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Research Paper

Terminalia catappa L.: A medicinal plant from the *Caribbean pharmacopeia* with anti-*Helicobacter pylori* and antiulcer action in experimental rodent models

Laísa Pinheiro Silva [a], Célio Damacena de Angelis [a], Flavia Bonamin [a], Hélio Kushima [a], Francisco José Mininel [b], Lourdes Campaner dos Santos [b], Flavia Karina Delella [c], Sergio Luis Felisbino [c], Wagner Vilegas [d], Lucia Regina Machado da Rocha [a], Matheus Aparecido dos Santos Ramos [f], Tais Maria Bauab [f], Walber Toma [e,*,1], Clelia Akiko Hiruma-Lima [a,*,1]

[a] Univ. Estadual Paulista-UNESP – Departamento de Fisiologia, Instituto de Biociências, CEP 18618-970 Botucatu, SP, Brazil
[b] Univ. Estadual Paulista-UNESP – Departamento de Química Orgânica, Instituto de Química, CEP 14800-900, Araraquara, SP, Brazil
[c] Univ. Estadual Paulista-UNESP – Departamento de Morfologia, Instituto de Biociências, CEP 18618-970, Botucatu, SP, Brazil
[d] Univ. Estadual Paulista-UNESP – Campus Experimental do Litoral Paulista, CEP 11330-900 São Vicente, SP, Brazil
[e] Universidade Santa Cecília – Pós-Graduação em Sustentabilidade de Ecossistemas Costeiros e Marinhos, Rua Oswaldo Cruz, 266, Boqueirão, CEP 11045907 Santos, SP, Brazil
[f] Univ. Estadual Paulista-UNESP – Departamento de Ciências Biológicas, Faculdade de Ciências Farmacêuticas, CEP 14801-902, Araraquara, SP, Brazil

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ABSTRACT

Ethnopharmacological relevance: *Terminalia catappa* L. (Combretaceae) is a medicinal plant listed as a pharmacopeia vegetable from Caribbean to treat gastritis. The objective of this study was to evaluate the gastroprotective and healing effect of the aqueous fraction (FrAq) obtained from the leaves of *Terminalia catappa* and to determine the antiulcer mechanism of action in experimental rodent models and its activity to *Helicobacter pylori*.

Material and methods: In rodents, the FrAq was challenged by different necrotizing agents, such as absolute ethanol and ischemia–reperfusion injury. The antiulcer mechanism of action of FrAq was assessed and the healing effects of the fraction after seven and 14 days of treatment was evaluated by matrix metalloproteinase activity (MMP-2 and MMP-9). The toxicological effect of subacute treatment with FrAq during 14 days of treatment was also analyzed. The anti-*Helicobacter pylori* activity was determined by microdilution. The phytochemical study of the fraction was analyzed by experiments with FIA-ESI-IT-MS^n (Direct Flow Analysis-ionization Electrospray Ion Trap Tandem Mass Spectrometry) and high performance liquid chromatography (HPLC) coupled to a photodiode array (PDA).

Results: Oral treatment with FrAq (25 mg/kg) significantly decreased the number of ulcerative lesions induced by ethanol and ischemia/reperfusion injury. The action of FrAq was mediated by the activation of defensive mucosa-protective factors, such as increases in mucus production, the nitric oxide (NO) pathway and endogenous prostaglandins. Oral treatment with FrAq for seven and 14 days significantly reduced the lesion area (80% and 37%, respectively) compared to the negative control group. Analyses of MMP-9 and MMP-2 activity from gastric mucosa confirmed the accelerated gastric healing effect of FrAq. This extract also presented considerable activity against *Helicobacter pylori*. The mass spectrum and MS/MS of the aqueous fraction indicates the existence of many different phenolic compounds, including punicalagin, punicalin, and gallagic acid, among others.

Conclusions: We concluded that FrAq from *Terminalia catappa* leaves has excellent preventive and curative effects on acute and chronic induced gastric ulcers and showed an important profile against *Helicobacter pylori*.

* Corresponding authors. Tel.: +55 14 38800312; fax: +55 1438153744.
E-mail addresses: walbertoma@gmail.com (W. Toma), hiruma@ibb.unesp.br (C. Akiko Hiruma-Lima).
[1] Contributed equally to the supervision of this study.

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1. Introduction

Terminalia catappa is a species of the family Combretaceae and popularly known in Brazil as “amendoeira”, “amendoeira-da-praia”, “amendoeira-da-Índia”, “cuca”, “guarda-sol”, “castanheira da Índia”, “castanhola” and “chapéu-de-Sol”. This plant is widely distributed in countries with tropical and subtropical climates, especially in coastal regions due to the plant's ability to easily adapt to salinity and winds (Thomson and Evans, 2006). In Asian countries, the leaves of this species are commonly used for the treatment of dermatitis, hepatitis, diarrhea and pyresis (Chen et al., 2000). This plant was also listed in Pharmacopeia vegetables of the Caribbean, where the leaves of this plant are used in a decoction for gastritis and urinary infection (Germosén-Robineau, 2014). The literature also shows that the polar extract from different parts (leaves, fruits and bark) of *Terminalia catappa* have shown the following biological activities: antimicrobial, antifungal (Fyhrquist et al., 2002), antioxidant (Masuda et al., 1999; Chen and Li, 2005; Pandya et al., 2013), antimetastatic (Yeh et al., 2012, 2014), anti-inflammatory (Fan et al., 2004; Lin et al., 1999), hepatoprotective (Lin et al., 1997; Chen et al., 2000; Tang et al., 2006; Chen and Li, 2005), mutagenic (Mininel et al., 2014), aphrodisiac (Ratnasooriya and Dharmasiri, 2000) and antidiabetic (Nagappa et al., 2003). Nunes et al. (2012) described the gastroprotective effect of the ethanolic extract obtained from bark of this species and Kumar et al. (2014) previously reported the antisecretory effect of the ethanolic extract from leaves, although the mechanism responsible for this preventive effect remains unknown, thus the use of this specie against gastric bacteria is relevant. Infection caused to *Helicobacter pylori* is considered the most prevalent cause of gastric diseases such as ulcers, dyspepsia and stomach cancer (Camargo et al., 2014). The drug therapy available for the treatment of diseases caused by this microorganism has limitation such as the high rate of resistance to conventional drugs and inappropriate patient treatment, as presented to numerous side effects (Megraud et al., 2013). In this sense, the use of natural products as an alternative to control this bacterium has shown to be satisfactory, what drives the improvement of investigations of medicinal plants as adjuvant or new antimicrobial drugs (Parreira et al., 2014; Takeuchi et al., 2014).

The aims of this present work were to characterize the anti-*Helicobacter pylori* activity and antiulcer effect of the aqueous fraction obtained from leaves of *Terminalia catappa* and determine the mechanism of action for this medicinal species.

2. Material and methods

2.1. Preparation of the aqueous fraction

The leaves of *Terminalia catappa* were collected in January (2010) by Msc. Laísa Pinheiro Silva in Santos/SP, Brazil. The specimen was identified by Msc. Paulo Salles Penteado Sampaio, and the voucher specimen was deposited to the Herbarium at the Universidade Santa Cecília, Santos, SP, Brazil, under the register M. Tomaz 01, for future reference. The leaves from *Terminalia catappa* (378.54 g) were dried for six days at 50 °C, powdered (3 μm) and subjected to percolation with absolute ethanol (2 L) for 2 h with a flux of 2.0 mL/min/kg. The hydroalcoholic extract submitted to the rotary evaporator (45 °C) resulted in 33.1 g of product (a yield of 8.75%). This extract was partitioned into three fractions: a hexane fraction – FrHex (7.09 g, 24.96%), an ethyl acetate fraction – FrEtOAc (12.22 g, 43.02%) and an aqueous fraction – FrAq (8.43 g, 26.16%). As the commonly used formulation of this medicinal plant is a decoction, we chose to utilize the FrAq in the experimental pharmacological protocols.

2.2. Chemicals and reagents

HPLC–grade methanol (MeOH) and acetonitrile were purchased from J.T. Baker (Baker-Mallinckrodt, Phillipsburg, NJ, USA). HPLC–grade water (18 MΩ cm) was obtained using a direct Milli-Q purification system (Millipore Co., Bedford, MA, USA). Sep-Pak RP18 cartridges (500 mg/mL) for solid-phase extraction (SPE) were purchased from Phenomenex Co. (Torrance, CA, USA).

2.3. Apparatus

The mass spectrometry experiments were performed on LCQ Fleet equipment (Thermo Scientific®) equipped with the dispersal of the directly introduced sample via flow injection analysis (FIA). The studied matrix was analyzed by electrospray ionization (ESI), and multiple stages of fragmentation (MS^2, MS^3, MS^n) were performed at an ion trap (IT) interface. The negative mode was selected for the generation and analysis of the mass spectra for the first order (MS) and for the remaining multi-stage experiments under the following conditions: capillary voltage, −25 V; voltage spray, −5 kV; capillary temperature, 275 °C. A carrier gas (N_2) with a flow of 8 arbitrary units (A.U.) was used, and the collision gas was helium (He). The track acquisition was 100–2000 m/z. *Xcalibur* version 1.3 software (Thermo Finigan®) was used to acquire and process the data.

For the FIA-ESI-IT-MS^n assay, 10 mg of the aqueous fraction was dissolved in 1 mL of MeOH:H_2O (1:1, v/v) after using an ultrasonic bath for 5 min. The samples were then filtered through a 0.22 μm PTFE filter, and aliquots of 20 μL were directly injected into the FIA-ESI-IT-MS^n system.

For the HPLC–PDA a *clean-up* step was performed to remove any contaminants; the solution was purified by solid phase extraction (SPE) using Phenomenex Strata C_{18} cartridges (500 mg of stationary phase) that were previously activated with 5 mL of MeOH and equilibrated with 5 mL of MeOH:H_2O (1:1, v/v). The dried aqueous fraction was diluted to 10 mg/mL in HPLC solvent. A 20 μL aliquot was injected directly into the HPLC–PDA with detection at 270 nm. The identification of the different compounds in the chromatographic profile of the aqueous fraction was done by comparing their retention times (t_r) and the UV spectra with those isolates previously described in the literature (Mininel et al., 2014).

2.4. Animals

Male Wistar rats weighing 180–250 g were obtained from breeding at the Biotério Central at the Universidade Estadual Júlio de Mesquita Filho (UNESP), Botucatu-SP. Animals were fed with a certified Nuvilab CR-diet, with free access to tap water and were housed on a 12 h light/dark cycle at 60 ± 1% humidity and a temperature of 22 ± 2 °C. The UNESP Institutional Animal Care and Use Committee, following the recommendations of the Canadian Council on Animal Care (Olfert et al., 1993), approved all of the employed protocols under number 18/05.

2.5. Evaluation of the gastroprotection activity of the FrAq

2.5.1. Gastric ulcer induced by absolute ethanol

The rats were divided into five treatment groups (n=7–14) and fasted for 12 h prior to receiving an oral dose of the vehicle (saline 10 mL/kg), carbenoxolone (100 mg/kg) and FrAq (12.5, 25 and 100 mg/kg). After 60 min, all groups were orally treated with 1 mL of absolute ethanol for the gastric ulcer induction. One hour later, animals were killed and their stomachs excised. The incision into each stomach was performed along the greater curvature and examined for linear hemorrhagic lesions in the glandular region (Morimoto et al., 1991). Then, the stomachs were photographed and