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Activity of Marine Sponge-Derived Extracts against Trypanosoma Cruzi is Associated with Apoptosis-Like Death

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Abstract: *Trypanosoma cruzi is the aetiological agent of Chagas disease that affects 6–7 million people in Latin America. Chemotherapy for this disease is unsatisfactory; therefore, it requires the development of new drugs. Marine sponges are known to be important sources of bioactive natural products. This present study investigated the activity of organic (E1) and aqueous (E2) extracts obtained from the sponge Amphimedon viridis against T. cruzi as well as their toxicity to mammalian cells. E1 and E2 displayed a dose-dependent lytic effect on bloodstream trypomastigotes with IC50 values of 10.80 and 0.57µg/mL, respectively, being the aqueous extract ~19-fold more active than benznidazole; the corresponding values for effects on intracellular amastigotes were 44.85 and 21.37 µg/ mL, respectively. Both A. viridis extracts were nontoxic to cardiac cells at a concentration of 100 µg/ mL for up to 72 h of incubation. Trypomastigotes treated with 8 µg/ mL E2 and labelling with TRME led to mitochondrial membrane potential dissipation on 78.7±26.8% of the parasites, whereas labelling with 7-AAD induced apoptosis-like death in 50.9±16.8% of parasites. These data suggest a potential trypanocidal activity of A. viridis, which is partially associated with the ability to induce apoptosis-like death. The bioactive compounds in E2 are currently being chemically characterized to further discriminate the active components involved.*

Keywords: *Trypanosoma cruzi, marine sponge, Amphimedon viridis, trypanocidal activity, apoptosis-like death.*

1. INTRODUCTION

Chagas disease is caused by the protozoan *T. cruzi* and affects ~8 million individuals in Latin America, of whom 30-40% either have or will develop cardiomyopathy, digestive mega syndromes, or both pathologies [1]. Although the rates of vectorial (*Triatoma infestans*) and transfusional transmissions have steadily declined [2], this disease can also be orally transmitted through the ingestion of contaminated food and transmitted via blood transfusions, organ transplantation, laboratory accidents and congenital routes [1]. Recently, a major concern has been the emergence of Chagas disease in non-endemic areas, such as North America and Europe, which is a consequence of the immigration of infected individuals [3]. The available chemotherapy for this illness includes two nitroheterocyclic agents-nifurtimox and benznidazole-which are effective against acute infections but

show poor activity in the late chronic phase, with severe collateral effects and limited efficacy against different parasitic isolates. These drawbacks support the urgent need to identify better drugs to treat chagasic patients [4].

Marine organisms are rich in diverse chemical compounds that carry out various pharmacological and ecological functions. Over 60% of bioactive compounds are from marine fauna, and 70% of these compounds come from sponges [5]. Pharmaceutical companies have been interested in marine products since the 1950's with the discovery of spongothymidine and spongouridine, which can be isolated from the marine sponge *Cryptotethya crypta* [6]. These two nucleosides were the basis for the synthesis of cytarabine arabinoside (Ara-C), an anti-leukemic agent, adenine arabinoside (Vidarabine or Ara-A), an antiviral agent, and azidothymidine (AZT), which is used in the treatment of HIV [7].

Different classes of compounds isolated from different species of marine sponges have been assayed against pathogenic trypanosomatids: polyketides (*Plakortis* sp) [8], steroids (*Haliclona* sp) [9] and seterterpenes (*Hyrtios cf. erecta*) [10] were active against *T. brucei*; and the sesquiterpene curcuphenol (*Myrmekioderma styx*) [11] and the alkaloid araguspongin C (*Haliclona* sp) were active against *L. donovani*. Moreover, bromopyrrole alkaloids (from Axinella and Agelas) [12], the steroidal glycoside pandaroside (*Pandaros acanthifolium*) [13], terpenoids (*Spongia* sp. and *Ircinia* sp.) [14], and valinomycin, staurosporine and tetromycin 3 (from *Streptomyces* sp. isolated from Mediterranean sponges) [15] were active against both *T. brucei* and *L. donovani*.

Against *T. cruzi*, bromotyrosine derivatives (*Verongula rigida*) were inactive [16], while the terpenoids heptaprenyl-p-quinol and 11-β-acetoxyspongi-12-en-16-one (*Spongia* sp. and *Ircinia* sp.) [14] and the polyketide plakortide P (*Plakortis angulospiculatus*) were active, with plakortide P being 15-fold more active than benznidazole, the standard drug [17]. A semisynthetic derivative of agelasine D, isolated from *Agelas* sp., was considered a lead compound for the development of drugs for Chagas disease [18].

To date, the trypanocidal activity of *Amphimedon viridis* sp. has not yet been well explored, but different biological activities of extracts from this sponge have been reported, which have been associated with the presence of halitoxin and also other bioactive compounds [19]. An alkaloidal substance obtained from an aqueous extract from *A. viridis* inhibited the proliferation of *Leishmania mexicana* promastigotes with loss of plasma membrane selective permeability [20]. Brazil presents a great biodiversity; however, few studies have explored its rich marine fauna and flora as a source of bioactive products. Herein, we investigated for the first time the activity of *A. viridis*-derived extracts against *T. cruzi*. This study showed the promising trypanocidal potential of this natural organism, which was partially associated with the ability to induce apoptosis-like death.

2. METHODS

2.1. Sponge Collection

The *A. viridis* sponge was collected by one of the authors (CCC) by diving in shallow waters in the Atlantic Ocean near Arraial do Cabo, Rio de Janeiro, Brazil (GPS: 22° 57′ 58″ S; 42° 1′ 44″ W). Taxonomic identification of the biological materials was performed at the University Federal do Rio de Janeiro, Brazil. After collection, the sponge was refrigerated through ice packages, superficially cleaned and frozen (-20°C).

2.2. Extract Preparation

The preparation of the extracts was carried out by incubating samples in a mixture of water/chloroform/methanol (1:1:2) for 2 h at room temperature. After separation of the solid residue by centrifugation (3000 rpm/15 min), the soluble portion was separated in hydrophilic and hydrophobic phases by centrifugation (3000 rpm/15 min). The hydrophobic phase (E1) was evaporated at 35°C for 18 h and subjected to overnight lyophilization. The aqueous extract (E2) was obtained after solvent evaporation at 60°C/10 min and was also lyophilized. Stock solutions of both extracts were prepared at a concentration of 100 mg/mL, with E1 dissolved in 100% dimethylsulfoxide (DMSO, Merck Darmstadt) and E2 in 100% water. Immediately before use, test solutions were prepared in Dulbecco's modified Eagle medium (DMEM), with the final concentration of solvent used in experiments never exceeding 0.1%. Preliminary experiments showed that concentrations of up to 0.5% DMSO caused no deleterious effect on the parasites.

2.3. Parasites and Cell Cultures

The Y strain (DTU II) of *T. cruzi* was used throughout the experiments. Bloodstream trypomastigotes were obtained at the peak of parasitaemia from infected Swiss Webster mice, isolated by differential centrifugation and resuspended in DMEM. Primary cultures of mouse embryo heart muscle cells (HMC) were used for toxicity assays. Briefly, hearts of 18-day-old mouse embryos were isolated, and the ventricular muscle was cut into small pieces before undergoing enzymatic dissociation with 0.01% collagenase and 0.025% trypsin diluted in phosphate-buffered saline. Thereafter, cells were resuspended in DMEM supplemented with 10% foetal calf serum (FCS), 2% chicken embryo extract, 2.5 mM $CaCl_2$ and 1 mM L-glutamine; then, cells were plated for 20 min in 0.01% gelatin-coated culture flasks to allow the adhesion of fibroblasts in DMEM. Next, HMC were plated onto gelatin-coated glass coverslips in 24-well plates (10^5 cells/ well) and maintained at 37°C in a 5% CO2 atmosphere.

All procedures were carried out in accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals resolution 242/99 (LW-16/13).

2.4. Effects on Bloodstream Trypomastigotes

Parasites were resuspended at a concentration of 107 cells/mL in DMEM supplemented with 5% foetal bovine serum and 1 mM L-glutamine (DMES). This suspension (100 µL) was added to the same volume of A. viridis extracts, which had been previously prepared at twice the desired final concentrations. Incubations were performed in 96-well microplates (Nunc Inc., Rochester, NY, USA) at 37°C for 24 h. Benznidazole was used as a reference drug. Cell counts were performed in a Neubauer chamber, and the trypanocidal activity is expressed as the IC50, which corresponds to the concentration that leads to the death of 50% of parasites [21].

2.5. Effects on Intracellular Amastigotes

At 24 h after plating, HMCs were infected with bloodstream forms of T. cruzi at a 10:1 parasite: host cell ratio. After 24 h of incubation, cultures were washed to remove free parasites, and sponge extracts were added a concentrations ranging from 5 to 50 µg/ mL. After 72 h of infection, cultures were fixed in Bouin's solution, stained with Giemsa and counted to assess the following parameters: percentage of cells infected, number of parasites/infected cells and the infection index, which refers to the number of parasites/100 cells. The IC50 values corresponding to the concentration that led to a 50% inhibition of the infection index was calculated. Triplicate coverslips were analysed for each plate, and at least two assays were performed in each analysis.

2.6. Toxicity Assays

To rule out toxic effects of extracts on mammalian cells, HMCs were seeded into 96-well microplates ($5x10^4$ cells/ plate) and incubated for 24 and 72 h at 37°C in the presence or absence of E1 or E2 up to 100 µg/ mL diluted in DMEM. Then, cell morphology was evaluated by light microscopy, and a methyl thiazolyl tetrazolium (MTT) colorimetric assay was performed. Briefly, 5 mg/mL MTT (Sigma, St. Louis, MO, USA) was added to each well for 4 h at 37°C- the cellular metabolic activity converts the reagent from yellow to a purple formazan. DMSO was then added to solubilize the formazan crystals and the optical density was measured at 490 nm with a spectrophotometer (VERSAmax, Molecular Device, USA) to determine the LD50 values (the concentration that reduces mammalian cell viability by 50%) and respective selectivity indexes (SI=LD50/IC50).

2.7. Apoptogenic Activity of Extracts Against *T. Cruzi*

Trypomastigote forms of *T. cruzi* (10^6 parasites/ mL) were treated with 8 µg/ mL E2 for 24 h. Then, parasites were washed twice with PBS, permeabilized for 20 min at room temperature with 0.01% saponin, rinsed again, and further incubated for 30 min at 37°C with 50 nM tetramethylrhodamine (TMRE) (Molecular Probes, Carlsbad, CA, USA) for mitochondrial membrane potential ($\Delta\Psi m$) analysis or with 20 µg/mL 7-aminoactinomycin D (7AAD) (Southern Biotech) for apoptosis-like death analysis. For cell death induction (positive control), parasites were maintained at 60°C for 10 min and then were immediately analysed by flow cytometry. In all assays, data acquisition and analysis were performed using a FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA, USA) equipped with Cell Quest software (Joseph Trotter, Scripps Research Institute, San Diego, CA, USA). A total of 10,000 events were acquired in the region previously established as that

corresponding to parasites. Data, histograms, and dot plots were obtained using Summit 4.3 software. All assays were run at least three times (each in duplicate).

3. RESULTS AND DISCUSSION

Concerning antiprotozoal activity, previous reports have demonstrated the potential antiparasitic activity of marine organism-derived products, including marine sponges [14]. However, few studies have investigated the actions of these compounds on *T. cruzi*. In this present study, the activity of organic and aqueous extracts from the marine sponge *A. viridis* was tested against *T. cruzi* forms that are relevant to Chagas disease in humans. Initially, we evaluated its direct lytic action on bloodstream trypomastigotes by quantifying motile parasites by light microscopy. Both extracts caused dose-dependent effects, with IC_{50} values reaching 10.80 μg/ mL for E1 and 0.57 μg/ mL for E2. The aqueous extract was 19-fold more active than either E1 or the reference drug benznidazole, which displayed an IC_{50} value of 10.9 μg/ mL [21] (Table 1, Figure 1A-B). These results suggest that a combination of these extracts, especially E2, with benznidazole might be efficacious. Combination therapy has been proven to be more effective than monotherapies for several infectious diseases and also minimizes the risk of drug resistance. For example, the sesquiterpene lactone parthenolide has previously shown a synergistic effect with Bz [22], allowing the dosage of this drug to be reduced and lowering the severity of its side effects.

Table1. *Effects of A. viridis extracts on trypomastigotes and intracellular amastigotes of T. cruzi on cardiac cells and the selectivity index*[a]

		24 h			72 h	
	BTF[b]	HMC[c]	SI	AMA[d]	HMC	SI
	IC_{50} (μg/mL)	LD_{50} (μg/mL)		IC_{50} (μg/mL)	LD_{50} (μg/mL)	
E1	10.80 ± 3.35[e]	>100	>11.8	44.85 ± 10.82[e]	>100	>2.4
E2	0.57 ± 0.13	>100	>189.3	27.37 ± 5.57	>100	>4.6
Bz[f]	10.9	1000	77	2.8	1000	360

[a] *Selectivity index (SI) = LD_{50}/IC_{50};* [b] *Bloodstream trypomastigote forms;* [c] *Heart muscle cells,* [d] *Intracellular amastigotes;* [e] *Mean ± standard deviation of three independent experiments;* [f] *Reference values for benznidazole [21].*

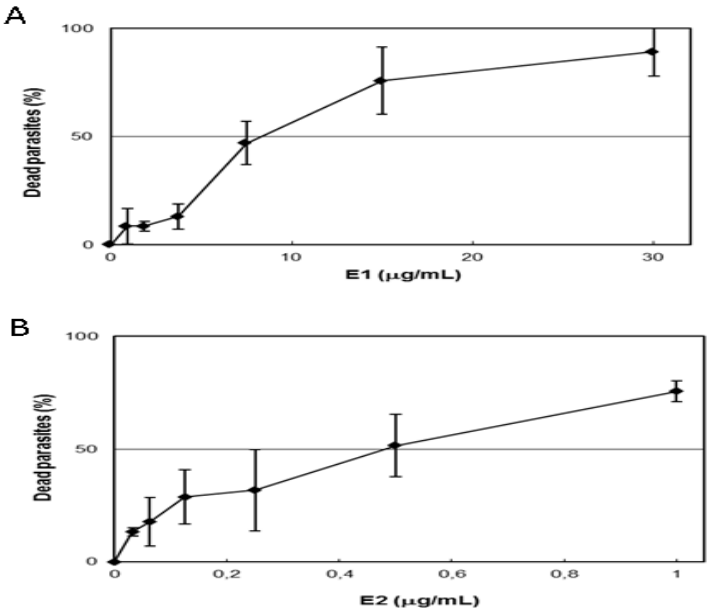


Figure1. *Trypanocidal activity of extracts isolated from A. viridis against bloodstream trypomastigotes of T. cruzi. Treatments were performed for 24 h at 37°C, and the percentages of dead parasites were determined. Results are expressed as the mean ± standard deviation of one representative of two duplicate experiments; (A) organic extract (E1) and (B) aqueous extract (E2).*

Next, the toxicity of A. viridis extracts to mammalian host cells was evaluated using primary cultures of cardiac cells, which are an important target of *T. cruzi*. Cellular viability was monitored by morphological analysis and a MTT colorimetric assay. After 72-h incubation with concentrations of up to 100 μg/ mL, both E1 and E2 maintained cellular viability at values greater than 90% (data not shown) (Table 1). To analyse the effects of *A. viridis* on intracellular amastigotes, cardiac cells

infected by *T. cruzi* were treated with E1 or E2 up to 50 µg/ mL, which are non-toxic concentrations for the host cell, until 72 h post-infection. As also observed in experiments with trypomastigotes, the aqueous extract E2 was the most active against amastigotes, with an IC_{50} value of 27.37 µg/ mL, while the corresponding value for E1 was 44.85 µg/ mL (Table 1, Figure 2). The excellent results obtained for the extracts provoked our interest in studying their chemical makeup and isolating the bioactive substance(s) involved in the trypanocidal activity.

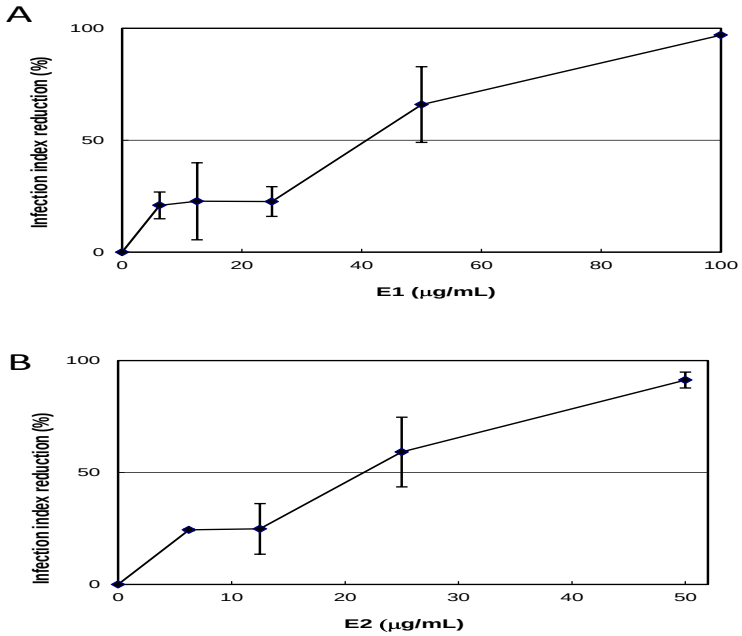


Figure2. *Trypanocidal activity of extracts isolated from A. viridis against intracellular amastigotes of T. cruzi. Treatments were performed for 72 h at 37°C, and the percentage of infection index reduction was measured. Results are expressed as the mean ± standard deviation of one representative of two duplicate experiments; (A) organic extract (E1) and (B) aqueous extract (E2).*

Apoptosis is a type of programmed cell death involved in the growth regulation of multicellular organisms and also in host defence against infections [23]. In the 1990's, cell death markers typical of apoptosis in metazoans, such as the loss of mitochondrial membrane potential (ΔΨm), cytochrome c release, abnormal DNA condensation and fragmentation, and phosphatidylserine externalization, were reported in unicellular eukaryotes, including pathogenic trypanosomatids [24]. For *T. cruzi*, apoptosis-like death has been shown to be involved in the control of proliferation and differentiation and in the response to stress conditions and drugs [25-27]. Recently, it has been reported that memantine, an NMDA receptor antagonist, and aryl thiosemicarbazones can induce apoptosis-like death in *T. cruzi* [28,29]. Similar to what occurs with metazoans, the mitochondrion of trypanosomatids is a central organelle involved in cell death pathways and can drive ROS production [30]. To investigate the potential effects of A. viridis on mitochondrial function, trypomastigotes were treated with E2, the most active extract, and then labelled with TMRE, which enables the discrimination of small variations in ΔΨm. Indeed, E2 induced an important ΔΨm loss (TMRElow) in 78.7±26.8% of parasites, whereas in the untreated population, the corresponding percentage was 20.0±12.5%. For a positive control for apoptosis (60°C/10 min), the percentage of ΔΨm loss was 94.5±2.7% (Table 2 and Figure 3, representative experiment).

Table2. Flow cytometry results of the evaluation of morphological and functional characteristics of apoptosis-like death of trypomastigotes of *T. cruzi* treated with a bioactive aqueous extract from *A. viridis*

Marker	Control-[a]	Control+[b]	E2[c]
% cells+$TMRE_{high}$	77.3±14.0[d]	5.0±1.8	14.0±19.8
% cells$TMRE_{low}$	20.0±12.5	94.5±2.7	78.7±26.8
% cells +7-AAD	6.5±7.5	52.9±11.0	50.9±16.8

[a] untreated parasites; [b] 60°C/10 min; [c] 8 µg/mL E2 for 24 h; [d] mean ± standard deviation of three independent experiments.

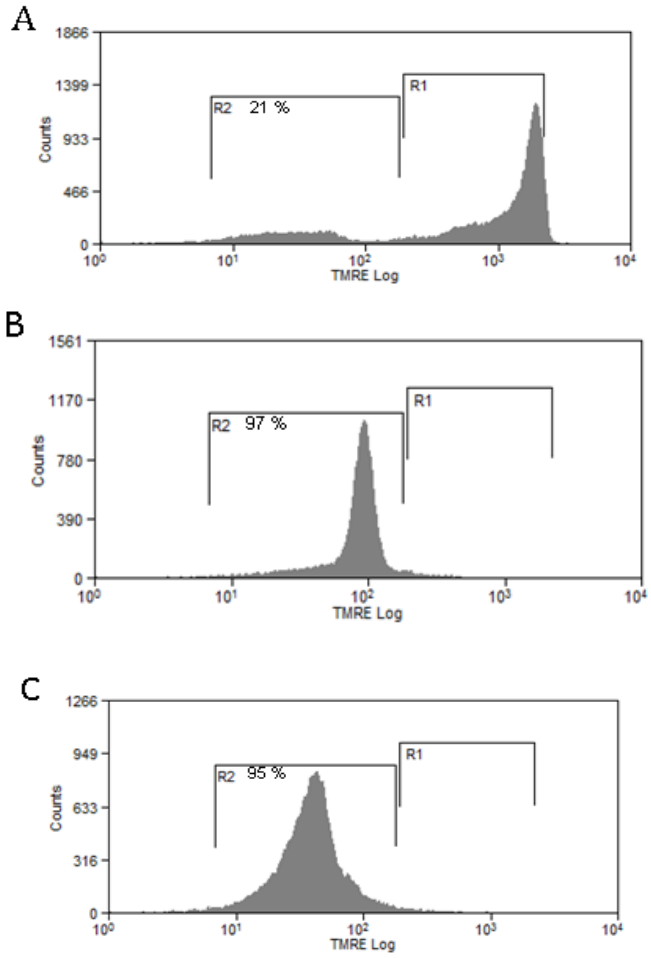
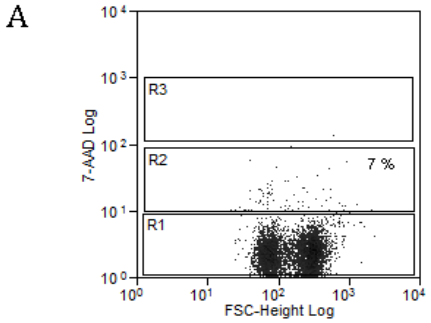


Figure3. *Flow cytometry analysis of mitochondrial membrane potential (ΔΨm) in T. cruzi trypomastigotes after TMRE staining. Histograms show the fluorescence intensity of (A) untreated parasites (negative control), (B) apoptotic parasites (positive control) induced by high temperature (60°C/10 min) and (C) parasites treated with 8 µg/mL E2 from A. viridis (24 h/37°C). The high fluorescence intensity peaks are labelled as R1, whereas the low fluorescence intensity peaks are labelled as R2, which represent the dissipation of ΔΨm. Plots from a representative experiment are shown.*

The percentage of live, apoptotic and necrotic parasites was determined by staining with 7-AAD, which can be used to discriminate different types of cell death. In this type of analysis, apoptotic cells appear in the R2 region of the dot-plot graph based on 7-AAD labelling and a retracted cell shape (based on Forward Scatter, FSC) (Figure 4, a representative experiment). Interestingly, E2 induced apoptosis in 50.9±16.8% of parasites (Table 2 and Figure 4C-R2); in the untreated group, only 6.5±7.5% of parasites exhibited apoptotic-like death features (Table 2 and Figure 4A-R2). In this assay, a positive control obtained by heating non-treated parasites at 60°C for 10 min led to 52.9±11.0% of parasites in R2 (Table 2 and Figure 4B-R2). Our present data corroborated previous reports that showed the collapse of mitochondrial membrane potential [24,26,27,30] in addition to morphological alterations in the kinetoplast and mitochondria of trypanosomatids treated with aromatic diamidines [27,31,32] or Bothrops jararaca venom [33].



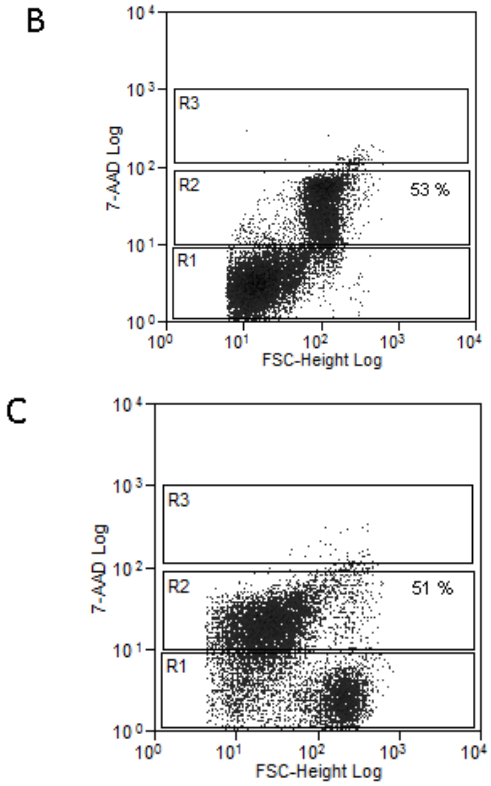


Figure 4. *Flow cytometry analysis of apoptosis-like death in T. cruzi trypomastigotes stained with 7-AAD. The dot-plot graphs show three regions representative of the populations of viable (R1), apoptotic (R2) and necrotic (R3) cells: (A) untreated parasites (negative control), (B) apoptotic parasites (positive control) induced by high temperature (60°C/10 min) and (C) parasites treated with 8 µg/mL E2 from A. viridis (24 h/37°C). Note the enrichment in the R2 population after treatment with E2 (51%) when compared with untreated parasites (7%). Plots are from a representative experiment.*

4. CONCLUSIONS

In the present study, we demonstrated, for the first time, the activity of *A. viridis* extracts against *T. cruzi*, which was more potent than the reference drug benznidazole against trypomastigotes. This potential activity suggests a possible combinatorial therapy to reduce toxic effects and increase treatment efficacy. Moreover, also for the first time, we showed that treatment with *A. viridis* aqueous extract induces *T. cruzi* apoptosis-like death. The parasite dies exhibiting mitochondrial membrane potential loss and cellular retraction, which are important apoptotic death features. Thus, the induction of apoptosis cell death might represent a mode of action for the extract. The apoptosis occurrence during an infectious process results in the downregulation of the immune response [34]. Interestingly, it is known that during the course of *T. cruzi* infection, an intense inflammatory process occurs that contributes to the generation of cellular damage, especially in the heart, which can be observed in Chagas heart disease [1]. In this context, in continuity with the present work, the chemical characterization of bioactive molecules from *A. viridis* extracts with apoptogenic activity could reveal novel bioactive molecules for the development of new lead compounds for treating Chagas' disease.

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REFERENCES

[1]. Rassi Júnior A., Rassi A., Rezende J.M. American trypanosomiasis (Chagas disease). Infect Dis Clin. North. Am. 26, 275-291 (2012)

[2]. Schofield C.J., Jannin J., Salvatella R. The future of Chagas disease control. Trends Parasitol. 22, 583-588 (2006).

[3]. Coura J.R., Viñas P.A. Chagas disease: a new worldwide challenge. Nature 465, S6-7 (2010).

[4]. Urbina J.A. Recent clinical trials for the etiological treatment of chronic Chagas disease: advances, challenges and perspectives. J Eukaryot. Microbiol. 62, 149-156 (2015).

[5]. Blunt J.W., Copp B.R., Keyzers R.A., Munro M.H., Prinsep M.R. Marine natural products. Nat. Prod. Rep. 32, 116-211 (2015).

[6]. Bergmann W., Feeney R.J. Contributions to the study of marine products. XXXII. The nucleosides of sponges. J. Org. Chem. 16, 981-987 (1951).

[7]. Cragg G.M., Grothaus P.G., Newman D.J. New horizons for old drugs and drug leads. J. Nat. Prod. 77, 703-723 (2014).

[8]. Oli S., Abdelmohsen U.R., Hentschel U., Schirmeister T. Identification of plakortide E from the Caribbean sponge *Plakortis halichondroides* as a trypanocidal protease inhibitor using bioactivity-guided fractionation. Mar. Drugs 12, 2614-2622 (2014).

[9]. Viegelmann C., Parker J., Ooi T., Clements C., Abbott G., Young L., Kennedy J., Dobson A.D., Edrada-Ebel R. Isolation and identification of antitrypanosomal and antimycobacterial active steroids from the sponge *Haliclona simulans*. Mar. Drugs 12, 2937-2952 (2014).

[10]. Kirsch G., Köng G.M., Wright A.D., Kaminsky R. A new bioactive sesterterpene and antiplasmodial alkaloids from the marine sponge Hyrtios cf. erecta. J. Nat. Prod. 63, 825-9 (2000).

[11]. Gul W., Hammond N.L., Yousaf M., Peng J., Holley A., Hamann M.T. Chemical transformation and biological studies of marine sesquiterpene (S)-(+)-curcuphenol and its analogs. Biochim. Biophys. Acta 1770, 1513-1519 (2007).

[12]. Scala F, Fattorusso E, Menna M, Taglialatela-Scafati O, Tierney M, Kaiser M, Tasdemir D. Bromopyrrole alkaloids as lead compounds against protozoan parasites. Mar. Drugs 8, 2162-2174 (2010).

[13]. Regalado E.L, Tasdemir D., Kaiser M., Cachet N., Amade P., Thomas O.P. Antiprotozoal steroidal saponins from the marine sponge *Pandaros acanthifolium*. J. Nat. Prod. 73, 1404-1410 (2010).

[14]. Orhan I., Sener B., Kaiser M., Brun R., Tasdemir D. Inhibitory activity of marine sponge-derived natural products against parasitic protozoa. Mar. Drugs 8, 47-58 (2010).

[15]. Pimentel-Elardo S.M., Kozytska S., Bugni T.S., Ireland C.M., Moll H., Hentschel U. Anti-parasitic compounds from *Streptomyces* sp. strains isolated from Mediterranean sponges. Mar. Drugs 8, 373-80 (2010).

[16]. Galeano E., Thomas O.P., Robledo S., Munoz D., Martinez A. Antiparasitic bromotyrosine derivatives from the marine sponge *Verongula rigida*. Mar. Drugs 9, 1902-1913 (2011).

[17]. Kossuga M.H., Nascimento A.M., Reimão J.Q., Tempone A.G., Taniwaki N.N., Veloso K., Ferreira A.G., Cavalcanti B.C., Pessoa C., Moraes M.O., Mayer A.M., Hajdu E., Berlinck R.G. Anti-parasitic, anti-neuroinflammatory, and cytotoxic polyketides from the marine sponge *Plakortis angulospiculatus* collected in Brazil. J. Nat. Prod. 71, 334-339 (2008).

[18]. Vik A., Proszenyák A., Vermeersch M., Cos P., Maes L., Gundersen L.L. Screening of agelasine D and analogs for inhibitory activity against pathogenic protozoa; identification of hits for visceral leishmaniasis and Chagas disease. Molecules 14, 279-288 (2009).

[19]. Berlinck R.G., Ogawa C.A., Almeida A.M., Sanchez M.A., Malpezzi E.L., Costa L.V., Hajdu E., de Freitas J.C. Chemical and pharmacological characterization of halitoxin from *Amphimedon viridis* (Porifera) from the southeastern Brazilian coast. Biochem. Physiol. C. Pharmacol. Toxicol. Endocrinol. 115, 55-63 (1996).

[20]. Marchán E, Arrieche, D, Henríquez W, Crescente O. *In vitro* effect of an alkaloid isolated from *Amphimedon viridis* (Porifea) on promastigots of *Leishmania mexicana*. Rev Biol Trop 2000; 1: 31-38.

[21]. De Souza E.M., Silva P.B., Nefertiti A.S., Ismail M.A., Arafa R.K., Tao B., Nixon-Smith C.K., Boykin D.W., Soeiro M.N. Trypanocidal activity and selectivity *in vitro* of aromatic amidine compounds upon bloodstream and intracellular forms of *Trypanosoma cruzi*. Exp. Parasitol. 127, 429-435 (2011).

[22]. Pelizzaro-Rocha K.J., Tiuman T.S., Izumi E., Ueda-Nakamura T., Dias Filho B.P., Nakamura C.V. Synergistic effects of parthenolide and benznidazole on *Trypanosoma cruzi*. Phytomedicine 18, 36-39 (2010).

[23]. Kerr J.F. History of the events leading to the formulation of the apoptosis concept. Toxicology 181/182, 471-474 (2002).

[24]. Soeiro M.N.C, De Souza E.M. Programmed cell death in protozoa. In: Programmed Cell Death & Trypanosomatids: A Brief Review. Martin J.M.P., Landes Bioscience, Chapter 3, 24-38 (2007).

[25]. Ameisen J.C., Idziorek T., Billaut-Mulot O., Loyens M., Tissier J.P., Potentier A., Ouaissi A. Apoptosis in a unicellular eukaryote (*Trypanosoma cruzi*): implications for the evolutionary origin and role of programmed cell death in the control of cell proliferation, differentiation and survival. Cell. Death. Diff. 2, 285-300 (1995).

[26]. De Souza E.M., Araujo-Jorge T.C., Bailly C., Lansiaux A., Batista M.M., Oliveira G.M., Soeiro M.N. Host and parasite apoptosis following *Trypanosoma cruzi* infection in *in vitro* and *in vivo* models. Cell Tissue Res. 314, 223-235 (2003).

[27]. De Souza E.M., Menna-Barreto R., Araújo-Jorge T.C., Kumar A., Hu,Q., Boykin D.W., Soeiro M.N.C. Antiparasitic activity of aromatic diamidines is related to apoptosis-like death in *Trypanosoma cruzi*. Parasitology 133, 75-79 (2006).

[28]. Damasceno F.S., Barisón M.J., Pral E.M., Paes L.S., Silber A.M. Memantine, an antagonist of the NMDA glutamate receptor, affects cell proliferation, differentiation and the intracellular cycle and induces apoptosis in *Trypanosoma cruzi*. PLoS Negl. Trop. Dis. 8, e2717 (2014).

[29]. Magalhães-Moreira D.R., Oliveira A.D., Teixeira de Moraes Gomes P.A., de Simone C.A., Villela F.S., Ferreira R.S., Silva A.C., Santos T.A., Brelaz-de-Castro M.C., Pereira V.R., Leite A.C. Conformational restriction of aryl thiosemicarbazones produces potent and selective anti-*Trypanosoma cruzi* compounds which induce apoptotic parasite death. Eur. J. Med. Chem. 75, 467-478 (2014).

[30]. Piacenza L., Irigoín F., Alvarez M.N., Peluffo G., Taylor M.C., Kelly J.M., Wilkinson S.R., Radi R. Mitochondrial superoxide radicals mediate programmed cell death in *Trypanosoma cruzi*: cytoprotective action of mitochondrial iron superoxide dismutase overexpression. Biochem. J. 403, 323-334 (2007).

[31]. Croft S.L., Brazil R.P. Effect of pentamidine isethionate on the ultrastructure and morphology of *Leishmania mexicana amazonensis in vitro*. Ann. Trop. Med. Parasitol. 76, 37-43 (1982).

[32]. De Souza E.M., Lansiaux A., Bailly C., Wilson W.D., Hu Q., Boykin D.W., Meuser-Batista M., Araújo-Jorge T.C., Soeiro M.N.C. Phenyl substitution of furamidine markedly potentiates its anti-parasitic activity against *Trypanosoma cruzi* and *Leishmania amazonensis*. Biochem. Pharmacol. 68, 593-600 (2004).

[33]. Deolindo P., Teixeira-Ferreira A.S., Melo E.J., Arnholdt A.C., Souza W., Alves E.W., DaMatta R.A. Programmed cell death in *Trypanosoma cruzi* induced by *Bothrops jararaca* venom. Mem. Inst. Oswaldo Cruz 100, 33-38 2005.

[34]. Barcinski M.A., DosReis G.A. Apoptosis in parasites and parasite-induced apoptosis in the host immune system: a new approach to parasitic diseases. Braz. J. Med. Biol. Res. 32, 395-401(1999).

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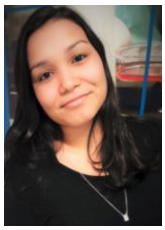

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