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Research Brief

Different susceptibilities of *Leishmania* spp. promastigotes to the *Annona muricata* acetogenins annonacinone and corossolone, and the *Platymiscium floribundum* coumarin scoparone

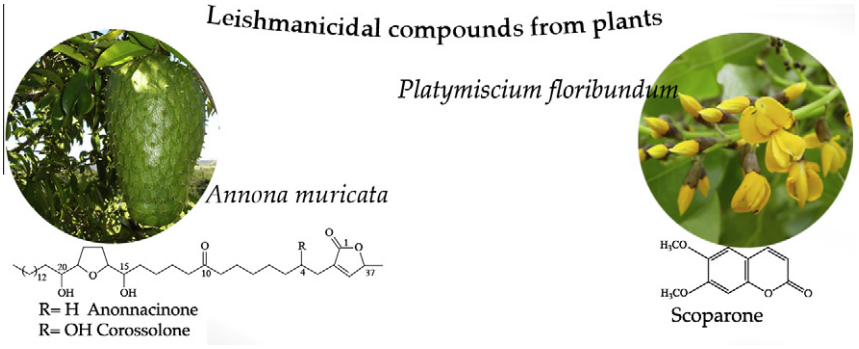
Nadja Soares Vila-Nova [a], Selene Maia de Morais [a,b,*], Maria José Cajazeiras Falcão [b], Terezinha Thaize Negreiros Alcantara [b], Pablito Augusto Travassos Ferreira [b], Eveline Solon Barreira Cavalcanti [b], Icaro Gusmão Pinto Vieira [c], Cláudio Cabral Campello [a], Mary Wilson [d,e,f]

[a] *Programa de Pós-Graduação em Ciências Veterinárias, Universidade Estadual do Ceará, Avenida Paranjana, 1700, Campus do Itaperi, 60740-000 Fortaleza, Ceará, Brazil*
[b] *Curso de Química, Centro de Ciências e Tecnologia, Universidade Estadual do Ceara, Avenida Paranjana, 1700, Campus do Itaperi, 60740-000 Fortaleza, Ceará, Brazil*
[c] *PADETEC – Parque de Desenvolvimento Tecnologico, Universidade Federal do Ceará, Av. Humberto Monte, 2977, Bairro Parquelandia Bloco 310, CEP 60.440-593 Fortaleza, Ceará, Brazil*
[d] *Department of Internal Medicine, University of Iowa, Iowa City, IA 52242, USA*
[e] *Department of Microbiology, University of Iowa, Iowa City, IA 52242, USA*
[f] *VA Medical Center, Iowa City, IA 52242, USA*

HIGHLIGHTS

- Acetogenins were isolated from *A. muricata* and scoparone from *P. floribundum*.
- All compounds inhibited the promastigote growth in the three species.
- Annonacinone presented high leishmanicidal activity.
- Corossolone, and scoparone demonstrated moderate leishmanicidal actions.
- Toxicity was tested using *A. salina* assay, corossolone was the most toxic compound.

GRAPHICAL ABSTRACT



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ABSTRACT

Leishmaniasis is a zoonotic disease that can manifest itself in visceral and cutaneous form. The aim of this study was to search for new leishmanicidal compounds. Preliminarily, *Artemia salina* assay was applied to compounds from two plants found in Northeastern Brazil, *Platymiscium floribundum* and *Annona muricata.* Then these compounds were tested against three *Leishmania* species (*Leishmania donovani*, *Leishmania mexicana* and *Leishmania major*). A screening assay using luciferase-expressing promastigote form were used to measure the viability of promastigote One coumarin, scoparone, isolated from *P. floribundum* and two acetogenins, annonacinone and corossolone isolated from *A. muricata* showed leishmanicidal activity in all species tested. Nevertheless, *Leishmania* species indicated different susceptibilities in relation to the tested compounds: *L. mexicana* was more sensitive to scoparone followed by *L. major* and *L. donovani.* The three species presented similar inhibition to corossolone and annonacinone. Acetogenin annonacinone (EC_{50} = 6.72 – 8.00 μg/mL) indicated high leishmanicidal activity; corossolone

* Corresponding author at: Curso de Química, Universidade Estadual do Ceará, Av. Paranjana, 1700, Campus do Itaperi, CEP 60740-903, Serrinha, Fortaleza, Ceará, Brazil. Tel.: +55 85 31019933.
E-mail address: selenemaiademorais@gmail.com (S.M. de Morais).

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(EC_{50} = 16.14 − 18.73 μg/mL) and scoparone (EC_{50} = 9.11 − 27.51 μg/mL) moderate activity. *A. saline* larvae were less sensitive to the coumarin scoparone and acetogenin corossolone was the most toxic. In conclusion, the leishmanicidal activity demonstrated by the coumarin and acetogenins indicate these compounds for further studies aiming the development of new leishmanicidal agents.

1. Introduction

Leishmaniasis is a tropical zoonotic disease caused by more than 20 protozoa species of *Leishmania* genus (Singh et al., 2012) manifesting itself in visceral and cutaneous form. The geographical distribution of leishmaniasis relate to the growth of sandfly vector, which are dominant in tropical and temperate regions (Roy et al., 2012). The chemotherapy used in the treatment of leishmaniasis is based on the use of drugs that are toxic heavy metals, known as antimoniates, among which the most used are meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostan®). When this type of treatment is not effective, other medications such as pentamidine and amphotericin B and its lipossomal formulation, are also used. All these medications are administrated intravenously and/or intramuscular require clinical supervision or hospitalization due to the severity of side effects (Chan-Bacab and Pena-Rodríguez, 2001; Rath et al., 2003). In dogs, one of the main reservoirs, treatment is not a recommended measure in Brazil, since it does not diminish the importance of these animals as reservoir of the parasite. It only provides clinical remission and not a parasitological cure (Sessa et al., 1994; Alves and Bevilacqua, 2004; Ait-Oudhia et al., 2012).

In the early 1990s, the World Health Organization (WHO) reported that 65–80% of the population of developing countries depended on medicinal plants as the only form of access to basic health care (Veiga et al., 2005). A vast number of Brazilian plants are able to produce secondary metabolites with antiparasitic activity; many of these metabolites have not been properly isolated and chemically or biologically evaluated. The Annonaceae family is a great producer of acetogenins that has shown promising results in the search for new drugs against various protozoa, revealing good potential as a source of agents for the treatment of leishmaniasis (Rocha et al., 2005). *Annona muricata* is a well-known plant in Brazil, the compounds there are most found in this plant are acetogenins and alkaloids (Vila-Nova et al., 2011; Tempone et al., 2005), and has antiprotozoal, antioxidant and anticancer properties (Rondon et al., 2011; Boyom et al., 2009; Lima et al., 2011; Chen et al., 2012a). *Platymiscium floribundum* is a regional plant in Ceará state used by the population as anti-inflammatory and the isoflavonoids isolated from this plant have demonstrated activity against cancer cells (Militão et al., 2006; Falcao et al., 2005), and the crude extract demonstrated antifungal and acetylcholinesterase activity (Cardoso-Lopes et al., 2008), pterocarpans isolated from the heartwood demonstrated antimitotic effect on sea urchin eggs (Militão et al., 2005), but a few of studies have been developed in the search of others chemotherapeutic values as well as isolating others compounds from this plant.

Due to the shortcomings described above, there is a pressing need for new leishmanicidal treatments, and compounds from Northeastern Brazilian plants can be promising sources for future drugs.

2. Material and methods

2.1. Plants

The heartwood and sapwood of the stem of *P. floribundum* and leaves of *A. muricata* were collected in the state of Ceará, Brazil. Aerial parts of the two plants were deposited in the Prisco Bezerra Herbarium under the reference number 31052 and 43951, respectively.

2.2. Chromatographic procedures

The active chemical constituents were isolated from plant extracts by silica gel (δ 0.063–0.200 mm; 70–230 mesh) and Sephadex (LH-20) column chromatography and solvents for elution were petroleum ether, hexane, chloroform, ethyl acetate, acetone and methanol from VETEC (Brazil). The fractions collected in the columns were compared by thin layer chromatography (TLC), using silica gel (60 G F 254) on glass plates (3 cm × 8 cm). The TLC plates were analyzed in a iodine chamber, under UV Light (at 312 and 365 nm), Vilbert Loumart, CN-15 LM model, and by spraying the reagent 2.5% vaniline in sulfuric acid diluted in ethanol (1:1), followed by heating in an oven at 100 °C.

2.3. Isolation of compounds and spectroscopic identification

A. muricata seeds (2 kg) were triturated and left in contact with methanol for 1 week, then the solvent was filtered, evaporated to dryness and a light yellow solid was obtained (402 g). This material was chromatographed on a filtering silica gel column yielding two main compounds. The trunk heartwood of *P. floribundum* (800 g) was cold extracted using hexane, chloroform and ethanol successively. Respective extracts were obtained by elimination of solvents in a rotatory evaporator. The chloroform extract was introduced on the top of a glass chromatographic column, filled with silica gel, and the solvents hexane and ethyl acetate, in mixtures of increasing polarity, were used to elute the column. Fifty-six 10 mL fractions were collected and compared by TLC. This procedure conducted to the isolation of a unique compound, revealed by a simple spot on TLC plate.

The structures of compounds were determined by spectroscopic analysis of infrared spectra, recorded on a FT-IR PerkinElmer 1000 spectrophotometer, values expressed in cm^{-1}, and nuclear magnetic resonance spectra, recorded on a Bruker Avance DRX-500 spectrometer in $CDCl_3$.

2.4. Leishmanicidal assay

L. major, *L. mexicana* and *L. donovani* mCherry-Luciferase-expressing (mCherry-Luc) species were generated with the integrating vector pIR1SAT, provided by Dr. Steve Beverley, as described (Thalhofer et al., 2010). The promastigote forms were cultured in hemoflagellate-modified MEM (HOMEM) medium, supplemented with 10% fetal bovine serum at 26 °C. For the leishmanicidal assay, an adapted methodology from Tempone et al. (2005) was used. The compounds were dissolved in DMSO at a concentration of 0.2% and diluted with HOMEM medium in 96-well microplates. The assay was performed at concentrations of 100, 50, 25, 12.5 and 6.25 μg/mL. Control wells contained DMSO or no additives. Each concentration was tested in triplicate in replicate experiments. Promastigotes at a logarithmic phase were counted in a Neubauer hemocytometer and seeded at 1×10^6/well. The plates were incubated at 26 °C for 24 h, and the viability of the promastigotes, based on their morphology, was observed under a