



**BIOACTIVITY OF LEAF EXTRACTS FROM SPECIES OF *PALICOUREA* (RUBIACEAE)  
ON *TRYPANOSOMA CRUZI*, *CANDIDA SP.* AND *FUSARIUM SOLANI***

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**ABSTRACT**

Plants extracts, as well as pure substances, have been reported to possess significant antiparasitic and antimicrobial activity, with no side effects. Investigation into species that belong to families that are already known for having species with chemotaxonomic markers is of great importance. Such is the case with the family Rubiaceae, which, from the phytochemical point of view, is known for the production of bioactive metabolites with high pharmacological potential. We present the results of an investigation into the antiprotozoan and antifungal activity of three plant species of the genus *Palicourea* (Rubiaceae) by testing different concentrations of their extracts against epimastigotes of *Trypanosoma cruzi*, the yeasts *Candida albicans* and *C. tropicalis* and the filamentous fungus *Fusarium solani*. Plant leaves were dried, pulverized and submitted to exhaustive extraction with methanol. This extract was partitioned by ethyl acetate, water, butanol, dichloromethane and hexane, and the extracts tested *in vitro*. The kinetics of the destruction of the parasite were analyzed morphologically using standard techniques for light and transmission electron microscopy. The results were confirmed by morphological deformation of the protozoans and their organelles. The fractions produced by butanol, methanol and ethyl acetate of *P. rudgeoides* were the best at reducing epistomastigotes. None of the extracts exhibited inhibitory activity on the development yeast and filamentous fungus tested. Additional studies are needed, such as the spectrometric analysis of the extracts, in order to elucidate the metabolite(s) responsible for the results of this study.

**KEYWORDS:** biological activity, Chagas disease, *Candida albicans*, light microscopy.

**INTRODUCTION**

Protozoan parasites and yeasts are the causative agents of several diseases of variable risks to human health. Some of these infections can remain asymptomatic, but others can cause serious health problems or even death if not treated. However, it has become increasingly difficult to treat these infections because of the serious side effects to the chemotherapeutic agents currently being used or the resistance of these microorganisms to them.<sup>[1, 2, 3, 4]</sup>

*Trypanosoma cruzi* is the causative agent of Chagas disease, which affects about 25 million people living in risk areas and about 10 million people currently infected, and resulting in over 15,000 deaths and 50,000 new cases

each year.<sup>[5]</sup> The life cycle of this parasite involves two hosts; an invertebrate (vector) and a vertebrate, including humans, in which the three main developmental forms are found.<sup>[6]</sup> The form found in the invertebrate vector (usually an insect) is the epimastigote and the forms found in vertebrates (usually mammals) are the blood trypomastigote and the intracellular amastigote, the latter of which multiplies in the cytoplasm of a wide variety of cells, although they have a tropism for muscle cells.<sup>[7]</sup> At the end of the intracellular multiplication, the amastigotes transform into trypomastigotes and burst out of the host cell and enter the bloodstream, from which they invade new cells and perpetuate the infection.<sup>[8, 9]</sup>

Another global problem is the alarming increase in the number of fungal infections. Yeasts are considered opportunistic pathogens that can influence infectious processes to be clinically asymptomatic to severe and fatal disease.<sup>[10, 11, 12]</sup> Amphotericin B, the echinocandin caspofungin and the azole derivatives fluconazol, itraconazol and voriconazol are the agents available for the management of serious fungal infections.<sup>[13]</sup> However, side effects, the appearance of opportunistic fungal pathogens, an increase in the variety of mycoses and the recovery of clinical isolates resistant to antifungal agents have called for the development of new antifungal agents (triazoles and echinocandins) as well as lipid formulations of amphotericin B and nystatin.<sup>[14, 15]</sup> Medical treatment of fungal infections is a challenge because there exist few drugs, the treatment is long, the drugs have high toxicity and they are not always effective.<sup>[16, 17, 18]</sup>

There is an increase in research on natural products active against *Candida sp.*<sup>[19]</sup> There are about 200 described species of the genus, with about 10% being disease causing, of which *Candida albicans* is associated with serious fungal infections.<sup>[15]</sup> Although this is the yeast most commonly involved in pathological processes, there are other species such as *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. Lusitaniae* and *C. glabrata*.<sup>[11, 12, 20]</sup> Therefore, the resistance of parasites and fungi to medicines, as well as insect vector resistance to insecticides, justifies the search for new compounds capable of controlling them.<sup>[1, 21]</sup>

Among plants that are promising sources of new active compounds,<sup>[22, 23, 24, 25]</sup> investigations into species belonging to families described by chemotaxonomic markers, such as Rubiaceae, are interesting.<sup>[26, 27 28, 29]</sup>

*Palicourea* Aublet. is a Neotropical genus of Rubiaceae containing species with ornamental, medicinal and phytotoxicological potential. They are used in traditional medicine for treatment of fungal infections, coughing and stomachache, as well as being included on the list of plants used against cancer.<sup>[30]</sup> Chemical investigation of this genus has described various classes of substances such as coumarins, benzoic acids and terpenes,<sup>[31]</sup> as well as indolic and quinolinic alkaloids.<sup>[32, 33]</sup> However, little is known about the biological activity of the extracts of this genus. Therefore, we have chosen *T. cruzi*, *C. albicans*, *C. tropicalis* and *F. solani* as test subjects for assessing the antiparasitic and antifungal activity of extracts of species of *Palicourea*.

The objective of this work was to test the toxic effect of leaf extracts of *Palicourea marcgravii* A. St.-Hil.; *P. rudgeoides* (Müll. Arg.) Standl. and *P. tetraphylla* Cham. & Schltld. on the epimastigote of *T. cruzi* and to evaluate their antifungal activity against *C. albicans*, *C. tropicalis* and *F. solani*.

## MATERIAL AND METHODS

### Botanical material

Completely expanded leaves of *Palicourea marcgravii* A. St.-Hil., *Palicourea rudgeoides* (Müll. Arg.) Standl. and *Palicourea tetraphylla* Cham. & Schltld. (Rubiaceae) were collected in the state of Rio de Janeiro in July 2013. The material was identified by Dr. Sebastião da Silva Neto and deposited in the herbarium of the Universidade do Estado do Rio de Janeiro.

**Table 1: Studied species, herbarium number and collection site.**

Species	Voucher	Collection Site
<i>P. marcgravii</i>	HRJ-12442	Itatiaia National Park
<i>P. rudgeoides</i>	HRJ-12444	Itatiaia National Park
<i>P. tetraphylla</i>	HRJ-12445	Itatiaia National Park

### Drying and milling

The leaves of *P. marcgravii*, *P. rudgeoides* and *P. tetraphylla* were dried at ambient temperature and pulverized in a grinder machine set at granulation.

### Solvent extraction

The dried and pulverized plant material was submitted to exhaustive extraction in methanol. Filtration and extraction of the methanol in reduced pressure produced crude extract, which was used for fractionation by liquid-liquid partition using solvents such as methanol, hexane, dichloromethane, ethyl acetate, butanol and water. In total there were six extracts for each species: methanol extract (MeOHE), hexane extract (HexE), dichloromethane extract (DCME), ethyl acetate extract (AcOEtE), butyl alcohol extract (ButE) and water extract (H<sub>2</sub>OE). Biological tests used 0.004 g/ml of each extract.

### Parasite culture

Epimastigotes of *T. cruzi* (DM28 strain) were cultivated in 5 mL of liver infusion triptose (LIT) medium supplemented with 10% FCS (fetal bovine serum, Gibco) and 4% of hemine at 28°C. Every five days, 1 mL of parasites, still in exponential population growth, were placed in a fresh tube to which fresh culture medium was added to reach 5 mL. Much research has been performed using the epimastigote of *T. cruzi* because it is easily cultivated *in vitro* and, consequently, it is widely used in preliminary experimental studies.<sup>[34]</sup>

### Maintenance of fungi

The yeasts *Candida albicans* (CE022) and *Candida tropicalis* (CE017) and the filamentous fungus *Fusarium solani* were cultivated in Sabouraud medium [peptone 10g/L, D(+) glucose 20g/L, agar-agar 17g/L; Merck S/A], maintained at 23°C and stored in the Laboratório de Fisiologia e Bioquímica de Microrganismos (LFBM) of the Centro de Biociências e Biotecnologia (CBB) of the Universidade Estadual do Norte Fluminense Darcy Ribeiro (UENF), Campos dos Goytacazes, Rio de Janeiro, Brazil.

### Obtaining filamentous fungus spores and yeast cells

The fungus *F. solani* was transferred from stock and placed to grow on a Petri dish containing Sabouraud agar for about 15 days at 30°C. After this period, 10 mL of Sabouraud broth was poured over the plate with the fungus and the spores were released using a Drigalski spatula. This suspension was duly filtered in gases to prevent the passage of mycelial debris that might be in the solution with the spores. The spores were then quantified using a Neubauer chamber (Optik Labor) under a light microscope. Inoculated cells of the yeasts *C. albicans* and *C. tropicalis* were removed from the tubes containing Sabouraud agar and transferred to Petri dishes containing the same agar. The cells were maintained for a period of two days at 30°C. After this period, the cells were used in the assay by removing colonies, with an inoculation loop, and adding them to 10 mL of culture medium (Sabouraud broth) and quantifying them using a Neubauer chamber (same as used for quantifying fungal spores) under a Zeiss Axiovision 4 light microscope.

### Test of cytotoxicity of total extracts on epimastigotes

A stock solution of 8 mg/mL in growth medium and DMSO (1.5% V/V) was prepared for each extract. This mixture was stirred for the best dilution of the extracts and sterilized with 0.22 µm membrane filters. Different volumes of stock solution were incubated in a 96-well plate to give final extract concentrations of 25, 50, 100, 200, 400 µg/mL. In addition, 100 µL of parasites, at a density of  $3.6 \times 10^6$ , were added per well and the volume adjusted to 200 µL using culture medium. The plate containing the experiment was incubated at 28°C for 24h. Quantification of parasites was done in a Neubauer chamber. After the first test, the aqueous extracts of the three plant species were used to further investigate their effects on the parasites with additional time of 8h and 16h.

### Analysis of inhibition of fungal growth

After quantification, the cells of the filamentous fungus *F. solani* and the yeasts *C. albicans* and *C. tropicalis* ( $1 \times 10^4$  cells.mL<sup>-1</sup>) were incubated in 200 µL of Sabouraud broth containing 250 µg.mL<sup>-1</sup> samples of the six extracts of *P. marcgravii*, *P. rudgeoides* and *P. tetraphylla*. The assay was performed in culture plates (96 wells) and incubated at 30°C for a period of 48 h for the filamentous fungi and 24 h for the yeasts. To observe inhibition of fungal growth, the optical density was calculated from readings of a microplate reader (EZ Read 400, Biochrom), at 620 nm every 6 h. The entire assay was done in triplicate and under aseptic conditions in a laminar flow hood following an adaptation of the methodology of.<sup>[35]</sup>

### Light microscopy

For morphological analysis, epimastigotes of *T. cruzi* were centrifuged at 1700 rpm for 10 minutes, and washed with PBS, pH 7.2 at room temperature. The parasites were fixed in a solution of 4%

paraformaldehyde diluted in PBS and stained with Giemsa (10% v / v) for 2 h at room temperature. Aliquots of 100 µL were spread on a microscope slides, dried at 37°C and examined using a photo-equipped Zeiss Axioplan light microscope with a 40 x objective. The images were obtained using the software Analysis (USA).

### Transmission electron microscopy

For analysis of ultrastructure, the samples treated with 100 µg/ mL of aqueous extract for 24h were centrifuged for 10 minutes at 1700 RPM, washed with PBS for 10 more minutes and re-centrifuged at the same RPM. Fixation was performed using 4% formaldehyde, 1% glutaraldehyde, 0.2 M sodium cacodylate buffer, 1.5 ml distilled water and 5% sucrose for 1 h at room temperature. The samples were then centrifuged again for 10 minutes at 1700 RPM and washed with a 0.1 M solution of sodium cacodylate buffer. The resulting pellet was post-fixed with 2% osmium tetroxide and 0.8% potassium ferrocyanide for 1.5 h at ambient temperature and protected from light. The post-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 a solution of 100% acetone-epon resin in proportions of 2:1, 1:1, 1:2 and pure epon resin for 6 h each step. The material was then embedded in epon and polymerized in a kiln at 60°C for 48 h. Ultrathin sections were obtained using an ultramicrotome in Reichert Ultracuts Leica Instruments® and contrasted with 5% aqueous uranyl acetate for 20 min in the dark and lead citrate for 5 minutes. The material was observed and photographed with a Zeiss TEM 900 transmission electron microscope operating at an accelerating voltage of 80 kV.

## RESULTS AND DISCUSSION

To evaluate the extracts from species of the genus *Palicourea* derived from different solvents, eighteen samples from leaves of *P. marcgravii*, *P. rudgeoides* and *P. tetraphylla* were tested on epimastigotes of *T. cruzi*. Epimastigotes in exponential growth phase were exposed to the extracts at different concentrations (25-400 µg/mL) and analysis of toxicity was assessed by morphological alterations and the elimination of the parasite. Not all extracts had dose/time dependent effects, as noted by the resumption of growth of the parasite after a few of the treatments.

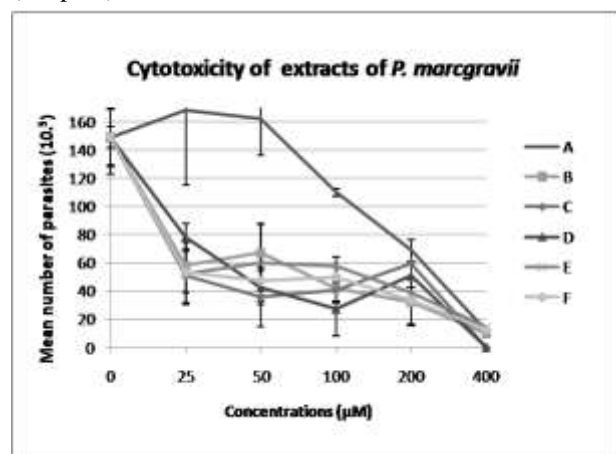
The first extract to be tested was an aqueous, by having contained all of the substances of the source plant. However, this extract was not the more efficient inhibitor. Butyl alcohol extract was the most significant inhibitor, reaching 100% elimination at 400 µg/mL of *P. marcgravii* extract and at 100, 200 and 400 µg/mL of *P. rudgeoides* extract. The second most efficient was crude extract methanol, which inhibited 100% at 200 and 400 µg/mL of *P. rudgeoides* and at 400 µg/mL of *P. tetraphylla*. Followed by dichloromethane extract, which inhibited 100% at 400 µg/mL of *P. rudgeoides* and *P.*

*tetraphylla*. Finally, inhibition at 200 and 400 µg/mL of *P. rudgeoides* the ethyl acetate extract.

According to the literature, the use of species of the family Rubiaceae has proved efficient in treatment against protozoans. Nevertheless, studies on the antiprotozoan activity with species of the *Palicourea* are scarce in the literature. Compounds of structures are already well established as antiprotozoan, such as quinine, an alkaloid of *Cinchona sp.* (Rubiaceae), artemisinin, a sesquiterpene lactone of *Artemisia annua* (Asteraceae) used to treat malaria and emetine, an alkaloid from *Cephaelis ipecacuanha* (Rubiaceae) used to treat amebiasis.<sup>[36]</sup> Quinine exhibited significant trypanocidal activity with an IC<sub>50</sub> of 1.2 µM, for *T. brucei* and artemisinin showed inactivity with IC<sub>50</sub> of 189 µM, whereas emetine had great results with IC<sub>50</sub> = 0.039 µM. The structural analyses of these compounds have been used for the development of synthetic or semi-synthetic drugs with improved efficiency and safety.<sup>[36]</sup> Ethanol, dichloromethane and petroleum ether extracts of leaves of *Morinda lucida* Benth. (Rubiaceae) have *in vitro* activity against *Plasmodium falciparum*.<sup>[37]</sup> Crude ethanol extract and its alkaloids of *Psychotria prunifolia* (Rubiaceae) showed that have toxic effects on the epimastigote of *T. cruzi*.<sup>[28]</sup>

#### Toxic effects of the species of the genus *Palicourea*

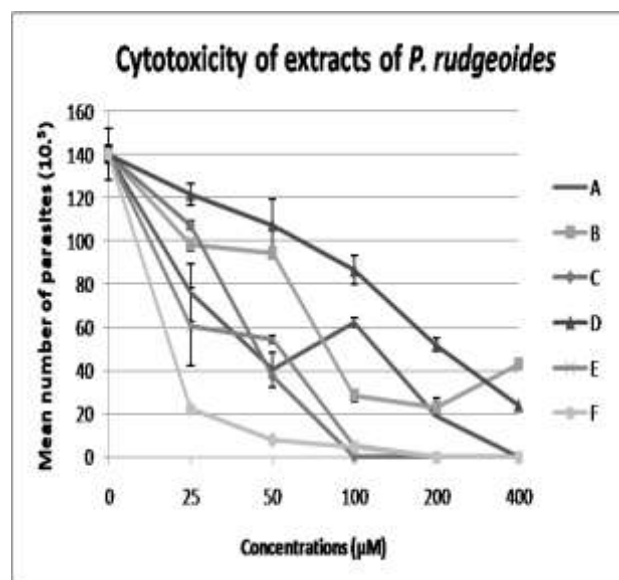
For *P. marcgravii*, AcOEtE reduced 65% of the parasites at 25 µg/mL, ranging from 67% to 78% in concentrations of 50, 100 and 200 µg/mL and reaching 92% at 400 µg/mL. H<sub>2</sub>OEtE at 25 µg/mL reduced 48% of the population, ranging from 67% to 72% in concentrations of 50-200 µg/mL and had linear toxic effects with all parasites being eliminated in 400 µg/mL. The same was observed for ButE in 400 µg/mL, ranging from 61% to 77% in concentrations of 25- 200 µg/mL. DCME began to take effect at 100 µg/mL, inhibiting 27% and continuing to increase with concentration until reaching 92% in 400 µg/mL. HexE ranged from 56% to 94%, for 25-400 µg/mL, while MeOHE exhibited a decrease, ranging from 60% to 91% for the same concentrations (Graph 1).



Graph 1: Effects of incubation for 24h in extracts of *P. marcgravii* on growth of epimastigotes.

A: ethyl acetate extract; B: water extract; C: butyl alcohol extract; D: dichloromethane extract; E: hexane extract; F: methanol extract.

For *P. rudgeoides*, AcOEtE was the most effective of the three species, eliminating 85% of the parasites at 25 µg/mL, 95% at 50 µg/mL, 98% at 100 µg/mL and reaching total elimination at 200 µg/mL. For H<sub>2</sub>OEtE, the reduction of the population ranged 14% to 39% in concentrations of 25, 50 and 100 µg/mL. ButE had significant results, with total death at 100 µg/mL. Butanolic extracts of *M. ciliata* inhibited the growth of *T. brucei* to 68.68% when administered orally in rats for five days.<sup>[38]</sup> DCME decreased the population linearly, ranging from 46% to 25 µg/mL for 100% to 400 µg/mL. For HexE the best result was at 200 µg/mL, with 84% elimination. MeOHE proved effective, reaching total inhibition at the concentration of 200 µg/mL (Graph 2). Methanolic extract of *Gardenia lutea* (Rubiaceae) was also efficient, caused lysis in 50% of the parasites below the trypanomastigote form in 22 µg mL<sup>-1</sup>, after 72 h of incubation.<sup>[39]</sup>

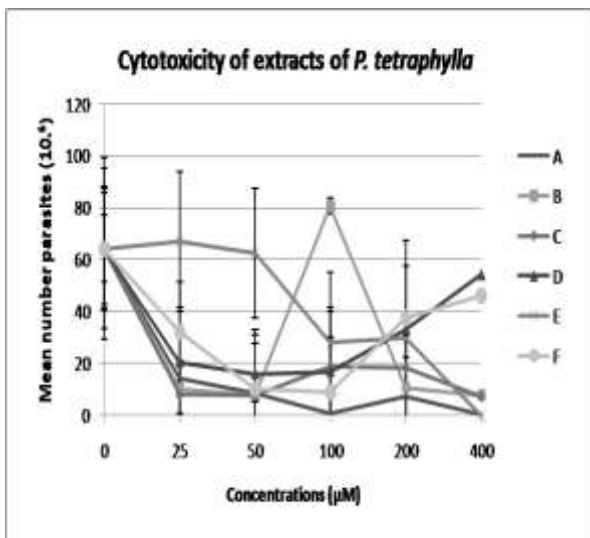


Graph 2: Effects of incubation for 24h in extracts of *P. rudgeoides* on growth of epimastigotes

A: ethyl acetate extract; B: water extract; C: butyl alcohol extract; D: dichloromethane extract; E: hexane extract; F: methanol extract.

For *P. tetraphylla*, AcOEtE reduced the parasites by 50, 85, 87, 42 and 28% in 25, 50, 100, 200 and 400 µg/mL, respectively. H<sub>2</sub>OEtE eliminated most parasites (76%) in 50 µg/mL. ButE varied little in its effects across concentrations, ranging 72 to 89% for 25-400 µg/mL. DCME was the most effective, eliminating 100% in 400 µg/mL, and ranged from 87% to 99% for the concentrations of 25-200 µg/mL. As for HexE, there were no significant differences among concentrations, which ranged between 84% and 88%. MeOHE was

effective at 100 µg/mL, reducing the parasites by 56% and reaching total elimination at 400 µg/mL (Graph 3).



Graph 3: Effects of incubation for 24h in extracts of *P. tetraphylla* on growth of epimastigotes

A: ethyl acetate extract; B: water extract; C: butyl alcohol extract; D: dichloromethane extract; E: hexane extract; F: methanol extract.

**The kinetics of the process of elimination of epimastigotes**

To understand the destruction kinetics of the parasite, we chose to use H2O at a concentration of 100 µg/mL, because it was intermediate and tested it at three incubation times: 8h, 16h and 24h (Fig. 1; Table 2).

Table 2: Kinetics of the elimination of epimastigotes using the aqueous extracts of species of *Palicourea*.

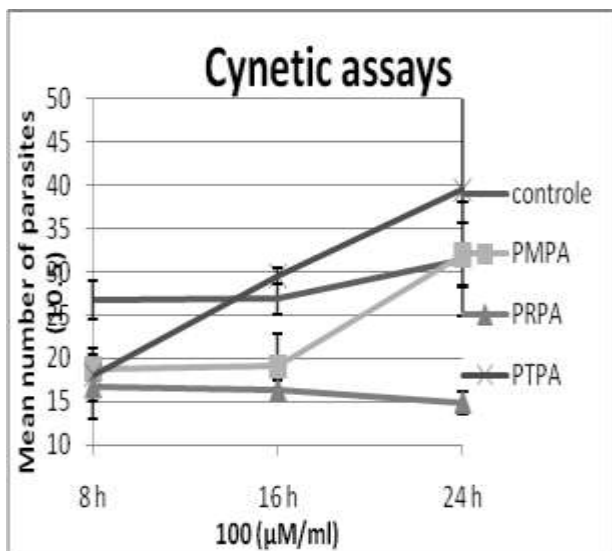
Time	Percentage of epimastigotes			
	Control	<i>P. marcgravii</i>	<i>P. rudgeoides</i>	<i>P. tetraphylla</i>
8 h	26.75	18.66	16.75	18.08
16 h	26.91	19.0	16.33	29.5
24 h	31.41	31.91	14.91	39.5

For *P. marcgravii*, the reduction at 8h was the same as at 16h, however, after this time the parasites began to multiply and returned to equal the control. As for *P. rudgeoides*, the effect was the same at all three treatment times, with the number of parasites remaining constant. For *P. tetraphylla*, a reduction in the number of epimastigotes was only observed at 8h, which was followed by proliferation 16h and continuing to 24h (Graph 4).

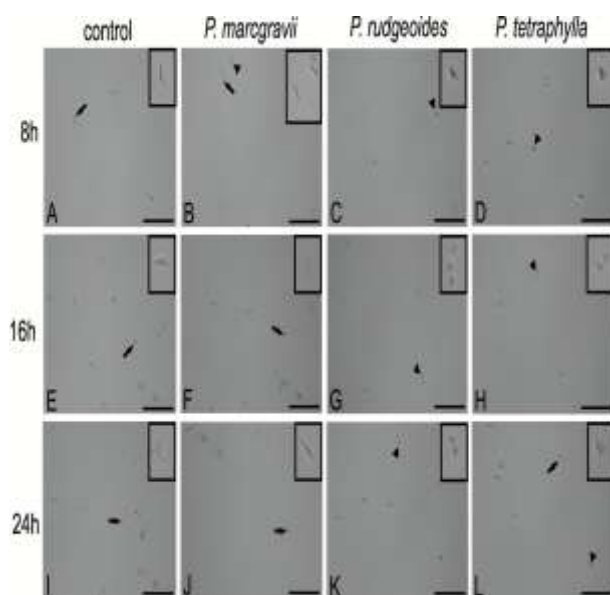
PMAP: *Palicourea marcgravii* aqueous part, PRAP: *Palicourea rudgeoides* aqueous part, PTPA: *Palicourea tetraphylla* aqueous part.

**Structural and ultrastructural analysis of epimastigotes**

Our results, using leaf extracts, clearly show their potential to inhibit the growth and to alter the morphology, of the epimastigote of *T. cruzi*. In the present work, complete elimination, when it occurred, started at 100 µg/mL of extract for three species, with the exception being hexane, within 24 hours. In general, the butanol extract of the three species eliminated the most parasites after 24 hours of incubation and *P. rudgeoides* had the most significant result with complete elimination. Light microscopy revealed that untreated epimastigotes had a typical elongate shape, a flagellum and no visible alterations (Fig. 1 A, E and I). However, in the presence of aqueous extracts incubated for different times at a concentration of a 100 µg/mL, the epimastigotes exhibit very condensed cytoplasm.



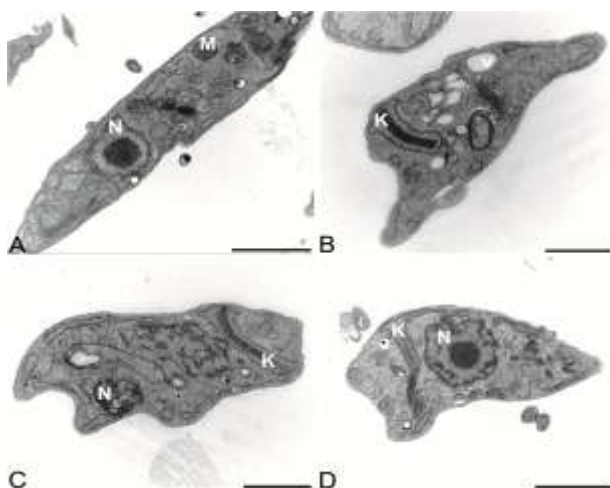
Graph 4: Kinetics of the elimination of epimastigotes using the aqueous extracts of species of *Palicourea*.



**Figure 1:**

Light microscopy of epimastigotes treated with different aqueous extracts from species of *Palicourea*: A, E and I = control. B, F and J = *Palicourea marcgravii*. C, G and K = *Palicourea rudgeoides*. D, H and L = *Palicourea tetraphylla*. Bars = 50  $\mu\text{m}$ .

Transmission electron microscopy found untreated epimastigotes to appear normal with an intact plasma membrane and cytoplasm and a nucleus showing no alterations (Fig. 2A). However, the treatment with aqueous extracts at 100  $\mu\text{g}/\text{mL}$  caused the loss of cytoplasmic contents with the retraction of the parasite body (Fig. 2B, C and D) and decomposition of the nuclear chromatin (Fig. 2C). Multiple cytoplasmic vacuolization (Fig. 2B) and alterations to the parasites plasma membrane were observed as was wrinkling of the plasmatic membrane (Fig. 2B, C and D).



**Figure 2:**

Transmission electron microscopy of epimastigotes treated for 24h with 100  $\mu\text{g}/\text{mL}$  aqueous extracts from different species of *Palicourea*: A. control, B. *Palicourea marcgravii*, C. *Palicourea rudgeoides* and D. *Palicourea tetraphylla*. Bars: A and D = 2  $\mu\text{m}$ , B and C = 1  $\mu\text{m}$ .

Assays of antifungal activity were also carried out with extracts of *P. marcgravii*, *P. rudgeoides* and *P. tetraphylla* against the yeasts *C. albicans* and *C. tropicalis* and the filamentous fungus *F. solani*. All of the extracts were tested at a concentration of 250  $\mu\text{g}/\text{mL}$ , but none of them exhibited any inhibitory effects on the fungi tested (data not shown). Studied the antifungal activity of the extracts of various species, including two species of the genus *Palicourea*. They found that aqueous and methanol extracts of *P. guianensis* and *P. corymbifera* were active against three strains of *Candida sp.*<sup>[40]</sup> The authors correlated the activity of these extracts with the possible presence of triterpene indole alkaloids, since these substances are commonly found in species of the family Rubiaceae, whose antimicrobial activity is well known.<sup>[26, 41]</sup> Also observed strong activity against *Candida sp.* by ethyl acetate extract of *Alibertia edulis* (Rubiaceae).<sup>[42]</sup> Different extracts from *Vangueria madagascariensis*<sup>[29]</sup> and from *Borreria laevicaulis*<sup>[21]</sup>, both of the family Rubiaceae, showed effectiveness against *C. albicans*.

## CONCLUSION

In conclusion, among the three species of *Palicourea* studied, *P. rudgeoides* was most effective at reducing epimastigotes, especially with extracts of butanol, methanol and ethyl acetate. Additional studies are needed, such as spectrometric analysis of extracts, in order to elucidate the metabolite(s) responsible for the results of this study.

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## REFERENCES

1. Dondorp, A.M., Nosten, F., Yi, P., Das, D., Phyto, A.P., Tarning, J., Lwin, K.M., Ariey, F., Hanpithakpong, W., Lee, S.J., Ringwald, P., Silamut, K., Herdman, T., An, S.S., Yeung, S., Socheat, D., White, N.J. Artemisinin Resistance in *Plasmodium falciparum* Malaria. *N. Engl. J. Med.*, 2009; 361: 455–467.
2. Albernaz, L.C., de Paula, J.E., Romero, G.A.S., Silva, M. do R.R., Grellier, P., Mambu, L., Espindola, L.S. Investigation of plant extracts in traditional medicine of the Brazilian Cerrado against protozoans and yeasts. *J. Ethnopharmacol.*, 2010; 131: 116–121.
3. Torrent, M., Pulido, D., Rivas, L., Andreu, D. Antimicrobial Peptide Action on Parasites. *Curr. Drug Targets*, 2012; 13: 1138–1147.
4. Pappas, P.G. The role of azoles in the treatment of invasive mycoses: review of the Infectious Diseases

- Society of America guidelines. *Curr. Opin. Infect. Dis*, 2011; 24: S1–S13.
5. Rassi, A., Marcondes de Rezende, J. American Trypanosomiasis (Chagas Disease). *Infect. Dis. Clin. North Am*, 2012; 26: 275–291.
  6. Noireau, F., Carbajal-De-La-Fuente, A.L., Lopes, C.M., Diotaiuti, L. Some considerations about the ecology of Triatominae. *An. Acad. Bras. Cienc*, 2005; 77: 431–436.
  7. Teixeira, A.R.L., Nascimento, R.J., Sturm, N.R. Evolution and pathology in Chagas disease - A Review. *Mem. Inst. Oswaldo Cruz*, 2006; 101: 463–491.
  8. Deane, M.P., Lenzi, H.L., Jansen, A. Trypanosoma cruzi: vertebrate and invertebrate cycles in the same mammal host, the opossum *Didelphis marsupialis*. *Mem. Inst. Oswaldo Cruz*, 1984; 79(4): 513-515.
  9. Telleria, J., Lafay, B., Virreira, M., Barnabé, C., Tibayrenc, M., Svoboda, M. Trypanosoma cruzi: sequence analysis of the variable region of kinetoplast minicircles. *Exp. Parasitol*, 2006; 114: 279–88.
  10. Repentigny, L. De, Lewandowski, D., Jolicoeur, P. Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection. *Immunopathogenesis of Oropharyngeal Candidiasis in Human Immunodeficiency Virus Infection. Society*, 2004; 17: 729–759.
  11. Erköse, G., Erturan, Z. Oral Candida colonization of human immunodeficiency virus infected subjects in Turkey and its relation with viral load and CD4+ T-lymphocyte count. *Mycoses*, 2007; 50: 485–490.
  12. Höfling, J.F., Anibal, P.C., Obando-Pereda, G. a, Peixoto, I. a T., Furletti, V.F., Foglio, M. a, Gonçalves, R.B. Antimicrobial potential of some plant extracts against Candida species. *Braz. J. Biol*, 2010; 70: 1065–1068.
  13. Sanguinetti, M., Posteraro, B., Lass-Flörl, C. Antifungal drug resistance among Candida species: Mechanisms and clinical impact. *Mycoses*, 2015; 58: 2–13.
  14. Denning, D.W. Echinocandins and pneumocandins- a new antifungal class with a novel mode of action. *J. Antimicrob. Chemother*, 1997; 40: 611–4.
  15. Sandven, P. Epidemiology of candidemia. *Rev. Iberoam. Micol*, 2000; 17: 73–81.
  16. Portillo, A., Vila, R., Freixa, B., Adzet, T., Cañigual, S. Antifungal activity of Paraguayan plants used in traditional medicine. *J. Ethnopharmacol*, 2001; 76: 93–98.
  17. Duraipandiyar, V., Ignacimuthu, S. Antifungal activity of traditional medicinal plants from Tamil Nadu, India. *Asian Pac. J. Trop. Biomed*, 2011; 1: S204–S215.
  18. Spampinato, C., Leonardi, D. Candida Infections , Causes, Targets and Resistance Mechanisms : Traditional and Alternative Antifungal Agents. *Biomed Res. Int*, 2013; 13 pages.
  19. Duarte, M.C.T., Leme, E.E., Delarmelina, C., Soares, A.A., Figueira, G.M., Sartoratto, A. Activity of essential oils from Brazilian medicinal plants on *Escherichia coli*. *J. Ethnopharmacol*, 2007; 111: 197–201.
  20. Sardi, J.C.O., Duque, C., Mariano, F.S., Peixoto, I.T. a, Höfling, J.F., Gonçalves, R.B. Candida spp. in periodontal disease: a brief review. *J. Oral Sci*, 2010; 52: 177–185.
  21. Wong, K.Y., Vikram, P., Chiruvella, K.K., Mohammed, A. Phytochemical screening and antimicrobial potentials of *Borreria* sps (Rubiaceae). *J. King Saud Univ. - Sci*, 2015; 27: 302–311.
  22. Espindola, L.S., Rossy E Vasconcelos, J., De Mesquita, M.L., Marqui??, P., De Paula, J.E., Mambu, L., Santana, J.M. Trypanocidal activity of a new diterpene from *Casearia sylvestris* var. *lingua*. *Planta Med*, 2004; 70: 1093–1095.
  23. Rodrigues, A.M.S., De Paula, J.E., Degallier, N., Molez, J.F., Espíndola, L.S. Larvicidal activity of some cerrado plants extracts against *Aedes aegypti*. *J. Am. Mosq. Control Assoc*, 2006; 22: 314–317.
  24. Coelho, V.P.D.M., Agra, M.D.F., Barbosa, M.R.D.V. Estudo farmacobotânico das folhas de *Tocoyena formosa* (Cham. & Schldtl.) K.Schum. (Rubiaceae). *Rev. Bras. Farmacogn*, 2006; 16: 170–177.
  25. de Mesquita, M.L., Grellier, P., Mambu, L., de Paula, J.E., Espindola, L.S. In vitro antiplasmodial activity of Brazilian Cerrado plants used as traditional remedies. *J. Ethnopharmacol*, 2007; 110: 165–170.
  26. Figueiredo, A.D.L., Bustamante, K.G.L., Soares, M.L., Pimenta, F.C., Bara, M.T.F., Fiuza, T.S., Tresvenzol, L.M.F., De Paula, J.R. Avaliação da atividade antimicrobiana das partes aéreas (folhas e caules) e raízes de *Richardia brasiliensis* Gomez (Rubiaceae). *Rev. Ciencias Farm. Basica e Apl*, 2009; 30: 193–196.
  27. Touré, A., Bahi, C., Bagré, I., N'Guessan, J., Djama, A., Coulibaly, A. *In vitro* antifungal activity of the soap formulation of the hexane leaf extract of morinda morindoides (morinda; rubiaceae). *Trop. J. Pharm. Res*, 2010; (9): 237–241.
  28. Kato, L., De Oliveira, C.M.A., Faria, E.O., Ribeiro, L.C., Carvalho, B.G., Sa Silva, C.C., Schuquel, I.T.A., Santin, S.M.O., Nakamura, C. V., Britta, E.A., Miranda, N., Iglesias, A.H., Delprete, P.G. Antiprotozoal alkaloids from *psychotria prunifolia* (kunth) steyererm. *J. Braz. Chem. Soc.*, 2012; (23): 355–360.
  29. Ramalingum, Nelvana & Mahomoodally, M.F. *Vangueria madagascariensis* J.F. Gmelin (Rubiaceae) - an under-utilised African traditional medicinal food plant with potential applications. *J. Intercult. Ethnopharmacol*, 2014; (3): 45.
  30. Hartwell, J. L. *Lloydia*, 1972; (34): 1-150.
  31. El-Seedi, Hesham R. Coumarins, benzoic acids and terpenoids from *Palicourea demissa*. *Rev. Latinoam. Quim*. 1999; 27(1): 13-16.
  32. Valverde, J., Tamayo, G., Hesse, M.  $\beta$ -Carboline monoterpene glucosides from *Palicourea adusta*.

- Phytochem., 1999; 52: 1485–1489.
33. Carvalho, F., Nascimento, M., Braga, J.M.D.A., Rodrigues, P.J.F.P. Estrutura da comunidade arbórea da Floresta Atlântica de baixada periodicamente inundada na Reserva Biológica de Poço das Antas, Rio de Janeiro, Brasil. *Rodriguésia*, 2006; 57: 503–518.
  34. Tyler, K., Olson, C., Engman, D. The Life Cycle Of *Trypanosoma Cruzi*. *Am. Trypanos*, 2002; (7): 1–11.
  35. Broekaert, W., Terras, F. An automated quantitative assay for fungal growth inhibition. *FEMS Microbiol*, 1990.
  36. Hoet, S., Opperdoes, F., Brun, R., Quetin-Leclercq, J. Natural products active against African trypanosomes: a step towards new drugs. *Nat. Prod. Rep*, 2004; (21): 353–364.
  37. Cimanga, R.K., Tona, G.L., Mesia, G.K., Kambu, O.K., Bakana, D.P., Kalenda, P.D.T., Penge, A.O., Muyembe, J.-J.T., Totté, J., Pieters, L., Vlietinck, A.J. Bioassay-Guided Isolation of Antimalarial Triterpenoid Acids from the Leaves of *Morinda lucida*. *Pharm. Biol.*, 2006; 44: 677–681.
  38. Ogbunugafor, H.A.O., Veronica I., Okpuzor, J.T., Adedayo and Stephen, E. *Mitragyna ciliata* and its trypanocidal activity. *J. Biotechnol*, 2007; 6: 2310–2313.
  39. Ali, H., König, G.M., Khalid, S.A., Wright, Kaminsky, A.D.R. Evaluation of selected Sudanese medicinal plants for their in vitro activity against hemoflagellates, selected bacteria, HIV-1-RT and tyrosine kinase inhibitory and for cytotoxicity. *J. Ethnopharmacol.*, 2002; (83): 219–228.
  40. Rodrigues, K., Ramos, D.F., Carrion, L.L., Cursino, L.M.C., Jefreys, M.F., Pedroza L.S., Osório, M.I.C., Oliveira, J.L., Andrade, J.I.A., Fernandes, C.C., Nunez, C.V., Silva, P.E.A. Antifungal activity of Brazilian amazon plants extracts against some species of *Candida SPP*, *Int. J. Phytopharmacol.*, 2014; 5(6): 445–453.
  41. Nunez, C.V., Roumy, V., Mesquita, D.W.O., Mesquita, A.S.S., Sahpaz, S., Bailleul, F., Hennebelle, T. Indole alkaloids from *Duroia macrophylla* (Rubiaceae). *Planta Med.* (internet), 2012; 78: 287.
  42. Valli, M., Young, M.C.M., Bolzani, V.S. The Invisible Beauty of the Biodiversity: The Rubiaceae Taxon. *Rev. Virtual Química*, 2016; 8: 296–310.