, Brazil

ARTICLE INFO

Article history:
Received 17 March 2016
Received in revised form
14 June 2016
Accepted 1 July 2016
Available online 1 July 2016

Keywords:
Plinia edulis
Myrtaceae
Anti-inflammatory
Antinociceptive
Flavonoids
Triterpenoids

ABSTRACT

Ethnopharmacological relevance: Plinia edulis (Vell.) Sobral (Myrtaceae) is native and endemic to the Brazilian Atlantic Rainforest. Popularly known as "cambucá", it has been used in folk medicine for the treatment of stomach disorders, diabetes, bronchitis, inflammation and as tonic. Although there are numerous records concerning its popular use as analgesic and anti-inflammatory, scientific information regarding these pharmacological activities is limited. Therefore, the aim of this study was to characterize the anti-inflammatory and antinociceptive activity of *P. edulis* leaf infusion (AEPe) in mice.

Materials and methods: The acetic acid-induced writhing response and mechanical nociceptive paw tests were used to evaluate the antinociceptive activity. Carrageenan-induced paw edema and lipopolysaccharide-induced peritonitis were used to investigate the anti-inflammatory activity. The substances in AEPe were identified by HPLC-MS analysis.

Results: At the test doses 30–300 mg/kg *p.o.*, AEPe has clearly exhibited anti-inflammatory effects, reducing carrageenan-induced paw edema and inhibiting leukocyte recruitment into the peritoneal cavity. The infusion has shown significant antinociceptive activity in both models of nociception. Gallic acid, myricitrin, guaijaverin, quercitrin, quercetin, corosolic acid, maslinic acid, oleanolic acid and ursolic acid were identified in AEPe.

Conclusion: P. edulis infusion presented antinociceptive and anti-inflammatory activities in all experiments realized in this study, which could be related to the presence of triterpenoids and flavonoids. These results provide scientific support for the traditional use of this species in the management of pain and inflammation.

1. Introduction

Myrtaceae comprises about 130 genera and 4600 species, many of them which have been proven to have anti-inflammatory and antinociceptive properties, such as *Myrcia pubiflora* (Andrade et al., 2012), *Plinia glomerata* (Fischer et al., 2008) and *Campomanesia xanthocarpa* (Viecili et al., 2014).

Plinia edulis (Vell.) Sobral, popularly known as "cambucá", is considered beneficial to health and used in Brazilian folk medicine to treat inflammatory conditions, diarrhoea, bronchitis, diabetes and as tonic, antipyretic and diuretic (Carvalho et al., 2012; Donato and Morretes, 2013). It is employed in the treatment of gastric disorders and previous experimental studies have shown that the ethanol leaf extract has an important anti-ulcer activity correlated with the presence of flavonoids and triterpenoids, without showing acute toxicity *in vivo* (Ishikawa et al., 2014, 2008).

Considering the use of the species in Indigenous "Caiçara" folk medicine, the aim of the present study was to evaluate the anti-inflammatory and antinociceptive effects of the *P. edulis* leaf infusion and to identify its phytochemical compounds with pharmacological properties.

2. Materials and methods

2.1. Plant material

Leaves of *P. edulis* were collected during the summer in Trindade, Rio de Janeiro State, Brazil. Dr. Lúcia Rossi (Botanic Institute of São Paulo, São Paulo State, Brazil) identified the plants; the

* Correspondence to: Departamento de Alimentos e Medicamentos, Faculdade de Ciências Farmacêuticas, Universidade Federal de Alfenas, R. Gabriel Monteiro da Silva, 700, Centro, CEP 37130-000 Alfenas, MG, Brazil.
E-mail address: tati.unifal@gmail.com (T. Ishikawa).

http://dx.doi.org/10.1016/j.jep.2016.07.001
0378-8741/

voucher specimen has been deposited in the Herbarium of the same Institute (SP 356.472).

2.2. Leaf infusion (AEPe)

The air-dried powdered leaves (400 g) were extracted with deionized water (4 L) by infusion, at 90 °C for 30 min (Carvalho et al., 2012). The infusion was freeze dried to afford 77.7 g of AEPe.

2.3. Phytochemical analysis

AEPe was analysed by Prominence HPLC system (Shimadzu) coupled to a MicroTOFQ-II mass spectrometer (Bruker). A reverse phase column Luna 3 μm PFP2 100A, 150 × 2 mm (Phenomenex) was used and chromatography was performed with a flux of 0.2 mL/min using acetonitrile: H2O (+0.1% formic acid) as mobile phase in a gradient of 0–2 min 40% of acetonitrile, from 2 to 22 min 40–100% of acetonitrile and kept at a plateau for 6 min. The column oven was set to 40 °C; UV detector was recorded at 254 nm. The mass spectrophotometer was operating in electrospray negative mode, with N_2 as nebulizer gas and dried at 4 bar and 8 L/min. Capillar voltage was set to 4500 V and drying temperature in 200 °C. Collision cell and quadrupole energy were set to 12 eV and 6 eV, respectively.

2.4. Test samples

AEPe was resuspended in 1% sodium carboxymethylcellulose (CMC) suspension in distilled water to obtain doses of 30, 100 and 300 mg/kg, which were based on previous assays (Ishikawa et al., 2014, 2008; Orlandi et al., 2011). The animals in the control group were treated with appropriate volumes of vehicle. Indomethacin (10 mg/kg, *p.o.*) was resuspended in sterile saline (0.9% NaCl) and used as reference drug. Test drugs, vehicle and the reference drugs were orally administered in an equivalent volume of 10 mL/kg/body weight of each animal.

2.5. Animals

Adult male Swiss mice (25–30 g) were obtained from Central Animal Facility of the Federal University of Alfenas. The animals were kept in a 12:12 h light: dark cycle; lights on at 6:00 a.m., temperature 23 ± 1 °C, with access to water and food *ad libitum*. The mice were acclimatized for at least one week before the experiments were started and fasted for 12 h prior to the experiments. The experimental protocol followed the principles and guidelines suggested by the Brazilian Society of Laboratory Animal Science (SBCAL) and were approved by the local ethical committee (352/2011).

2.6. Anti-inflammatory assays

2.6.1. Carrageenan-induced paw edema

The mice (n=8) were orally treated with test samples 1 h before intraplantar injection of 1% carrageenan (50 μL) as previously described (Passos et al., 2007). The right hind paw volume was measured using a plethysmometer 7140 (Ugo Basile). The basal paw volume was determined before administration of vehicle, AEPe or indomethacin and at 1–4 h after the carrageenan injection. Paw edema was calculated as the difference between the paw volume (mL) after inflammatory injury (V_T) and the basal paw volume (V_0). The percentages of inhibition were calculated according to the formula (Bhandare et al., 2010):

$$\%\text{Inhibition} = \left[(V_T - V_0)_{\text{control}} - (V_T - V_0)_{\text{treated group}} \right] \times 100 / (V_T - V_0)_{\text{control}}$$

2.6.2. Peritonitis induced by lipopolysaccharide

Mice (n=8) were treated with test samples 30 min before the *i.p.* injection of lipopolysaccharide (LPS) from *Escherichia coli* serotype 026: B6 (500 μg/kg) according to the protocol described by Vilela et al. (2010) with modifications. At 4 h after LPS administration, the animals were sacrificed by halothane overdose (6%, flow rate 1 L/min). Cells from the peritoneal cavity were harvested by injection of 5 mL of PBS containing 0.5% of sodium citrate. The suspensions of blood cells were aspirated and the total numbers of cells were counted using Humacount equipment (Human).

2.7. Antinociceptive assays

2.7.1. Acetic acid-induced writhing response

According to the protocol of Araújo et al. (2014), mice (n=8) were orally treated with test samples 30 min prior to *i.p.* injection of acetic acid (0.6%, v/v in saline, 0.1 mL/10 g). The writhing response was measured by counting the number of writhes during the 20 min following the *i.p.* injection of the acid. The results were expressed as the total writhes.

2.7.2. Mechanical nociceptive paw test

Hind paw flexion was induced with a handheld force transducer (Electronic Anesthesiometer Insight Model EFF-302) equipped with a 0.5 mm^2 polypropylene tip (Orlandi et al., 2011). The animals (n=8) were orally treated with test samples at 1 h before the intraplantar injection of carrageenan (100 μg/paw). The results are expressed as the delta (Δ) withdrawal threshold (in g), which is calculated by subtracting the zero-time mean measurements from the mean measurements obtained at 30–180 min after carrageenan injection.

2.8. Statistical analysis

The data were analysed using GraphPad software program version 4.0 and expressed as the mean ± S.E.M. Statistical significance was determined by One way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test. *P*-values below 0.05 ($p < 0.05$) were considered significant.

3. Results

3.1. Phytochemical analysis

Through LC-MS analysis of AEPe and co-elution with authentic samples previously isolated (Ishikawa et al., 2014) were identified gallic acid, myricitrin, guaijaverin, quercitrin, quercetin, corosolic acid, maslinic acid, oleanolic acid and ursolic acid.

3.2. Anti-inflammatory assays

Fig. 1A shows that AEPe (30–300 mg/kg) produced a significant reduction of carrageenan-induced mice paw edema ($p < 0.001$) with peak effect (96.8% reduction) produced at the dose of 100 mg/kg at 4 h after the carrageenan injection. The anti-edematous effect of AEPe is significant, once the reference drug indomethacin (10 mg/kg) exhibited 100% of inhibition ($p < 0.001$) at the same time.

On the peritonitis induced by LPS, oral treatment with 30 mg/kg of AEPe significantly inhibited leukocyte recruitment by 67.7% ($p < 0.001$), a percentage very similar to that obtained with indomethacin (67.4% inhibition; $p < 0.001$). In addition, the treatment with 300 mg/kg inhibited leukocyte recruitment to the peritoneal cavity by 45.1% ($p < 0.05$). The leukocyte recruitment at