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Effect of extracts and isolated compounds from *Chresta scapigera* on viability of *Leishmania amazonensis* and *Trypanosoma cruzi*

Elisandra Cristina Schinor[1]*, Marcos José Salvador[2,5], Elisabeth Mieko Furusho Pral[3], Silvia Celina Alfieri[3], Sérgio Albuquerque[4], Diones Aparecida Dias[1,5]

[1]Departamento de Química da Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, [2]Universidade do Vale do Paraíba, Instituto de Pesquisa e Desenvolvimento, [3]Departamento de Parasitologia do Instituto de Ciências Biomédicas, Universidade de São Paulo, [4]Departamento de Análises Clínicas, Toxicológicas e Bromatológicas da Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, [5]Departamento de Física e Química da Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo

*Correspondence:
E. C. Schinor
Departamento de Física e Química
Faculdade de Ciências Farmacêuticas
de Ribeirão Preto
Universidade de São Paulo
Av. do Café, s/n
14040-903 - Ribeirão Preto - SP, Brasil
E-mail: ec.schinor@uol.com.br

Fractionation of bioactive crude extracts of Chresta scapigera *led to the isolation of four triterpenes and five flavonoids, among them β-amyrin acetate (**1**), 11α,12α-oxidetaraxeryl acetate (**2**) and lupeol (**3**), as well as the flavonoids apigenin (**6**), kaempferol (**7**), crysoeriol (**8**) and luteolin (**9**) were active against* Leishmania amazonensis *amastigotes-like stages, while only the flavonoids (**6**), (**7**) and (**9**) showed toxicity towards bloods trypomastigote forms of* Trypanosoma cruzi.

Uniterms
- *Chresta scapigera*/ trypanocidal activity
- Flavonoids
- Triterpenes
- *Chresta scapigera*/ leishmanicidal activity

INTRODUCTION

Plants have been traditionally used for the treatment of diseases of different aethiology. Especially in the last decade, phytotherapy has received considerable attention in the search for alternatives to chemotherapy in parasitic diseases control (Muelas-Serrano *et al*., 2000). Many of these studies involve species of the Asteraceae family, among which can be mentioned *Lychnophora granmongolense* (Grael *et al*., 2000), *Munnozia maronii* (Fournet *et al*., 1993), *Vernonia brachycalyx* (Oketch-Rabah *et al*., 1998), *Neurolaena lobata* (Berger *et al*., 2001), whose crude extracts and isolated compounds presented antiprotozoal activity.

Leishmaniasis is a protozoan disease and occurs widely in tropical areas in South America (Sauvain *et al*., 1996). According to the WHO there are 2 million new cases of leishmaniasis per year worldwide (Barata *et al*., 2000). This disease is caused by haemoflagellate protozoan parasites that survive and multiply in magrophages in the mammalian host and are transmitted by phlebotomine sandflies (Salvador *et al*., 2002). The incidence of the disease has increased in the last years, and the therapy available mainly relies on antimonials, pentamidine and amphotericin B, which are toxic, difficult to apply in the field and not always effective (Mafezoli *et al*., 2000).

Another protozoan disease is South America trypanosomiasis caused by *Trypanosoma cruzi* and widespread though out the subcontinent with an estimated 18 million people infected (Barata *et al*., 2000). In Brazil about 5-6 million people are infected, and of which 300,000 are located in São Paulo State. The population has been infected mainly by contaminated blood transfusion for the control of the insect vector (Ribeiro, Veloso, 1997). Gentian violet is the only effective chemoprophylatic agent available in blood banks. However, it is not completely accepted by

clinicians or patients because of its undesirable effects such as coloring the skin and possible mutagenic effects (Grael *et al*., 2000; Coura, Castro, 2002). For the acute and chronic diseases, the treatment is still a challenge today, since the available drugs possess severe side effects and are not completely efficient (Coura, Castro, 2002).

Thus, there is an urgent need for new drugs active against *Leishmania* spp and *Trypanosoma cruzi*.

Chresta, belonging to Asteraceae family, is a small genus that comprises 12 endemic species of the "Cerrado brasileiro" (Robinson, 1999). There are no prior studies about biological activity of this genus. The present studies, crude extracts (roots, stem, leaves and inflorescences) and some compounds isolated from *Chresta scapigera* were evaluated for their ability to reduce viability of axenic *Leishmania amazonensis* amastigotes and *T. cruzi* trypomastigotes forms, *in vitro*.

MATERIAL AND METHODS

Plant material

Chresta scapigera was collected in Furnas (MG-Brazil) in July, 1998, and identified by Dr. João Semir (Instituto de Biologia, Unicamp, Campinas, SP-Brazil). A voucher of each specimen is deposited in the Herbarium of FFCLRP/USP (SPFR6874).

Extraction and isolation process

Roots (188.0 g), stem (252.0 g), leaves (313.0 g) and inflorescences (99.0 g) were separated, dried, pulverized and stored in dark bags to protect them from humidity and light. The powdered material was extracted by maceration with *n*-hexane, dichloromethane and ethanol, respectively, at room temperature. The *n*-hexanic extract of roots (9.521 g) was chromatographed on a column of silica gel 60 (0.063-0.200 mm, Merck), eluted with hexane, ethyl acetate, methanol and mixtures of these solvents of increasing polarity. This extract furnishes 18 fractions and the fractions 3 and 4 were purified by preparative TLC yielding β-amyrin acetate (1) (16 mg) and 11α,12α-oxidetaraxeryl acetate (2) (10 mg), respectively. The *n*-hexanic extract of inflorescences (2.594 g) was chromatographed in the same conditions and furnishes 14 fractions. Preparative TLC was used to purify the fractions 4 and 7, which yield lupeol (3) (30 mg) and its acetate (4) (6 mg), respectively. The ethanolic crude extract of inflorescence (2.6 g) was partitioned between dichloromethane and methanol. The hidroalcoholic phase (1.3 g) was submitted to filtration on sephadex LH-20 and the fractions 6 and 7 were purified by preparative HPLC in reverse phase (column ODS Shimadzu 5 μm, 20 x 250 mm, eluent: methanol:water in gradient, flow 9mL/min, UV detection: 280 nm) affording tiliroside (5) (22 mg), apigenin (6) (12 mg) and kaempferol (7) (10 mg), respectively. While the dichloromethanic phase (391 mg) was chromatographed on a silica gel column eluted with hexane, ethyl acetate, methanol and mixtures of these solvents of increasing polarity. The fraction 3 was purified by preparative TLC affording crysoeriol (8) (3 mg). The ethanolic crude extract of stem was chromatographed on PVP by vacuum liquid chromatography eluted with $CHCl_3$, methanol and mixtures of these solvents of increasing polarity. Filtration on sephadex LH-20 was used to purify the fraction 1, which yield crysoeriol (8) (3 mg), apigenin (6) (2 mg) and luteolin (9) (3 mg).

The structures of all compounds were determined by spectroscopic methods (IR, ^{1}H- and ^{13}C-NMR). All extracts and isolated compounds were evaluated against axenic *L. amazonensis* amastigotes and *T. cruzi* trypomastigotes forms, *in vitro*, according to a previous report (Mafezoli *et al*., 2000; Bastos *et al*., 1999).

Trypanocidal activity

For the trypanocidal activity, blood of Swiss albino mice infected with *Trypanosoma cruzi* (Y strain) was used, which was collected by cardiac puncture at the peak of parasitemia and was diluted to contain 10^6 trypomastigotes/mL. Stock solutions (crude extracts) were prepared in 2.5% of dimethyl sulfoxide (DMSO) and were added to blood samples to provide a final concentration of 4000 μg/mL. After incubation for 24 hours at 4 °C, the number of parasites was determined according to Brener (1962). In the tests, gentian violet (250 μg/mL) was used as positive control and DMSO 2.5% as negative control. All experiments were performed in triplicate.

Leishmanicidal activity

Axenic *Leishmania (L.) amazonensis* (strain designation MPRO/BR/72/M 1841) amastigotes were serially cultivated at 33 °C in modified UM-54 mediun (Pral et al., 2003) and were used at the beginning of the stationary phase. Washed parasites were resuspended in RPMI-1640 medium supplemented with 4% fetal calf serum, pH 5.0 and incubated at 33 °C for 24 hours with crude extracts (1000 μg/mL) dissolved previously in RPMI-1640. As controls, parasite suspensions were incubated in RPMI alone or RPMI containing 0.1%

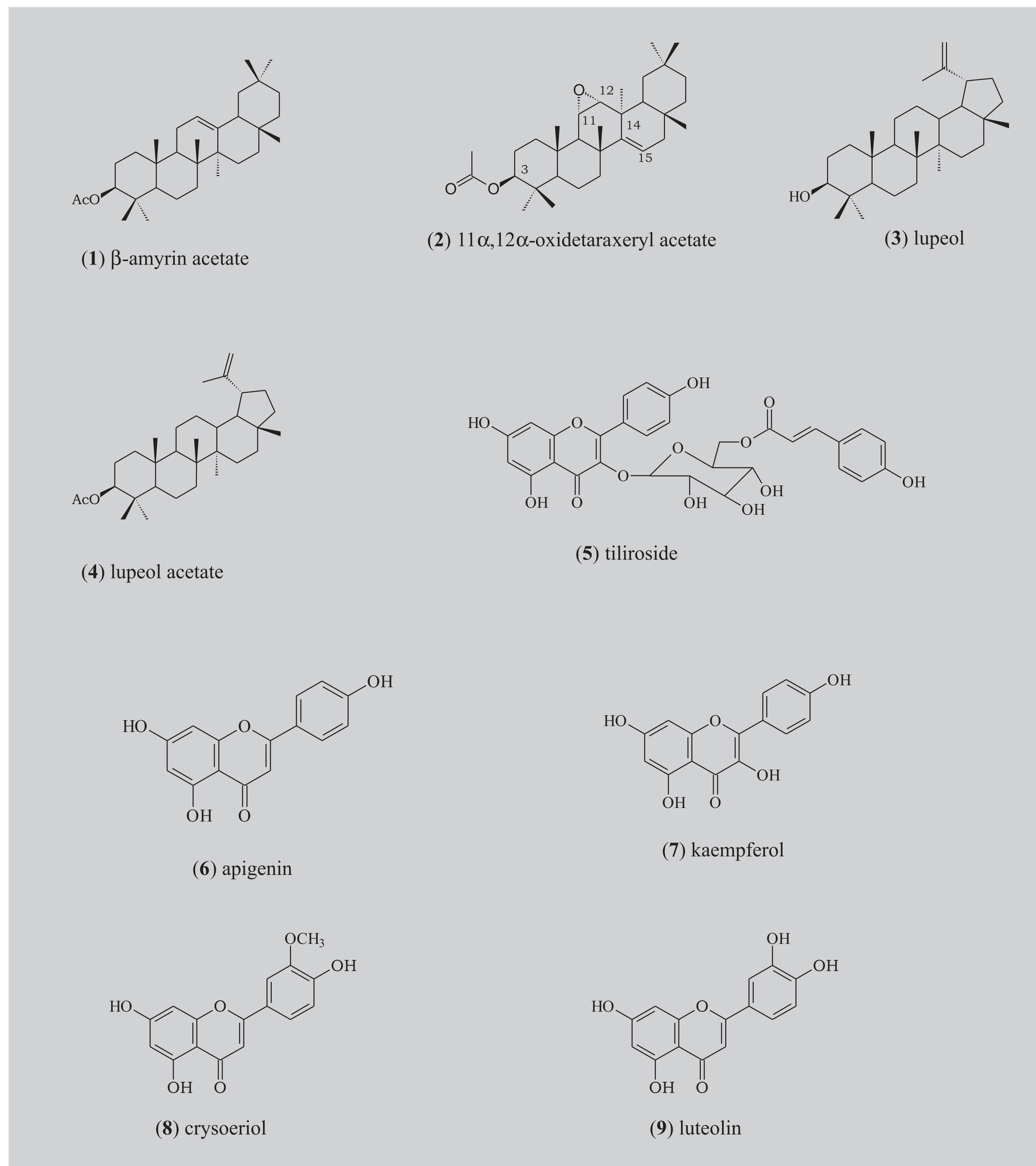

FIGURE 1 - Structures of isolated compounds from *C. scapigera*.

DMSO. Amastigote viability was assessed colorimetrically by reduction of a tetrazolium salt (MTT) as described by Mosmann (1983). Amphotericin B (20 µg/mL) was used as the positive control and DMSO/RPMI-1640 (1:99) negative control. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

The results are showed in Tables I and II. All the crude extracts evaluated markedly reduce the viability of *L. amazonensis* amastigotes (at 1000 µg/mL), while only the extracts hexanic of inflorescence, dichloromethanic of root and ethanolic (root and stem) did not interfere appreciably with lysis percentages of *T. cruzi* trypomastigotes forms (at 4000 µg/mL).

Data in Tables I and II show that effect of the evaluated compounds varies depending on the different concentrations used in the bioassay.

Compound **(5)** was not active against *L. amazonensis* and *T. cruzi* at the concentrations evaluated, while the compound **(1)** reduced the viability of *L. amazonensis* at the lower concentration used (at 14 µg/mL), but not interfere with lysis percentages of *T. cruzi* trypomastigote forms. Flavonoids **(6)** and **(9)** were the most active ones when assayed at 84 µg/mL against *L. amazonensis*, but a slight activity was observed against *T. cruzi*.

Compound **(6)** presents a 5,7,4'-hydroxylation pattern and showed active since 100 µg/mL with 54% of lysis. Ribeiro *et al.* (1997) and Grael *et al.* (2000) have demonstrated that hydroxylated flavonoids were more active than methoxylated flavonoids when evaluated against trypomastigote forms of *T. cruzi*. The authors believed that the probable mechanism of action for this activity might be explained from drugs that are able to generate reactive oxygen species in which *T. cruzi* is susceptible.

The other compounds interfered with viability of *L. amazonensis* and *T. cruzi* at concentration evaluated.

The results presented can be considered promising and these extracts may represent a source of active substances against mainly amastigote forms of *L. amazonensis*.

TABELA I - *In vitro* leishmanial activity of crude extracts and compounds from *C. sacpigera* against axenic *Leishmania amazonensis* amastigotes

Evaluated material	Viability % of axenic *Leishmania amazonensis* amastigotes (SD)[a] Concentration (µg/mL)			
	14	84	500	1000
hexanic extract of root	—	—	—	0.96 ± 0.00
stem	—	—	—	1.25 ± 0.00
leaves	—	—	—	2.50 ± 0.00
inflorescence	—	—	—	1.25 ± 0.00
dichloromethanic extract of root	—	—	—	1.83 ± 0.00
stem	—	—	—	3.08 ± 0.00
leaves	—	—	—	0.77 ± 0.00
inflorescence	—	—	—	3.27 ± 0.00
ethanolic extract of root	—	—	—	2.31 ± 0.00
stem	—	—	—	2.12 ± 0.00
leaves	—	—	—	2.12 ± 0.00
inflorescence	—	—	—	3.56 ± 0.00
Apigenin (6)	87.50 ± 0.01	16.23 ± 0.00	3.09 ± 0.00	—
Tiliroside (5)	100.00 ± 0.00	100.00 ± 0.00	67.8 ± 0.00	—
Kaempferol (7)	30.00 ± 0.56	21.00 ± 0.70	20.00 ± 0.17	—
Crysoeriol (8)	86.44 ± 0.02	73.10 ± 0.01	4.37 ± 0.00	—
Luteolin (9)	60.69 ± 0.02	16.32 ± 0.02	12.87 ± 0.00	—
β-amyrin acetate (1)	46.69 ± 0.04	36.17 ± 0.00	23.65 ± 0.00	—
11α,12α-oxidetaraxeryl acetate (2)	79.28 ± 0.04	50.16 ± 0.01	2.36 ± 0.00	—
Lupeol (3)	90.89 ± 0.08	71.02 ± 0.02	2.36 ± 0.00	—
Amphotericin B (20 µg/mL)[b]	3.37 ± 0.00			
DMSO/RPMI-1640 (1:99)[c]	100.00 ± 0.02			

[a] Parasite viability was determined by the MTT reduction assay; O.D. values are expressed as percent of those of untreated (negative control) ± standard deviation; [b] positive control; [c] negative control; —: concentration did not evaluated. All experiments were run in triplicate

TABELA II - In vitro trypanocidal activity of crude extracts and isolated compounds from *C. scapigera* against the trypomastigote forms of *Trypanosoma cruzi*

Evaluated material	Lysis % of *T. cruzi* trypomastigote ± (SD)[a] Concentration (µg/mL) 100	250	500	4000
hexanic extract of root	—	—	—	62.77 ± 2.19
stem	—	—	—	100.0 ± 0.00
leaves	—	—	—	100.0 ± 0.00
inflorescence	—	—	—	24.82 ± 4.55
dichloromethanic extract of root	—	—	—	42.37± 3.59
stem	—	—	—	61.86 ± 7.62
leaves	—	—	—	92.80 ± 2.64
inflorescence	—	—	—	77.96 ± 6.39
ethanolic extract of root	—	—	—	36.43 ± 2.54
stem	—	—	—	44.91 ± 1.46
leaves	—	—	—	70.76 ± 2.20
inflorescence	—	—	—	50.85 ± 10.78
Apigenin (6)	54.57 ± 1.56	55.50 ± 2.41	62.46 ± 1.28	—
Luteolin (9)	44.88 ± 5.84	52.57 ± 8.00	58.98 ± 2.22	—
Tiliroside (5)	12.08 ± 2.60	17.08 ± 7.53	45.00 ± 0.00	—
11α,12α-oxidetaraxeryl acetate (2)	1.67 ± 3.53	17.08 ± 0.88	23.33 ± 3.53	—
β-amyrin acetate (1)	6.57 ± 0.92	20.61 ± 2.74	20.61 ± 0.76	—
lupeol acetate (4)	41.81 ± 5.14	78.80 ± 3.85	79.40 ± 2.09	—
lupeol (3)	6.67 ± 3.53	23.33 ± 4.38	23.75 ± 8.19	—
Gentian violet (250 µg/mL)[b]	0.00 ± 0.00			
DMSO (2.5%)[c]	100.00 ± 0.00			

[a] Results are expressed as lysis % of *T. cruzi* trypomastigote forms ± standard deviation (SD); [b] positive control (IC_{50}= 31 µg/mL); [c] mice infected blood containing the same DMSO concentration used in the stocks solution (negative control) did not interfere with parasite viability; —: concentration did not evaluated. All experiments were run in triplicate.

However, further studies (*in vitro* and *in vivo*) should be accomplished in order to understand the mechanisms of action, as well as to evaluate the toxicity, looking for a clinical employment of those bioactive compounds.

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RESUMO

Efeito dos extratos e compostos isolados de *Chresta scapigera* sobre a viabilidade de *Leishmania amazonensis* e *Trypanosoma cruzi*

O fracionamento dos extratos bioativos de Chresta scapigera *proporcionou o isolamento de triterpenos e flavonóides, dentre os quais acetato de β-amirina (**1**), acetate de 11α,12-oxidotaraxeril (**2**) e lupeol (**3**), assim como os flavonóides apigenina (**6**), caenferol (7), crisoeriol (**8**) e luteolina (**9**) mostraram-se ativos contra formas amastigotas de* Leishmania amazonensis*, enquanto, apenas os flavonóides (**6**), (7) e (**9**) apresentaram toxicidade contra as formas tripomastigotas de* Trypanosoma cruzi.

***UNITERMOS**:* Chresta scapigera/*atividade tripanocida. Flavonóides. Triterpenos* . Chresta scapigera/*atividade leishmanicida*

REFERENCES

BARATA, L.E.S.; SANTOS, L.S.; FERRI, P.H.; PHILLIPSON, J.D.; PAINE, A.; CROFT, S.L. Anti-leishmanial activity of neolignans from *Virola* species and synthetic analogues. *Phytochemistry,* v. 55, p. 589-595, 2000.

BASTOS, J.K.; ALBUQUERQUE, S.; SILVA, M.L.A. Evaluation of the trypanocidal activity of lignans isolated from leaves of *Zanthoxylum naranjillo*. *Planta Med.*, v. 65, p. 541-544, 1999.

BERGER, I.; PASSREITER, C.M.; CACERE, A.; KUBELKA, W. Antiprotozoal activity of *Neurolaena lobata*. *Phytother. Res.*, v. 15, p. 327-330, 2001.

BRENER, Z. Therapeutic activity on criterion of cure on mice experimentally infected with *Trypanosoma cruzi*. *Rev. Inst. Med. Trop. São Paulo*, v. 4, p. 389-396, 1962.

COURA, J.R.; DE CASTRO, S.L. A critical review on Chagas disease chemotherapy. *Mem. Inst. Oswaldo Cruz*, v. 97, p. 3-24, 2002.

FOURNET, A.; MUÑOZ, V.; ROBLOT, F.; HOCQUEMILLER, R.; CAVÉ, A.; GANTIER, J.C. Antiprotozoal activity of dehydrozaluzanin C, a sesquiterpene lactone isolated from Munnozia maronii (Asteraceae). *Phytother. Res.,* v. 7, p. 111-115, 1993.

GRAEL, C.F.F.; VICHNEWSKI, W.; DE SOUZA, G.E.P.; LOPES, J.L.C.; ALBUQUERQUE, S.; CUNHA, R.W. A study of the trypanocidal and analgesic properties from *Lychnophora granmongolense* (Duarte) Semir & Leitão Filho. *Phytother. Res.*, v. 14, p. 203-206, 2000.

MAFEZOLI, J.; VIEIRA, J.C.; FERNANDES, J.B.; DA SILVA, M.F.G.F.; ALBUQUERQUE, S. In vitro activity of Rutaceae species against the trypomastigote form of *Trypanosoma cruzi*. *J. Ethnopharmacol.*, v. 73, p. 335-340, 2000.

MOSMANN, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Imm. Method,* v. 65, p. 55-63, 1983.

MUELAS-SERRANO, S.; NOGAL, J.J.; MARTINEZ-DÍAZ, R.A.; ESCARIO, J.A.; MARTINEZ-FERNANDEZ, J.R.; GÓMEZ-BARRIO, A. In vitro screening of American plant extracts on *Trypanosoma cruzi* and *Trichomonas vaginalis*. *J. Ethnopharmacol.*, v. 71, p. 101-107, 2000.

OKETCH-RABAH, H.A.; CHRISTENSEN, S.B.; FRYDENVANG, K.; DOSSAJI, S.F.; THEANDER, T.G.; CORNETT, C.; WATKINS, W.M.; KHARAZMI, A.; LEMMICH, E. Antiprotozoal properties of 16,17-dihydrobrachycalyxolide from *Vernonia brachycalyx*. *Planta Med.*, v. 64, p. 559-562, 2001.

RIBEIRO, A.; VELOSO, D.P. Trypanocidal flavonoids from Trixis vauthieri. *J. Nat. Prod.*, v. 60, p. 836-838.

ROBINSON, H. Generic and subtribal classification of American Vernonieae, Smithsonian contributions to Botany, number 89. Washington: Smithsonian Institution Press, 1999. p. 116.

SALVADOR, M.J.; FERREIRA, E.O.; PRAL, E.M.F.; ALFIERI, S.C.; ALBUQUERQUE, S.; ITO, I.Y.; DIAS, D.A. Bioactivity of crude extracts and some constituents of Blutaparon portulacoides (Amaranthaceae). *Phytomedicine*, v. 9, p. 566-571, 2002.

SAUVAIN, M.; POISSON, J.; KUNESCH, N.; GANTIER, J.C.; GAYRAL, P.; DEDET, J.P. Osilation of leishmanicidal triterpenes and lignans from Amazonian Liana *Doliocarpus dentatus* (Dilleniaceae). *Phytother. Res.*, v. 10, p. 1-4, 1996.

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