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Fungicidal and trypanocidal activity of aqueous extracts of leaves and inflorescences of *Verbesina macrophylla* (Asteraceae)

Laís de Almeida Bezerra[1], Lais Pessanha Carvalho[2], Érica de Oliveira Mello[3], Gabriel Bonan Taveira[3], Edésio José Tenório de Melo[1], Valdirene Moreira Gomes[3] and Maura da Cunha[1]*

[1]Laboratório de Biologia Celular e Tecidual, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Av. Alberto Lamego, 2000, Parque California, 280103-602, Campos dos Goytacazes, Rio de Janeiro, Brasil. [2]Intitut fur Tropenmedizin, University of Tubingen, Tuebingen, Germany. [3]Laboratório de Fisiologia e Bioquímica de Microrganismos, Universidade Estadual do Norte Fluminense Darcy Ribeiro, Campos dos Goytacazes, Rio de Janeiro, Brasil. *Author for correspondence. E-mail: maurauenf@gmail.com

ABSTRACT. Among the several native Brazilian plant species that have yet to be studied for their medicinal properties is *Verbesina macrophylla*, popularly known as "assa-peixe". The popular use of this species for medicinal purposes has been reported in southeastern Bahia State, with leaf tea being used to treat renal and urethral problems with anti-inflammatory and antifebrile activities, and flower tea to treat inflammation. Despite the potential medicinal importance of *V. macrophylla*, studies of its chemical composition and possible activity in controlling fungi and parasites are scarce and recent. This study tested the biological activity of aqueous extracts of leaves and inflorescences of *V. macrophylla* on the yeasts *Candida albicans* and *Candida tropicalis*, the filamentous fungus *Fusarium oxysporum,* and the epimastigote form of *Trypanosoma cruzi*. Inflorescence extract showed an inhibitory activity of 41% for cells of *C. tropicalis*, causing changes in the plasma membrane, resulting in fungicidal activity. Both aqueous extracts eliminated epimastigotes with a dose/time-dependent relationship, reaching 100% elimination after treatment with 200 µg mL^{-1} of either, causing cytoplasmic and nuclear disorganization, vacuolization and enlargement of the reservosomes. Although the studied extracts did not present considerable inhibitory activities against the filamentous fungus and yeasts, promising results were seen with the epimastigote form of *T. cruzi*. These data add industrial and medicinal value to the species. Additional studies are needed, particularly spectrometric analysis of extracts, to elucidate the metabolite(s) responsible for the results of this study.

Keywords: *Trypanosoma cruzi*; *Candida*; *Fusarium oxysporum*; Assa-peixe; Medicinal plant; Biological test.

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Introduction

Parasites, such as some yeasts and protozoans, are causative agents of various health risks. Some of these infections may remain asymptomatic, while others may cause serious health problems or even death. It has become increasingly challenging to treat such infections due to the side effects of currently used chemotherapy products (Altamura, Rajesh, Catta-Preta, Moretti, & Cestari, 2022; Kaur & Nobile 2023).

Several fungal species of the genus *Fusarium* are commonly phytopathogens and soil saprophytes. Individuals of *Fusarium oxysporum* (Schlecht.) Emendo Snyd. & Hans. parasitize cereal seeds and other fruits in the field, causing damage before and after harvest. Controls for this phytopathogen include the use of healthy bulbs, chemical treatment before planting, and crop treatments such as the elimination of infected plants and crop rotation (Silva et al., 2022). Researchers have been searching for chemical compounds in natural sources that can control the action of this phytopathogen (Rongai, Pulcini, Pesce, & Milano, 2015; Satish, Raghavendra, & Raveesha, 2009).

The yeasts *Candida albicans* (Robin) Berkhout and *C. tropicalis* (Castellani) Berkhout cause candidiasis, an opportunistic fungal infection that affects immunosuppressed people (Rodrigues et al., 2022). Oral and topical antifungals may be used to treat this infection, but there is still a need for new treatments, such as herbal medicines, for example, with lower toxicity and greater selectivity (Santos et al., 2022).

Protozoans are unicellular microorganisms with complex life cycles with distinct morphological states during development in vertebrate and invertebrate hosts that can cause severe disease. Some species of the family Trypanosomatidae Doflein (Estevam et al., 2018) cause diseases in humans, such as Chagas disease, a zoonosis caused by the protozoan *Trypanosoma cruzi* (Zuma, Barrias, & Souza, 2021). The life cycle of this

parasite involves two hosts: an invertebrate (vector) and a vertebrate, including humans. The forms found in vectors are epimastigotes and trypomastigotes, while trypomastigotes and amastigotes are found in vertebrate hosts (Zuma et al., 2021). The drugs available to treat this infection have limited therapeutic potential and severe side effects (Mazzeti, Capelari-Oliveira, Bahia, & Mosqueira, 2021). Thus, there is an urgent need to develop new trypanocidal agents with lower toxicity and greater activity, especially for the chronic phase of the disease (Kourbeli et al., 2021).

The resistance of parasites and fungi to drugs, and the resistance of insect vectors to insecticides, justify the search for new compounds capable of controlling them (Altamura et al., 2022). Modern drug research has found natural products to be a rich source of therapies for various human diseases and disorders (Ferreira et al., 2023, Gárcia-Bores et al., 2020, Trindade et al., 2023). Nonetheless, many Brazilian plant species have yet to be studied chemically, even though they represent significant economic and medicinal potential in a global context.

The plant family Asteraceae has been highlighted as a target of investigation because it possesses several species with biological activities (Giordani, Santin, & Cleff, 2015, Trindade et al., 2023). *Verbesina* L. (Asteraceae) is the largest genus of the subtribe Verbesininae, tribe Heliantheae. Studies carried out to date with species of this genus have aroused interest due to observations of various biological activities, including nematocidal activity (Oka 2012), hypoglycemic effect in rats (Toribio, Oriani, Fernández, & Skliar, 2005), and antimicrobial activity against human pathogens (Rodríguez-Valdovinos et al., 2021). Additionally, these species exhibit anti-inflammatory activity, antioxidant properties (Amaro-Luis, Ramirez, Delgado-Mendez, & Jorge, 2002; Lobitz et al., 1998; Rodríguez-Valdovinos et al., 2021), cytotoxic potential (Al-Oqail et al., 2016), and the promotion of wound healing (Gárcia-Bores et al., 2020). Chemical studies within the genus include those with *Verbesina sphaerocephala* A. Gray, which have shown that all extracts possess high levels of phenolics and flavonoids, with the flavonoid rutin being particularly notable (Rodríguez-Valdovinos et al., 2021). Studies have also identified the presence of terpenes/steroids, alkaloids, phenols, and glycosides, as well as glycosylated derivatives of catechin (Gárcia-Bores et al., 2020).

Verbesina macrophylla (Cass.) S. F. Blake is one of the several native Brazilian species of *Verbesina* that have yet to be studied for their medicinal properties. Popularly known in the country as "assa-peixe", it is a tree that can reach about three meters in height (Agra, Freitas, & Barbosa-Filho, 2007; Moreira, Costa, Costa, & Rocha, 2002). The species is reported to be used popularly for medicinal purposes in southeastern Bahia State, in the form of leaf tea to treat renal and urethral problems (Moreira et al., 2002) and flower tea to treat inflammations (Agra et al., 2007). Veras et al., (2021) validated the ethnopharmacological properties of *V. macrophylla*, finding that the essential oil possesses antimicrobial activity and anti-inflammatory and antipyretic effects, with confirmed toxicological safety.

Nevertheless, only 13% of the species in the genus *Verbesina* have been chemically studied so far (Arciniegas et al., 2020). Thus, the importance of seeking new information on the biological properties of isolated compounds of species of the genus and searching for new biologically active metabolites in uninvestigated species, is evident. The present study aimed to test the biological activity of two different concentrations of the aqueous extracts of leaves and inflorescences of *V. macrophylla* against the yeasts *C. albicans* and *C. tropicalis*, the filamentous fungus *F. oxysporum* and the protozoan *T. cruzi*.

Material and methods

Plant material and extract preparation

Leaf and inflorescence samples were collected from adult specimens of *V. macrophylla* grown in the Medicinal Plant Garden (14˚, 47' and 56" S and 39˚, 10' and 36" W) of *Universidade Estadual de Santa Cruz* (UESC), municipality of Ilhéus, Bahia, Brazil. The region where UESC is located has a humid tropical climate, with regular rainfall throughout the year and rich biodiversity associated with the Atlantic Forest biome. It is characterized by high temperatures for most of the year, with an annual average around 24 to 26 °C.

The data collection was carried out in 2014, in the month of August, around 8 AM. Exsiccates were deposited at the UESC Herbarium under registration number 19101, and the species was identified by the herbarium curator, taxonomist Luiz Alberto Mattos Silva. The material was oven dried at 40 °C and ground. A total of 100 g of dried leaves was used to produce the extract, with the powder then submerged in demineralized water at a rate of 10 g 100 mL^{-1} and stirred for 24 hours to produce the aqueous extract, which was subsequently filtered and concentrated in a lyophilizer. The obtained dry extract yielded 28.3 g.

Scanning electron microscopy (SEM)

To assess micromorphology of *V. macrophylla*, fragments of leaves and inflorescences were fixed in an aqueous solution of 2.5% glutaraldehyde, 4% formaldehyde and 0.05 M sodium cacodylate buffer, pH 7.2 (Karnovsky 1965 modified by Da Cunha et al., 2000) then post-fixed in 1% osmium tetroxide and 0.05 M sodium cacodylate buffer for 2 hours at room temperature. After fixation, the samples were submitted to acetone dehydration, followed by CO_2 critical point drying (CPD 030, Baltec). The samples were then fixed to stubs with carbon tape and covered with a layer of approximately 20 nm gold (SCD 050, Baltec, Switzerland). Images were obtained using a Zeiss EVO 40 (Germany) SEM at a voltage of 15 kV.

Microorganisms

The yeasts *Candida albicans* (CE022) and *Candida tropicalis* (CE017) were obtained from *Departamento de Biologia, Universidade Federal do Ceará*, Fortaleza, Brazil. The fungus *Fusarium oxysporum* (5845) was obtained from Micoteca URM of *Universidade Federal de Pernambuco*, Recife, PE, Brazil. The fungi were maintained on Sabouraud agar (1% peptone, 2% glucose, and 1.7% agar-agar) (Merck) in the *Laboratório de Fisiologia e Bioquímica de Microrganismos*, from *Centro de Biociências e Biotecnologia, Universidade Estadual do Norte Fluminense Darcy Ribeiro* (UENF), Campos dos Goytacazes, RJ, Brazil.

The fungus *F. oxysporum* was transferred from stock and placed on a Petri dish containing Sabouraud agar for approximately 15 days at 30 °C. After this period, 10 mL of Sabouraud broth was poured onto the dish containing the fungi, and the spores released with the aid of a Drigalski spatula. The suspension was subsequently filtered on gauze to prevent the passage of any mycelial remains present in the solution. Spores were then quantified in a Neubauer chamber (Optik Labor) under light microscopy (Axiovison, Zeiss).

Inoculation of yeast cells of *C. albicans* and *C. tropicalis* were removed from the inclined Sabouraud agar tubes transferred to Sabouraud agar Petri dishes and kept for two days at 30 °C. The cells were subsequently used in the assay by removing colonies with the aid of a seeding loop and adding 10 mL of culture medium (Sabouraud broth) for quantification using a Neubauer chamber under light microscopy (Broekaert, Terras, Cammue, & Vanderleyden, 1990).

The epimastigote form of *T. cruzi* (strain DM28) was maintained in glass tubes containing 5 mL LIT (liver infusion tryptose, liver broth, and tryptose, both from Fluka Analytica) supplemented with 10% fetal bovine serum (GIBCO) in a BOD incubator at 28 °C. For culture maintenance, every five days, 1 mL of the supernatant was removed from the tubes and placed in a new tube, which was completed to 5 mL with fresh medium.

Fungal growth inhibition assay

After quantification, cells *F. oxysporum, C. albicans* and *C. tropicalis* (1×10^4 cells mL^{-1}) were incubated in 200 µL of Sabouraud broth containing 200 µg mL^{-1} and 400 µg mL^{-1} of aqueous leaf extract or aqueous inflorescence extract of *V. macrophylla* diluted in 10% v/v DMSO. The assay was performed in cell culture plates (96 wells) incubated at 30°C for 48 hours for filamentous fungus and 24 hours for yeasts. Optical densities were measured at 620 nm in a microplate reader (EZ Read 400, Biochrom) after 48 or 24 hours. Untreated fungal cells were used as a positive control and the culture medium was used as a negative growth control. The percentage of growth of the different fungal cells was calculated as follows: % growth = (absorbance of treated cells - absorbance of negative control)/(absorbance of positive control - absorbance of negative control). The maximum final volume of DMSO was 2% and did not affect fungal growth. The entire assay was performed under aseptic laminar-flow conditions, according to an adaptation of the methodology of Broekaert et al. (1990). All data analyzed were obtained from experiments carried out in triplicate. The data from the fungal growth inhibition assay were evaluated using the unidirectional ANOVA test Tukey's. The mean differences were considered significant at $p < 0.05$. All statistical analysis was performed using the GraphPad Prism software (version 6.0 for Windows).

Anti-*Trypanosoma cruzi* activity

For the experiments, the contents of the tubes were centrifuged, and the parasites diluted in 1 mL of medium. A volume of 10 µL was taken and mixed with 90 µL of counting medium (40% formaldehyde + 60% PBS). A volume of 10 µL was then taken from this mixture and counted in a Neubauer chamber. Assays were performed with an initial amount of approximately 2×10^6 parasites mL^{-1}.

Extracts were diluted in 1.5% v/v DMSO (dimethyl-sulfoxide, Merck) and LIT medium to determine a stock concentration. The parasites were placed in 96-well plates at a volume of 100 µL $well^{-1}$. Samples of the extracts

were diluted, again in LIT medium, to the concentrations of use and placed in the wells of the plates for a total volume of 200 µL well^{-1}. The final volume of DMSO did not affect the parasites. Parasites were again quantified in a Neubauer chamber after 24 h.

Membrane permeabilization of *Candida tropicalis* cells

Membrane permeabilization of *C. tropicalis* cells was evaluated by measuring Sytox Green fluorescence as previously described by Thevissen, Terras, & Broekaert, (1999), with some modifications. Sytox Green is a dye that only penetrates cells when the plasma membrane is structurally compromised. Once inside the cytoplasm, it binds to nucleic acids, resulting in a fluorescent complex. After the growth, cells of *C. tropicalis* were incubated with 0.2 µM Sytox Green in 1.5 ml microcentrifuge tubes for 30 minutes at 25 °C with periodic shaking. The cells were then examined using a differential interference contrast (DIC) microscope (Axiophoto, Zeiss) equipped with a set of filters for fluorescence detection (excitation wavelengths, 450-490 nm, emission wavelength, 500 nm). Negative controls (which were not subjected to the extract) were also performed to assess basement membrane permeability.

For morphological observation of *T. cruzi* epimastigotes, they were centrifuged at 1,700 rpm for 10 minutes and washed with PBS pH 7.2 at room temperature. The parasites were then fixed in a 4% paraformaldehyde solution diluted in PBS and stained with Giemsa (10% v/v) for two h at room temperature. Aliquots of 100 µL were spread on microscope slides, dried at 37 °C, and examined using a Zeiss Axioplan photographic microscope with a 40x objective. Images were obtained using Analysis software (USA).

For ultrastructure analysis, *T. cruzi* samples treated with 50 µg mL^{-1} aqueous extract for 16 hours were centrifuged for 10 minutes at 1,700 RPM, washed with PBS for a further 10 minutes, and re-centrifuged at the same RPM. Fixation was performed with 4% formaldehyde, 1% glutaraldehyde, 0.2 M sodium cacodylate buffer, 1.5 mL distilled water, and 5% sucrose for 1 hour. The samples were then centrifuged for 10 minutes at 1,700 rpm and washed with 0.1 M sodium cacodylate buffer solution. The resulting pellet was post-fixed with 2% osmium tetroxide and 0.8% potassium ferrocyanide for 1.5 hours at room temperature and protected from light. Fixed samples were washed in sodium cacodylate buffer, centrifuged twice and dehydrated in an increasing series of acetone. After dehydration, the samples were incubated in solutions of acetone and epoxy resin (Epon®) in ratios of 2:1, 1:1, 1:2 for 6 hours each step. The material was then embedded in epon and polymerized in an incubator at 60°C for 48 hours. Ultrathin sections (60 nm) were obtained using a Reichert Ultracuts Leica Instruments® ultramicrotome and stained with 5% aqueous uranyl acetate for 20 minutes and lead citrate for 5 minutes (Reynolds 1963). Sections were observed at 80 kV using a transmission electron microscope (Zeiss TEM 900).

Statistical analysis

Results were reported as mean ± standard deviation of three independent replicates. An analysis of variance (ANOVA) was used to assess statistical significance. Differences between values with a $p < 0.05$ were considered statistically significant. For the comparison of means for the corresponding results, Tukey's test was performed. These analyses were performed with the software Prism-GraphPad 8.

Results

Verbesina macrophylla (Asteraceae) is a tree that can reach three meters in height (Figure 1A). It has sessile, alternate leaves with a membranous consistency, palmate venation, hairy surface and dentate blade, ranging from two to four entrances (Figure 1B). It exhibits heterophily during the flowering period, as leaves closer to capitulescence have an entire blade. There are no stipulations.

Verbesina macrophylla has a whitish and aromatic capitulescence-type inflorescence that is very attractive to pollinators (Figure 1C). The capitulum is a condensed type of indeterminate inflorescence, in which all the flowers are sessile and attached to the inflorescence axis, surrounded by an envelope of bracts, known as a pseudant (Figure 1D). Involucral bracts are modified capitulum leaves that functionally act as sepals, free from each other, protecting the young capitulum throughout its development. The head is disc-shaped and heterogamous, with two types of tubular flowers on the disc: the outermost are tubular-filiform, pistillate and zygomorphic (Figure 1F), while the innermost, in the center, are tubular, bisexual and actinomorphic.

The innermost flowers have a five-lobed tubular corolla, which is distinctly divided into tube and limbus, the latter containing the anther tube. There are five stamens with free filaments inserted directly into the

corolla tube (epipetalous stamens). The anthers are linked together by their lateral edges, forming a tube that surrounds the style and stigma (synanthers). The anthers are black, which is unique to Heliantheae. The apex of the anther possesses a sterile extension called the connective appendix.

The style is bifid, being divided at the apex into two long branches (Figure 1E). The stigmatic areas are internal and uniformly cover the entire surface of the stylet branches, with the presence of papillae. The inflorescence axis possesses paleae, which are laminar, membranous, scale-shaped organs characteristic of the tribe of this species (Figure 1G). The pollen grains are echinate, characterized by exine ornamentation in the form of spines (Figure 1H).

The species has a single flowering period per year. The flower buds begin development in the beginning of June, and their production extends until August, for a total reproductive period of three months.

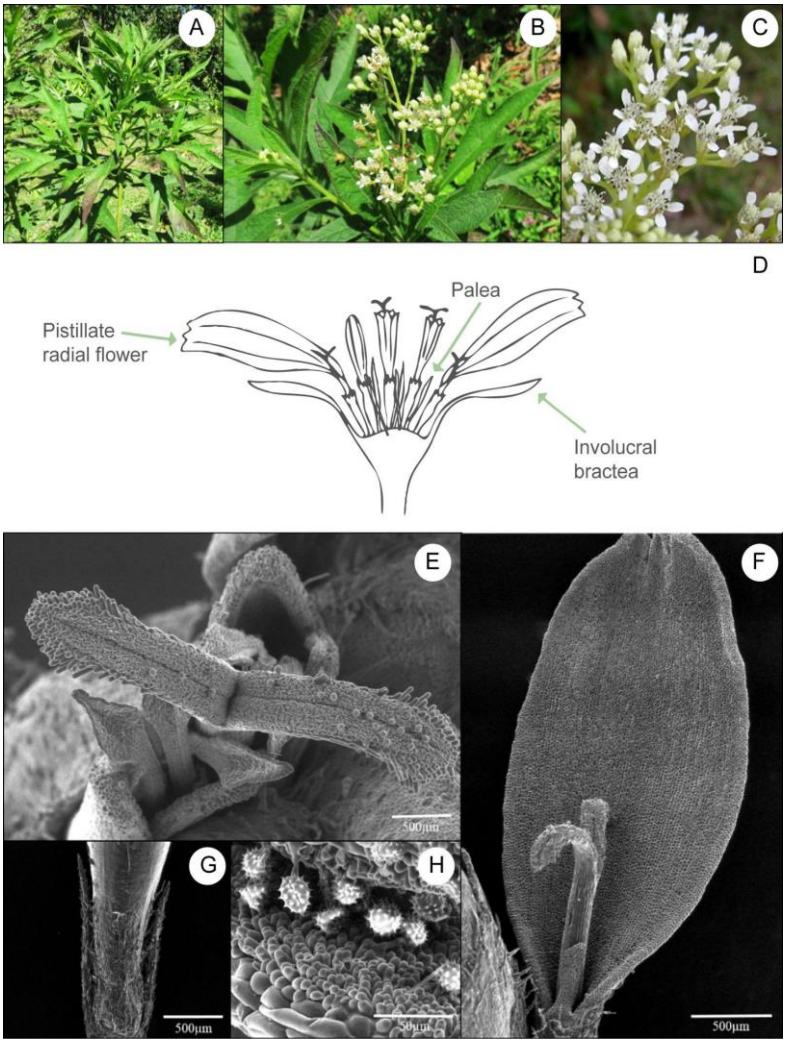

Figure 1. A-C: Macro view of *Verbesina macrophylla*. D: Representative diagram of the arrangement of parts in the capitulum. E-H: Flowers observed using a scanning electron microscope. (E) Detail of the stigma of a bisexual flower. (F) Flower of the outermost region of the capitulum, which are tubular-filiform and pistillate. (G) Evidencing the paleae present at the base of the bisexual flower. (H) Echinate pollen.

Antifungal activity

Figure 2 shows the results of tests for antifungal activity by aqueous extracts of leaves and inflorescences of *V. macrophylla* at two concentrations. Leaf extract did not inhibit the growth of any of the tested fungi (filamentous and yeast). Inflorescence extract did inhibit the growth of *F. oxysporum* by 12 and 14% at concentrations of 200 and 400 µg mL^{-1}, respectively, the two concentrations not differing significantly. Both concentrations of inflorescence extract also inhibited the growth of *C. tropicalis* by 41% (Figure 2) relative to the control. These findings suggest that the inflorescence extract does not act on *F. oxysporum* and *C. tropicalis* cells in a dose-dependent manner. Microscopic analysis revealed a decrease in cell number, corroborating the growth inhibition of *C. tropicalis* treated with 200 µg mL^{-1} of inflorescence extract measured by optical density (Figure 3). No significant differences were observed in cell number for *C. albicans* (Figure 3).

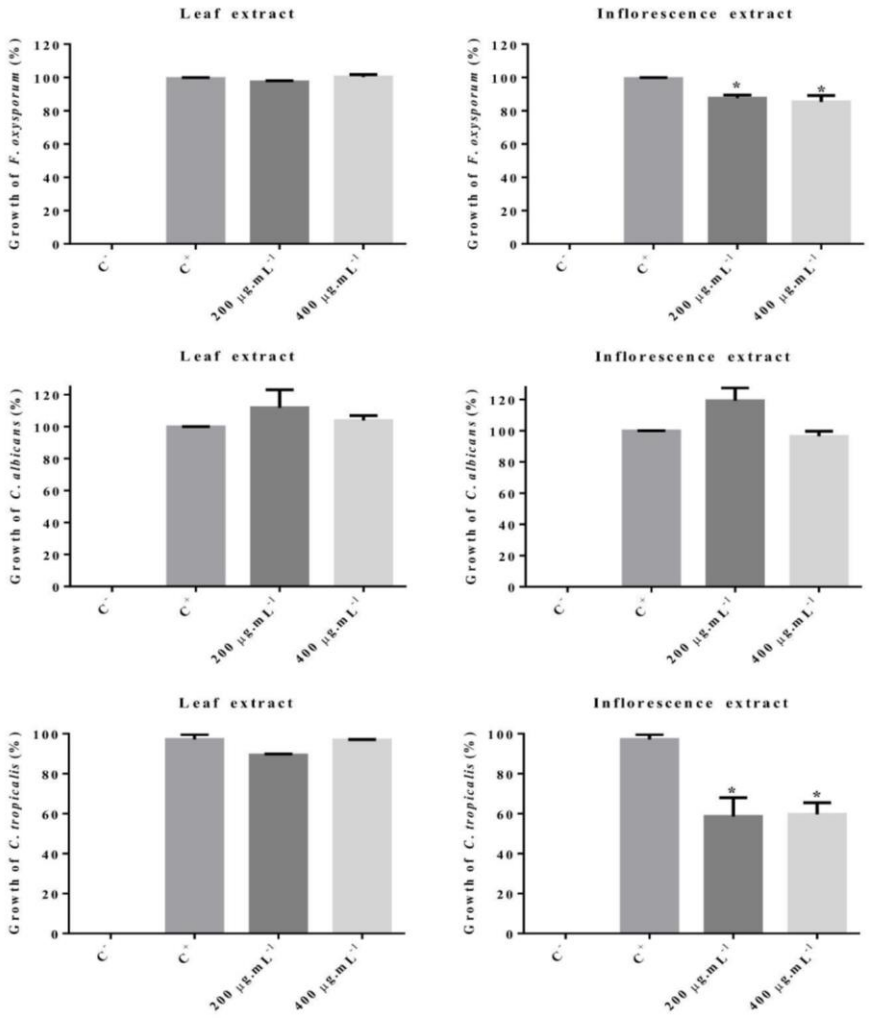

Figure 2. Effects of *Verbesina macrophylla* extracts on fungal growth. Cells of *Fusarium oxysporum, Candida albicans* and *Candida tropicalis* were treated with of 200 or 400 µg mL^{-1} of leaf or inflorescence extract of *Verbesina macrophylla* with incubation for 48h for the filamentous fungus and 24h for the yeasts, Negative Control (C^-) - Sabouraud Broth; Positive Control (C^+) untreated yeast suspensions. The presented results are mean values obtained in triplicate. * $p < 0.05$ compared to positive control as determined by Tukey's test.

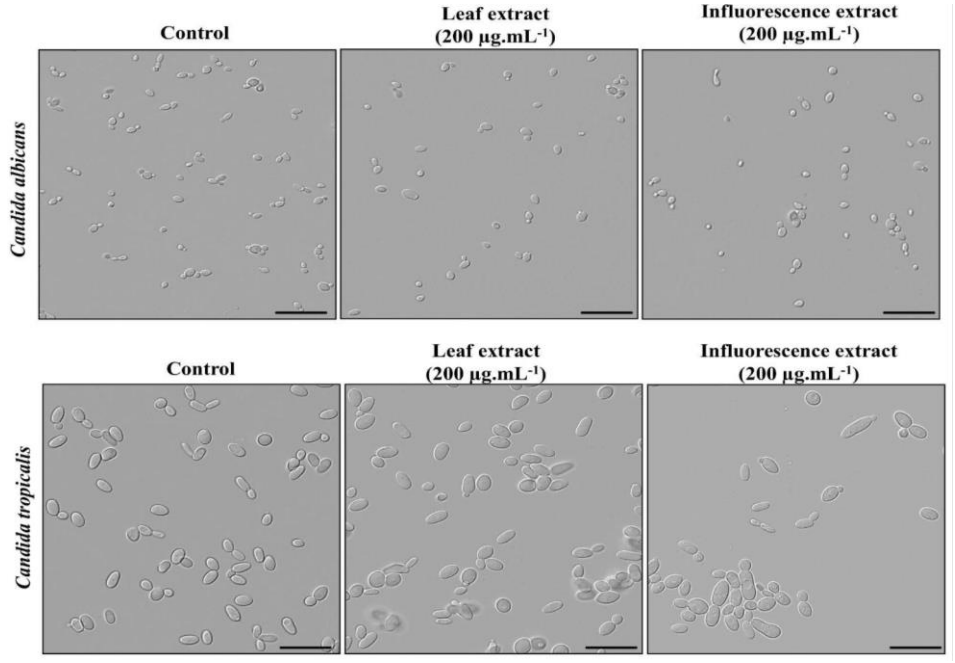

Figure 3. Optical microscopy images of cells of *Candida albicans* and *Candida tropicalis* treated with 200 µg mL^{-1} of leaf or inflorescence extract of *Verbesina macrophylla* or untreated (control). Bar = 20 µm.

Candida tropicalis cells emitted internal fluorescence when treated with 200 µg mL^{-1} of inflorescence extract, showing that the plasma membrane was structurally compromised (Figure 4). Optical microscopy assays revealed morphological changes in *C. tropicalis* cells treated with inflorescence extract, which were not visible in the control cells. The treated cells presented difficulties in releasing the buds from the mother cell, cytoplasm with a granular aspect, and cellular shrinkage (Figure 5). Taken together, the obtained data showed that the inflorescence extract can cause significant morphological alterations that compromise the cell membrane, resulting in growth inhibition of *C. tropicalis*.

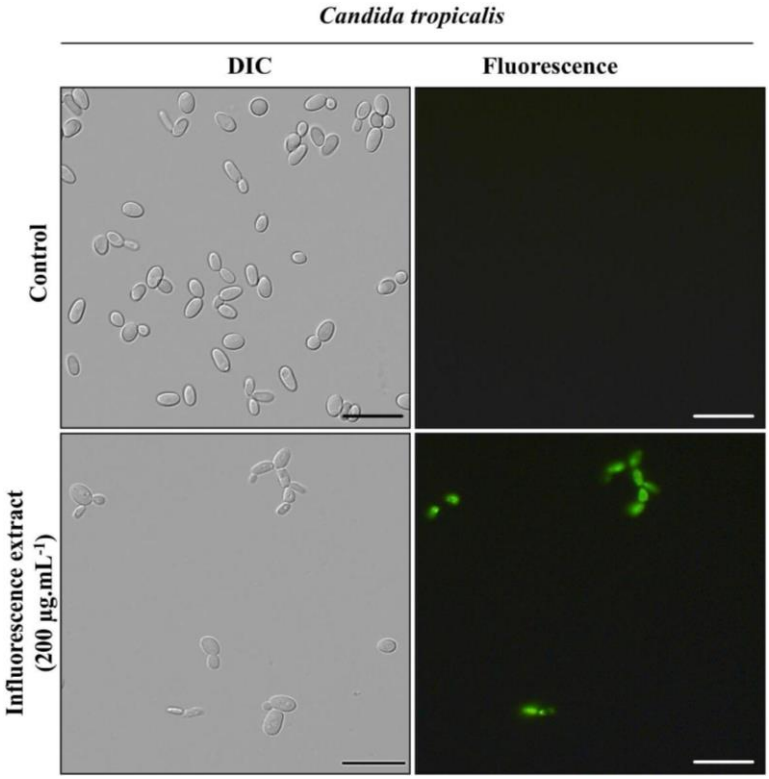

Figure 4. Images of *Candida tropicalis* cells after membrane permeabilization assay by fluorescence microscopy using a Sytox fluorescent green probe. Cells were treated with 200 µg mL^{-1} of inflorescence extract of *Verbesina macrophylla* and then assayed for membrane permeabilization. Control cells were treated only with Sytox green Bar = 20 µm.

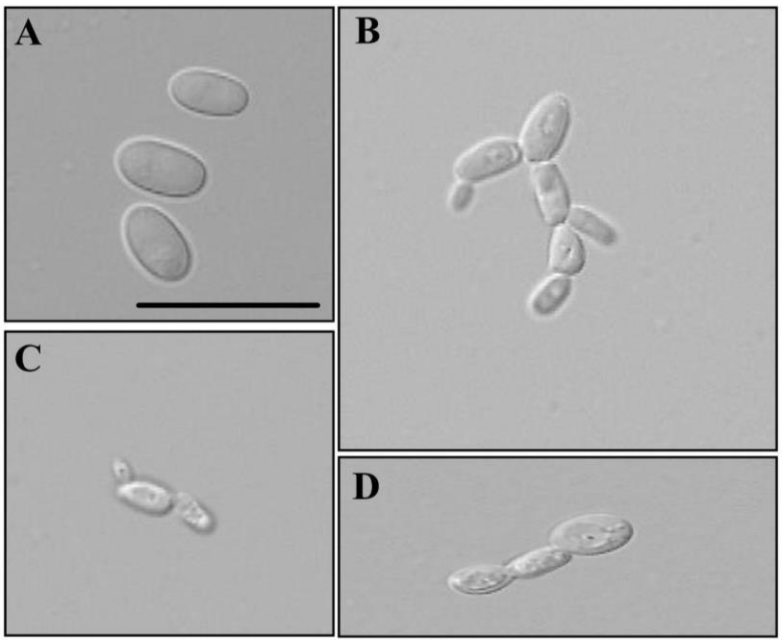

Figure 5. Cells of *Candida tropicalis* under light microscopy after the growth inhibition test. A - control cells; B-D - cells treated with aqueous inflorescence extract of *Verbesina macrophylla* for 24 h. Bar = 20 µm.

Trypanocidal activity

Aqueous leaf (Figure 6A) and inflorescence (Figure 6B) extract of *V. macrophylla* at different concentrations eliminated the epimastigote form of *T. cruzi*, reaching 100% elimination after treatment with 200 µg mL^{-1}. The toxic effect on the studied protozoan resulting from the extract remained throughout the test period (48 hours).

The inflorescence extract (Figure 6B) had higher inhibitory action on parasite growth than the leaf extract, especially at higher concentrations. Even the highest concentration of aqueous leaf extract did not inhibit the parasites until 16 hours, with inhibition occurring mainly after this period. In contrast, inflorescence extract achieved total inhibition at 8 h for 200 and 400 µg mL^{-1}.

Analysis using light microscopy revealed that untreated epimastigotes had a typical elongated shape with a flagellum and no visible alterations (black arrows) (Figure 7A). On the other hand, when subjected to 50 µg mL^{-1} of aqueous leaf (Figure 7B) or inflorescence (Figure 7C) extract for 16 hours, epimastigotes acquired an irregular, condensed shape and compromised structure, rendering them unviable (white arrows).

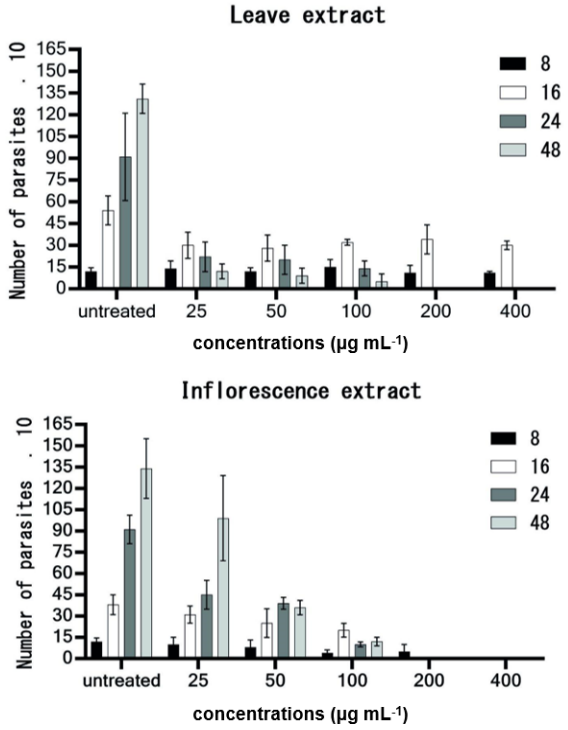

Figure 6. Effects of aqueous leaf and inflorescence extracts of *Verbesina macrophylla* at different concentrations on the growth of the epimastigote form of *Trypanosoma cruzi*. Incubation times were from 8 to 48 hours.

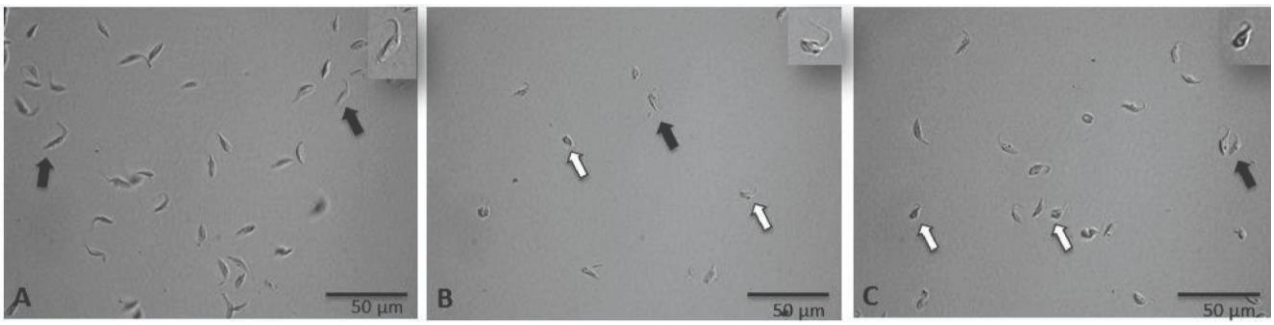

Figure 7. Effects of aqueous inflorescence (B) and leaf (C) extracts of *Verbesina macrophylla* at different concentrations on the growth of the epimastigote form of *Trypanosoma cruzi*, as visualized by optical microscopy. A - control; B – inflorescence extract; C - leaf extract. Both treatments were subjected to 50 µg mL^{-1} aqueous extract for 16 hours. Black arrows indicate viable parasites; white arrows indicate unviable parasites.

Transmission electron microscopy to observe the action of the extracts against the epimastigote form of *T. cruzi* at the ultrastructural level revealed cytoplasmic and nuclear disorganization (Figure 8B), vacuolization, and swelling of reservosomes (Figure 8C). The control parasites, on the other hand, exhibited typical morphology with organized cytoplasm and kinetoplast, mitochondria, and intact nuclei (Figure 8A).

Figure 8. Effects of aqueous extract of *Verbesina macrophylla* on the ultrastructure of the epimastigote form of *Trypanosoma cruzi*, as visualized by transmission electron microscopy. (A) Control, (B) leaf extract and (c) inflorescence extract. K: kinetoplast; N: nucleus; white arrow: mitochondria; black arrow: cytoplasmic vesiculations; star: disorganization of chromatin. Bar = 2 µm.

Discussion

Phenological investigations can make important contributions to the conservation of native plant species, such as *Verbesina macrophylla*, mainly because its flowers are used to prepare teas. The species flowers from June to August, reflecting a water requirement as this period corresponds to increased rainfall in the region.

Unlike the present study, trials performed with methanolic extract of the aerial parts of *V. nudipes* S. F. Blake and *V. encelioides* (Cav.) Benth. & Hook. f. ex A. Gray, showed inhibition for *Candida albicans* (Giordani et al., 2015; Toribio et al., 2005). Among studies with other species of *Verbesina*, experimental results indicate that *V. sphaerocephala* A. Gray may have ethnopharmacological usage as a potential source of natural antioxidants. The methanolic extracts possessed notable antioxidant power and strong inhibitory activity toward the test bacteria (Rodríguez-Valdovinos et al., 2021).

Duarte, Figueira, Sartoratto, Rehder, and Delarmelina, (2005) screened, for anti-*C. albicans* activity, the essential oils and ethanolic extracts from the leaves and/or roots of 35 medicinal plant species commonly used in Brazil, with the essential oils of 13 species showing activity. Ethanolic extracts were not effective at any of the tested concentrations. Chemical analyses showed the presence of compounds with known antimicrobial activity, including 1,8-cineol, geranial, d-germacrene, limonene, linalol, and menthol (Duarte et al., 2005). Antifungal analyses using the essential oil of *V. macrophylla* are recommended since Bezerra, Mangabeira, Oliveira, Costa, and Da Cunha (2018) reported the presence of components with potential microbial action, such as germacrene-D, in its essential oil. Veras et al. (2021) suggest that essential oil from *V. macrophylla* may be used by industry to develop drugs with natural antimicrobial, anti-inflammatory, and antipyretic effects.

Techniques that employ fluorescent dyes to visualize cell integrity are advantageous, as the widely used plaque counting method may include cells that are no longer viable due to membrane damage (Sung, Lee, & Lee, 2007). The cell membrane is an active structure that acts as a barrier between the cytoplasm and the extracellular medium, making it essential for maintaining optimal internal conditions for metabolism (Sánchez, García, & Heredia, 2010). Thus, by disrupting the cell membrane, the inflorescence extract studied here resulted in bactericidal activity.

The most appropriate clinically known antibiotics act mainly at the membrane level by inhibiting membrane proteins associated with cell wall synthesis (Nelson, Grier, Barbaro, & Ismail, 2009). Antibiotic resistance has limited the use of these drugs, reinforcing the importance of finding new compounds that also act at the membrane level. The goal of future efforts in medicinal chemistry will be to enhance biological activity while minimizing toxicity against mammalian cell membranes.

The present study revealed promising results, demonstrating alterations to the plasma membrane of *Candida tropicalis*. These findings suggest that the sesquiterpene lactones present in *V. macrophylla* may contribute to its biological activities, like those observed in *Wunderlichia azulensis* (Trindade et al., 2023). Sesquiterpene lactones are terpenoid compounds with a wide variety of chemical structures and are characteristic of the family Asteraceae (Arciniegas et al., 2020). Therefore, the present findings with *V. macrophylla* complement and reinforce the understanding of the therapeutic potential of sesquiterpene

lactones in plants of Asteraceae, promoting new perspectives for the development of antifungal and antiparasitic treatments.

The trypanocidal activity observed here might also be related to the characteristic chemical properties of the family Asteraceae. Tests using extract of *Ambrosia tenuifolia* Sprengel led to the isolation of two bioactive sesquiterpene lactones with significant trypanocidal activity. The development of an antimalarial drug isolated from *Artemisia annua* L., increased interest in investigating this class of substances as antiprotozoal agents. In addition, other sesquiterpene lactones with antiprotozoal activity have been described (Grael, Albuquerque, & Lopes, 2005; Kayser, Kiderlen, & Croft, 2003; Schmidt, Brun, Willuhn, & Khalid, 2002; Tiuman et al., 2005).

Encapsulated sesquiterpene lactones have shown high therapeutic efficacy in combating Chagas disease (Branquinho et al., 2020). Experimental studies with mice infected with a *T. cruzi* strain resistant to benznidazole and nifurtimox, found that orally-administered sesquiterpene lactones cured 75% of the animals in the acute phase of infection and 88% in the chronic phase. These results indicate that nanotechnology can significantly improve the efficacy of treatment against Chagas disease, even for infections resistant to conventional drugs (Branquinho et al., 2020). Compared to other methods of treating Chagas disease, the use of sesquiterpene lactones has proven to be the most promising (Mazzeti et al., 2021). Thus, the present study demonstrated that *V. macrophylla* has trypanocidal action and may be a source of new sesquiterpene lactones of biological importance.

The present results are similar to those reported by Izumi et al. (2008), who verified the activity of crude extracts from *Tanacetum parthenium* (L.) Sch. Bip., also of the family Asteraceae, against epimastigote and amastigote stages of *T. cruzi*. Scanning and transmission electron microscopy observed morphological modifications and ultrastructural alterations. As the number of nuclei increased, so did the number of reservosomes and surrounding membrane formation, as well as swelling of mitochondria and distortion of inner membranes, along with an excess of vacuoles (Izumi et al., 2008). Increased swelling of the lumen of reservosomes may indicate a direct relationship with increased cysteine proteinase activity caused by parthenolide, as previously reported with *Leishmania* Ross (Tiuman et al., 2005).

The results presented here clearly show the potential of aqueous extracts of *V. macrophylla* to alter the morphology of, and eliminate, the epimastigote form of *T. cruzi*. The extracts also showed moderate activity against *C. tropicalis*, and less activity against *C. albicans* and *F. oxysporum*. Although the studied extracts did not present considerable inhibitory activities against the filamentous fungus and yeasts, promising results were seen with the epimastigote form of *T. cruzi*, which adds industrial and medical value to *V. macrophylla*. We suggest that biological activity assays and subsequent assays with *T. cruzi* in intracellular form be performed to obtain results more relevant to the action of the chemical components of *V. macrophylla* extracts as controls of Chagas disease.

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