Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Antitrypanosomal and Antileishmanial Effects of the Hydroalcoholic Extract of *Croton cajucara* Benth and its 19-*nor*-Clerodane Chromatographic Fractions

Gerson S. Lima[1,2], Gerzia C. Machado[3], Maria A. M. Maciel[4,5], Aurea Echevarria[1]

[1]Department of Organic Chemistry, Institute of Chemistry, Federal Rural University of Rio de Janeiro, [2]Biomanguinhos, FIOCRUZ, [3]Biochemistry Laboratory of Trypanosomatids, FIOCRUZ, Rio de Janeiro, [4]Potiguar Laureate International Universities University, Postgraduate Program in Biotechnology, Campus Salgado Filho, [5]Federal University of Rio Grande do Norte, Postgraduate Program in Biotechnology (RENORBIO), Campus Lagoa Nova,Natal-RN, Brazil

Submitted: 13-Aug-2020 Revised: 23-Nov-2020 Accepted: 09-Mar-2021 Published: 12-Jul-2021

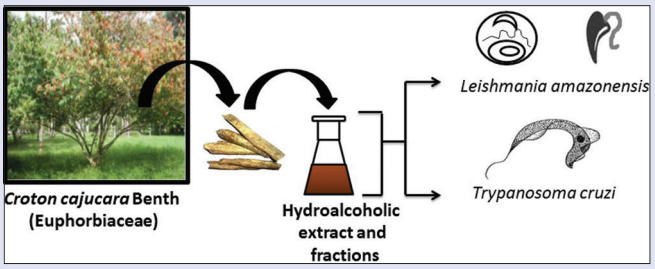
ABSTRACT

Context: *Croton cajucara* Benth has been widely used in folk medicine, especially in the Amazonian region of Brazil, to treat several illnesses. **Objectives:** The objective of the study is to evaluate the stem bark hydroalcoholic extract (CC-EHA) of *C. cajucara* and their clerodane-type diterpene fractions (F1-7, F25-27, and F28) on promastigotes and axenic amastigotes of *Leishmania amazonensis* and trypomastigotes and epimastigotes of *Trypanosoma cruzi*. **Materials and Methods:** The extract was obtained in ethanol: water and the fractions with solvents of increasing polarity. The antiparasitic activities were assessed by 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide method against promastigotes and axenic amastigotes from *L. amazonensis* in 24-h cultures and trypomastigotes and epimastigotes of *T. cruzi* in 72-h cultures. The experiments in triplicate were made in quadruplicate way in each time. The statistical tests used were t-Students and ANOVA. **Results:** Among those evaluated samples, the CC-EHA extract showed the higher antileishmanial activity of promastigote cultures (IC_{50} = 18.00 ± 0.01 μg/mL at 24 h). However, against axenic amastigotes, the polar fraction (F28), rich in diterpene transdehydrocrotonin (*t*-DCTN), showed the highest effect with an IC_{50} = 6.18 ± 0.02 μg/mL in culture of 24 h. In the *T. cruzi* assays, F28 also showed the greatest effect against trypomastigotes and epimastigotes, IC_{50} = 0.43 ± 0.02 μg/mL and 0.27 ± 0.02 μg/mL, respectively, at 72 h of culture. The results showed that the diterpene *t*-DCTN is the most important antiparasitic component in the hydroalcoholic extract obtained from *C. cajucara*, specifically against *L. amazonensis* and *T. cruzi*. **Conclusion:** Our results contribute to knowledge of these folk medicinal species as a promising antiparasitic phytotherapeutic alternative.

Key words: Antiparasitic activity, *Euphorbiaceae, Leishmania amazonensis*, sacaca, *Trypanosoma cruzi*

SUMMARY

- The hydroalcoholic extract done with the stem bark of *Croton cajucara* and the fractions rich in clerodane-type terpenes were investigated about their antiparasitic activities
- The hydroalcoholic crude extract showed higher activity against promastigotes of *Leishmania amazonensis*
- The polar fraction rich in the diterpene transdehydrocrotonin was more active against axenic amastigotes of *Leishmania amazonensis* and epimastigotes and trypomastigotes of *Trypanosoma cruzi*
- The fractions rich in diterpene transdehydrocrotonin showed that this is the most important metabolite to antiparasitic activity



Abbreviations used: *t*-DCTN: *trans*-dehydrocrotonin; CC-EHA: hydroalcoholic extract; F1-7, F25-27 and F28: fractions rich in clerodane-type diterpenes; MTT: 3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide; AcOEt: ethyl acetate; TLC: thin-layer chromatography; AAA: acetyl aleuritolic acid; *t*-CTN: *trans*-crotonin; FBS: fetal bovine serum; LIT: cs8g medium; DMSO: dimethyl sulfoxide.

Correspondence:

Prof. Aurea Echevarria,
Department of Organic Chemistry, Institute of Chemistry, Federal Rural University of Rio de Janeiro, 23.890 000, Seropédica, Rio de Janeiro, Brazil.
E-mail: echevarr@ufrrj.br

DOI: 10.4103/pm.pm_345_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Croton cajucara Benth (*Euphorbiaceae*) is an Amazonian species largely used in folk medicine. Sacaca, as commonly renowned, is used in tea or pills of stem bark to treat liver, stomach, and kidney diseases and to control the cholesterol and diabetes.[1,2] Further, the leaf tea and its conventional capsules are used to control weight, but for this propose, toxic side effects have been reported.[3]

The *C. cajucara* leaves contain flavonoids and steroids, and the stem bark is an abundant font of clerodane-type diterpenes, of which the most representative in their tested pharmacologic potential is *trans*-dehydrocrotonin (*t*-DCTN) and *trans*-crotonin (*t*-CTN), which are 19-*nor*-clerodane-furan diterpene-type and acetyl aleuritolic acid (AAA), a triterpene [Figure 1].[3,4] Specifically, pharmacological

Cite this article as: Lima GS, Machado GC, Maciel MA, Echevarria A. Antitrypanosomal and antileishmanial effects of the hydroalcoholic extract of *Croton cajucara* Benth and its 19-*nor*-clerodane chromatographic fractions. Phcog Mag 2021;17:302-6.

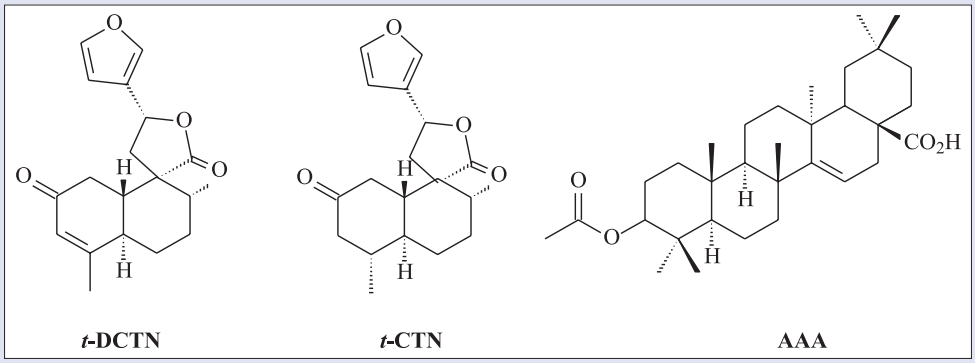


Figure 1: Chemical structures of the terpenoid *trans*-dehydrocrotonin, *trans*-crotonin, and acetyl aleuritolic acid isolated as major compounds from the stem bark of *Croton cajucara* Benth

properties of these terpenoids have shown a remarkable relation with the beneficial uses of *C. cajucara*; among them, hypoglycemic, cardiovascular, antiulcer, anti-inflammatory, antinociceptive, and antispasmodic activities were reported.[1,2,5-7]

Recently, the antiparasitic effects of several plants have been investigated, demonstrating a successful approach to obtaining new treatments. Several studies have indicated that terpenes possess antiparasitic activity, such as antitrypanosomal[8-10] and antileishmanial activity,[11] among others.

Infirmities caused by parasites such as leishmaniasis and Chagas disease are culpable for death in tropical and subtropical regions of the world. Trypanosomatids are protozoa (*Kinetoplastida* class), who's *Trypanosoma cruzi* species causes Chagas disease by and leishmaniasis by species of the *Leishmania* genus. Chagas disease affects approximately 7 million people in the world with due to various effects such as cardiac, neurological, or liver disorders.[12,13] Leishmaniasis includes several other diseases characterized by diversified clinical manifestations, and today approximately 1.3 million persons are newly infected and approximately 30,000 people expire from it every year, causing serious public health problems.[14]

The treatments for these diseases include a limited group of drugs that present some serious drawbacks, such as length of treatment, toxicity, and high cost. In this manner, there is a crucial demand to obtain alternatives for a more effective and safe treatment for these diseases.

The aim of this work was to evaluate the antileishmanial activity on promastigotes and axenic amastigotes of *Leishmania amazonensis* by the non-polar and polar chromatographic fractions obtained from a hydroalcoholic extract, so called CC-EHA, isolated of *C. cajucara*. Furthermore, the effects of CC-EHA and its fractions were evaluated against trypomastigotes, epimastigotes, and axenic amastigotes of *T. cruzi*

MATERIALS AND METHODS

Plant material

C. cajucara Benth (*Euphorbiaceae*) was gather in Belém, Pará (Brazil) and recognized by Nelson Rosa of Museu Paraense Emílio Goeldi (Belém, Brazil), in which was deposited a voucher specimen, n° 247.

Crude extract and chromatographic fractions

The hydroalcoholic extraction (ethanol: H_2O at an 8:2 ratio) of the pulverized stalk of *C. cajucara* Benth was performed in a Soxhlet apparatus as previously reported.[4] After reduction of the solvent, the hydroalcoholic extract (CC-EHA) was submitted to chromatographed using silica gel as stationary phase affording several fractions eluted with mixtures of hexane: AcOEt at different gradient of polarity. Specifically, CC-EHA was submitted to chromatographic fractionation using silica gel (70–230 mesh) as the adsorbent according to the previous phytochemical studies of *C. cajucara*.[1,4,15] The tested terpenoid fractions F1-7 corresponding to non-polar fractions were eluted with hexane, and fractions F25-27 and F28 were eluted with mixtures of hexane: AcOEt in ratios of 9:1 and 8:2, respectively, and then tested for bioactivity.

Thin-layer chromatography (TLC) was made on silica gel PF_{254} plates (Merck, Darmstadt, Germany) using hexane: AcOEt (8:2–6:4) as the elution solvent and compounds were revealed with sulfuric acid: methanol (1:1), Ehrlich (furyl moiety) and Dragendorff (α,β-unsaturated ketone and/or lactone moiety) reagents. TLC samples were also revealed by UV radiation at wavelengths of 254 and 360 nm. Authentic samples were used to identify AAA, *t*-CTN, and *t*-DCTN as well as the other minor content compounds *cis*-and *trans*-cajucarin B. The NMR spectra of crude fractions were obtained on Varian-Gemini and Bruker-Advance spectrometers (300 MHz for ^1H and 75 MHz for ^{13}C) and the HRGC-MS analyses agreed with our previously reported data.[7,16,17]

Parasite cultures

Y strain of *T. cruzi* was firstly isolated from a human infection[18] which was used in all experiments. Epimastigotes grew to 28°C in liver infusion tryptose (LIT) medium with 10% fetal bovine serum plus penicillin 100 U/mL and streptomycin 100 µg/mL. The culture was conserved in log expansion once a week passage. Trypomastigotes were obtained from Vero cells infected by epimastigotes after 14-day culture in stationary phase, cultivating in DMEM with supplement of 10% calf fetal serum and gentamicin (100 µg/mL) and incubating at 34°C for 24 h under 5% CO_2 in humid conditions. The medium of culture was substituted every 2 days and after 7 days of infection, and the trypomastigotes were collected in the culture supernatant. The trypomastigotes were checked in a Neubauer chamber using crescent dilutions.

L. amazonensis promastigotes, MHOM/BR/77/LTB0016 strain characterized according literature,[19] were maintained at 25°C in Schneider medium with supplement of FCS (20%, v/v). Cells were collected in the tardy log-phase, resuspended in new medium, computed in a Neubauer chamber, and altered to a final of 4×10^6/mL.

The axenic amastigotes were prepared from culture of promastigotes in Schneider medium (pH = 7.2). Subsequent to 3 days, the culture was centrifuged, was resuspended in the same medium, and after 5 days, was centrifuged. The adjusted to 5×10^5 parasites/mL was made by addition trypan blue dye (0.1% PBS) to calculate the viable parasites. After, an aliquot was resuspended in Schneider medium supplemented with 20%

FCS (pH = 5.5). The samples were maintained at 26°C for 10 days and the process was continuous of equal form, and after 5 days, the sample was incubated at 32°C.

Leishmania promastigote assays

The tests were performed in 96-well plates and the fractions solubilized in dimethyl sulfoxide (DMSO) (1.6%, v/v) added to a parasite culture at 150–9.38 µg/mL of range concentration. After incubation at 26°C (24 h), the surviving parasites were counted and calculated the percentage of inhibition. The IC_{50} ± standard deviation (SD) values were obtained from the plot of inhibition percent × log (dose). All assays were made for each concentration in triplicate and three independent tests. The positive control was the pentamidine isethionate (May and Baker Lab., England).

Leishmania amastigote axenic assays

The amastigote culture was ready to use at the 16th day and after shock by heat, it was utilized.[20] The IC_{50} ± SD values were determined from the plot of inhibition percent × log (dose).

Trypanosoma cruzi epimastigote assays

T. cruzi epimastigotes at 5 × 10^6/mL in LIT medium were incubated with the *C. cajucara* stem bark extract and fractions, using benzonidazol (Rochagan®) as reference. The fractions and extract were diluted in DMSO (1.5% v/v) making concentrations of 100–3.125 µg/mL. The cultures were incubated at 26°C in 96-well plates by 24 h. The survival epimastigotes were counted, and the EC_{50} values, relating to the effectual dose that kills 50% of the parasites, were determined by a plot of survival parasites *versus* log (dose). Untreated and benznidazole-treated parasites were used as controls. All tests were performed in triplicate.

Trypanosoma cruzi trypomastigote assays

The trypomastigotes at 4 × 10^5 trypomastigotes well-1 in 96-well microplates were incubated with fractions in LIT medium supplemented with 50 mg/mL gentamicin and 10% calf fetal serum. DMSO (1.5% v/v) was used to solubilize all samples and the concentration of 150, 75, 37.5, 18.76, 9.38, 4.69, 2.34, 1.18, and 0.586 µg/mL for CC-EHA (F1-7 and F25-27) and at 50, 25, 12.5, 6.25, 3.125, 1.56, 0.8, 0.38, and 0.19 µg/mL for F28. After 24, 48, and 72 h, the living parasites were counted and the EC_{50} values were determined in the same way that to the epimastigotes. Untreated and benznidazole-treated parasites were used as controls. All tests were performed in triplicate.

Statistical analysis

The assays were made in triplicate, each time with four repetitions. The nonpaired Student's *t*-test was utilized, and the variation was judged as statistically significant when $P < 0.05$.

RESULTS

Extract and fractions

The hydroalcoholic extract, CC-EHA, was prepared using a solvent mixture (ethanol: H_2O) and showed a non-polar terpenoid fraction (F1-7) which contains sesquiterpenes, fatty acids, and steroids and small amounts of *t*-CTN, *cis*-cajucarin B, and *trans*-cajucarin B. The more polar fractions (F25-27 and F28) contain a mixture of *t*-DCTN, *t*-CTN, and the diastereoisomeric pair *cis*-and *trans*-cajucarin B [Figure 2].

The non-polar terpenoid fraction F1-7 was submitted to an esterification procedure and after evaluated by HRGC-MS. The chemical characterizations of these fractions were carried by correlation with mass spectra of literature, compared to the Wiley database and by their Kovats indices. The observed analyses revealed 70% sesquiterpenes (α-copaene and cyperene as major compounds and linalool as a minor oxygenated sesquiterpene), and the remaining 30% was detected to be a mixture of steroids, fatty acids, and clerodane diterpenes (*t*-CTN, *cis*-cajucarin B, and *trans*-cajucarin B) [Figures 1 and 2] that is according with our earlier data.[17]

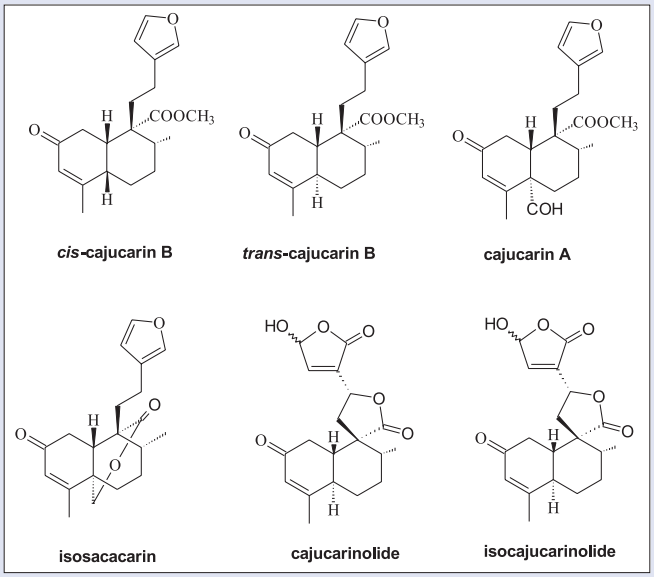


Figure 2: Chemical structures of the minor clerodane-type diterpenes isolated from the stem bark of *Croton cajucara* Benth

Comparative NMR analyses for fractions F25-27 and F28 with reported data[4,15] showed the presence of the triterpene AAA and clerodane-type 19-*nor*-diterpenes such as *trans*-crotonin (*t*-CTN), *cis*-cajucarin B (*c*-CJC B), *trans*-cajucarin B (*t*-CJC B), and *trans*-dehydrocrotonin (*t*-DCTN) [Figures 1 and 2]. The terpenoid total amounts for the CC-EHA extract were observed by chromatographic procedures with contents (0.8% for *t*-DCTN, 0.08% for AAA, and 0.002% for *t*-CTN; 0.001% of *c*-CJC B; and 0,005% of *t*-CJC B) concordant with reported data[1-4,7,16] in which the clerodane diterpene *t*-DCTN is the major compound followed by the acid triterpene AAA. The CC-EHA chromatographic fractions (F1-7, F25-27, and F28) showed different contents of AAA, *t*-CTN, and *cis*-and *trans*-CJC B or *t*-DCTN. Comparatively, the fraction F28 showed the greatest amount of the *t*-DCTN

Antiparasitic activities

The higher polar extract CC-EHA prepared from *C. cajucara* and its terpenoid fractions F1-7 (non-polar and non-volatile fraction) and the 19-*nor*-clerodane-rich fractions (F25-27 and F28) were assayed for antileishmanial effect against promastigotes and axenic amastigotes of *L. amazonensis*. Promastigotes revealed a greater activity to CC-EHA, with IC_{50} = 18.0 ± 0.01 µg/mL at 24 h of culture, but the fractions F1-7, F25-27, and F28 did not present any significant antipromastigote effect.

The hydroalcoholic extract (CC-EHA) and its terpenoid fractions were after tested with *L. amazonensis* axenic amastigotes in cultures of 24 h, 48 h, and 72 h [Table 1]. The most active fraction was the *t*-DCTN-rich fraction (F28) showing IC_{50} = 6.18 ± 0.02 µg/mL (24 h culture).

The assays with axenic amastigotes in culture for 48 h and 72 h confirmed the higher activity of the *t*-DCTN-rich fraction F28 (IC_{50} = 2.75 ± 0.07 µg/mL and 1.14 ± 0.03 µg/mL, respectively) and for the non-polar fractions (F1-7), in which *t*-DCTN was not detected, and

IC_{50} = 5.49 ± 0.14 μg/mL (48 h) and 2.54 ± 0.23 μg/mL (72 h) were observed [Table 1].

After these promising results, the hydroalcoholic CC-EHA extract and its terpenoid fractions (F1-7, F25-27, and F28) were assayed against trypomastigotes, epimastigotes, and axenic amastigotes of *T. cruzi* in 24, 48, and 72 h of culture. The IC_{50} values were evaluated in assays using a range concentration of 150 μg/mL to 9.38 μg/mL of CC-EHA, F1-7 and F25-27 and 50 μg/mL to 3.125 μg/mL of F28 against *T. cruzi* trypomastigotes and epimastigotes [Table 2].

The results of IC_{50} values to *T. cruzi* epimastigotes and trypomastigotes indicated a significant activity at 24 h of culture confirmed by 48 and 72 h of culture. The most active fraction was F28, with an IC_{50} = 3.23 ± 0.24 μg/mL, similar to that against *L. amazonensis*.

DISCUSSION

Leishmanicidal activity

Previously, we reported the significant antileishmanial activity of 19-*nor*-clerodane diterpene *trans*-dehydrocrotonin on promastigotes (IC_{50} = 6.30 ± 0.06 μg/mL) and axenic amastigotes (IC_{50} = 19.98 ± 0.05 μg/mL) of *L. amazonensis* and as none toxic for macrophages (0% of macrophage destroyed at >100 μg/mL).[21] These results reinforce the current result for F28 from which a clerodane mixture containing minor content of *t*-CTN, *c*-CJC B, *t*-CJC B, and greater content of *t*-dehydrocrotonin (*t*-DCTN) was shown to be more effective, indicating a possible synergic effect of these bioactive compounds.

Several essential oils obtained from diversified plants have shown antiparasitic effects on *Leishmania* species.[22,23] Among these studies, the report of *C. cajucara* essential oil, a rich source of linalool, showed antiparasitic effect on promastigotes and amastigotes of *L. amazonensis*,[24] reinforcing the importance of *C. cajucara* as a potential antiparasitic medicinal plant.

Trypanocidal activity

The hydro alcoholic extract of C. cajucara showed IC50 = 26.72±0.04 μg/mL in a 24 h culture against T. cruzi. In previous work,[8] the methanol extract of C. cajucara showed IC50 = 49.4±5.6 μg/mL value against T. cruzi, reaffirming the importance of the polar extract for trypanocidal activity. Further, for epimastigote cultures for 96 h, the methanolic extract demonstrated an IC_{50} = 109.1 ± 11.5 μg/mL and for 72 h of culture, the hydroalcoholic extract showed IC_{50} = 1.50 ± 0.03 μg/mL.

These results may be related to higher polar clerodanes which present in higher polar extract as well as aromatic metabolites as vanillic acid and 4-hydroxy-benzoic acid, eluted with EtOAc-EtOH (at polar gradient) and/or alkaloids compounds (magnoflorine and *N*, *N*-dimethyl-lindicarpine) and also, an amino acid (*N*-methyltyrosine) (eluted with EtOH: H_2O) isolated from the hydroalcoholic extract of *C. cajucara*.[3,7]

C. cajucara Benth has been shown to improve the availability of bioactive clerodane compounds. In this sense, *t*-DCTN is the major natural occurrence, and also, the most bioactive target compound isolated from this plant. Instead of that, for further investigations, largely parasitic pharmacological assays on the higher polar CC-EHA extract using a more polar biocomponents such as cajucarin A, isosacacarin, cajucarinolide, and isocajucarinolide [Figure 2], which are present in higher polar fractions,[1,3,7] or alkaloid compounds with are present in hydroalcohol fractions (ethanol: H_2O), should be performed in accordance to demonstrate the biological effects of *C. cajucara*.

The findings of this work compared to previous reports[8,21] showed that the 19-*nor*-clerodane-furan diterpene-type *trans*-DCTN is the most important antiparasitic component in the hydroalcoholic CC-EHA extract, specifically against *L. amazonensis* and *T. cruzi*. Further, the antipromastigote effect of CC-EHA showed higher activity (IC_{50} = 18.0 ± 0.01 μg/mL), but the terpenoid fractions (F1-7, F25-27, and F28) lack efficacy. In the other hand, when assayed with *L. amazonensis* axenic amastigotes, the most active fraction was F28, which is a rich source of *t*-DCTN, showing IC_{50} = 6.18 ± 0.02 μg/mL. Reinforcing the *t*-DCTN antiparasitic importance, the assays with axenic amastigotes in culture for 48 h and 72 h confirmed the higher activity of the fraction F28 (IC_{50} = 2.75 ± 0.07 μg/mL and 1.14 ± 0.03 μg/mL, respectively) and for the non-polar fractions (F1-7), in which *t*-DCTN was not detected, IC_{50} = 5.49 ± 0.14 μg/mL (48 h) and 2.54 ± 0.23 μg/mL (72 h). This result suggests that the other terpenoid compounds, such as sesquiterpenes, fatty acids, and steroids; small amounts of *t*-CTN, *c*-CJC B, and *t*-CJC B (observed in the terpenoid fractions F1-7); and the other 19-*nor*-clerodanes, present in the fraction F25-27, contribute less than *t*-DCTN, and strongly present in the F28 fraction.

Table 1: IC_{50} values (fraction concentration required to kill 50% ± standard deviation of the parasite) of CC-EHA, F1-7, F25-27, and F28 against *Leishmania amazonensis* axenic amastigotes in 24, 48, and 72 h of culture

Fractions	IC_{50} (μg/mL)		
	24 h	48 h	72 h
CC-EHA	14.74±0.05	19.17±0.14	7.12±0.01
F1-7	14.70±0.04	5.49±0.14	2.54±0.23
F25-27	12.45±0.71	8.39±0.25	4.28±0.16
F28	6.18±0.02	2.75±0.07	1.14±0.03

IC_{50}: Half-maximal inhibitory concentration

CONCLUSION

Finally, the results available in this work indicate that C. cajucara hydro alcoholic extract is a rich source of t DCTN and the stem bark of this plant constituted a favorable and useful in the therapy of parasitic diseases.

Table 2: IC_{50} values (fraction concentration required to kill 50% ± standard deviation of the parasite) of CC-EHA, F1-7, F25-27, and F28 against *T. cruzi* trypomastigotes and epimastigotes in 24, 48, and 72 h of culture

Fraction	IC_{50} (μg/mL)					
	Trypomastigotes			Epimastigotes		
	24 h	48 h	72 h	24 h	48 h	72 h
CC-EHA	26.72±0.04	6.48±0.06	1.94±0.02	13.74±0.30	5.93±0.02	1.59±0.03
F1-7	13.98±0.02	11.35±0.05	1.35±0.03	7.61±0.06	1.97±0.33	n.d.
F25-27	20.30±0.02	11.82±0.10	1.11±0.11	11.78±0.16	6.43±0.01	1.77±0.04
F28	3.23±0.24	2.45±0.11	0.43±0.02	7.92±0.67	1.78±0.16	0.27±0.02

n.d: Not determined; IC_{50}: Half-maximal inhibitory concentration

Financial support and sponsorship

The authors would like to thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPERJ (Fundação de Amparo a Pesquisa do Estado do Rio de Janeiro), CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), and FIOCRUZ (Fundação Oswaldo Cruz) for financial support.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Maciel MA, Pinto AC, Arruda AC, Pamplona SG, Vanderlinde FA, Lapa AJ, *et al.* Ethnopharmacology, phytochemistry and pharmacology: A successful combination in the study of *Croton cajucara*. J Ethnopharmacol 2000;70:41-55.
2. Maciel MA, Dantas TN, Câmara JK, Pinto AC, Veiga VF Jr., Kaiser CR, *et al.* Pharmacological and biochemical profiling of lead compounds from traditional remedies: The case of *Croton cajucara*. In: Khan MT, Ather A, editors. Advances in Phytomedicine -Lead Molecules from Natural Products, Discovery and New Trends. The Netherlands: Elsevier; 2006. p. 229-57.
3. Maciel MA, Gomes FE, Soares BA, Grynberg NF, Echevarria A, Cólus IM, *et al.* Biological effectiveness and recent advancing of natural products on the discovery of anticancer agents. In: Gupta VK, editor. Bioactive Phytochemicals: Perspectives for Modern Medicine. Vol. 2., Ch. 12. Nova Delhi: Daya Pulishing House; 2014. p. 239-93.
4. Maciel MA, Pinto AC, Brabo SN, Silva MN. Terpenoids from *C. cajucara*. Phytochemistry 1998;49:823-8.
5. Khan MT, Ather A, Pinto AC, Maciel MA. Potential benefits of the 19-nor-clerodane trans-dehydrocrotonin on the central nervous system. Braz J Pharmacog 2009;19:7-13.
6. Perazzo FF, Carvalho JC, Rodrigues M, Morais EK, Maciel MA. Comparative anti-inflammatory and antinociceptive effects of terpenoids and an aqueous extract obtained from *Croton cajucara* Benth. Braz J Pharmacog 2007;17:521-8.
7. Maciel MA, Martins JR, Pinto AC, Kaiser CR, Esteves-Souza A, Echevarria A. Natural and semi-synthetic clerodanes of *Croton cajucara* and their cytotoxic effects against Ehrlich carcinoma and human K562 leukemia cells. J Braz Chem Soc 2007;18:391-6.
8. Campos MC, Salomão K, Castro-Pinto DB, Leon LL, Barbosa HS, Maciel MA, *et al. Croton cajucara* crude extract and isolated terpenes: Activity on Trypanosoma cruzi. Parasitol Res 2010;107:1193-204.
9. Otoguro K, Iwatsuki M, Ishiyama A, Namatame M, Nishihara-Tukashima A, Kiyohara H, *et al. In vitro* antitrypanosomal activity of plant terpenes against Trypanosoma brucei. Phytochemistry 2011;72:2024-30.
10. Izumi E, Ueda-Nakamura T, Veiga VF Jr., Pinto AC, Nakamura CV. Terpenes from Copaifera demonstrated *in vitro* antiparasitic and synergic activity. J Med Chem 2012;55:2994-3001.
11. Ramírez-Macías I, Marín C, Chahboun R, Olmo F, Messouri I, Huertas O, *et al. In vitro* evaluation of new terpenoid derivatives against Leishmania infantum and Leishmania braziliensis. Mem Inst Oswaldo Cruz 2012;107:370-6.
12. WHO. Investing to Overcome the Global Impact of Neglected Tropical Diseases. Third WHO Report on Neglected Tropical Diseases. Geneva, Switzerland: World Health Organization; 2015. p. 75-81.
13. Coura JR, Viñas PA, Junqueira AC. Ecoepidemiology, short history and control of Chagas disease in the endemic countries and the new challenge for non-endemic countries. Mem Inst Oswaldo Cruz 2014;109:856-62.
14. WHO. Vector Borne Diseases. Leishmaniasis, Media Centre. Fact sheet Nº 375. Region office for Europe. World Health Organization; 2016. Available from: http://www.who.int/mediacentre /factsheets/fs387/en/index7.html. [Last access on 2019 Sep 25].
15. Maciel MA, Pinto AC, Kaiser CR. NMR and structure review of some natural furoclerodanes. Magn Reson Chem 2003;41:278-82.
16. Farias RA, González RP, Leyva A, Maia LS, Maciel MA, Pinto AC, *et al.* Chromatographic fractions from *Croton cajucara* inhibit cell proliferation and induce differentiation in a human leukemia cell line. J Soc Integr Oncol 2005;3:75-80.
17. Souza MA, Souza SR, Veiga VF Jr., Cortez JK, Leal RS, Dantas TN, *et al.* Chemical composition of Croton cajucara oil and determination of its fungicidal properties. Braz J Pharmacog 2006;16:599 610.
18. Silva LH, Nussenszweig V. On a strain of Trypanosoma cruzi highly virulent for the white mouse. Folia Clin Biol 1953;20:191 208.
19. Temporal RM, Cysne-Finkelstein L, Echevarria A, de Souza MA, Sertà M, da Silva-Gonçalves AJ, *et al.* Effects of amidine derivatives on parasite-macrophage interaction and evaluation of toxicity. Arzneimittelforschung 2002;52:489-93.
20. Castro-Pinto DB, Echevarria A, Genestra MS, Cysne-Finkelstein L, Leon LL. Trypanothione reductase activity is prominent in metacyclic promastigotes and axenic amastigotes of Leishmania amazonensis. Evaluation of its potential as a therapeutic target. J Enzyme Inhib Med Chem 2004;19:57-63.
21. Lima GS, Castro-Pinto DB, Machado GC, Maciel MA, Echevarria A. Antileishmanial activity and trypanothione reductase effects of terpenes from the Amazonian species *Croton cajucara* Benth (Euphorbiaceae). Phytomedicine 2015;22:1133-7.
22. Tariku Y, Hymete A, Hailu A, Rohloff J. *In vitro* evaluation of antileishmanial activity and toxicity of essential oils of Artemisia absinthium and Echinops kebericho. Chem Biodivers 2011;8:614-23.
23. Machado M, Pires P, Dinis AM, Santos-Rosa M, Alves V, Salgueiro L, *et al.* Monoterpenic aldehydes as potential anti-Leishmania agents: Activity of *Cymbopogon citratus* and citral on L. infantum, L. tropica and L. major. Exp Parasitol 2012;130:223-31.
24. do Socorro S Rosa Mdo S, Mendonça-Filho RR, Bizzo HR, de Almeida Rodrigues I, Soares RM, Souto-Padrón T, *et al.* Antileishmanial activity of a linalool-rich essential oil from *Croton cajucara*. Antimicrob Agents Chemother 2003;47:1895-901.