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Leishmanicidal activity of the alkaloid-rich fraction from *Guatteria latifolia*

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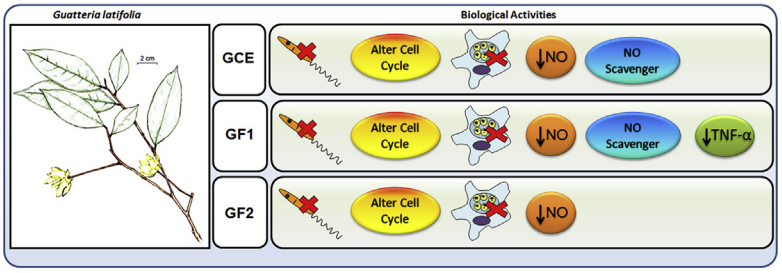
HIGHLIGHTS

- The crude branches extract (GCE), subfractions 1 and 2 (GF1, GF2) of *G. latifolia* have anti-*Leishmania amazonensis* activity.
- GCE, GF1 and GF2 decrease NO production in IFN-γ and LPS-stimulated macrophages.
- GF2 decrease TNF-α production in IFN-γ and LPS-stimulated macrophages.
- GCE, GF1 and GF2 affected the cell cycle of promastigotes without changes in the mitochondrial membrane potential.
- Puterine, oxoputerine and lysicamine are presents in GF1 and, in the GF2 only oxoputerine and lysicamine are present.

GRAPHICAL ABSTRACT



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ABSTRACT

Leishmaniasis is caused by protozoan parasites belonging to the genus *Leishmania* and includes cutaneous, mucocutaneous and visceral clinical forms. The drugs currently available for leishmaniasis treatment are pentavalent antimonials, amphotericin B and miltefosine, which present high toxicity, elevated cost and development of parasite resistance. The natural products constitute an important source of substances with leishmanicidal potential. Here we evaluated *in vitro* the anti-*Leishmania amazonensis* activity of crude extracts of branches, leaves and fruits of *Guatteria latifolia*. The branch extract (GCE) exhibited promising leishmanicidal activity against promastigotes (IC_{50} 51.7 μg/ml), and was submitted to fractionation guided by *in vitro* assays. Among the seven subfractions obtained, GF1

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Leishmania amazonensis
Leishmanicidal activity

and GF2 were the most actives against promastigotes with IC_{50} 25.6 and 16 μg/ml, respectively. Since GCE, GF1 and GF2 were not toxic for macrophages, next, we tested their effect on intracellular amastigotes, and the IC_{50} values obtained were, respectively 30.5, 10.4 and 7.4 μg/ml, after 24 h treatment. The selectivity index for GCE, GF1 and GF2 were >6.5, >19.2 and > 27, respectively. Additionally, GCE, GF1 and GF2 affected the division pattern of the promastigotes by increasing 6.7, 9.4 and 7-fold the cells in Sub-G0/G1 phase, and decreasing 1.6, 2.5 and 1.8-fold the cells in G0/G1 phase, respectively. To assess the GCE and GFs capacity to modulate microbicidal mechanisms of macrophages, nitric oxide (NO) and TNF-α production were tested. Our results indicated that at the IC_{50s} GCE, GF1 and GF2 decreased NO production of infected macrophages stimulated with IFN-γ and LPS, besides, only GF1 decreased the production of TNF-α. Our data warrant further studies of GCE, GF1 and GF2 to identify active compounds against *Leishmania* parasites.

1. Introduction

Leishmaniasis is a public health problem that affected 98 countries with approximately 1.7 million cases worldwide each year (Alvar et al., 2012). The drugs currently available for leishmaniasis treatment are pentavalent antimonials, amphotericin B and its lipid formulations, pentamidine and miltefosine (Croft et al., 2006; Ouellette et al., 2004). However, these drugs need to be administered by intravenous infusion and the treatment has poor patient compliance because many of them require daily systemic administration for long periods. These drugs are toxic and they have side effects such as chills, fever, thrombophlebitis, myocarditis, nephrotoxicity and ultimately death (Andrews et al., 2014; Singh et al., 2014; Croft et al., 2006; Ouellette et al., 2004). Miltefosine is the first oral drug approved for treatment of leishmaniasis in India. However, it has been reported, its low efficacy against cutaneous leishmaniasis, besides its teratogenicity (Sánchez-Cañete et al., 2009; Sindermann and Engel, 2006). Moreover, the emergence of drug-resistant strains of *Leishmania* sp. is rapidly increasing worldwide. Therefore, there is an urgent need for new therapies against leishmaniasis (Andrews et al., 2014; Sánchez-Cañete et al., 2009; Sindermann and Engel, 2006).

Different studies have demonstrated that natural products are a promising source to search for novel drugs for the treatment of neglected tropical diseases such as leishmaniasis. Actually, leishmanial activity were already reported for genera (families) such as *Peschiera* (Apocynaceae) (Delorenzi et al., 2001), *Pourouma* (Moraceae) (Torres-Santos et al., 2004), *Kalanchoe* (Crassulaceae) (Muzitano et al., 2006), *Baccharis* (Asteraceae) (Tempone et al., 2008), *Physalis* (Solanaceae) (Guimarães et al., 2009), *Piper* (Piperaceae) (Ferreira et al., 2011), *Copaifera* (Fabaceae) (Soares et al., 2013), *Croton* (Euphorbiaceae) (Lima et al., 2015), *Lippia* (Verbenaceae) (Funari et al., 2016), and *Serjania* (Sapindaceae) (Passos et al., 2017), among several others (reviewed by Singh et al., 2014; Oliveira et al., 2016; Ullah et al., 2016). Among the *Annonaceae* species many secondary metabolites displaying antileishmanial activity were isolated (Siqueira et al., 2015; Montenegro et al., 2003; Vila-Nova et al., 2011). The family *Annonaceae* has about 135 genus and 2500 species of plants. In Brazil, the *Guatteria* genus is the most abundant with 88 species, from which 47 are endemic (Lobão et al., 2012; Lobão and Mello-Silva, 2007). Although considered endangered *G. latifolia* is a prevalent species in Minas Gerais and in Rio de Janeiro states (Lobão and Mello-Silva, 2007), for which any biological or chemical studies have been done. Previous phytochemical investigations of other species of this genus have revealed significant biological activities such as antimicrobial (Costa et al., 2010), antitumor (Fontes et al., 2013), antioxidant and antiparasitic (Mahiou et al., 2000).

In the present investigation, we report that a bioassay-guided fractionation of crude branches extract of *G. latifolia* led to the isolation of alkaloids rich fractions. *In vitro* activity of these fractions was assessed against promastigote and intracellular amastigote forms of *Leishmania amazonensis*, as well as for some macrophage toxicity and microbicidal mechanisms.

2. Materials and methods

2.1. Ethics statement

All animal experiments were performed in strict accordance with the Brazilian animal protection law (Lei Arouca number 11.794/08) of the National Council for the Control of Animal Experimentation (CONCEA, Brazil). Animals were housed in a temperature-controlled room (24 °C), with a 12 h light-dark cycle, with food and water *ad libitum* in mini-isolators (Alesco Brasil). This study protocol was approved by the Committee for Animal Use of the Universidade Federal do Rio de Janeiro (Permit Number: 128/15).

2.2. Plant material

Guatteria latifolia was collected on October 27, 2011, in Itatiaia National Park, at Serra da Mantiqueira, Rio de Janeiro state. Species identification was performed by Dr. Adriana Quintella Lobão (Universidade Federal Fluminense, Rio de Janeiro, Brazil). The voucher specimen (number RB 518836) was deposited in the herbarium of the Jardim Botânico of Rio de Janeiro.

2.3. Extracts and fractionation procedures

Branches, leaves and fruits were dried at 40 °C for 48 h, and then extracted with ethanol and water 9:1 (v/v) for six days with five solvent changes (first 48 h extraction was followed by four 24 h extractions). The ethanol/water extracts obtained after evaporation of solvent in vacuum were stored at −70 °C until analyzed. Since only the crude branches extract (GCE) showed leishmanicidal activity, this extract (3.0 g) was suspended in 400 ml of distilled water using ultrasound (Branson−1510) for 15 min. The aqueous solution was partitioned with the same volume of *n*-butanol (Tedia, Brazil) for three times. The *n*-butanol (m = 1400 g) phases was chromatographed over C-18 (reverse phase) column. Gradient elution was carried out with methanol/water (10–100% of methanol) and provided seven subfractions (GF1 to GF7). The subfractions GF1 (335.5 mg), GF2 (384.0 mg), GF3 (372.2 mg) and GF5 (134.2 mg) were tested for leishmanicidal activity. The subfractions GF4, GF6 and GF7 were not tested because of the low mass obtained.