Journal of Ethnopharmacology 165 (2015) 238–242

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

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

Antileishmanial activity of standardized fractions of *Stryphnodendron obovatum* (Barbatimão) extract and constituent compounds

Tatiana G. Ribeiro [a], André M. Nascimento [a], Bárbara O. Henriques [a], Miguel A. Chávez-Fumagalli [b], Juçara R. Franca [a], Mariana C. Duarte [b], Paula S. Lage [b], Pedro H.R. Andrade [c], Daniela P. Lage [c], Lívia B. Rodrigues [a], Lourena E. Costa [b], Vivian T. Martins [d], André A.G. Faraco [a,e], Eduardo A.F. Coelho [b,c], Rachel O. Castilho [a,e,*]

[a] Programa de Pós-Graduação em Ciências Farmacêuticas, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Programa de Pós-Graduação em Ciências da Saúde: Infectologia e Medicina Tropical, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Departamento de Patologia Clínica, COLTEC, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[d] Departamento de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[e] Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

ARTICLE INFO

Article history:
Received 26 May 2014
Received in revised form
18 December 2014
Accepted 21 February 2015
Available online 28 February 2015

Keywords:
Cerrado
Tannins
Gallic acid
Gallocatechin
Antioxidant
Leishmania amazonensis

ABSTRACT

Ethnopharmacological relevance: *Stryphnodendron obovatum* Benth. is a Brazilian tree used to treat skin ulceration, promote wound healing, and inhibit the growth of protozoa, including *Trypanosoma* and *Leishmania* species. Bioguided fractionation of the ethanol extract of *S. obovatum* stem bark was performed, and antileishmanial and antioxidant activities of the standardized fractions were analyzed.
Materials and methods: Stationary-phase *Leishmania amazonensis* promastigotes, murine macrophages, and human red blood cells (RBCs) were exposed to plant extract, standardized fractions or isolated compounds for 48 h at 37 °C to evaluate their antiparasitic activity and cytotoxicity. The 2,2-diphenyl-1-picryl-hidrazyl assay was used to evaluate antioxidant activity.
Results: The *S. obovatum* extract and fractions showed antileishmanial and antioxidant activity; however, the organic fraction (OF) showed the best efficacy. We identified gallic acid, gallocatechin, epigallocatechin, catechin, and epigallocatechin gallate in the OF fraction. These compounds effectively inhibited *L. amazonensis* activity, with gallic acid, gallocatechin, and epigallocatechin gallate showing the highest selectivity. Furthermore, the evaluated compounds had no significant effect on murine macrophages and human RBCs.
Conclusions: The compounds present in the *S. obovatum* plant bark ethanol extract may provide an alternative therapeutic approach for *L. amazonensis* treatment.

1. Introduction

Leishmaniasis is a protozoal disease with high morbidity and mortality worldwide. Over 350 million people in 98 countries are at risk of contracting leishmaniasis (WHOE, 2010), in addition to approximately 700,000–1.2 million cases of tegumentary leishmaniasis registered annually worldwide (Alvar et al., 2012).

Leishmaniasis has a wide spectrum of clinical manifestations, attributable to the different protozoa species belonging to the *Leishmania* genus (Desjeux, 2004). Cutaneous leishmaniasis first develops as a localized papule, which evolves into an ulcer upon the loss of the epidermis, resulting in skin barrier impairment. While parenteral administration of pentavalent antimony organic compounds remains the first-line therapy for all forms of leishmaniasis, increased drug resistance and adverse side effects, including arthralgia, myalgia, pancreatitis, leukopenia, and cardiotoxicity, have been reported (Oliveira et al., 2011). Additionally, as leishmaniasis has emerged as an opportunistic infection in human immunodeficiency virus (HIV)-infected patients, the development of new and cost-effective alternative therapeutic strategies has become a priority (Kedzierski et al., 2009).

In recent years, researchers have sought to identify plant-derived compounds for use as novel antileishmanial drugs (Ribeiro et al.,

Abbreviations: RBCs, red blood cells; OF, organic fraction; AF, aqueous fraction; GA, gallic acid; EGC, epigallocatechin; C, catechin; GC, gallocatechin; EGCG, epigallocatechin gallate

* Corresponding author at: Departamento de Produtos Farmacêuticos, Faculdade de Farmácia, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil. Tel.: +553134096936; fax: +553134096935.
E-mail address: rocastilho40@gmail.com (R.O. Castilho).

http://dx.doi.org/10.1016/j.jep.2015.02.047
0378-8741/

2014a). A wide variety of separation techniques have been developed to identify active molecules in plants, thereby supporting the development of new therapeutics. Although numerous studies have identified plant extracts and/or purified compounds with antileishmanial activity, effective alternative therapeutics for leishmania have not yet been developed (Lage et al., 2013).

The genus *Stryphnodendron,* belonging to the Fabaceae family, consists of approximately 48 species, including *Stryphnodendron obovatum* Benth., called "barbatimao" (Costa et al., 2013). *S. obovatum* is a tree found in the Cerrado, a savannah region of Brazil (Sanches et al., 2007). It has long been used in Brazil as an antiseptic and antiprotozoal agent, showing efficacy against *Trypanosoma* and *Leishmania.* Furthermore, *S. obovatum* has been used to treat skin ulceration and induce wound healing (Nascimento et al., 2013; Oliveira et al., 2014). Studies of *Stryphnodendron* species have identified anti-ulcerogenic, antioxidant, wound-healing, antimicrobial, and antileishmanial activity (Lopes et al., 2005; Luize et al., 2005; Souza et al., 2007). *Stryphnodendron* species contain tannins, including prodelphinidins and prorobinetinidins (Mello et al., 1999). Tannins are phenolic compounds that exhibit a remarkably wide range of bioactivities (Okuda, 2005). They are thought to mediate the curative and palliative efficacy, and health benefits of many traditional herbal medicines and foods (Kolodziej and Kiderlen, 2005). Owing to the beneficial effects *S. obovatum*, we sought to evaluate the antileishmanial and antioxidant activity of the ethanol extract (EE) of this plant. After bioguided fractionation, the organic fraction (OF) was analyzed and standardized to identify and quantify the compounds responsible for the antileishmanial activity. We identified five tannins that effectively inhibited *Leishmania amazonensis*. Further we established the 50% inhibitory concentrations (IC_{50}), the concentrations required to achieve 50% of the antioxidant effect (EC_{50}), and the concentrations required to achieve 50% cytotoxicity in murine macrophages (CC_{50}), and O^+ human red blood cells (RBC_{50}) of these compounds.

2. Materials and methods

2.1. Chemicals and reagents

High-performance liquid chromatography (HPLC)-grade acetonitrile and methanol were purchased from Tedia (Fairfield, OH, USA) and J.T. Baker (Phillipsburg, NJ, USA), respectively. Concentrated phosphoric acid (85% w/v, Merck, Darmstadt, Germany) and commercial ethanol (96% v/v) were used. Ultrapure water was obtained using a Milli-Q plus system from Millipore (Milford, MA, USA). The HPLC-grade reference substances used were as follows: gallic acid (GA, 98%, Acrós Organics, Geel, Belgium), epigallocatechin (EGC; 90%, Fluka, Milwaukee, WI, USA), catechin (C; 98%, Sigma, Milwaukee, WI, USA), gallocatechin (GC, 98%, Sigma), and epigallocatechin gallate (EGCG, 95%, Sigma). Schneider's medium, RPMI 1640 medium, fetal bovine serum (FBS), L-glutamine, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), and penicillin/streptomycin solution were purchased from Sigma-Aldrich (St. Louis, MO, USA). Amphotericin B (AmpB) was provided by Cristália Produtos Químicos Farmacêuticos Ltd. (São Paulo, Brazil).

2.2. Plant material

The stem bark from *S. obovatum* Benth. was collected in Mato Grosso do Sul (2006), in Campo Grande City (S20°24′37.4″ and WO54°36′52.5″), Brazil. The species was identified by Prof. Arnildo Pott at the Universidade Federal de Mato Grosso do Sul (UFMS), Brazil, and a voucher specimen (32997) was deposited at the CGMS herbarium, UFMS.

2.3. Preparation of the extract and fractions

The stem bark of *S. obovatum* was dried at 40 °C to constant weight. After grinding, stem bark (170 g) was extracted with 200 ml of 96% v/v commercial ethanol by percolation for two weeks at room temperature, changing the solvent every 24 h. The solvent was then removed in a rotary evaporator at 40 °C, yielding a dark brown solid (EE), representing 45.5% dry mass. The *S. obovatum* EE was fractionated by liquid–liquid extraction using ethyl acetate:butanol:2-propanol:water (3.5:0.5:1.0:4.5), yielding an organic fraction (OF) and an aqueous fraction (AF). The OF and AF were separately evaporated under a warm air stream (40 °C) to obtain dry residues. The OF was standardized by reversed phase (RP)-HPLC as described by Nascimento et al. (2013).

2.4. Chromatographic analysis (RP-HPLC)

The EE, OF, and reference compounds (GA, EGC, C, GC, and EGCG) were dissolved in methanol to concentrations of 10.0, 5.0, and 1.0 mg/ml, respectively. Chromatographic analyses were performed using an HP1100 system (Agilent, Santa Clara, CA, USA) coupled to a quaternary pump, an auto sampler, and a programmable ultraviolet photodiode array detector (UV/DAD). An HPChemStation for LC3D systems software (Rev. B.02.01-SR2 [260] 2001–2006) was used to evaluate the data. An RP C18 pre-column (XDB Zorbax®, $4 \times 4\ mm^2$ internal diameter [I.D.]; 5 μm, Agilent) was attached to a C18 column (LiChrospher®100, $250 \times 4\ mm^2$ I.D.; 5 μm, Merck) at 40 °C. After filtration using a 0.45 μm polytetrafluoroethylene (PTFE) membrane, 10 μl solutions were automatically injected into the system at a flow rate of 1 ml/min, with detection at λ 210 nm. UV/DAD spectra were recorded on-line for peak purity and identification from λ 190 to 400 nm, and compound identities were confirmed by co-elution of standards, using 100 μl of the standard solutions. The degassed mobile phase was composed of aqueous 0.1% phosphoric acid (solution A), and 0.1% phosphoric acid in acetonitrile (solution B), with a linear gradient from A–B (95:5, v/v) to A–B (60:40, v/v) over 60 min. Cleaning and reconditioning of the column was performed for 15 min.

2.5. Free radical scavenging activity

Free radicals were formed using 2,2-diphenyl-1-picryl-hidrazyl (DPPH) from Sigma. Samples (100 μl; 0.43–71.40 μg/ml) were added to each well of a 96-well culture plate (Nunc, Nunclon®, Roskilde, Denmark), using methanol as the diluent control and rutin (Sigma) as a positive control. The culture plate was shaken for 1 min, and then incubated for 30 min at 37 °C. Absorbance was measured using a multi-well scanning spectrophotometer (Tecan Infinity M200) at a wavelength of 517 nm. The results were presented as the EC_{50} of the radical scavenging capacity, corresponding to the concentration of sample required to decrease the initial DPPH absorbance by 50%. The data are representative of three independent experiments, performed in triplicate (Farias et al., 2013).

2.6. Parasites and mice

L. amazonensis (IFLA/BR/1967/PH-8) parasites were grown at 24 °C in Schneider's medium (Sigma-Aldrich), supplemented with 20% heat-inactivated FBS (Sigma), 20 mM L-glutamine, 200 U/ml penicillin, and 100 μg/ml streptomycin at pH 7.4. Stationary-phase promastigotes were prepared as described previously (Coelho et al., 2003).

Murine peritoneal macrophages were obtained from female BALB/c mice (aged 8 weeks), purchased from the Institute of Biological Sciences of the Universidade Federal de Minas Gerais (UFMG). The Animal Use Committee at UFMG approved the experimental protocols (code 136/2012).