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ORIGINAL ARTICLE

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Cytotoxicity and anti-*Leishmania amazonensis* activity of *Citrus sinensis* leaf extracts

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ABSTRACT

Context: *Leishmania amazonensis* is the main agent of diffuse cutaneous leishmaniasis, a disease characterized by lesional polymorphism and the commitment of skin surface. Previous reports demonstrated that the *Citrus* genus possess antimicrobial activity.

Objective: This study evaluated the anti-*L. amazonensis* activity of *Citrus sinensis* (L.) Osbeck (Rutaceae) extracts.

Materials and methods: *Citrus sinensis* dried leaves were subjected to maceration with hexane (CH), ethyl acetate (CEA), dichloromethane/ethanol (CD/Et – 1:1) or ethanol/water (CEt/W – 7:3). *Leishmania amazonensis* promastigotes were treated with *C. sinensis* extracts (1–525 μg/mL) for 120 h at 27 °C. Ultrastructure alterations of treated parasites were evaluated by transmission electron microscopy. Cytotoxicity of the extracts was assessed on RAW 264.7 and J774.G8 macrophages after 48-h treatment at 37 °C using the tetrazolium assay. In addition, *Leishmania*-infected macrophages were treated with CH and CD/Et (10–80 μg/mL).

Results: CH, CD/Et and CEA displayed antileishmanial activity with 50% inhibitory activity (IC_{50}) of 25.91 ± 4.87, 54.23 ± 3.78 and 62.74 ± 5.04 μg/mL, respectively. Parasites treated with CD/Et (131.2 μg/mL) presented severe alterations including mitochondrial swelling, lipid body formation and intense cytoplasmic vacuolization. CH and CD/Et demonstrated cytotoxic effects similar to that of amphotericin B in the anti-amastigote assays (SI of 2.16, 1.98 and 1.35, respectively). Triterpene amyrins were the main substances in CH and CD/Et extracts. In addition, 80 μg/mL of CD/Et reduced the number of intracellular amastigotes and the percentage of infected macrophages in 63% and 36%, respectively.

Conclusion: The results presented here highlight *C. sinensis* as a promising source of antileishmanial agents.

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Antileishmanial activity; macrophage infection; cutaneous leishmaniasis; citrus genus

Introduction

American tegumentary leishmaniasis (ATL) is a noncontagious infectious disease caused by parasites from the *Leishmania* genus, affecting the skin [cutaneous leishmaniasis (CL)] and mucosa [mucocutaneous leishmaniasis (MCL)] (Brazil, Ministry of Health 2010). In Brazil, *L. guyanensis*, *L. braziliensis* and *L. amazonensis* are the main dermotropic species. The last two species are widely distributed in the country, with cases of infection reported in all Brazilian states (Lima et al. 2017). In 2012, Brazil reported over 5000 new cases of tegumentary leishmaniasis (Carvalho et al. 2015). *Leishmania amazonensis* is also the aetiological agent of a much less common form of the disease known as diffuse cutaneous leishmaniasis (DCL), which is characterized by skin diffuse infiltration and the appearance of papules, tubers, infiltrated plaques and nodules (Barral et al. 1995).

Leishmaniasis treatment relies on the use of pentavalent antimonials (*N*-methylglucamine antimoniate and sodium stibogluconate). In addition, amphotericin B (liposomal and conventional) is used as an alternative chemotherapeutic drug in severe cases of unresponsive leishmaniasis. In uncomplicated CL cases, the use of antimony in combination with cryotherapy is often recommended (de Vries et al. 2015). Other drugs introduced for CL treatment include pentamidine and paromomycin. However, all of these drugs may lead to serious side effects, high toxicity or potentially induce parasite resistance (Berman 1998; Gontijo & Carvalho 2003; Jhingran et al. 2009).

Citrus sinensis (L.) Osbeck (Rutaceae), popularly known as sweet orange, is originally from Southern China, North Eastern India and Southern Asia (Ekwenye & Edeha 2010). It was first introduced in Brazil in the beginning of colonization. *C. sinensis* is a perennial tree of 5–10 m in height containing aromatic leaves, flowers and fruits (Lorenzi et al. 2006). It is widely cultivated for commercial purposes (fruit, juice, essential oil). Extracts obtained from its leaves or fruit peels have been used in folk

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medicine for the treatment of various ailments (Siddique et al. 2012). Previous studies have described diverse biological activities for different extracts and essential oils of *C. sinensis*, including antiosteoporotic (Shalaby et al. 2011), antifungal, anti-aflatoxigenic, antioxidant (Sharma & Tripathi 2008; Singh et al. 2010) and antibacterial (Pittman et al. 2011) activities. Here, we evaluated the antileishmanial activity and toxic potential of different extracts obtained from the leaves of *C. sinensis*.

Materials and methods

Chemicals

The solvents used in extraction procedures were of spectroscopic grade from Tedia Brazil (Rio de Janeiro, RJ, Brazil). Dulbecco's modified Eagle's medium (DMEM), Schneider's Drosophila medium, resazurin, amphotericin B and thiazolyl blue tetrazolium bromide (MTT) were obtained from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS) was purchased from LGC Biotecnologia (São José, Cotia, Brazil).

Plant material collection and identification

Citrus sinensis leaves were collected in Nova Friburgo (State of Rio de Janeiro, Brazil) between the months of June and July 2014 at 9–10 a.m. Samples were authenticated by Dr. Rosana C. Lopes at the RFA Herbarium, Department of Botany, Federal University of Rio de Janeiro (IB/UFRJ), where a voucher specimen is deposited (n. 39990). Intact leaves of *C. sinensis* were dried at 40 °C and kept intact under refrigeration (4 °C) until extraction procedures.

Extraction procedures

Citrus sinensis dried leaves were crushed and subjected to static maceration at room temperature for 120 h with different solvents: hexane (100%), dichloromethane/ethanol (1:1), ethyl acetate (100%) or ethanol/water (7:3). After the maceration period, solvents were removed using a rotary evaporator at 40 °C, giving rise to the CH, CD/Et, CEA and CEt/W extracts, respectively. The dried extracts were stored at 4 °C protected from light. Stock solutions (100 mg/mL) of each extract were prepared using dimethyl sulfoxide (DMSO). Then, subsequent dilutions were made so that the final DMSO concentration did not exceed 0.5% in the biological assays.

L. amazonensis *culture*

Promastigote forms of *L. amazonensis* (IFLA/BR/1967/PH8) were obtained from the *Leishmania* Type Culture Collection (LTCC) of Oswaldo Cruz Institute/Fiocruz (Rio de Janeiro, RJ, Brazil). Parasites were maintained by weekly subcultures in Schneider's Drosophila medium supplemented with 10% FBS.

Antipromastigote activity

Growth inhibition assays were carried out in 96-well microplates where the *C. sinensis* extracts CH, CD/Et, CEA and CEt/W were serially diluted in concentrations ranging from 1 to 525 μg/mL. Then, *L. amazonensis* promastigote forms collected in early stationary phase (96 h) were added to the microplate at the final concentration of 5.0×10^6 parasites/mL. The microplates were incubated at 27 °C for 120 h. At the end of the incubation period, resazurin at a concentration of 0.00083% was added to the plates and parasite viability was determined by measuring the absorbance at 490 and 595 nm in a SpectraMax M5 (Molecular Devices, Sunnyvale, CA), as previously described (Rolón et al. 2006). Amphotericin B (reference drug) was used as a positive control (concentrations ranging from 0.031 to 1 μg/mL). The 50% inhibitory concentration (IC_{50}) was calculated by logarithmic regression analysis using Microsoft Excel 2013. Alternatively, the wells that showed no cellular growth had their supernatants collected, centrifuged and washed twice with 0.9% saline buffer. The resulting pellets were resuspended in fresh culture medium in the absence of the extracts and incubated at 27 °C for 120 h in order to observe parasite growth. The lack of turbidity represented a leishmanicidal activity, while the presence of turbidity demonstrated that the extracts exerted a leishmanistatic effect. In addition, the lack or presence of growth was confirmed by light microscopy. The inhibitory assays were performed in triplicate.

Transmission electron microscopy (TEM)

Promastigote forms of *L. amazonensis* (10^6 parasites/mL) were harvested at the early stationary phase, washed twice with 0.9% saline buffer and treated with CD/Et (the most promising extract from *C. sinensis*) at MIC and subMIC (MIC/2) concentrations. Parasites were incubated at 27 °C for 24 h. Negative controls were made using untreated parasites. At the end of treatment, parasites were washed twice with 0.9% saline buffer and fixed with 0.9% glutaraldehyde solution (2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer containing 3.5% sucrose, pH 7.4) at 4 °C for 60 min. The samples were sent to the Rudolf Barth Platform (FIOCRUZ/RJ) and processed as previously described (Rodrigues et al. 2013). Image acquisition was performed in a JEOL JEM1011 transmission electron microscope (JEOL Inc., Peabody, MA).

Cytotoxicity assay

Cytotoxicity of CH and CD/Et extracts (the most active extracts) against J774.G8 and RAW 264.7 macrophages was evaluated. Cells were maintained in polystyrene culture flasks containing DMEM culture medium supplemented with 10% FBS and cultured at 37 °C and 5% CO_2. For the cytotoxic assays, 48-h cultured macrophages were washed twice with culture medium and then released from the culture flasks. A suspension of 10^6 cells/mL was prepared, from which 100 μL was transferred to a 96-well microplate. After 24 h of incubation, cell cultures were submitted to different concentrations (15 to 260 μg/mL) of *C. sinensis* crude extracts and incubated for additional 48 h. The minimal cytotoxic concentration (MCC) was determined by the MTT ((3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium reduction assay (Mosmann 1983). Absorbance was measured at 570 nm in a SpectraMax M5 spectrophotometer (Molecular Devices, CA). The 50% cytotoxic concentration (CC_{50}) was calculated by logarithmic regression analysis using Microsoft Excel 2013. The cytotoxicity assays were performed in triplicate.

Analysis of C. sinensis *CH and CD/Et extract by thin layer chromatography (TLC)*

TLC was performed using silica gel plates and 7:3 hexane:acetate as solvent system. The purple spots in the chromatogram,

indicative of terpenoids, were visualized after reaction with vanillin–sulphuric acid reagent.

Analysis of CH and CD/Et extract by gas chromatography-mass spectrometry (GC-MS)

The TLC analysis showed that the main substances present in CH and CD/Et were terpenoids with low polarity and probably volatiles. Therefore, the extracts were analyzed by GC-MS to determine its components. The heating temperature was ramped from 70 °C to 305 °C at 5 °C/min, and the helium carrier gas was set to1.5 mL/min. A 1% solution of each extract (1 µL) was injected in split mode. Mass spectra were obtained in an Agilent Technologies 6890 N system, adjusted with 5% diphenyl/95% dimethyl polysiloxane-fused silica capillary column (HP – 5 MS, 30 x 25 mm, 0.25 µm) operating in electron ionization mode at 70 eV with a scan mass range of 40–700 *m/z*. The identification of the extract components was made by comparing the obtained mass spectra with the apparatus Wiley library.

Anti-intracellular amastigote activity

Raw 264.7 macrophages were cultured in DMEM culture medium supplemented with 10% FBS at 37 °C and 5% CO_2 atmosphere. After 48-h culture, the cells were harvested, washed twice with PBS (150 mM NaCl; 20 mM phosphate buffer, pH 7.2) and allowed to adhere on coverslips placed in 24-well culture plates at the concentration of 2.0×10^5 cells/well. After 24-h incubation at 37 °C and 5% CO_2 atmosphere, *L. amazonensis* promastigotes were added in a ratio of 10 parasites/macrophage, and cells were allowed to interact for 4 h. Then, free parasites were removed by washing the plates with PBS, and the cultures were incubated for additional 24 h in order to enable promastigotes differentiation into amastigotes. The *L. amazonensis*-infected cultures were treated with CH or CD/Et extracts (10 to 80 µg/mL) for 48 h. Alternatively, infected macrophages were treated with the reference drug amphotericin B (0.125 to 1.0 µg/mL). Finally, the coverslips were fixed and Giemsa-stained and the number of intracellular amastigotes/100 macrophages as well as the % of infected cells was determined by light microscopy. The IC_{50} values were calculated as previously described in the antileishmanial activity assay. The anti-intracellular amastigote activity assays were performed in duplicate.

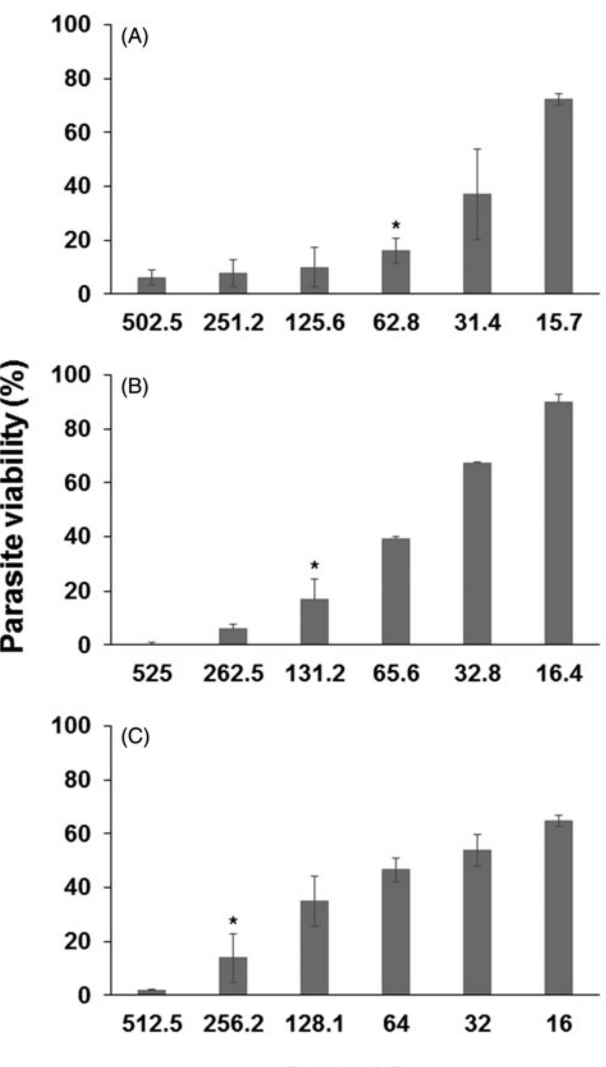

Figure 1. Effect of *C. sinensis* on *L. amazonensis* promastigote viability. Parasites were treated with several concentrations (1–525 µg/mL) of *C. sinensis* extracts for 120 h. The promastigote viability was determined by the resazurin reduction assay. Each bar represents the mean ± standard error of at least three independent experiments. Parasites treated with (A) CH, (B) CEt/W and (C) CEA extracts. The asterisk indicates the minimum inhibitory concentration of each extract.

Results and discussion

Inhibition of promastigote growth

In the present study, four crude extracts obtained from *C. sinensis* were tested in order to determine the *in vitro* antileishmanial effect against *L. amazonensis*, the causative agent of cutaneous and diffuse leishmaniasis. Figure 1 shows the viability of *L. amazonensis* promastigotes after treatment with CH, CD/Et and CEA. CEt/W was not active against the parasites. We observed a dose-dependent antileishmanial activity for the CH, CD/Et and CEA extracts. The MIC values for these extracts were 62.8, 131.2 and 256.2 µg/mL, respectively. It is worth mentioning that parasites treated with MIC values were no longer able to grow when they were reintroduced into fresh culture medium. This result demonstrates that CH, CD/Et and CEA extracts displayed leishmanicidal activity.

The 50% inhibitory concentrations (IC_{50}) for parasites treated with the extracts are shown in Table 1. CH and CD/Et presented the highest activity observed in the present study with IC_{50} values of 25.91 and 54.23 µg/mL, respectively. Indeed, *C. sinensis* has been described as an interesting source of antimicrobial agents. Previous studies have shown that the *C. sinensis* essential oil was able to inhibit growth and spore germination of *Aspergillus* spp. (Sharma & Tripathi 2006) and *Penicillium* spp. (Martos et al. 2008). Mehmood et al. (2015) reported the antibacterial activity of several *C. sinensis* crude extracts. In their study, the ethanol and methanol extracts displayed significant growth inhibition of *Escherichia coli*, *Klebsiella pneumonia* and *Serratia odorifera* (Mehmood et al. 2015). In regard to the antiprotozoal activity, Chenniappan and Kadarkarai (2010) demonstrated that the cyclohexane and methylene chloride extracts obtained from *C. sinensis* seeds were active against *Plasmodium falciparum* (IC_{50} = 7.34 and 8.63 µg/mL, respectively). Bagavan et al. (2011) reported that the hexane and ethyl acetate extracts obtained from the plant peel displayed antiplasmodial activity at 42.13 and 25.67 µg/mL (IC_{50} values), respectively. However, to date, reports

Table 1. *In vitro* antileishmanial and cytotoxic activities of *C. sinensis* extracts.

	L. amazonensis		J774.G8*	RAW 264.7**	SI*	SI**	
Extracts	*PRO* IC_{50} (±SE)	*AMA* IC_{50} (±SE)	CC_{50} (±SE)	CC_{50} (±SE)	*PRO*	*PRO*	*AMA*
CH	25.91 (± 4.87)	39.78 (± 2.45)	35.75 (± 11.51)	86.17 (± 9.93)	1.4	3.32	2.16
CD/Et	54.23 (± 3.78)	51.0 (± 0.16)	123.79 (± 20.95)	101.07 (± 7.09)	2.3	1.86	1.98
CEA	62.74 (± 5.04)	nd	nd	nd	nd	nd	nd
CEt/W	na	nd	nd	nd	nd	nd	nd
Amph. B	0.085 (± 0.001)	1.54 (± 0.42)	8.4 (± 0.44)	2.09 (± 0.69)	98.8	24.58	1.35

CH: *C. sinensis* hexane extract; CD/Et: *C. sinensis* dichloromethane/ethanol (1:1) extract; CEA: *C. sinensis* ethyl acetate extract; CEt/W: *C. sinensis* ethanol/water (7:3) extract; Amph. B: amphotericin B; PRO: promastigotes; AMA: amastigotes; SI: selective index; nd: not determined; na: not active; SE: standard error.

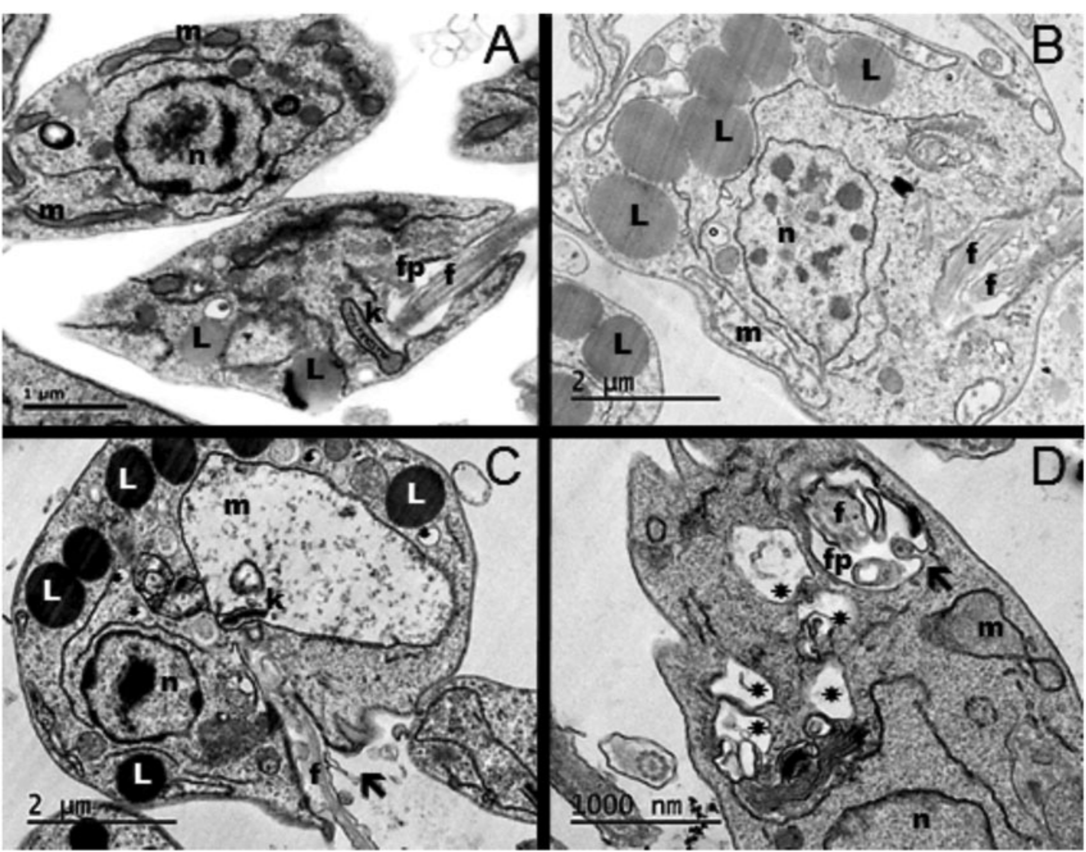

Figure 2. Ultrastructure alterations of *L. amazonensis* treated with CD/Et. (A) Parasites displaying normal morphology and intracellular structures: nucleus (n), mitochondrion (m), kinetoplast (k), flagellar pocket (fp), flagellum (f) and lipid bodies (L). (B-C) Cells treated with CD/Et subMIC concentration (65.6 1 µg/mL) showed an increase in cytoplasmic lipid bodies. (B) Parasite displaying nuclear chromatin condensation and two flagella inside the flagellar pocket. (C) Parasite showing extensive mitochondrion swelling with loss of mitochondrial cristae. (D) Cells treated with CD/Et MIC concentration (131.2 µg/mL) exhibited intense vacuolization (*) and loss of cytoplasmic content into the flagellar pocket (arrow).

of *C. sinensis* extracts activity against *Leishmania* species are completely missing.

Ultrastructure alterations

Figure 2 shows the ultrastructure damage caused by CD/Et on *L. amazonensis* promastigotes. After 24-h treatment, parasites presenting a rounded body and cytoplasmic lipid droplets were observed (Figure 2(B,C)). Increased mitochondrial volume with loss of the matrix content followed by increased cytoplasmic vacuolization is shown in Figure 2(C,D). Flagellar pocket alterations, such as the presence of double flagella (Figure 2(B)) and vacuoles containing cytoplasmic matrix, were also noted (Figure 2(B–D)). Similar results were described in a previous study reporting the antileishmanial activity of *Arrabidaea chica* (HBK) Verlot, Bignoniaceae, hexane extract (Rodrigues et al. 2014). The lipophilic character and the similar composition (sterols, fatty acids, vitamin E, terpenes) of the extracts may explain their similar effects. However, in the present work, we observed intense lipid body formation in the promastigote cytoplasm. Mitochondrial dysfunction and lipid accumulation suggest that CD/Et treatment may lead to lipid metabolism disturbance, a mechanism of action widely described in the literature (Medina et al. 2012; Godinho et al. 2013; Stefanello et al. 2014). A clerodane diterpene obtained from the hexane extracts of *Polyalthia longifolia* var. *pendula* Linn (Annonaceae) leaves induced *L. donovani* ultrastructure alterations typical of apoptosis, including mitochondrial disturbance, cytoplasmic vacuolization, increased accumulation of lipid droplets and multivesicular bodies (Kathuria et al. 2014).

Table 2. Chemical composition of the *Citrus sinensis* CH and CD/Et extracts.

	CH		CD/Et
Compounds	R_t	(%)	(%)
β-Elemene	13.9	0.46	1.09
Dihydroactinidiolide	17.3	0.59	–
Spathulenol	18.4	0.63	–
Caryophyllene oxide	18.6	2.54	–
β-Sinensal	21.0	0.98	–
6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-2-naphthalenol	21.7	3.81	–
Neophytadiene	24.0	2.83	–
Hexahydrofarnesyl acetone	24.2	1.01	–
Methyl palmitate	25.9	0.56	–
Ethyl palmitate	27.2	1.32	–
Phytol	29.3	1.71	5.23
Ethyl linoleate	30.3	1.61	–
4,8,12,16-Tetramethylheptadecan-4-olide	33.4	1.49	–
Eicosane	38.9	7.16	10.41
Squalene	43.3	–	2.19
Octadecane	44.4	4.83	–
Tocopherol	44.6	0.62	–
Nonacosane	44.8	–	3.76
Vitamin E	45.3	4.24	11.26
Campesterol	45.7	0.90	1.54
Stigmasterol	46.1	1.33	2.37
Sitosterol	46.9	6.97	8.71
Docosane	47.1	–	3.04
3-β-Lanost-8-en-3-ol	47.2	2.07	–
β-Amyrin	47.3	7.63	7.56
Lupen-3-one	47.4	2.35	1.62
α-Amyrin	47.9	30.5	25.56
Friedelin	49.6	4.07	–
Identified compounds		92.21	84.34

R_t: retention time; CH: *C. sinensis* hexane extract; CD/Et: *C. sinensis* dichloromethane extract.

Cytotoxic effects of active extracts

The most active extracts, CH and CD/Et, were selected for cytotoxicity evaluation. CH minimal cytotoxic concentrations were 125.6 (CC_{50} = 35.75 µg/mL) and 125 µg/mL (CC_{50} = 86.17 µg/mL) against J774.G8 and RAW264.7 macrophages, respectively. CD/Et presented minimal cytotoxic concentrations of 262.5 (CC_{50} = 123.79 µg/mL) and 250 µg/mL (CC_{50} = 101.07 µg/mL), respectively. The essential oil obtained from sweet orange peel displayed cytotoxic activity at 5.51 µg/mL (CC_{50}) against HeLa cells (Liu et al. 2012). Here, the leaf extracts CH and CD/Et were less toxic for both macrophage lineages tested. Nonetheless, considering the intracellular amastigote-based SI, CH and CD/Et exhibited low selectivity. However, the cytotoxic effect was similar to the reference drug amphotericin B (SI = 2.16, 1.98 and 1.35, respectively) (Table 1). Recently, the hexane extracts of different *Hypericum* species (Hypericaceae), *H. linoides* A. St.-Hil., *H. carinatum* Griseb. and *H. polyanthemum* Klotzsch ex Reichardt were described as low cytotoxic for murine macrophages, presenting SI at 1.2, 1.8 and 4, respectively (Dagnino et al. 2015).

Phytochemical profile of CH and CD/Et

Based on the preliminary analysis by TLC, which revealed the presence of volatile terpenoids, we subjected CH and CD/Et to CG-MS. The main substances present in the extracts are listed in Table 2. Among those, the triterpene α-amyrin, the major component found in the extracts, has been previously described as an antiprotozoal agent. Mwangi et al. (2010) reported the antiprotozoal activity of α-amyrin isolated from *Teclea trichocarpa* Enge. (Rutaceae) against *P. falciparum* and *L. donovani* (amastigote) with IC_{50} values of 0.96 and 7.90 µg/mL, respectively. The authors also demonstrated that α-amyrin presented CC_{50} values for myoblast (L-6) cells ≥90 µg/mL. In a previous study, the acetylated form of β-amyrin showed antileishmanial activity against *L. amazonensis* amastigotes with IC_{50} values of 14 µg/mL (Schinor et al. 2007). Those results further support the antileishmanial potential of triterpene amyrins.

CH and CD/Et effects on macrophage infection

Studies evaluating the efficacy of drug candidate substances against the intracellular stage of *Leishmania* are necessary, since this is the infective form for vertebrate hosts. CH and CD/Et were most active when compared to the other extracts. Thus, CH and CD/Et were selected for the evaluation of anti-intracellular amastigote activity. CH (40 µg/mL) was able to reduce the number of internalized parasites as well as the percentage of infected macrophages in 36.2% and 16.6%, respectively (Figure 3(A)). On the other hand, CD/Et displayed better results. We observed a reduction in the amastigote numbers of 16, 46 and 63% after treatment with 20, 40 and 80 µg/mL, respectively (Figure 3(B)). In addition, the percentage of infected macrophages reduced in 33% when cells were treated with 80 µg/mL of CD/Et. Amphotericin B was able to reduce macrophage infection by 48.5% when cells were treated with 1 µg/mL (Figure 3(C)). Extracts or purified substances from several plants of the Rutaceae family have been described as antileishmanial agents. *Zanthoxylum monophyllum* (Lam.) P. Wilson (Rutaceae) is a Colombian plant that has been shown to exhibit antileishmanial activity against *Leishmania panamensis* and *Leishmania major* amastigotes. The ethanol extract and its alkaloid fraction displayed 50% effective concentrations (EC_{50}) of 6.16 ± 0.115 and 24.95 ± 3.15 µg/mL, and 46.23 ± 1.05 and 30.135 ± 0.685 µg/mL for *L. panamensis* and *L. major*, respectively (Chávez Enciso et al. 2014).

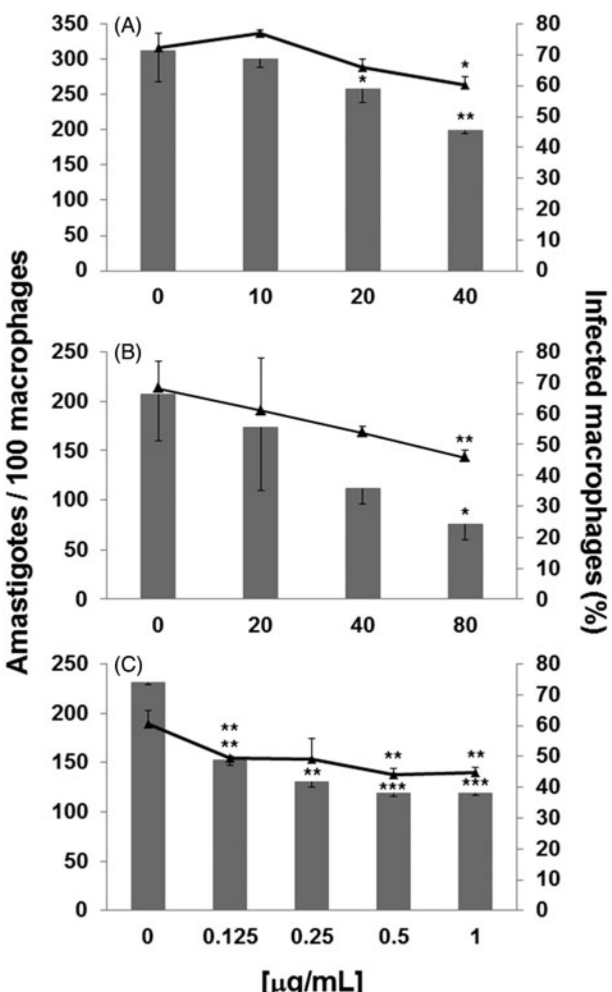

Figure 3. Effect of *C. sinensis* on *L. amazonensis* intracellular amastigotes. Raw 264.7 macrophages previously infected with *L. amazonensis* promastigotes were treated with *C. sinensis* extracts, CH (A) and CD/Et (B), or with the reference drug amphotericin B (C) for 48 h. The number of internalized amastigotes *per* 100 macrophages was determined by direct count under a light microscope. Each bar represents the mean ± standard error of at least two independent experiments. Statistical analysis of the differences between mean values obtained for the experimental groups was done by Student's *t*-test. *p*-Values ≤0.05 (one asterisk), ≤0.005 (two asterisks) and ≤0.0001 (three asterisks) were considered significantly different from that for the control.

Coy Barrera et al. (2011) showed that a *seco*-limonoid (compound 1) isolated from the stem bark of *Raputia heptaphylla* Pittier (Rutaceae) was active against *L. panamensis* amastigotes with EC_{50} of 8.7 ± 3.1 µg/mL. In the present study, *C. sinensis* CH and CD/Et extracts showed IC_{50} of 39.78 and 51 µg/mL, respectively (reference drug IC_{50} = 1.54 ± 0.42 µg/mL).

Conclusions

Here, we demonstrated the antileishmanial potential of *C. sinensis* extracts. The hexane (CH) and dichloromethane/ethanol (CD/Et) extracts displayed potential activity against *L. amazonensis* promastigotes and intracellular amastigotes. In addition, the extracts presented cytotoxic effect at the same level of the reference drug. The stereoisomeric α- and β-amyrins content of the extracts (over 38.13 and 33.12%, respectively) may be responsible for their antileishmanial activity. Nevertheless, further investigation will be necessary in order to establish the bioactive substance(s) found in CH and CD/Et. This study highlights the investigation of *C. sinensis* as a promising source of antileishmanial agents.

Disclosure statement

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