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Antiprotozoal activity of Jatrogrossidione from *Jatropha grossidentata* and Jatrophone from *Jatropha isabellii*

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The activity of jatrogrossidione, the main diterpene of *Jatropha grossidentata* and jatrophone from *Jatropha isabellii* was determined against *Leishmania* and *Trypanosoma cruzi* strains *in vitro* as well as against *Leishmania amazonensis* in vivo. Jatrogrossidione showed a strong *in vitro* leishmanicidal and trypanocidal activity with IC_{100} of 0.75 and 1.5–5.0 μg/mL, respectively. Under similar conditions, the IC_{100} of glucantime, ketoconazole and pentamidine towards *Leishmania* strains were >100, 50–100 and 1 μg/mL, respectively. The IC_{50} of jatrogrossidione was <0.25 μg/mL against amastigote forms of *Leishmania* infecting macrophages, with toxicity at concentrations higher than 0.5 μg/mL.

BALB/c mice infected with *L. amazonensis* strain PH 8 were treated 24 h after infection with jatrogrossidione and jatrophone for 13 consecutive days. Jatrophone at 25 mg/kg/day subcutaneously administered was significantly active ($p<0.05$) against the virulent strain PH 8 of *L. amazonesis*; it was more active than Glucantime at 112 mg Sb^v per kg/day. Subcutaneous administration of jatrophone, however, proved to be too toxic under our assay conditions. Assays of single local treatment on the footpad infection 2 weeks after inoculation of *L. amazonensis* indicated that jatrogrossidione and jatrophone were inactive at the selected doses.

Keywords: Jatropha grossidentata; *Jatropha isabellii*; Euphorbiaceae; antiprotozoal; *Leishmania*; *Trypanosona cruzi*; jatrogrossidione; jatrophone.

INTRODUCTION

Leishmaniasis is a tropical disease endemic in south and Central America and caused by the protozoan *Leishmania* spp. The chemotherapy of leishmaniasis involves the use of pentavalent antimonials and amphotericin B, which are expensive, toxic and sometimes inefficient drugs (Croft, 1988; Berman, 1988). Chagas' disease, a South American disease, is caused by the protozoan *Trypanosoma cruzi* and affects 15–20 million people from Argentine to Mexico. The treatment of acute Chagas' disease is performed with drugs such as nifurtimox and benznidazole, both of them toxic, with varying efficacy and with undesirable side effects. There is no effective cure for the chronic phase of the disease (WHO, 1991).

New drugs for the treatment of these protozoal diseases are urgently required and the ethnomedicine of the South American Indians is a valuable source of plant candidates for pharmacological and chemical studies (Wright and Phillipson, 1990).

Jatropha grossidentata Pax et Hoffm. (Euphorbiaceae) is a shrub known as 'Caniroja' by the Ayoreo Indians living in the central-northern part of the Paraguayan Chaco. The powdered roots are smoked in shamanic practices (Schmeda-Hirschmann, 1993). In a preliminary screening, the petroleum ether and ethyl acetate extract of Caniroja roots showed *in vitro* activity against *T. cruzi* and *Leishmania* strains at 10 μg/mL. Several diterpenes have been isolated from the roots, the main compound being the rhamnofolane jatrogrossidione (Jakupovic *et al.*, 1988; Schmeda-Hirschmann *et al.*, 1992). *Jatropha isabellii* Muell. Arg. is known as 'Yaguá rová' in Eastern Paraguay. An infusion, decoction or macerate of the rhizomes is used in Paraguayan traditional medicine as an abortifacient as well as to treat rheumatism and human gout.

The objective of this work was to assess the antiprotozoal activity of the diterpenes jatrogrossidione **1** and jatrophone **2** (see Fig. 1) towards *T. cruzi* and *Leishmania* strains.

MATERIALS AND METHODS

Plant material. *Jatropha grossidentata* Pax et Hoffm. was collected in Campo Loro, Paraguayan Chaco, while *Jatropha isabellii* Muell. Arg. was gathered in Altos, Departamento Cordillera, Paraguay, in December 1991.

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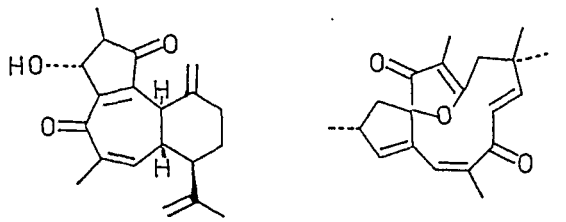

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Figure 1.

Voucher herbarium specimens (Schmeda 1289 and 1594, respectively) have been deposited at the Department of Botany, Smithsonian Institution, Washington, D.C., USA as well as at the Herbario de la Universidad de Talca, Chile. The collections were identified by Stephen Smith (US).

Extraction and isolation. Jatrogrossidione was extracted from *J. grossidentata* roots as previously described (Jakupovic *et al.*, 1988; Schmeda-Hirschmann *et al.*, 1992) and was identified by physical constants, high field NMR spectroscopy and direct comparison on TLC with an authentic sample.

Jatrophone was isolated as the main diterpene from *J. isabellii* rhizomes and was identified by spectroscopic methods and x-ray diffraction (Kupchan *et al.*, 1970).

Leishmanicidal activity. Culture and maintenance of the *Leishmania*. Parasite: *Leishmania amazonensis* strain MHOM/GF/84/CAY H-142 was originally isolated at the French Guyana Institut Pasteur. *Leishmania braziliensis* strain MHOM/BR/75/M2903 and *Leishmania chagasi* strain MHOM/BR/74/PP75 were obtained from IBBA, a WHO reference laboratory. Strain identification was confirmed by isoenzyme analysis (Sauvain *et al.*, 1993). *Leishmania* promastigotes were cultured in Novy, MacNeal and Nicolle medium with 10% heat inactivated fetal serum at 28 °C.

***In vitro* tests on promastigote cultures of *Leishmania* spp.** *J. grossidentata* fractions, jatrogrossidione and jatrophone were aseptically dissolved in liquid medium and DMSO and placed in 96-well microtitre plates (Flow Laboratories) to obtain final concentrations of 20, 10, 5, 3, 1.5 and 0.75 μg/mL. The final DMSO concentration did not exceed 0.1%. All assays were carried out in triplicate. Each cell was cultured with 50 000 parasites at 27 °C. The activity of the samples was evaluated after 72 h by optical observation on a culture drop with an inverted phase microscope, by comparison with control cells (without extracts). Pentamidine was used as a positive control (Sauvain *et al.*, 1993).

***In vitro* test on the amastigote forms.** Mouse peritoneal macrophages were obtained as described by Sauvain (1989). One million non-inflammatory macrophages were collected from each BALB/c mouse. The adherent cells were placed at 37 °C under 5% CO_2 for 2 h. The plates were then washed with RPMI+buffer (MOPS-Sigma, USA), without FCS to eliminate non-adherent cells. The supernatant was replaced by 0.5 mL/well of fresh RPMI medium+glutamine+FCS+antibiotics (100 IU penicillin and 100 μg streptomycin/mL) before infection by *L. amazonensis* amastigotes at a ratio of infecting organism to host cells of 5:1. Infection took place at 34°C for a minimum of 2 h. Compounds were then added to the culture maintained at 37°C under 5% CO_2 for 24 h. The medium was then renewed and the cells left to incubate for another 24 h before fixation. Plates were fixed with methanol and stained with 10% Giemsa's stain (CML, France). They were set up with Eukitt Resin (CML, France). Macrophages with and without parasites were counted under ×40 magnification. For each triplicate assay, the survival index (SI) of amastigotes was calculated relative to the control.

***In vivo* test procedure.** Eight-week-old BALB/c mice, 20 ± 2 g, $n = 10$ for each random group (IFFA Credo, France) were infected with 2.5×10^5 amastigotes of *L. amazonensis* (PH 8 strains) in one posterior foot (Trotter *et al.*, 1980). Growth of cutaneous lesions was monitored for 8 consecutive weeks, by measuring the diameter of the infected feet with a vernier caliper (Kroelin 1ODIOOT6) at weekly intervals. Results were compared with those obtained with N-methylglucamine antimoniate (Glucantime). For each experiment, the mean of the difference between the infected foot and the normal foot was calculated with standard error of the mean.

Mice in the first experiment were treated by the subcutaneous route. N-methylglucamine antimoniate was given at a dose of 112 mg of Sb^v per kg of body weight daily and jatrogrossidione and jatrophone were given at 25 mg/kg/day for 13 days. The compounds were dissolved in 75 μL of RPMI 1640 and 25 μL of DMSO. Drug treatment commenced 1 day after the inoculation of amastigotes and was continued once daily for 13 days. In the second experiment, the treatment of mice started 2 weeks after infection by a single intralesional injection. For this experiment, mice were treated with N-methylglucamine antimoniate at 112 mg of Sb^v per kg and with the diterpene at 50, 25 and 12.5 mg/kg. The Student's *t*-test with the program STAVIEW was used for the statistical analysis of the 14 day assay ($p < 0.05$).

Trypanocidal activity. Parasite: *Trypanosoma cruzi* strains Tulahuen, C8CL1, 1979CL1 and YC12 were used. The strains were obtained from IBBA and the identification was confirmed by isoenzyme analysis. *T. cruzi* epimastigotes were cultured in LIT (liver infusion tryptose) medium supplemented with 10% fetal calf serum at 28 °C with an inoculum of 10^6 cells/mL. Samples (4 mg) were aseptically dissolved in 50 μL DMSO and liquid medium to obtain final concentrations of 20, 10, 5, 3, 1.5 and 0.75 μg/mL. All assays were carried out in triplicate. Final DMSO concentration was less than 0.5%. Parasites were counted after 48 h of contact with the samples in a haemocytometer and the activity of the test substances was assessed by comparison with controls (without extract) and with nifurtimox containing cells (Fournet *et al.*, 1988b).

RESULTS AND DISCUSSION

The petroleum ether and ethyl acetate extracts of *Jatropha grossidentata* roots showed a significant activity against *Leishmania* promastigotes and *T. cruzi* epimastigotes *in vitro*. Bioassay-guided isolation identified jatrogrossidione as the main active *J. grossidentata* compound. Jatrogrossidione **1** and jatrophone **2** were quite active towards *Leishmania* promastigotes, showing IC_{100} of 0.75 μg/mL and 5 μg/mL, respectively, and similar activities against the

Table 1. ***In vitro*** **activity of jatrogrossidione, jatrophone and selected reference compounds on** ***Leishmania*** **(μg/mL, IC_{100}) and** ***T. cruzi*** **(IC_{90} μg/mL)**

	L. braziliensis	L. amazonensis	L. chagasi
IC_{100}	2903	H-142	PP-75
Jatrogrossidione	0.75	0.75	0.75
Jatrophone	5	5	5
Glucantime	>100	>100	>100
Ketoconazole	50	50	100
Pentamidine	1	1	1

T. cruzi	Tulahuen	C8CL1	1979	YC12
Jatrogrossidione	1.5	<5	<5	<5
Nifurtimox	25	25	50	25
Benznidazole	50	100	25	25

Tulahuen strain of *Trypanosoma cruzi* (Table 1). Under similar conditions, the IC_{100} of the reference compounds glucantime, ketoconazole and pentamidine were >100, 50 and 1 μg/mL, respectively (see Table 1).

Compound **1** is a diterpene belonging to the rhamnofolane type while jatrophone **2** is a jatrophane macrocycle, previously isolated from *Jatropha gossypiifolia* (Kupchan *et al.*, 1970). Acetylation of **1** sharply reduced the trypanocidal effect, the compound being active at concentrations >100 μg/mL.

Jatrogrossidione was active at 0.5 μg/mL against the amastigote forms of *Leishmania* infecting macrophages, with a reduced toxicity towards the host cells (macrophages) (Table 2). The *in vivo* activity of diterpenes **1** and **2** was evaluated against BALB/c mice infected with *L. amazonensis* strain PH 8. The compounds were administered subcutaneously at 25 mg/kg/day for 13 days. The LD_{50} was previously assessed for each diterpene by intraperitoneal injection. Results of this *in vivo* model are presented in Table 3. Jatrophone significantly reduced the diameter of lesions compared with control mice at weeks 1, 2, 3, 4 and 6 ($p<0.05$). Moreover, the lesions of jatrophone-treated mice were smaller than those treated with Glucantime. However, half of the mice receiving jatrophone at 25 mg/kg/day died during the experiment. In mice treated with a single intralesional injection in the infected footpad after inoculation of *L. amazonensis*, jatrogrossidione and jatrophone did not show statistically significant effects at 25 mg/kg (Table 4). The toxicity of jatrophone renders this diterpene unsuitable for use in the chemotherapy of leishmaniasis. Structure/activity relationships studies of jatrophone and their derivatives are necessary to identify compounds with reduced toxicity and increased leishmanicidal effects *in vivo*.

Table 2. ***In vitro*** **activity of jatrogrossidione and the most active** ***J. grossidentata*** **fraction on** ***L. amazonensis*** **amastigotes compared with their toxicity towards macrophages**

	Dose (μg/ml)			
	1.0	0.5	0.25	0.125
Fraction 12				
Survival of amastigotes, SI%	7	81	87	—
Viability of macrophages %	95	98	—	—
Jatrogrossidione				
Survival of amastigotes, SI%	0	0	2	62
Viability of macrophages %		74	91	100

Viability %, mean percentage of surviving macrophages.
SI%, percentage survival of intracellular amastigotes.

Table 3. Effect of N-methylglucamine antimonate (Glucantime) at 112 mg Sb^v per kg/day, jatrogrossidione and jatrophone at 25 mg/kg/day on the development of ***L. amazonensis*** **PH 8 in BALB/c mice (diameter of lesion±SEM). Treatments were given for 13 days starting 1 day after inoculation of** ***L. amazonensis***

	Diameter of lesion			
	Jatrogrossidione	Jatrophone	Glucantime	Untreated
Weeks post-infection				
1	0.75±0.09	0.16±0.05*	0.48±0.25	0.93±0.37
2	1.94±0.41	0.28±0.08*	1.48±0.40*	2.11±0.48
3	3.16±0.49	1.30±0.21*	2.58±0.43*	3.27±0.71
4	4.19±0.73	2.40±0.27*	3.68±0.45*	4.34±0.73
5	5.59±0.90	3.88±0.19	4.92±0.80*	5.65±1.11
6	6.64±0.81	5.08±0.40*	6.35±0.91	6.45±1.13
7	7.89±1.10	7.02±0.40	7.62±0.98	8.07±0.66
8	9.01±1.38	8.70±0.29	8.73±0.75	8.51±0.81
N	8	5	10	10

N number of animals surviving the assay.
* $p<0.05$.

Several natural products have been reported as displaying antiprotozoal activity towards *T. cruzi* and *Leishmania*. In a study of the antiparasitic effects of bisbenzylisoquinoline alkaloids, daphnandrine, gyrocarpine and obaberine were active towards *Leishmania* and *T. cruzi* strains, with IC_{100}>50 μg/mL (Fournet *et al.*, 1988a, 1988b). The same authors (1992) report the *in vitro* effect of *Pera benensis* naphthoquinones against the promastigote and epimastigote forms of *T. cruzi* and the amastigote form of *Leishmania* as well as of 2-substituted quinoline alkaloids on *T. cruzi* and *Leishmania*, with IC_{90} (μg/mL) between 25 and 100 μg/mL (Fournet *et al.*, 1993a). Sauvain *et al.* (1993) reported the effect of ursolic acid and jacaranone from *Jacaranda copaia* on Leishmania, with ED_{50} of 0.02 mM *in vitro* and weak *in vivo* activity.

Dehydrozaluzanin C inhibited *in vitro* the growth of 12 *Leishmania* and 15 *T. cruzi* strains with IC_{90} between 2.5

Table 4. Effect of N-methylglucamine antimonate at 112 mg of Sb^v per kg/day, jatrogrossidione and jatrophone at 50, 25 and 12.5 mg/kg/day on the development of ***L. amazonensis*** **PH 8 in BALB/c mice (diameter of lesion±SEM). Treatments were given on the infected footpad with a single intralesional injection 14 days after the inoculation of** ***L. amazonensis***

	Jatrogrossidione			Glucantime
Dose (mg/kg)	50	25	12.5	200
Weeks post-infection				
1	1.21±0.53	1.26±0.28	1.29±0.23	0.90±0.32
2	2.96±0.44	2.64±0.52	2.44±0.37	1.83±0.37
3	3.99±0.57	3.71±0.56	3.71±0.53	3.05±0.46
4	5.34±0.59	5.13±0.68	5.04±0.57	3.85±0.82
5	6.90±0.72	6.74±0.93	6.92±1.00	5.54±0.66
6	7.88±0.88	7.39±0.78	8.03±0.81	6.89±0.70
7	9.29±0.71	7.58±0.78	9.54±0.80	8.66±0.74
8	10.23±0.72	7.60±1.00	9.07±0.96	9.44±0.46
		Jatrophone		Untreated
Dose (mg/kg	50	25	12.5	
1	1.30±0.25	1.10±0.16	1.14±0.15	1.23±0.17
2	3.01±0.28	2.49±0.21	2.38±0.23	2.66±0.20
3	3.60±0.63	3.49±0.36	3.47±0.32	4.03±0.29
4	4.77±0.61	4.66±0.63	4.35±0.90	5.49±0.39
5	6.22±0.82	6.02±0.71	6.37±0.63	6.53±0.69
6	7.34±0.88	7.46±0.81	7.31±0.31	7.33±0.43
7	8.89±1.07	9.14±0.62	8.61±0.58	7.31±0.91
8	9.80±0.58	9.59±0.70	9.23±0.75	8.06±1.01

and 50 μg/mL. Under the same experimental conditions, pentamidine and ketoconazole showed IC_{90} of 0.5–2.5 and 50–>100 μg/mL, respectively (Fournet *et al.*, 1993b). A review of the antileishmanial activity of medicinal plants and isolated compounds has been published by Iwu *et al.* (1994).

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