

# *In vitro* evaluation of the cytotoxic and trypanocidal activities of *Ampelozizyphus amazonicus* (Rhamnaceae)

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## Abstract

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*Ampelozizyphus amazonicus* Ducke is a tree commonly found in the Amazon region and an extract of its stem bark is popularly used as an antimalarial and anti-inflammatory agent and as an antidote to snake venom. Ursolic acid; five lupane type triterpenes: betulin, betulinic acid, lupenone, 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid, and 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid, and three phyto-steroids: stigmasterol, sitosterol and campesterol, have been isolated from stem extracts of *A. amazonicus* Ducke. Their structures were characterized by spectral data including COSY and HMQC. In an *in vitro* biological screening of the isolated compounds, 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid was cytotoxic against the SKBR-3 human adenocarcinoma cell line (1 to 10 mg/mL), while 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid exhibited cytotoxicity against both SKBR-3 human adenocarcinoma and C-8161 human melanoma tumor cell lines (>0.1 mg/mL). In the present study, different extracts and some fractions of this plant were also investigated for trypanocidal activity due to the presence of pentacyclic triterpenes. The triterpene classes are potent against *Trypanosoma cruzi*. The bioassays were carried out using blood collected from Swiss albino mice by cardiac puncture during the parasitemic peak (7th day) after infection with the Y strain of *T. cruzi*. The results obtained showed that *A. amazonicus* is a potential source of bioactive compounds since its extracts and fractions isolated from it exhibited *in vitro* parasite lysis against trypomastigote forms of *T. cruzi* at concentrations >100  $\mu$ g/mL. Fractions containing mainly betulin, lupenone, 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid, and 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid showed more activity than crude extracts.

### Key words

- *Ampelozizyphus amazonicus*
- Rhamnaceae
- Pentacyclic triterpenes
- Cytotoxic activity
- Trypanocidal activity
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## Introduction

*Ampelozizyphus amazonicus* Ducke (Rhamnaceae) is a native tree of the Amazon forest that is popularly known in Brazil as *saracura-mirá* or *cerveja de índio*. Infusions of *A. amazonicus* roots are commonly used in traditional medicine as anti-inflammatory preparations, as an antidote to snake venoms and for the treatment and prevention of malaria (1,2). Earlier chemical investigations have reported the presence of saponins in *A. amazonicus* (1,3). Additionally, Rhamnaceae species have shown an inhibitory effect on alcohol-induced muscle relaxation and cytotoxic, genotoxic, neurotoxic, anti-inflammatory, antipyretic, and hepatoprotective activities have been associated with its extracts and pure compounds (4-8).

The transmission of Chagas' disease has become a major health problem in South and Central America, with the transfusion of infected blood being the most significant transmission mechanism (9). The lack of effective medicines against acute and chronic diseases justifies the search for more efficient and less toxic drugs than those currently used. At present, the only trypanocidal compound used to prevent infection in blood banks is gentian violet, but due to its toxic effects and the alarming color acquired by the skin and urine of transfusion recipients, its use is limited (10). Since most of the synthetic compounds used thus far to treat parasitic diseases produce toxic side effects, the nitroheterocycles nifurtimox and benzimidazole used to be the only drugs prescribed in the early stages of trypanosomiasis in America (11,12), but more recently nifurtimox has been banned from the market. For this reason, a continuous search for bioactive trypanocidal compounds, in combination with studies on their mechanism of action to validate the rational development of lead compounds, has motivated the screening of natural products active against *Trypanosoma cruzi* and related organisms.

In the present study, we report the isolation and structural elucidation of constituents of extracts from *A. amazonicus* stems collected in Manaus, AM, Brazil. The EtOAc extract and its fractions inhibited the growth of two tumor cell lines and showed potential *in vitro* lysis of *A. amazonicus* extracts and fractions against trypomastigote forms of *T. cruzi*.

## Material and Methods

*Ampelozizyphus amazonicus* stems were collected in the Ducke Reserve (Manaus, AM, Brazil). A voucher specimen was identified by Mad de Souza INPA, AM, Brazil) and deposited in the herbarium of the Instituto Nacional de Pesquisa da Amazônia, INPA, under code number 189,858. The bioassay procedures adopted were approved by the Ethics Committee of Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, which follows the rules recommended by International Guiding Principles for Biochemical Research Involving Animals (CIOMS), Geneva, 1985.

General  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded in pyridine- $d_5$  at 300 and 75 MHz (Bruker DPX-300, Departamento de Química, FFCLRP, USP, Ribeirão Preto, SP, Brazil), respectively, using tetramethylsilane as internal standard.  $^{13}\text{C}$  NMR chemical resonances were qualified for multiplicity by the DEPT sequence. Thin layer chromatography (TLC) precoated plates with silica gel PF<sub>254</sub> (Aldrich®, Milwaukee, WI, USA) using the following solvent systems: hexane:EtOAc (7:3),  $\text{CHCl}_3$ :MeOH (4:1) and n-butanol:acetic acid:water, 4:1:5, upper phase. TLC color reagent: vanillin-sulfuric acid followed by heating. CC: silica gel (230-400 mesh; Merck, Darmstadt, Hessen, Germany). Preparative thin layer chromatography (PTLC) precoated plates with silica gel G (1.0 mm) using solvent system  $\text{CHCl}_3$ :MeOH (95:5). HPLC was performed under isocratic conditions

(MeOH:H<sub>2</sub>O, 4:1, v/v) using a diode array detector (Shimadzu, Kyoto, Japan) and a Supelcosil™ LC-18 column (10 x 250 mm, 5 µm) and a flow rate of 3.0 mL/min.

### Extraction and isolation

Air-dried and powdered plant material (900 g) was macerated and successively extracted (1:2 kg/L) with organic solvents (hexane, EtOAc, EtOH, and MeOH) and water at room temperature for each 7 days. Next, the material was filtered, the solvent evaporated, water was added and the sample was lyophilized. Crude extracts were concentrated to yield hexane (E<sub>1</sub>, 1.74 g), EtOAc (E<sub>2</sub>, 17.33 g), EtOH (E<sub>3</sub>, 42.09 g), MeOH (E<sub>4</sub>, 11.02 g), and aqueous (E<sub>5</sub>, 1.83 g) extracts. A second extract was obtained by maceration of 2500 g air-dried and powdered *A. amazonicus* stems in 5 L methanol. This MeOH extract (E<sub>6</sub>, 50 g) was concentrated under vacuum and submitted to partition with *n*-butanol (E<sub>7</sub>, 45.23 g). The insoluble fraction was separated and dried (E<sub>10</sub>, 3.84 g).

Extract E<sub>2</sub> and fraction E<sub>7</sub> were submitted to successive steps of column chromatography (silica gel) eluted with a step gradient of hexane, EtOAc and/or chloroform and methanol. Fractions E<sub>2</sub>P (10.63 g), E<sub>2</sub>P<sub>3</sub> (0.97 g), E<sub>2</sub>P<sub>9</sub> (0.17 g), and E<sub>7(5/6)</sub> (5.78 g) were obtained as above. PTLC and HPLC were used for purification. Fractions were collected and monitored by TLC. Compounds 2 (38.6 mg), 5 (95.1 mg) and 6 (23.9 mg) were obtained from the E<sub>2</sub> extract and compounds 1 (18.1 mg), 3 (4.5 mg) and 4 (36.5 mg) from the E<sub>7</sub> fraction. The E<sub>3</sub> extract (15.0 g) was submitted to partition with *n*-butanol (EEBAa, 4.15 g).

The stem bark aqueous extract was prepared with 1000 g air-dried stem bark in 2 L water under mechanical shaking and the extracts with no froth (E<sub>8</sub>) and with froth (E<sub>9</sub>) were separated and lyophilized, yielding 12.27 and 16.65 g, respectively.

### Tumor cell line culture

The SKBR-3 human breast adenocarcinoma and C-8161 human melanoma tumor cell lines were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA). The tumor cell lines were cultured and maintained in RPMI 1640 medium supplemented with 2 mM L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate, 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin. All cell lines were maintained at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% air and 95% humidity.

### Cytotoxic activity of tumor cells

The cytotoxic activity of tumor cells was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method (13). Briefly, tumor cells cultured in appropriate flasks and maintained in continuous exponential growth were detached with 0.05% trypsin, 0.02% EDTA in calcium-free phosphate-buffered saline and washed three times with RPMI medium at 1000 rpm/15 min at 10°C. Cells were placed on 96-well plates at a density of 1 x 10<sup>5</sup> cells per well. After 24 h of culture, the medium was removed and fresh medium, with or without different concentrations of the indicated extracts/compounds/or the control drug methotrexate (10-0.01 mg/mL), was added to the wells and incubated for 24 h. At the end of treatment, 10 µL MTT (10 mg/mL) was added to the wells, and cells were incubated for a further 4 h. Finally, 50 µL 20% sodium dodecyl sulfate solution was added to each well. Formazan crystals were dissolved at 37°C overnight. The absorbance of each well was read on a microplate reader at 540 nm. The cytotoxic rate was calculated as follows: % cytotoxicity of compounds = 1 - absorbance drug treated/absorbance control x 100.

### Trypanocidal assay

Crude extracts and their subfractions were tested *in vitro* against trypomastigote blood forms of *T. cruzi* (Y strain). The bioassays were carried out using blood collected from Swiss albino mice by cardiac puncture during the parasitemic peak (7th day) after infection with the Y strain of *T. cruzi*. The blood was diluted in non-infected murine blood to give a concentration of ca.  $2 \times 10^6$  trypomastigote forms/mL. Stock solutions of the extracts and fractions to be tested were prepared by dissolving dimethyl sulfoxide to a final concentration of 25 mg/mL. The bioassays were performed in triplicate on 96-well microtiter plates containing 200  $\mu$ L of mixture/well. For each sample, aliquots of the stock solutions were added to the diluted blood in such quantities as to give final concentrations of 100, 250 and 500  $\mu$ g/mL sample per mL of mixture in the wells. The plates were incubated at 4°C for 24 h and the number of parasites was determined (14). Blood of infected mice and infected blood with dimethyl sulfoxide added in

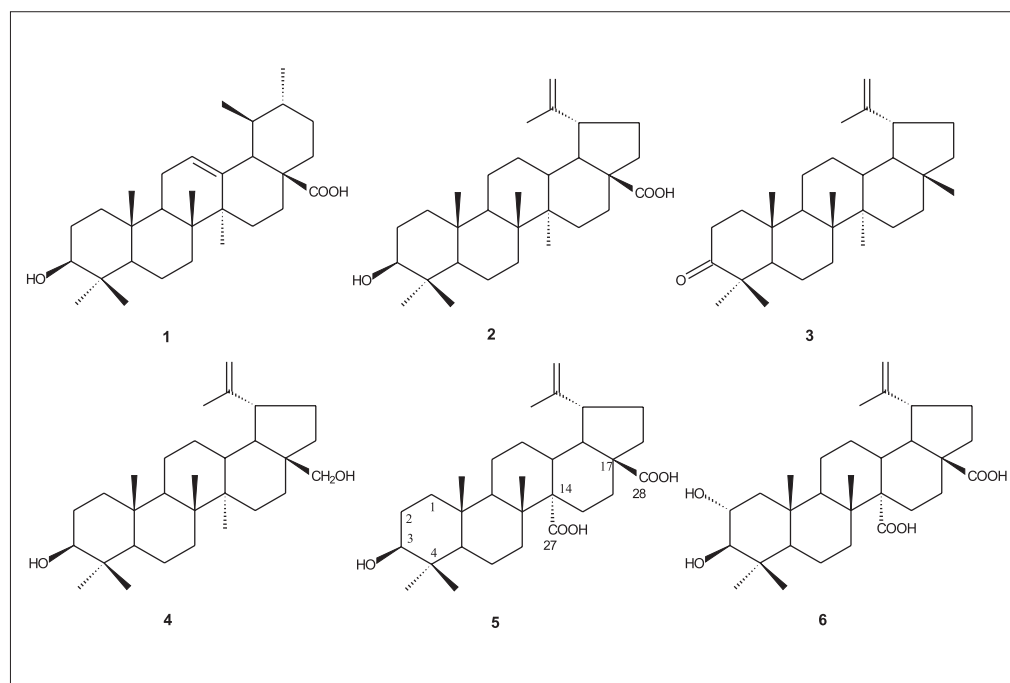
amounts equivalent to the samples were used as control, and infected blood plus gentian violet (250  $\mu$ g/mL) was used as positive control.

### Results and Discussion

Pentacyclic lupane- and ursane-like triterpene skeletons were detected in *A. amazonicus* stem crude extracts and fractions. Some of them were previously reported in root extracts of the same species (1).

Bioassay-guided fractionation of the EtOAc (E<sub>2</sub>) and MeOH (E<sub>6</sub>) extracts using a combination of column chromatography, PTLC and HPLC led to the isolation of ursolic acid (1), betulinic acid (2), lupenone (3), betulin (4), 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid (5), and 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid (6) (Figure 1), as well as the well-known phytosteroids stigmasterol, sitosterol and campesterol. Compounds 2 and 4 have been isolated from *A. amazonicus* root bark extract (1) whereas the others are reported here for the first time for this species. Compounds 5 and 6 are

Figure 1. Structures of pentacyclic triterpenes. Ursolic acid (1), betulinic acid (2), lupenone (3), betulin (4), 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid (5), and 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid (6).



correlated, at least in part, to the cytotoxic activity of the extract.

The structures of the isolated compounds were determined using spectroscopic methods (MS,  $^1\text{H}$  and  $^{13}\text{C}$  NMR, DEPT, COSY, and HMQC) in comparison with previously published data (1,15-19). Both 1-D and 2-D NMR experiments ( $^1\text{H}$ ,  $^{13}\text{C}$ , DEPT,  $^1\text{H}$ - $^1\text{H}$  COSY, and HMQC) were performed in order to fully assign  $^1\text{H}$  and  $^{13}\text{C}$  chemical shifts to compounds 1-6. The  $^{13}\text{C}$  NMR spectra of compounds 1-6 revealed 30 carbon signals whose multiplicities were sorted by DEPT spectrum (Table 1).

The  $\Delta^{20,29}$ -functionality of a lupane skeleton was inferred for compounds 2-6 from the resonance of the  $\text{sp}^2$  carbons at C-29 (secondary carbon signal deduced by DEPT pulse sequence) at 110 ppm and C-20 (quaternary carbon) at 151 ppm.

A detailed analysis of the  $^1\text{H}$  NMR of 2-6 confirmed the characteristic features of a lupane parent structure, with some modifications. The spectra of 5 and 6 were characterized by signals of four tertiary methyls ( $\delta$  0.8-1.2, Me-23-Me-26) and one vinylic methyl ( $\delta$  1.9, Me-30), two protons of an isopropenyl moiety at  $\delta$  4.7 and 5.0 (1 H each, s,  $\text{H}_a$ -29 and  $\text{H}_b$ -29), and one carbinolic proton for 5 and two carbinolic protons for 6. The  $^1\text{H}$  NMR spectrum of 5 showed a signal at  $\delta$  3.2 (dd with  $J = 10.5$  and  $5.3$  Hz) due to coupling with two methylene protons. The values of the chemical shift and  $J$  couplings (diaxial and axial/equatorial interactions) suggested the presence of  $\beta$ -OH substituents at C-3 (15).

The  $^1\text{H}$  NMR spectrum of 6 showed a signal for a carbinyl proton at  $\delta$  3.2 (d with  $J = 9.0$  Hz) due to coupling with one proton, a diaxial interaction. A signal at  $\delta$  4.0 (1 H, ddd) was also present in the same region of the  $^1\text{H}$  NMR spectrum of 6. The COSY spectrum showed the correlation between H-2 and H-3. The chemical shifts and  $J$  couplings were typical for a  $2\alpha,3\beta$ -dihydroxy substitution pattern (16).

The  $^{13}\text{C}$  NMR spectrum of compound 5 revealed 30 carbon signals. The signals of 5 were sorted by DEPT spectrum as 5 methyls, 10 methylenes, 5 methines, 5 quaternaries, 1 alcoholic methine, 2 carboxylic acids, and 2 olefinic carbons (1 =  $\text{CH}_2$  and one quaternary). The signals of 6 were similar to those of 5, except for the substitution of one proton of methylene carbon with 1 OH.

The substitution at C-14 was revealed by a shift of the carbon signal at carboxylic group at  $\delta$  42.8 of betulinic acid to  $\delta$  60.0 and by the deshielded of signals of C-7, C-9, C-12, C-13, C-16 and C-26, and by the shielded

Table 1.  $^{13}\text{C}$  NMR chemical shifts of pentacyclic triterpenes 1-6 (pyridine- $d_5$ ) with tetramethylsilane as internal standard.

Carbon	Pentacyclic triterpenes					
	1	2	3	4	5	6
1	38.9	39.1	39.6	39.4	39.2	48.0
2	27.9	28.1	34.1	28.1	28.0	68.7
3	78.0	78.0	216.4	77.9	77.7	83.3
4	39.2	39.3	47.6	39.1	39.1	39.6
5	55.7	55.7	54.8	55.7	55.8	55.9
6	18.6	18.6	19.7	18.6	18.6	18.7
7	33.4	34.7	33.8	34.7	35.2	35.5
8	39.8	40.9	40.8	41.1	40.6	40.6
9	47.9	50.8	49.9	50.6	51.5	51.1
10	37.3	37.3	36.9	37.4	37.5	38.8
11	23.5	21.0	21.5	20.9	21.1	21.3
12	125.5	25.9	25.9	25.6	26.7	26.8
13	139.1	38.4	38.5	37.3	40.1	40.0
14	42.3	42.7	42.7	42.8	59.8	60.0
15	28.5	30.1	27.4	27.4	28.5	28.6
16	24.8	32.7	34.1	30.2	38.2	38.0
17	47.9	56.4	42.7	48.2	56.4	56.6
18	53.4	47.6	47.1	48.4	52.2	52.3
19	39.2	49.6	47.1	49.0	47.6	47.7
20	39.3	151.1	151.1	151.1	150.9	151.3
21	30.9	31.0	30.1	29.9	30.9	31.0
22	37.1	37.4	36.9	34.5	37.6	37.7
23	28.7	28.5	26.6	28.5	28.3	28.8
24	16.4	16.2	21.0	16.0	16.8	17.4
25	15.5	16.2	15.8	16.3	16.2	17.4
26	17.3	19.3	15.9	16.2	17.3	18.0
27	23.8	14.7	14.6	14.8	178.3	179.0
28	179.7	178.7	17.3	59.3	179.3	180.1
29	17.4	109.8	109.8	109.8	110.1	109.9
30	21.3	19.3	19.3	19.1	19.1	19.1

1 = ursolic acid; 2 = betulinic acid; 3 = lupenone; 4 = betulin; 5 = 3 $\beta$ -hydroxylup-20(29)-ene-27,28-dioic acid; 6 = 2 $\alpha$ ,3 $\beta$ -dihydroxylup-20(29)-ene-27,28-dioic acid.

of signals of C-8 and C-15 due to  $\delta$  and  $\gamma$  steric effects of this substitute.

Other triterpenoids isolated from this plant were identified as ursolic acid (1), betulinic acid (2), lupenone (3), and betulin (4) by their spectral data as well as by comparison with spectral data from the literature (16,17). Besides these triterpenes, three phytosteroids were identified by GC/MS: sitosterol, stigmasterol and campesterol.

The cytotoxic activities of 5 and 6 were evaluated on the SKBR-3 and C-8161 human tumor cell lines. Both compounds exhibited cytotoxic activity against SKBR-3 breast adenocarcinoma tumor cells. Compound 6, even at moderate concentrations, also showed cytotoxic activity against C-8161 melanoma tumor cells (Table 2).

Previous study reported that *A. amazonicus* stem extracts are sources of pentacyclic triterpenes that exhibit selective cytotoxicity against several melanoma-derived cell lines (20). It has been recently reported that betulinic acid is cytotoxic against non-melanoma (neuroectodermal and malignant brain tumor) human tumor varieties (21).

The present results support previous data showing that compounds found in *A. amazonicus* extracts exhibit tumor cytotoxicity. We also suggest that the compounds 5 and 6 isolated from *A. amazonicus* stem extracts

and described here play a role in the tumor cytotoxicity exhibited by such extracts. In particular, the present results show that compound 5 has selective cytotoxicity against breast adenocarcinoma tumor cells and that compound 6 displays cytotoxic activity against both tumor cell lines assayed at the tested concentration. Compounds 5 and 6 did not show significant cytotoxic activity on peripheral blood mononuclear cells (data not shown). This observation agrees with data showing that lupane-type triterpenes such as betulinic acid present specific cytotoxicity against tumor cells and a favorable therapeutic index even at doses up to 500 mg/kg body weight. Betulinic acid is a very promising new chemotherapeutic agent for the treatment of cancer (20-23). The mechanisms of triterpene cytotoxic activity on tumor cells have not been fully elucidated. Betulinic acid seems to function by inducing apoptosis of cells irrespective of their p53 status (21). Further experiments are necessary to check the apoptosis-inducing ability of compounds 5 and 6. The activity of extracts and pure compounds seems to be much lower than established by the criteria of the American National Cancer Institute. However, activity should not be judged by direct comparison of numerical values because the cell lines and the bioassay experimental con-

Table 2. Characterization of the *in vitro* cytotoxicity-related anti-tumor effects of the crude extract of *Ampelozizyphus amazonicus* stem bark and of compounds 5 and 6.

Compounds	% Cytotoxicity					
	SKBR-3 human adenocarcinoma cell lines			C-8161 human melanoma cell lines		
	0.1 mg/mL	1 mg/mL	10 mg/mL	0.1 mg/mL	1 mg/mL	10 mg/mL
E <sub>2</sub> extract	30.5 ± 1.8	44.3 ± 2.2	53.3 ± 0.9	1.3 ± 0.5	45.5 ± 1.7	76.3 ± 2.1
5	0.0	39.4 ± 1.9	45.6 ± 2.5	0.0	0.0	14.3 ± 0.4
6	30.6 ± 2.7	32.5 ± 1.0	42.5 ± 3.1	14.3 ± 1.7	35.5 ± 2.3	68.1 ± 3.2
Methotrexate	3.5 ± 0.2	10.8 ± 1.1	36.8 ± 3.6	1.66 ± 0.7	10.5 ± 0.8	63.7 ± 2.4

Data are reported as the means ± SD of triplicate samples and are representative of at least three independent experiments. The cytotoxic activity of the extract or isolated compounds was analyzed by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay as described in Material and Methods. Methotrexate was used as positive control.

ditions, and probably the criteria for the judgment of activity used by different laboratories might not be the same.

The trypanocidal activity of crude extracts and fractions of *A. amazonicus* is shown in Table 3. The results demonstrated that all samples tested were effective against the Y strain of *T. cruzi*; however, the E<sub>0</sub> and EEBAa extracts and fractions E<sub>2</sub>P, E<sub>2</sub>P<sub>3</sub>, E<sub>2</sub>P<sub>9</sub>, and E<sub>7(5/6)</sub> were more efficient and exhibited more than 50% parasite lysis at a concentration of 500 µg/mL. Though the other extracts and fractions were less effective they presented active compounds. Fraction E<sub>2</sub>P showed activity mainly due to compounds 2, 5 and 6. After chromatographic purification, two fractions, E<sub>2</sub>P<sub>3</sub> and E<sub>2</sub>P<sub>9</sub>, were obtained, containing compounds 5 and 6, respectively. Fraction E<sub>7(5/6)</sub>, obtained by *n*-butanol partition of E<sub>7</sub>, contains lupenone (3) and betulin (4).

The lupene- and ursane-like skeletons of pentacyclic triterpenes were identified in the extracts and fractions, as well as in the roots of *A. amazonicus* (1). Ursolic and oleanolic acids and their derivatives showed trypanocidal activity, with ursolic acid being the most active (24). The presence of a free hydroxyl group at C-3 and/or the carboxyl group at C-17 is required for trypanocidal activity (24). The

Table 3. Percent parasite lysis induced by extracts and fractions of *Ampelozizyphus amazonicus* against the trypomastigote form of *Trypanosoma cruzi* Y strain.

Extracts and fractions	Concentration (µg/mL)		
	100	250	500
E <sub>1</sub>	4.86 ± 0.87	19.63 ± 7.44	25.16 ± 4.61
E <sub>2</sub>	33.73 ± 0.00	34.97 ± 0.89	42.94 ± 1.51
E <sub>3</sub>	5.53 ± 1.74	17.20 ± 4.49	32.53 ± 6.24
E <sub>4</sub>	17.2 ± 4.49	17.2 ± 3.96	46.03 ± 3.48
E <sub>5</sub>	11.06 ± 5.28	17.16 ± 1.51	25.77 ± 5.66
E <sub>6</sub>	19.63 ± 4.84	23.93 ± 1.74	23.93 ± 2.28
E <sub>7</sub>	19.03 ± 6.00	22.70 ± 4.49	39.86 ± 1.74
E <sub>8</sub>	8.60 ± 3.78	20.23 ± 1.74	30.06 ± 9.02
E <sub>9</sub>	2.80 ± 2.37	17.26 ± 3.94	66.50 ± 5.93
E <sub>10</sub>	11.16 ± 7.99	30.80 ± 0.00	43.46 ± 5.10
E <sub>2</sub> P	38.20 ± 5.39	55.00 ± 5.31	82.00 ± 5.58
E <sub>2</sub> P <sub>3</sub>	26.70 ± 7.85	46.06 ± 2.64	57.06 ± 5.36
E <sub>2</sub> P <sub>9</sub>	12.56 ± 5.15	49.70 ± 0.00	53.43 ± 7.71
E <sub>7(5/6)</sub>	61.33 ± 6.52	60.73 ± 1.26	62.36 ± 5.22
EEBAa	59.53 ± 7.59	62.30 ± 8.90	61.26 ± 1.46

Data are reported as the means ± SD of triplicate samples and are representative of at least three independent experiments. Positive control: gentian violet (100% lysis). The entire extraction and purification processes are described in Material and Methods.

results obtained demonstrate that *A. amazonicus* extracts contain potential compounds for use as chemoprophylactic agents against *T. cruzi*. In addition, triterpenes, compounds such as phenols, saponins, peptide alkaloids, and others have been identified in Rhamnaceae species (5,25-28).

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