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In vitro evaluation of traditionally used Surinamese medicinal plants for their potential anti-leishmanial efficacy

D.R.A. Mans [a,*], T. Beerens [b], I. Magali [a], R.C. Soekhoe [a], G.J. Schoone [b], K. Oedairadjsingh [a], J.A. Hasrat [a], E. van den Bogaart [b], H.D.F.H. Schallig [b]

[a] *Department of Pharmacology, Anton de Kom University of Suriname, Paramaribo, Suriname*

[b] *Koninklijk Instituut voor de Tropen (KIT)/Royal Tropical Institute, KIT Biomedical Research, Parasitology Unit, Amsterdam, The Netherlands*

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ABSTRACT

Ethnopharmacological relevance: Plant-based preparations are extensively used in Surinamese folk medicine for treating leishmaniasis, but often without a scientific rationale.
Aim of the study: To evaluate 25 Surinamese medicinal plants for their potential efficacy against leishmaniasis.
Materials and methods: Concentrated plant extracts were evaluated for their effect on the viability of *L. (V.) guyanensis* AMC, *L. (L.) major* NADIM5, and *L. (L.) donovani* GEDII promastigotes, as well as intracellular amastigotes of *L. (L.) donovani* BHU814 in infected THP-1 cells. Selectivity was assessed by cytotoxicity against THP-1 cells.
Results: The only plant extract that showed potentially meaningful anti-leishmanial activity was that from *Solanum lycocarpum* that displayed mean IC_{50} values of about 51, 61, and <16 μg/mL against *L. (V) guyanensis*, *L. (L) major*, and *L. (L) donovani* promastigotes, respectively; about 374 μg/mL against *L. (L) donovani* amastigotes; and >500 μg/mL against THP-1 cells. The *Bryophyllum pinnatum*, *Inga alba*, and *Quassia amara* extracts displayed moderate to high IC_{50} values against promastigotes (about 51 to >500 μg/mL) and/or amastigotes (about 224 to >500 μg/mL) but were relatively toxic to THP-1 cells (IC_{50} values <16 to about 42 μg/mL). The remaining plant extracts exhibited in many cases IC_{50} values close to, around, or above 500 μg/mL against promastigotes, amastigotes, and THP-1 cells.
Conclusions: The *S. lycocarpum* preparation may be useful against leishmaniasis and may have a good safety index, warranting further investigations into its active constituents and mechanism(s) of action.
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1. Introduction

Leishmaniasis is a parasitic disease caused by approximately twenty different protozoan species of the genus *Leishmania* which is transmitted by female sand flies from at least thirty species of *Phlebotomus* in the Old World and *Lutzomyia* in the New World to a number of mammalian reservoirs including man (Desjeux, 2004; Reithinger et al., 2007; World Health Organization, 2014). All *Leishmania* species develop as promastigotes and amastigotes. The promastigotes are the infectious, motile flagellated forms inside the sand fly that are expelled into the host when the fly draws a blood meal (Dostálová and Volf, 2012). The amastigotes do not have a flagellum, reside and multiply inside mammalian host macrophages, and eventually spread throughout the host's reticulo-endothelial system (Vannier-Santos et al., 2002). Clinical disease becomes apparent within weeks to months after infection, depending on the (sub-)species of *Leishmania* and the host's immune status (Vannier-Santos et al., 2002).

The clinical manifestations following *Leishmania* infection may range from localized, single lesions to multiple cutaneous ulcers, satellite lesions, or nodular lymphangitis, to disease forms with mucosal and even potentially fatal systemic visceral involvement (Desjeux, 2004; Reithinger et al., 2007). Currently, an estimated 12 million individuals in 98 countries throughout the world suffer from one of these forms of leishmaniasis, approximately 350 million people are at risk of contracting the infection, and more than 2 million disability-adjusted life years are lost annually due to this disease (World Health Organization, 2014). These considerations have led to the characterization of leishmaniasis as a WHO category-1 disease, *i.e.*, an uncontrolled and severely neglected disease requiring increased efforts to improve vector control, diagnostics,

* Correspondence to: Department of Pharmacology, Faculty of Medical Sciences, Anton de Kom University, Kernkampweg 5, Paramaribo, Suriname.
E-mail addresses: dennis_mans@yahoo.com, dennis.mans@uvs.edu (D.R.A. Mans).

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Table 1
Relevant information about the plants evaluated in the current study. All reference vouchers are stored at the National Herbarium of Suriname (BBS) at the Anton de Kom University of Suriname, Paramaribo, Suriname (UvS: Universiteit van Suriname; LBB: 's Lands Bosbeheer; TvA: Tinde van Andel; MJJ: Marjon Jansen–Jacobs).

Plant species (vernacular name in English; Surinamese)	Plant family	Herbarium voucher	Plant part used and traditional method of use[a]	Plant part used in current study	Preparation of extract for current study
Allium sativum L. (Garlic; knoflook)	Amaryllidaceae	None[b]	Crushed cloves are applied to sore	Cloves	Crushed cloves soaked for 2 h at 45 °C in water
Aloe vera (L.) Burm.f. (Aloe; aloe)	Xanthorrhoeaceae	UVS-17469	Crushed leaves are smeared on sore	Leaves	Juice collected from crushed leaves
Anacardium occidentale L. (Cashew; kasyu)	Anacardiaceae	UVS-17493	Bark boiled with water and decoction applied to sore	Bark	Boiled for 1 h in water
Azadirachta indica A. Juss. (Nimtree; neem)	Meliaceae	UVS-17486	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 1 h in water
Bryophyllum pinnatum (Lam.) Oken (Mother of thousands; wonderblad)	Crassulaceae	UVS-17498	Leaves heated above fire and sore covered with them when still hot	Leaves	Boiled for 40 min in water
Cecropia peltata L. (Trumpet tree; uma busipapaya)	Urticaceae	TvA-4868	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 30 min in water
Citrus aurantifolia (Christm.) Swingle Key lime; limoen)	Rutaceae	UVS-17472	Juice dripped on sore	Fruits	Juice collected at room temperature
Colocasia esculenta (L.) Schott (Taro; tayerblad)	Araceae	UVS-17502	Leaves heated above fire and sore covered with them when still hot	Leaves	Crushed leaves soaked for 2 h at 45 °C in water
Crescentia cujete L. (Calabash; kalebas)	Bignoniaceae	UVS-17436	Crushed leaves applied to sore	Fruits	Collect juice at room temperature (25–26 °C)
Dieffenbachia seguine (Jacq.) Schott (Dumbcane; donke)	Araceae	UVS-17430	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 1 h in water
Eclipta prostrata (L.) L. (False daisy; lowisawiwiri)	Asteraceae	UVS-17464	Crushed leaves applied to sore	Leaves	Crushed leaves soaked for 2 h at 45 °C in water
Gossypium barbadense L. (Sea island cotton; redi katun)	Malvaceae	UVS-17433	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 1 h in water
Abelmoschus esculentus (L.) Moench (Okra; oker)	Malvaceae	UVS-17504	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 30 min in water
Hymenaea courbaril L. (Jatobá; loksi)	Fabaceae	LBB-5821	Macerated bark boiled with water and decoction applied to sore	Bark	Boiled for 2 h in water
Inga alba (Sw.) Willd. (Guavo; rode prokini)	Fabaceae	BBS-1075		Bark	Boiled for 2 h in water
Ipomoea batatas (L.) Lam. (Sweet potato; switi patata)	Convolvulaceae	LBBG-16823	Mashed tubers applied to sore (for 24 h)	Tubers	Crushed tubers soaked for 40 min in water at 45 °C
Mikania micrantha Kunth (Bitter vine; brokobaka)	Asteraceae	LBB-12491	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 30 min in water
Morinda citrifolia L. (Cheese fruit; noni)	Rubiaceae	LBB-12811	Leaves heated above fire and sore covered with them when still hot	Leaves	Crushed leaves soaked for 2 h at 45 °C in water
Musa sp. (Banana; banana)	Musaceae	None[c]	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 20 min in water
Quassia amara L. (Amargo; kwasibita)	Simaroubaceae	UVS-17475	Bark boiled with water and decoction applied to sore	Bark	Boiled for 3 h in water, then left for 1 day
Senna alata (L.) Roxb. (Candle bush; slabrikiwiri)	Fabaceae	TvA-5088	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 1 h in water
Solanum lycocarpum A.St.-Hil. (Wolf apple; uma parabita)	Solanaceae	UVS-17489	Juice from leaves applied to sore	Leaves	Leave juice collected at room temperature
Spondias mombin L. (yellow mombin; mope)	Anacardiaceae	LBB-12479	Bark boiled with water and decoction applied to sore	Bark	Boiled for 1 h in water
Uncaria guianensis (Aubl.) J.F.Gmel. (Cat's claw; popokay nangra)	Rubiaceae	UVS-16826	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 2 h in water
Vismia guianensis (Aubl.) Pers. (Bloodwood; pinya wiwiri)	Hypericaceae	6308-MJJ	Leaves boiled with water and decoction applied to sore	Leaves	Boiled for 1 h in water

[a] According to Ramdas (2015).
[b] The current study used commercially available garlic cloves.
[c] No voucher number available as the exact species could not be determined.

and therapeutic arsenal (World Health Organization, 2014).

Cutaneous leishmaniasis (CL) is endemic and the principal form of leishmaniasis in the Republic of Suriname (South-America), where it is locally known as *bosyaws* or *busiyasi* (Flu, 1911; Van der Meide et al., 2008c). This condition is mainly caused by *Leishmania (Viannia) guyanensis* (Lai et al., 2002; Van der Meide et al., 2008c), but other *Leishmania* species such as *L. (V) naiffi* and *Leishmania (Leishmania) amazonensis,* have also been identified in the country (Van der Meide et al., 2008a, 2008b; Van Thiel et al., 2010; Hu et al., 2012). CL is particularly prevalent in Suriname's rural-interior hinterland. Its victims are in general individuals who intrude into the parasites' habitat such as residing Indigenous and Maroon people as well as eco-tourists, recreational hunters and fishers, and personnel of gold mining, bauxite mining, and logging companies (Van der Meide et al., 2008c). This has led in the year 2008 to 5.32–6.13 infections per 1000 inhabitants for Suriname's hinterland, and 0.64–0.74 per 1000 for the entire country (Van der Meide et al., 2008c).

The current first-line drug and the only treatment option for CL caused by *L. (V.) guyanensis* in Suriname is pentamidine isethionate administered with three intramuscular injections of 300 mg each during a seven-day period (Lai et al., 2002). Unfortunately, this drug - developed more than fifty years ago - is rather toxic and prone to cause resistance (Croft et al., 2006). Furthermore, poor compliance - probably attributable to the lack of adequate health education - may lead to incomplete therapy and sub-therapeutic drug levels in the plasma, another reason for the development of drug-tolerant parasites (Croft et al., 2006). Notably, the substantial expenses for treatment and transportation to health centers in Paramaribo together with loss of income due to the inability to work, often represent considerable obstacles for affected individuals to seek medical treatment.

These considerations may lead patients to attempt self-treatment, using, among others, traditional plant-based remedies (Ramdas, 2015). Contributing to this inclination is Suriname's comprehensive ethnobotanical knowledge (Van Andel and Ruysschaert, 2011) and unique plant biodiversity that provides ample raw material to be processed into ethnopharmacological preparations for treating CL (DeFilipps et al., 2004). So far, however, the scientific evidence to support the therapeutic usefulness of these substances is scant. If efficacious, some of them may represent lead compounds for the development of novel drugs for treating CL. For this reason, a number of plants that are used in Suriname against this disease were evaluated for their activity against cultured promastigotes and amastigotes from various *Leishmania* species including *L. (V.) guyanensis*. To obtain an indication about the selectivity of the plant preparations, their potential anti-leishmanial effect was compared with their cytotoxicity against cultured human THP-1 monocytic cells. In all cases, the well-established anti-leishmanial drug amphotericin B served as a reference compound.

2. Materials and methods

2.1. Plant collection and preparation of plant extracts

The plants investigated in the current study were selected on the basis of a previous comprehensive survey on ethnopharmacological remedies for treating CL (Ramdas, 2015). The survey involved a series of open non-structured interviews with members, key informants, and traditional practitioners of Indigenous and Maroon communities as well as gold mining workers in the interior of Suriname who are knowledgeable about traditional forms of treatment of CL (Ramdas, 2015). The interviews were conducted in the period 2010–2011, and provided information 25 plants that are ethnopharmacologically used for treating CL, the plant parts used, their use as dried or fresh preparations, and the methods of extraction (Table 1). This information was cross-checked with previously published data on plants that are traditionally used for treating CL compiled by De Filipps et al. (2004) and Van Andel and Ruysschaert (2011).

On the basis of this information, the indicated plant parts were collected in Suriname's hinterland in areas that had been free of herbicidal or pesticidal use for at least the preceding six months. The collections were done in close collaboration with the National Herbarium of Suriname that is in the possession of a collection permit from the Surinamese Ministry of Physical Planning, Land- and Forestry Management. Notably, none of the collected plants are on the International Union for Conservation of Nature's Red List of endangered or threatened species (IUCN, 2015).

The collected samples were thoroughly washed with distilled water, dried in open air, washed again, processed as indicated in Table 1, filtered, freeze-dried so as to obtain a stable powder, and divided in aliquots of 2 g which were stored at -20 °C before being shipped on dry ice to The Netherlands. The plant extracts were subsequently liquefied in ultra-pure water to a concentration of 10 mg/mL and further dissolved by shaking for 1 h at 50 °C. The suspensions were then centrifuged for 5 min at 3000 rpm after which the supernatants were collected, sterilized through 0.22 μM filters (Millipore), and stored at -20 °C until tested.

2.2. Drugs and chemicals

Fetal bovine serum (FBS), phorbol 12-myristate 13-acetate (PMA), tris(hydroxymethyl)-aminomethane) (Tris), ethylenediamminetetraacetic acid (EDTA), phenylmethanesulfonyl fluoride (PMFS), nicotinamide adenine dinucleotide phosphate (NADPH), and 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were from Sigma-Aldrich Co (St. Louis, MO, USA). Alamar Blue dye was from AbD Serotec (Düsseldorf, Germany), RPMI 1640 medium from Life Technologies (Bleiswijk, The Netherlands), Triton X-100 from BDH Laboratory Supplies (Poole, UK), and trypanothione disulfide ($T[S]_2$) from Bachem AG (Budendorf, Switzerland). All other chemicals used were from the laboratory stock of the Parasito logy Unit, KIT Biomedical Research, Royal Tropical Institute (Amsterdam, The Netherlands), and were of the highest grade available.

2.3. Maintenance and culture of leishmania parasite isolates and THP-1 cells

The promastigote forms of *L. (V.) guyanensis* (strain AMC 2014), *L. (L.) major* (strain MHOM/IR/1972/NADIM5), and *L. (L.) donovani* (strain GEDII, a fresh isolate from Gaderif State, Sudan maintained in the KIT Biomedical Research laboratory since 2013, or strain BHU-814, a recent isolate from Bihar State, India, which is only used for the amastigote drug susceptibility assay) were cultured axenically in RPMI 1640 medium containing 25 mM HEPES and 2 mM *L*-glutamine and supplemented with 15% (v/v) heat-inactivated FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin. The promastigotes were maintained at 27 °C by weekly sub-culturing. The *L. guyanensis* AMC 2014 strain (donated by the Academic Medical Centre, Amsterdam, The Netherlands) which was recently isolated from a patient in Suriname, was originally maintained in NNN medium but adapted to the supplemented RPMI 1640 medium for the current study.

THP-1 human acute monocytic leukemia cells (Tsuchiya et al., 1980) were from ATCC (no. TIB 202) and maintained in RPMI 1640 medium containing 25 mM HEPES and 2 mM *L*-glutamine and supplemented with 10% (v/v) heat-inactivated FBS, 100 IU/mL penicillin, and 100 μg/mL streptomycin at 37 °C at a minimum

relative humidity of 95% and in a 5%-CO_2 atmosphere.

2.4. Promastigote and THP-1 cell drug susceptibility assay

Susceptibility of *Leishmania* promastigotes and THP-1 monocytic cells to the plant extracts was assessed as previously described by van den Bogaart et al. (2014). In brief, triplicate cultures of promatigotes of *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII, at densities of 2×10^5 parasites/100 µL, or 2×10^4 THP-1 monocytic cells/100 µL medium per well in 96-well flat-bottomed microplates, were exposed to the plant extracts at serial dilutions of 500–15,625 µg/mL. *L. (L.) major* also causes CL but is not present in Suriname, while *L. (L.) donovani* causes visceral leishmaniasis and is also not present in Suriname. However, the promastigotes from *L. major* NADIM5 and *L. donovani* GEDII have been incorporated in the study besides those from *L. guyanensis* AMC, in order to determine whether or not the test compounds exhibited specificity for (a) particular *Leishmania* species. Incubations were for 72 h in final volumes of 200 µL per well. Amphotericin B at serial dilutions ranging from 0.0007 to 0.231 µg/mL served as a reference compound, and untreated parasites or THP-1 cells incubated with medium only were used as controls. The use of amphotericin B as a reference compound rather than pentamidine isethionate was based on the previous assessment of the efficacies of miltefosine, pentamidine, amphotericin B, and paromomycin as standard drugs against *L. donovani* and *L. major* promastigotes and intracellular amastigotes that showed that the two former compounds - unlike the two latter - exhibited differential effects toward the different parasite species and developmental stages (van den Bogaart et al., 2014).

At the end of the incubations, responses of the parasite and monocyte cultures were assessed using a method based on staining with Alamar Blue dye (Mikus and Steverding, 2000). A number of pilot experiments performed in our laboratory indicated that the plant extracts (alone) interfered with the Alamar Blue reduction. For this reason, the method was slightly modified in order to enhance its performance. Thus, the contents of the flat-bottomed plates was transferred to V-bottomed plates which were centrifuged for 10 min at 3000*g*, the supernatants were discarded, and the pelleted parasites or THP-1 cells were re-suspended in 100 µL complete RPMI medium containing 10% (w/v) Alamar Blue dye. After thorough mixing, the contents of the V-bottomed plates was transferred back to new flat-bottomed plates which were incubated for 4.5 h at 27 °C in the case of the promastigotes and for 2 h at 37 °C and 5% CO_2 in the case of the THP-1 cells.

After incubation, fluorescence was measured with an Infinite M200Pro multimode plate reader (Tecan, Männedorf, Switzerland) using excitation and emission wavelengths of 560 and 590 nm, respectively, and the values obtained were plotted against plant extract concentrations after correction for background fluorescence. The latter was determined from blank wells which had received medium only. Dose–response profiles were constructed and a non-linear regression analysis (GraphPad Prism 5.03, San Diego, CA) was performed to obtain the IC_{50} values *i.e.*, plant extract concentrations resulting in 50% inhibition of the growth of the parasites or the monocytes when compared to untreated controls.

2.5. Amastigote drug susceptibility assay

This procedure has been described in detail by van den Bogaart et al. (2014). It should be noted that this assay has been set-up for *L. donovani* BHU-814 strain only as it was previously shown that *L. major*, *L. guyanensis* and *L donovani* GEDII failed to efficiently infect the THP-1 cells (infection grade less than 30%). Therefore, the amastigote-phagocyte assay could not be validated with these strains, and the *L. donovani* BHU814 was used instead.

In brief, triplicate cultures of THP-1 monocytic cells were inoculated at densities of 1×10^5/200 µL per well in 96-well flat-bottomed microplates and stimulated for 48 h with 10 ng/mL PMA to obtain differentiated, non-dividing macrophage-like cells. After removing the PMA by three times washing with medium, the THP-1 cells were subjected to infection by stationary-growth phase promastigotes of *L. donovani* BHU-814 (5-day old cultures) at a ratio of 10 parasite per THP-1 cell. The use of stationary growth phase promastigotes was based on the fact that there are more metacyclic promastigotes in this growth phase which can infect the macrophages more efficiently (Inocêncio da Luz et al., 2009). Sixteen hours later, promastigotes that had failed to infect the macrophages were removed by washing three times with RPMI 1640 medium. Next, infected cells were exposed to the plant extracts at serial dilutions (range 500–15,625 µg/mL) or to medium only (untreated control). A blank comprising uninfected THP-1 cells with no exposure to plant extracts was also included. Amphotericin B was used as a reference compound (positive control for drug action) at serial dilutions ranging from 0.007 to 0.231 µg/mL. All incubations were for 96 h at 37 °C and at 5% CO_2.

At the end of the incubations, the number of infected monocytes was assessed using the *Leishmania*-specific trypanothione reductase (TryR)-based assay (van den Bogaart et al., 2014). To this end, cells were first washed and then incubated for 15 min with a lysing buffer consisting of 2% (w/v) Triton X-100 in PBS supplemented with 1 mM EDTA, 40 mM HEPES, and 1 mM PMFS and buffered with 50 mM Tris pH 7.5. Of each lysate, 75 µL was transferred to the wells of 96-well microplates and incubated for 3 h at 27 °C with 200 µM NADPH (25 µL), 75 µ M $T[S]_2$ (75 µL), and 100 µM DTNB (25 µL). Absorbances were measured with an Infinite M200Pro multimode plate reader (Tecan, Männedorf, Switzerland) at a wavelength of 412 nm, and corrected for the corresponding blanks where $T[S]_2$ was replaced by Tris 0.05 M buffer pH 7.5. The inhibition of parasite growth was determined by comparison of the signal produced by drug-treated parasites with that of untreated control parasites. Non-linear regression was used for curve fitting and calculation of 50% inhibitory concentrations (IC_{50} values).

2.6. Data processing

All experiments have been carried out at least three times, and the data obtained have been expressed as means with 95% confidence intervals (CIs) and are given in Table 2. Comparisons for statistically significant differences have been done with Student's *t* test, and p values < 0.05 have been taken to indicate statistically significant differences.

2.7. Ethical considerations

The studies described by Ramdas (2015) including the inquiries about the plants traditionally used for treating CL, have been approved by the Surinamese national health and village authorities. This included approval from the Committee for Research Involving Human Subjects from the Surinamese Ministry of Health (file number VG 006–2009), as well as from the captains and traditional chiefs of the villages where the interviews with individuals holding information about traditional forms of treatment of CL took place. Prior to each interview, an explanatory text was read to each village head and each informant in his/her native language, and an informed consent form was signed to confirm that the person's participation was voluntary.

Table 2
IC_{50} values (95% CI) of *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII promastigotes, *L. donovani* BHU814 amastigotes, and THP-1 cells in response to exposure to amphotericin B or plant extracts.

	IC_{50} (μg/mL)				
	L. guyanensis AMC promastigotes	*L. major* NADIM5 promastigotes	*L. donovani* GEDII promastigotes	*L. donovani* BHU814 amastigotes	THP-1 cells
Amphotericin B	0.012 (0.011–0.013)	0.032 (0.028–0.034)	0.035 (0.024–0.052)	0.012 (0.011–0.046)	> 0.231
A. esculentus	> 500	> 500	> 500	> 500	> 500
A. sativum	340 (208–555)	> 500	> 500	396 (7–20,863)	364 (155–856)
A. vera	> 500	> 500	> 500	> 500	> 500
A. occidentale	> 500	230 (133–396)	395 (277–562)	> 500	169 (113–252)
A. indica	> 500	187 (152–231)	247 (170–358)	451 (112–1,821)	352 (299-85)
B. pinnatum	> 500	> 500	> 500	> 500	42 (21–85)
C. peltata	354 (299–419)	424 (344–524)	321 (278–370)	> 500	312 (115–415)
C. aurantifolia	> 500	> 500	> 500	> 500	> 500
C. esculenta	> 500	> 500	> 500	> 500	> 500
C. cujete	> 500	> 500	> 500	> 500	> 500
D. seguine	> 500	> 500	> 500	> 500	> 500
E. prostrata	> 500	> 500	> 500	> 500	> 500
G. barbadense	> 500	> 500	> 500	> 500	156 (116–211)
H. courbaril	306 (211–443)	341 (287–405)	297 (203–435)	> 500	130 (95–177)
I. alba	435 (312-606)	298 (230–385)	205 (49–2339)	224 (96–524)	< 16
I. batatas	> 500	427 (347–526)	> 500	> 500	> 500
M. micrantha	> 500	> 500	402 (351–460)	403 (283–825)	128 (43–378)
M. citrifolia	> 500	> 500	> 500	> 500	> 500
Musa sp.	> 500	> 500	> 500	> 500	> 500
Q. amara	> 500	51 (44–60)	68 (60–77)	288 (10–8477)	< 16
S. alata	> 500	> 500	> 500	> 500	> 500
S. lycocarpum	51 (36-73)	61 (45–178)	< 16	374 (289–405)	> 500
S. mombin	> 500	> 500	> 500	> 500	209 (169–260)
U. guianensis	> 500	> 500	> 500	> 500	337 (251–452)
V. guianensis	> 500	426 (329–554)	401 (359–449)	> 500	153 (67–352)

3. Results

3.1. Growth responses of promastigotes, amastigotes, and THP-1 cells to amphotericin B

As shown in Table 2, amphotericin B was active against *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII promastigotes, as well as against *L. donovani* BHU814 amastigotes, displaying IC_{50} values that ranged from about 0.012 to about 0.035 μg/mL, but inhibited the growth of THP-1 cells at 12- to 72-fold higher concentrations. This observation is in agreement with previous observations (van den Bogaart et al., 2014) and validates the use of the promastigote and amastigote-phagocyte assay for evaluating the potential anti-leishmanial efficacy of the plant extracts.

3.2. Growth responses of promastigotes, amastigotes, and THP-1 cells to the extract from Solanum lycocarpum A.St.-Hil.

Table 2 summarizes the growth responses of *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII promastigotes, *L. donovani* BHU814 amastigotes, and THP-1 cells to the 25 plant extracts evaluated in the current study.

The preparation from *Solanum lycocarpum* A.St.-Hil. elicited the greatest anti-leishmanial activity, displaying cytotoxic effects against *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII promastigotes at IC_{50} values of 51 (36–73), 61 (45–178), and < 16 μg/mL, respectively. This preparation produced a much higher IC_{50} value in *L. donovani* BHU814 amastigotes-374 (289–405) μg/mL-but had hardly any effect on the growth of THP-1 cells at the concentration tested (IC_{50} value > 500 μg/mL). These observations suggest that the *S. lycocarpum* extract was relatively toxic to the *Leishmania* promastigotes and did not affect normal human cells, but was only moderately active against *Leishminia* amastigotes.

3.3. Growth responses of promastigotes, amastigotes, and THP-1 cells to the extracts from Bryophyllum pinnatum (Lam.) Oken, Inga alba (Sw.) Willd., and Quassia amara L.

The extract from *Bryophyllum pinnatum* (Lam.) Oken had almost no effect on the growth of *L. guyanensis* AMC, *L. major* NADIM5, and *L. donovani* GEDII promastigotes as well as that of *L. donovani* BHU814 amastigotes (IC_{50} values > 500 μg/mL; Table 2) but was relatively toxic towards the THP-1 cells (IC_{50} value of 42 (21–85) μg/mL; Table 2).

The extra ct from *Inga alba* (Sw.) Willd. also inhibited the growth of the promastigotes and amastigotes at relatively high IC_{50} values (205–435, and 224 μg/mL, Table 2), but was highly toxic towards the THP-1 cells (IC_{50} value of < 16 μg/mL).

And the *Quassia amara* L. sample had almost no effect on the growth of *L. guyanensis* AMC promastigotes (IC_{50} value > 500 μg/mL), but inhibited that of *L. major* NADIM5 and *L. donovani* GEDII promastigotes at IC_{50} values of 51 and 68 μg/mL, respectively (Table 2), and that of *L. donovani* BHU814 amastigotes at the relatively high IC_{50} value of 288 μg/mL (Table 2). However, this preparation was, similarly to the *I. alba* extract, highly cytotoxic against THP-1 cells (IC_{50} value < 16 μg/mL.

Together, these observations suggest that the *B. pinnatum*, *I. alba*, and *Q. amara* extracts were (much) more toxic to normal cells than to *Leishmania* promastigotes or amatigotes, and/or that their toxicity to normal human cells surpassed their potential anti-leishmanial activity.

3.4. Growth responses of promastigotes, amastigotes, and THP-1 cells to the remaining plant extracts

The remaining plant extracts were hardly cytotoxic to promastigotes, amastigotes, and THP-1 cells, displaying IC_{50} values that ranged from 100 μg/mL to (far) in excess of 500 μg/mL (Table 2).

4. Discussion

In this study, 25 plant extracts from Suriname have been evaluated for their potential efficacy against *Leishmania* parasites using a promastigote and an amastigote–macrophage assay, as well as against cultured human THP-1 monocytic cells to assess their selectivity. Unfortunately, the *Leishmania* species most important for Suriname - *L. (V.) guyanensis* - showed a very low THP1-infecting grade and could therefore not be incorporated in the amastigote–macrophage assay. *L. (V.) guyanensis* was earlier reported as notoriously inefficient to infect macrophages (Inocêncio da Luz et al., 2009), and therefore we had to use the *L. donovani*-based amastigote assay in this case. Nevertheless, the most promising outcome of the present study was that the extract from *S. lycocarpum* leaves was relatively toxic towards the promatigotes while hardly affecting the viability of the THP1 cells. This suggests that this phytocomplex may possess useful anti-leishmanial properties while exhibiting low cytotoxicity against human cells.

Partial support for the apparent anti-leishmanial activity of the *S. lycocarpum* preparation highlighted by the current study is provided by the significant anti-leishmanial effect of solamargine and solasonine - the principal glycoalcaloids in the fruits of *S. lycocarpum* - displayed *in vitro* activity towards promastigotes of *L. (L.) amazonensis* (Miranda et al., 2013). These compounds exhibited IC_{50} values that were lower than those found in parallel experiments against cultured LLC-MK2 rhesus monkey kidney cells (Miranda et al., 2013), indicating that the cytotoxic effects of the glycoalkaloids were fairly *Leishmania*-specific. Notably, solamargine and solasonine are also present in the leaves of *S. lycocarpum* (Tiossi et al., 2012) as well as in various parts of many other members of the plant family Solanaceae (Bajak et al., 1979; Jaggi and Kapoor, 1994).

However, it should not be overlooked that the *S. lycocarpum* extract was only modestly cytotoxic against *L. donovani* amastigotes in the current study. Indeed, *in vitro* activity against this mammal-infective stage of *Leishmania* is clinically much more more relevant than activity against the insect-infective stage of the parasite (De Muylder et al., 2011). Furthermore, screening of candidate compounds against the promastigote assay reportedly leads to the identification of 50% false positives, necessitating re-evaluation of the test compounds with the more appropriate amastigote assay (De Muylder et al., 2011). For these reasons, the current results with the *S. lycocarpum* preparation, even though encouraging, should be regarded with caution.

Still, it is important to note that preparations from *Solanum* species are extensively used in various parts of the world for treating CL. Examples are the leaves of *S. americanum (Mill.)* in an endemic area in Bahia, Brazil (França et al., 1996); the leaves and fruits of *S. nigrum* L, *Nicotiana tabacum* L., and/or *Capsicum annuum* L. in rural Ecuador (Weigel and Armijos, 2001); the fruits of *S straminifolium* var. *straminifolium* Jacq. and the aerial part of *S. peruvianum* L. by the Chayahuita and the Yanesha ethnic groups, respectively, in Peru (Estevez et al., 2007; Valadeau et al., 2009); and the bark of *S. crinitum* Lam. and *S. subinerme* Jacq. by the Wayãpi Amerindian of the Oyapock basin in French Guiana and other parts of the Guiana Shield (Odonne et al., 2011). Testing of ethanol extracts of the two latter samples along with chloroform extracts of the aerial parts of *S. sisymbriifolium* Lam. revealed moderate to high activity against amastigotes and/or promastigotes of *L. (L.) amazonensis* and/or *L. (V.) braziliensis* (Estevez et al., 2007; Valadeau et al., 2009; Cechinel Filho et al., 2013). These findings further support the potential anti-leishmanial activity of the *S. lycocarpum* leaf extract observed in the current study. More comprehensive *in vitro*, *in vivo*, and clinical studies should more definitely establish the value of such a preparation in the treatment of CL.

Preparations from *B. pinnatum*, *I. alba*, and *Q. amara* are used against CL by, among others, certain rural communities in Ecuador (Gachet et al., 2010), an Indigenous community in Junín, Peru (Luziatelli et al., 2010), the above-mentioned Wayãpi Amerindians from the Guiana Shield (Odonne et al., 2011), and the inhabitants of the Bandundu province in DR Congo (Musuyu Muganza et al., 2012). Furthermore, there is some preclinical and clinical evidence for anti-leishmanial activity of the two former preparations (Rossi-Bergmann, 2000; Torres-Santos et al., 2003; Bhattacharjee et al., 2009).

However, the results from the current study showed that these plant extracts affected the viability of the THP-1 cells at relatively low concentrations while displaying only modest or hardly any activity against *Leishmania* promastigotes or amatigotes. This suggests that these samples contain substances that are highly cytotoxic but lack meaningful leishmanicidal activity. The former supposition is supported by the presence - at least in *Q. amara* preparations - of highly cytotoxic quassinoids that often exert *in vitro* cytotoxic effects against cancer cells at (much) lower concentrations when compared to, for instance, their antimalarial and antileishmanial effects (Xu et al., 2000; Guo et al., 2005; Muhammad and Samoylenko, 2007; Houël et al., 2009). Support for the latter suggstion comes from reports mentioning that the active constituents of *B. pinnatum* and *Q. amara* extracts - flavonoids (Muzitano et al., 2006, 2009) and quassinoids (Bhattacharjee et al., 2009), respectively - do not exert direct cytotoxicity against the parasites, but improve the host's immune response to *Leishmania* infection (Bhattacharjee et al., 2009) and activate the reactive nitrogen intermediates pathway of macrophages (Da Silva et al., 1995, 1999; Kolodziej et al., 2001), respectively. Future studies should verify these suppositions and account for the apparently high cytotoxicity of the *I. alba* preparation.

The remaining plant extracts were virtually ineffective towards *Leishmania* promastigotes and amastigotes as well as THP-1 cells. Thus far, there are no experimental data available about a possible anti-leishmanial activity of preparations from *C. esculenta*, *C. cujete*, *G. barbadense*, *A. esculentus*, *I. batatas*, and *U. guianensis*. However, the negative results with preparations from *A. occidentale*, *C. peltata*, *D. seguine*, *E. prostrata*, *M. paradisiaca*, *S. alata*, and *V. guianensis* are in accordance with the nonexistent or relatively modest anti-leishmanial effects in cultured promastigotes and/or amastigotes from various *Leishmania* species exposed to an ethanolic extract from the stem bark of *A. occidentale* (Braga et al., 2007); alcoholic extracts of *C. peltata* (García et al., 2012); preparations from *D. seguine* and its family members *D. aff. costata* H. Karst. ex Schott and *D. williamsii* Croat (Grenand et al., 2004; Estevez et al., 2007; Valadeau et al., 2009); saponins from *E. prostrata* (Khanna et al., 2014); fractions of an ethanolic extract of *M. paradisiaca* (Accioly et al., 2012); ethanolic extracts from leaves and roots of *S. alata* (Espinoza et al., 2009); and ethanolic extracts from the bark of the *Vismia* species *V. tomentosa* Ruiz and Pav. (Estevez et al., 2007) and from bark, leaves, and stem of *V. baccifera* (L.) Triana and Planch, *V. pozuzoensis* Engl., and *Vismia sp.*, respectively (Valadeau et al., 2009).

On the other hand, the lack of anti-leishmanial effects of the *A. sativum*, *A. vera*, *A. indica*, *C. aurantifolia*, *H. courbaril*, *M. micrantha*, and *S. mombin* extracts noted in the current study is at variance with literature data reporting the opposite (Da Silva et al., 1995; Torres-Santos et al., 2003; Khalid et al., 2005; Arruda et al., 2009; Accioly et al., 2012; Carneiro et al., 2012; Iqbal et al., 2012; Laurella et al., 2012; Ribeiro et al., 2014). However, in at least some of these cases, these apparent discrepancies might be attributed to differences in experimental conditions. For instance, rather than with water, various of the samples mentioned in the literature had been extracted with methanol (*A. vera* leaves; Iqbal et al., 2012), ethanol

(*H. courbaril* leaves, Ribeiro et al., 2014; *S. mombin* leaves; Accioly et al., 2012), or apolar solvents (*A. indica* fruits, Carneiro et al., 2012; *M. micrantha* leaves, Laurella et al., 2012; *H. courbaril* leaves, Ribeiro et al., 2014). Also, instead of a preparation from the stem bark, the current study evaluated an extract from the leaves of *M. citrifolia*, while the former rather than the latter sample was effective in patients with CL caused by *L. (L.) major* (Sattar et al., 2012; Siddiqui et al., 2014).

Summarizing, the results from this study suggest that preparations from *S. lycocarpum* could potentially be used for treating CL while causing tolerable side-effects. In light of the lack of efficacious forms of CL treatment, this observation makes this plant an interesting candidate for new drug development programs. On the other hand, preparations from *B. pinnatum*, *I. alba*, and *Q. amara* should be avoided because of apparently little therapeutic benefit but the possibility of excessive toxicity. At this moment, preparations are being made to identify the pharmacologically active principle(s) in the *S. lycocarpum* extract applying bioassay-guided purification. Also, the plant extracts that turned out inactive in the current study but have been reported by others to possess leishmanicidal properties, will be retested under more appropriate experimental conditions.

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