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RESEARCH ARTICLE

Trypanocidal and leishmanicidal activities of flavonoids isolated from *Stevia satureiifolia* var. *satueiifolia*

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ABSTRACT

Context Chagas' disease and leishmaniasis produce significant disability and mortality with great social and economic impact. The genus *Stevia* (Asteraceae) is a potential source of antiprotozoal compounds.

Objective Aerial parts of four *Stevia* species were screened on *Trypanosoma cruzi*. *Stevia satureiifolia* (Lam.) Sch. Bip. var. *satueiifolia* (Asteraceae) dichloromethane extract was selected for a bioassay-guided fractionation in order to isolate its active compounds. Additionally, the antileishmanial activity and the cytotoxicity of these compounds on mammalian cells were assessed.

Materials and methods The dichloromethane extract was fractionated by column chromatography. The isolated compounds were evaluated using concentrations of 0–100 µg/mL on *T. cruzi* epimastigotes and on *Leishmania braziliensis* promastigotes for 72 h, on trypomastigotes and amastigotes of *T. cruzi* for 24 h and 120 h, respectively. The compounds' cytotoxicity (12.5–500 µg/mL) was assessed on Vero cells by the MTT assay. The structure elucidation of each compound was performed by spectroscopic methods and HPLC analysis.

Results The dichloromethane extracts of *Stevia* species showed significant activity on *T. cruzi* epimastigotes. The flavonoids eupatorin (1.3%), circimaritin (1.9%) and 5-desmethylinensetin (1.5%) were isolated from *S. satureiifolia* var. *satueiifolia* extract. Eupatorin and 5-desmethylinensetin showed IC₅₀ values of 0.2 and 0.4 µg/mL on *T. cruzi* epimastigotes and 61.8 and 75.1 µg/mL on trypomastigotes, respectively. The flavonoid 5-desmethylinensetin showed moderate activity against *T. cruzi* amastigotes (IC₅₀ value = 78.7 µg/mL) and was the most active compound on *L. braziliensis* promastigotes (IC₅₀ value = 37.0 µg/mL). Neither of the flavonoids showed cytotoxicity on Vero cells, up to a concentration of 500 µg/mL.

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Leishmania braziliensis; phenolic compounds; *Stevia* spp.; *Trypanosoma cruzi*

Introduction

Chagas' disease results in significant disability with great social and economic impact, including under- and unemployment. About 6–7 million people are estimated to be infected worldwide, mostly in Latin America with almost 12 000 deaths annually (DNDi 2015a; WHO 2015). Although endemic in 21 countries across Latin America, the number of affected people is growing in non-endemic, developed countries due to increased migration of Latin American people unknowingly carrying the parasite (Rodríguez Coura & Albajar Viñas 2010). Chagas' disease is the most important

endemic disease in Argentina with 1.6–2 million people being infected. This parasite disease affects mainly people living in rural areas, where the vector lives, and people from urban centres due to migration phenomena (Franco-Paredes et al. 2009, Rassi et al. 2010).

Leishmaniasis is another poverty-associated disease responsible for different clinical forms, the most common being visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL). The most common form in our country is the CL caused mainly by *L. braziliensis*, but since 2006, human and canine VL cases have been reported (Gould et al. 2013). Mixed infections caused by

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Leishmania spp. and *T. cruzi* have been reported in Argentina and Brazil (Chiaramonte et al. 1999; Frank et al. 2003).

Current treatment for Chagas' disease is limited to two drugs, benznidazol and nifurtimox, which were developed around 30 years ago. These two drugs are effective in the acute phase of infection and present drawbacks associated to side effects, lack of effectiveness and long treatment periods (DNDi 2015b). Leishmaniasis treatment includes pentavalent antimonials (sodium stibogluconate and meglumine antimoniate), amphotericin B, miltefosine and paromomycin. These drugs have limitations due to serious side effects, resistance, long duration of treatment, low tolerability, difficult administration and high cost (DNDi 2015b).

In recent years, considerable attention has been given to secondary metabolites from plants in the search for new trypanocidal and leishmanicidal compounds. Among these metabolites, terpenes and flavonoids have been pointed out as promising molecules (Schmidt et al. 2012).

The genus *Stevia* (Asteraceae) is a New World genus, distribution ranging from the Southern United States to Northeastern Paraguay, Southeastern Brazil and the South American Andean region (Kinghorn 2002). One member of this family, *Stevia rebaudiana* (Bertoni) Bertoni (Asteraceae) is of worldwide economic importance today since its *ent*-kaurene diterpene glycosides are used as non-nutritive natural sweeteners, mainly in Japan. This genus is rich in sesquiterpene lactones and flavonoids among which, methoxylated aglycones at the C-6 position have been reported (Cerda-García Rojas & Pereda-Miranda 2002). Flavonoids of this type, such as hispidulin and santin, have been reported to have trypanocidal and leishmanicidal activities (Sülsen et al. 2007).

In Argentina, the genus *Stevia* is represented by more than 30 species and several varieties growing in the Northern and Central areas of the country (Zuloaga et al. 2008). Among the native species, the chemical composition and bioactivity of *Stevia aristata* D. Don ex Hook. & Arn., *S. multiaristata* Spreng., *S. entriensis* Hieron. and *S. satureiifolia* (Lam.) Sch. Bip. var. *satureiifolia* have not been investigated so far. The only literature data available on the chemical composition of *S. aristata* were published by Zdero et al. (1987) in which beyerene derivatives and other terpenoids were reported. Thus, the potential of these species is still unexplored.

In this work, four native *Stevia* species were screened for their antiprotozoal activity on *Trypanosoma cruzi*. One of the most active species was selected for a bioassay-guided fractionation in order to isolate its active compounds. Additionally, the antileishmanial activity and the cytotoxicity of these compounds on mammalian cells were assessed.

Materials and methods

General experimental procedures

UV spectra were recorded on a Jasco-630 spectrophotometer (Jasco Tools, Inc., Rochester, NY). MS spectra were obtained in an Agilent 5973 spectrometer (Agilent Technologies, Inc., Santa Clara, CA). HPLC analyses were performed in a Varian Pro Star instrument (Beckman Instruments Inc., Irvine, CA) equipped with a Rheodyne injection valve (20 μ L) and a diode array detector (DAD) set at 340 nm. Chromatograms were recorded and processed using the Varian Star Chromatography Workstation version 6.x (Beckman Instruments Inc., Irvine, CA). The water employed to prepare the mobile phase was of ultrapure quality (Milliq). Methanol (J. T. Baker) was of HPLC grade. Eupatorin and circimaritin (Sigma, St. Louis, MO) were used as standards for HPLC analysis. Silicagel (Merck, Darmstadt, Germany, 230–400 mesh) was used for column chromatographic separation and Silicagel 60 F254 (Merck, Darmstadt, Germany) was used for TLC analysis.

Plant material

The aerial parts of *Stevia aristata* D. Don ex Hook. & Arn. (Asteraceae) (741) and *Stevia entriensis* Hieron. (Asteraceae) (762) were collected in the province of Entre Ríos, Argentina in November 2011 and March 2012, respectively. The aerial parts of *Stevia satureiifolia* (Lam.) Sch. Bip. var. *satureiifolia* (Asteraceae) (744) and *Stevia multiaristata* Spreng. (Asteraceae) (742) were collected in the province of Buenos Aires, Argentina, in February 2012.

The plant material was collected and identified by one of the authors (Gustavo Giberti Ph.D.) and voucher specimens were deposited at the Museo de Farmacobotánica, Facultad de Farmacia y Bioquímica (BAF), Universidad de Buenos Aires.

Parasites

Trypanosoma cruzi epimastigotes (RA strain) were grown in a biphasic medium. *Leishmania braziliensis* promastigotes (MHOM/BR/75/M2903) were grown in liver infusion tryptose medium (LIT). Cultures were routinely maintained by weekly passages at 28 °C and 26 °C, respectively.

Plant extracts

For the preparation of plant extracts for screening, 10 g of the dried aerial parts of each *Stevia* species were extracted by maceration (24 h) twice with 100 mL of

dichloromethane (DCM). The extracts were filtered and taken to dryness. The extraction of *S. satureiifolia* var. *satureiifolia* (aerial parts) for bioassay-guided fractionation was performed using 500 g of plant material and DCM (5 L) as described before above.

HPLC analysis of *S. satureiifolia* var. *satureiifolia* dichloromethane extract (DCME)

A chromatographic profile of *S. satureiifolia* var. *satureiifolia* DCME was obtained using a Kinetex XB-C-18 100 Å (250 mm × 46 mm × 5 µm) column eluted with a gradient of H₂O:AcOH (98:2) (A) and MeOH:AcOH (98:2) (B) from 50% B to 80% B in 40 min. The flow rate employed was 1.2 mL/min. The DCME (5 mg) was dissolved in MeOH:H₂O (9:1) to a final concentration of 5 mg/mL.

Fractionation and isolation of compounds from *S. satureiifolia* var. *satureiifolia* extract

Stevia satureiifolia var. *satureiifolia* DCME (2.5 g) was fractionated by column chromatography (CC) (400 mm × 30 mm) on Silicagel 60 (50 g) with a gradient of DCM and ethyl acetate (EtOAc): DCM 100%, DCM:EtOAc 9:1, 3:1, 2:1, 1:1, 1:3, EtOAc 100%. Seven fractions (SS₁–SS₇) of 100 mL each were collected and taken to dryness in a rotatory evaporator. Each fraction was tested for trypanocidal activity on *T. cruzi* epimastigotes. A yellow precipitate (compound 3) (10 mg) was obtained from fraction SS₂ eluted with DCM: EtOAc 9:1. The TLC analysis of fractions SS₄ and SS₅, eluted with DCM:EtOAc 2:1 and 1:1, respectively, was performed on Silicagel 60 F254, developed with hexane:EtOAc (1:9) and Natural Products Reagent (Sigma, ST. Louis, MO) as a revealing agent. Fraction SS₅ (50 mg) was subjected to CC (240 mm × 20 mm) on Silicagel 60 (10 g) eluted with a gradient of DCM and EtOAc: DCM 100%, DCM:EtOAc 9:1, 7:3, 5:5, 3:7, 2:8, 1:9, EtOAc 100%, to obtain eight subfractions (SS_{5A}–SS_{5H}). From subfractions SS_{5B}–SS_{5D} eluted with DCM:EtOAc 9:1, 7:3, 5:5, 3:7, an amorphous powder (6 mg) was obtained (compound 1). This compound was filtered and washed three times with hexane and EtOAc. The purity of compounds 1 and 3 was assessed by HPLC and the compounds were assayed for trypanocidal and leishmanicidal activities.

Identification of compounds

The structure elucidation of compound 3 was performed by UV spectroscopy with the addition of diagnostic reagents (Mabry et al. 1970), by proton nuclear magnetic

resonance (¹H NMR) and by electron impact-mass spectrometry (EI-MS). Spectra were compared with those found in the literature.

Identification of compound 1 was performed by HPLC/DAD against a eupatorin standard. Each sample was dissolved in MeOH:H₂O (9:1). Three different chromatographic systems were used: (1) a Phenomenex column C-18 Luna (2) (250 mm × 4.6 mm × 5 µm) eluted with a gradient of H₂O:HCOOH (19:1) (A) and MeOH (B) from 50% B to 100% B in 35 min. The flow rate was set at 1 mL/min. (2) The same column as that used in system 1 eluted with a gradient of H₂O:AcOH (98:2) (A) and MeOH:AcOH (98:2) (B) from 15% B to 100% B in 60 min. The flow rate was set at 1.2 mL/min. (3) The system was the same as that used for HPLC analysis of *S. satureiifolia* var. *satureiifolia* DCME.

Compound 2 was identified in the DCME by HPLC/DAD against a cirsimaritin standard, using the same three solvent systems as those used for compound 1.

Quantification of compounds 1–3 in *S. satureiifolia* var. *satureiifolia* DCME

The quantification of compounds 1–3 was performed by HPLC/DAD using system 3. Eupatorin, cirsimaritin (Sigma, St. Louis, MO) and the isolated compound 3 were used as a reference and 1 mg of each compound was dissolved in 25 mL of a mixture MeOH:H₂O (9:1). The DCME (5 mg) was dissolved in the same solvent mixture to a final concentration of 5 mg/mL.

Activity assay on *T. cruzi* and *L. braziliensis*

The growth inhibition of *T. cruzi* epimastigotes and *L. braziliensis* promastigotes was evaluated by a [³H]-thymidine uptake assay as previously described (Sülsen et al. 2008). Parasites were adjusted to a cell density of 1.5 × 10⁶/mL and cultured in the presence of *Stevia* spp. extracts, *S. satureiifolia* var. *satureiifolia* fractions (100 and 10 µg/mL) and compounds 1 and 3 (100–0.1 µg/mL) for 72 h. Benznidazole (5–20 µM; Elea) and Amphotericin B (0.27–1.6 µM; ICN) were used as positive controls. Radioactivity was measured as counts per minute (cpm). The percentage of inhibition was calculated as 100 – [(cpm of treated parasites)/(cpm of untreated parasites)] × 100.

The trypanocidal effect of compounds 1 and 3 was also tested on bloodstream trypomastigotes as previously described (Sülsen et al. 2008). Briefly, mouse blood containing trypomastigotes was diluted in complete LIT medium to adjust the parasite concentration to 1.5 × 10⁶/mL. Parasites were seeded (150 µL/well) by duplicate in a 96-well microplate, and 2 µL of each

compound (0–100 µg/mL, final concentration) were added per well. Plates were incubated for 24 h at 4 °C and the remaining live parasites were counted in a haemocytometer. Live trypomastigotes were calculated as $\{[(\text{live parasites in wells after compound treatment})/(\text{live parasites in untreated wells})] \times 100\}$.

To evaluate the effect of the compounds **1** and **3** on intracellular forms of *T. cruzi*, 96-well plates were seeded with the non-phagocytic Vero cells at 5×10^3 per well in 100 µL of culture medium and incubated for 2 h at 37 °C in a 5% CO₂ atmosphere. Cells were washed and infected with transfected bloodstream trypomastigotes expressing β-galactosidase (Sülsen et al. 2013) at a parasite:cell ratio of 10:1. After 24 h of co-culture, plates were washed twice with PBS to remove extracellular parasites and each pure compound was added at 1–100 µg/mL per well in 150 µL of fresh complete RPMI medium without phenol red (Gibco, Rockville, MD). Controls included infected non-treated cells (100% infection control) and uninfected cells (0% infection control). The assay was developed 5 d later by the addition of chlorophenolred-β-D-galactopyranoside (CPRG) (100 µM) and 1% Nonidet P40. Plates were then incubated for 4–6 h at 37 °C and the absorbance was measured at 570 nm in a microplate reader (Bio-Rad Laboratories, Hercules, CA). The percentage inhibition was calculated as $100 - \{[(\text{absorbance of treated infected cells})/(\text{absorbance of untreated infected cells})] \times 100\}$ and the IC₅₀ value was estimated.

Cytotoxicity assay

Vero cells were employed to determine the viability by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method (Sülsen et al. 2011). Cells (5×10^5) were settled at a final volume of 150 µL in a flat-bottom 96-well microplate and were cultured at 37 °C in a 5% CO₂ atmosphere in the absence or presence of increasing concentrations of the pure compounds **1** and **3** (12.5–500 µg/mL). After 24 h, MTT was added at a final concentration of 1.5 mg/mL. Plates were incubated for 2 h at 37 °C. The purple formazan crystals were dissolved by adding 150 µL of ethanol and the absorbance was read at 570 nm in a microplate reader. Results were calculated as the ratio between the optical density in the presence and absence of the compound multiplied by 100.

Statistical analysis

Results are presented as mean ± SEM. The statistical significance was determined by using one-way analysis of variance (ANOVA) employing the GraphPad Prism 5.0

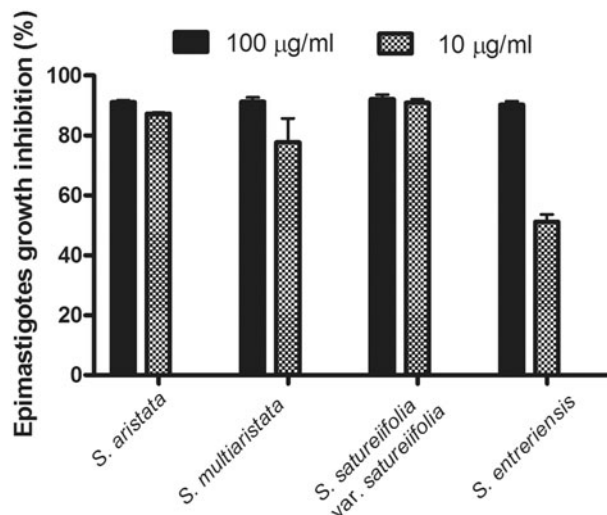


Figure 1. Trypanocidal activity of organic extracts of *Stevia aristata*, *Stevia aristata*, *Stevia satureiifolia* var. *satureiifolia*, *Stevia multiaristata* against *T. cruzi* epimastigotes. Epimastigotes were cultured for 72 h at 28 °C in the presence of the extracts at concentrations of 100 and 10 µg/mL. Values represent mean ± SEM from three independent experiments carried out in triplicate.

software (GraphPad Software Inc., San Diego, CA). Comparisons were referred to the control group. *p* values of <0.05 were considered significant.

Results

Trypanocidal activity of plant extracts

All the tested dichloromethane extracts showed parasiticidal activity on *T. cruzi* epimastigotes at concentrations of 100 and 10 µg/mL. *Stevia aristata* and *S. satureiifolia* var. *satureiifolia* extracts induced growth inhibition percentages of $87.3 \pm 0.3\%$ and $90.9 \pm 1.2\%$ at a concentration of 10 µg/mL (Figure 1).

HPLC analysis of *S. satureiifolia* var. *satureiifolia* dichloromethane extract (DCME)

The DCME was analyzed by HPLC and three major compounds were detected: compounds **1** (rt: 14.4 min), **2** (rt: 15.5 min) and **3** (rt: 18.1 min) in order of elution (Figure 2). The UV spectra indicated that these compounds had a flavonoid structure.

Bioassay-guided fractionation of *S. satureiifolia* var. *satureiifolia*

The DCME was fractionated using column chromatography. Seven fractions were obtained and assayed on *T. cruzi* epimastigotes. All the fractions (SS₁–SS₇) exerted significant trypanocidal activity with growth inhibition

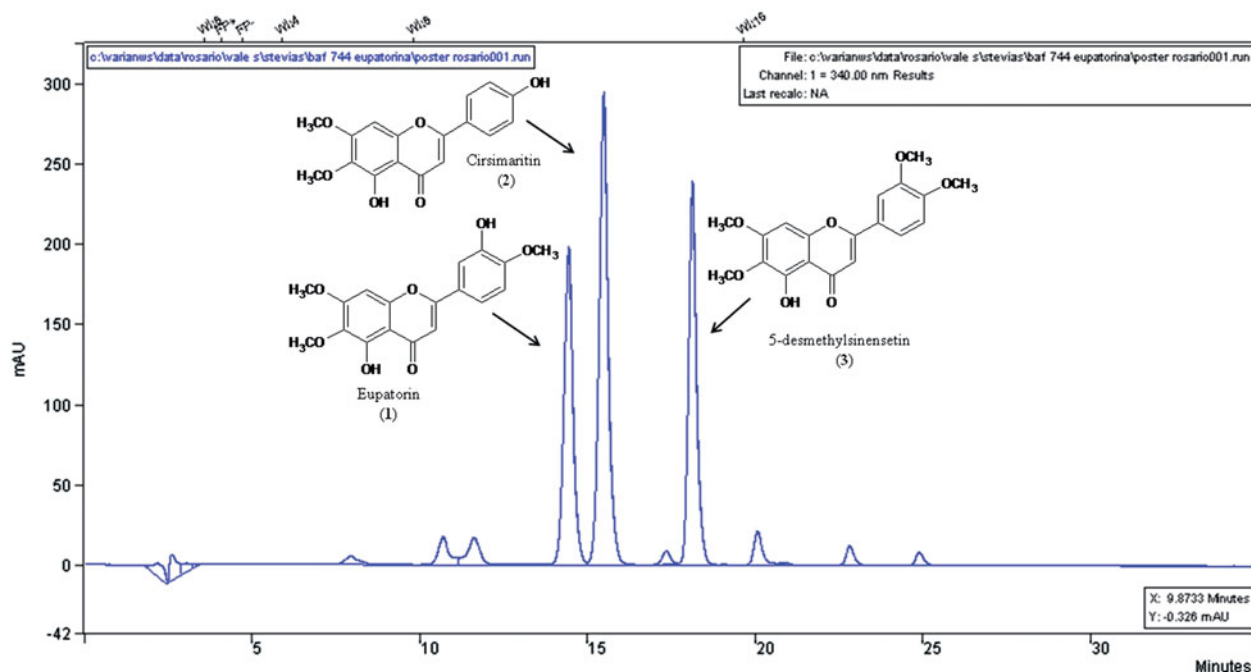


Figure 2. HPLC profile of the dichloromethane extract of *Stevia satureifolia* var. *satueifolia*.

percentages that were higher than 90% at 100 $\mu\text{g/mL}$. At a concentration of 10 $\mu\text{g/mL}$, fractions SS₁–SS₅ were the most active ones with inhibition percentages that were higher than 80% (data not shown). Compound **3** was obtained as a yellow precipitate from fraction SS₂. The purification of fraction SS₅ by CC yielded an amorphous powder (compound **1**).

Identification and quantification of compounds

Compound **3** was identified as 5-hydroxy-6,7,3',4'-tetramethoxyflavone (5-desmethylinensetin). Amorphous powder. UV λ_{max} (MeOH) nm: 338, 275, 250, 240. EIMS (70 eV), m/z (rel. int.): 358: [M]⁺ (100), 343 (54), 329 (19), 315 (12), 298 (3), 257 (1), 181 (8), 167 (2), 153 (16), 149 (3). ¹H-NMR (600 MHz, CDCl₃): δ 3.96 (3H, s), δ 4.00 (3H, s), δ 4.01 (3H, s), δ 4.02 (3H, s), δ 6.58 (1H, s, H-3), δ 6.63 (1H, s, H-8), δ 7.01 (1H, d, J = 8.6 Hz, H-5'), δ 7.37 (1H, d, J = 2.1 Hz, H-2'), δ 7.56 (1H, dd, J = 2.0 and 8.5 Hz, H-6'), 12,78 (1H, s). These data are in accordance with those reported in the literature (González et al. 1978, 1988).

Compound **1** was identified as 5,3'-dihydroxy 6,7,4'-trimethoxyflavone (eupatorin) by HPLC employing a commercial standard. Compound **2**, which had been detected in the DCME, was identified as cirsimaritin by HPLC employing a commercial standard. The structures of eupatorin, cirsimaritin and 5-desmethylinensetin are shown in Figure 2. The content of these flavonoids was 1.3, 1.9 and 1.5% in the DCME, and 0.047, 0.068 and 0.054%, respectively, calculated on dry plant material.

Antiprotozoal activity of compounds

The effect of eupatorin and 5-desmethylinensetin on *T. cruzi* epimastigotes is shown in Figure 3. IC₅₀ values, calculated for both flavonoids, were 0.2 and 0.4 $\mu\text{g/mL}$, respectively. IC₅₀ values for both compounds on *T. cruzi* trypomastigotes were 75.1 $\mu\text{g/mL}$ (5-desmethylinensetin) and 61.8 $\mu\text{g/mL}$ (eupatorin) (Figure 4). On one hand, the flavone 5-desmethylinensetin showed moderate activity on amastigotes, with an IC₅₀ value of 78.7 $\mu\text{g/mL}$. On the other hand, eupatorin was not active against this parasite stage (Figure 5). The flavones, 5-desmethylinensetin and eupatorin, showed activity against *L. braziliensis* promastigotes (IC₅₀ value = 37.0 and 55.1 $\mu\text{g/mL}$, respectively) (Figure 6). The reference drug benznidazole showed IC₅₀ values of 1.3, 9.5 and 0.98 $\mu\text{g/mL}$ against epimastigotes, trypomastigotes and amastigotes, respectively. Amphotericin B had an IC₅₀ value of 0.12 $\mu\text{g/mL}$ for *L. braziliensis* promastigotes.

Cytotoxicity

Eupatorin and 5-desmethylinensetin showed no cytotoxicity on Vero cells up to a concentration of 500 $\mu\text{g/mL}$ (Table 1).

Discussion

In the search for new natural antiprotozoal compounds, four native *Stevia* species were evaluated for their activity on *T. cruzi* epimastigotes. All the tested dichloromethane

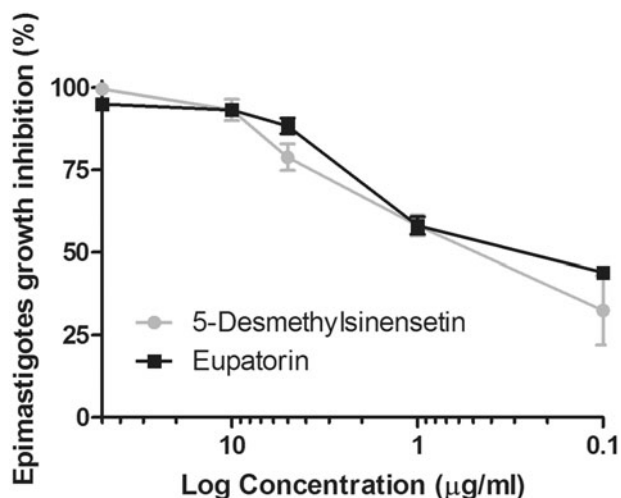


Figure 3. Effect of eupatorin and 5-desmethylinensetin on *T. cruzi* epimastigotes. Parasites were cultured for 72 h at 28 °C in the presence of final compound concentrations ranging from 0.1 to 50 µg/mL. Results are expressed as mean ± SEM.

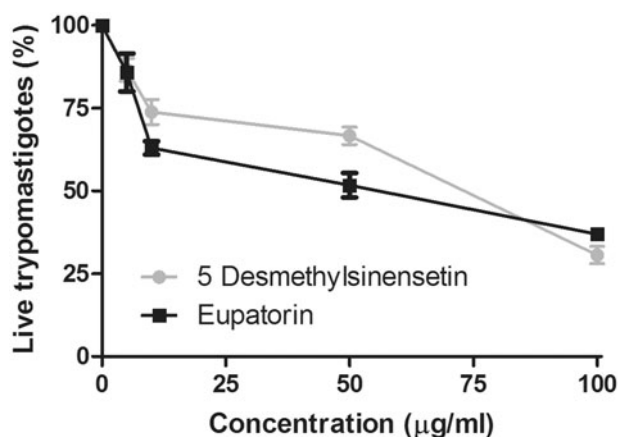


Figure 4. Trypanocidal activity of eupatorin and 5-desmethylinensetin on *T. cruzi* trypomastigotes. Parasites were seeded in a 96-well microplate over 24 h at 4 °C in the presence of the compounds (0–100 µg/mL), and the remaining live parasites were counted in a Neubauer chamber. Results are expressed as mean ± SEM.

extracts showed parasiticidal activity. *Stevia satuireifolia* var. *satuireifolia* dichloromethane extract was selected for bioassay-guided fractionation in order to isolate its bioactive compounds. Three 6-methoxy flavones, eupatorin, cirsimaritin and 5-desmethylinensetin, were identified in this extract, being cirsimaritin the major compound, as determined by HPLC. Eupatorin and cirsimaritin have been previously reported in *S. satuireifolia* (Sosa et al. 1984). However, this is the first report on the presence of 5-desmethylinensetin in this plant species.

Eupatorin and 5-desmethylinensetin were isolated by bioassay-guided fractionation and tested for their trypanocidal and leishmanicidal activities. Cirsimaritin

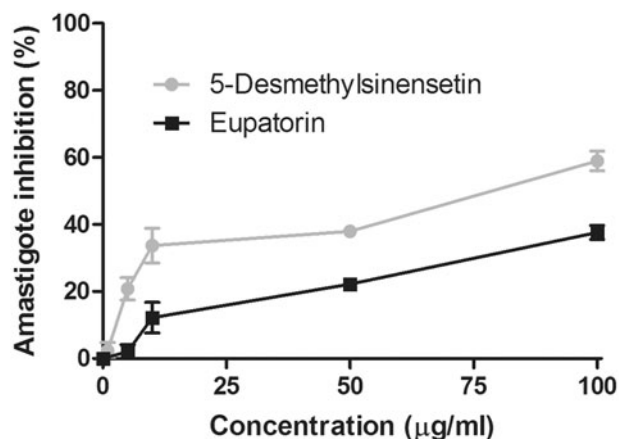


Figure 5. Trypanocidal activity of eupatorin and 5-desmethylinensetin on *T. cruzi* amastigotes. Vero cells infected with transfected bloodstream trypomastigotes expressing β -galactosidase were incubated for 5 d in the presence of the compounds (0–100 µg/mL). The presence of amastigotes was developed later by adding chlorophenolred- β -D-galactopyranoside in the presence of Nonidet P40 and incubated for 4 h. The absorbance was measured at 595 nm. Results are expressed as mean ± SEM.

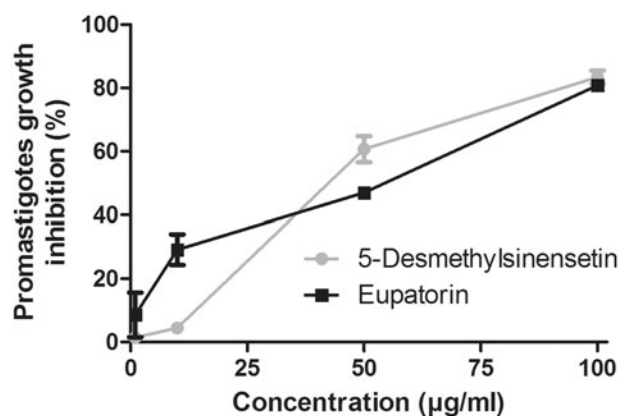


Figure 6. Leishmanicidal activity of eupatorin and 5-desmethylinensetin on *L. braziliensis*. Parasites were cultured for 72 h at 26 °C in the presence of final compound concentrations ranging from 0.1 to 100 µg/mL. Results are expressed as mean ± SEM.

Table 1. Vero cells viability after treatment with eupatorin and 5-desmethylinensetin.

Concentration (µg/mL)	Viability (media ± SEM)	
	Eupatorin	5-Desmethylinensetin
500	88.4 ± 1.3	90.7 ± 1.1
100	96.4 ± 3.2	97.7 ± 1.2
50	95.0 ± 4.5	92.3 ± 3.7
25	99.6 ± 0.8	96.4 ± 1.2
12.5	98.7 ± 5.9	98.5 ± 1.3

was not included in this study since it has been previously reported to be active against *T. cruzi* and *Leishmania* sp. with moderate cytotoxicity on Vero cells (Tasdemir et al. 2006). *Trypanosoma cruzi* epimastigotes and trypomastigotes proved to be susceptible to both

eupatorin and 5-desmethylinensetin. When these compounds were assayed against the intracellular stage of the parasites in mammalian cells, only 5-desmethylinensetin was moderately active. This compound was also the most active against *L. braziliensis* promastigotes. Nevertheless, the isolated compounds were less active than the reference drugs.

In order to determine the selectivity of the isolated compounds, the cytotoxicity of eupatorin and 5-desmethylinensetin on Vero cells was evaluated. Neither of the flavonoids showed cytotoxicity, indicating that these compounds have the capacity to inhibit the parasite growth without displaying a significant toxicity on the host's cell.

According to the literature, 6-methoxyflavones have been reported as having antiprotozoal activity (Schmidt et al. 2012). In this sense, we have previously found that hispidulin and santin were active *in vitro* against *T. cruzi* epimastigotes and trypomastigotes as well as on *L. mexicana* promastigotes, with low cytotoxicity against murine T cells (Sülsen et al. 2007). Our present findings support the importance of these kinds of flavonoids as trypanocidal and leishmanicidal leads.

Conclusion

The antiprotozoal activity of 5-desmethylinensetin and eupatorin, together with their low toxicity on Vero cells, makes them attractive leads for the discovery of new drugs against *T. cruzi* and *Leishmania* spp.

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Disclosure statement

The authors report that they have no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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