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Effect of Essential Oil of *Ocimum gratissimum* on the Trypanosomatid *Herpetomonas samuelpessoai*

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Summary. In this study, we reported the effect of the essential oil of *Ocimum gratissimum* on *Herpetomonas samuelpessoai*, a non-pathogenic trypanosomatid. Parasites were grown at 28 or 37°C, in a chemically defined or a complex medium, containing essential oil obtained from *Ocimum gratissimum.* At concentrations from 20 to 250 µg/ml, the essential oil, progressively inhibited the protozoan growth. The IC_{50}, in defined and complex media, at 28°C were 100 and 91 µg/ml, respectively. Cells cultivated in chemically defined medium were more sensitive to essential oil at concentration of 50, 62.5 and 100 µg/ml in relation to those cultured in complex medium at 37°C. In addition, ultrastructural and enzymatic alterations of the trypanosomatid were also evaluated. *H. samuelpessoai* exposed to 100 µg/ml of essential oil, in chemically defined medium at 28°C for 72 h, presented considerable ultrastructural alteration, mainly at mitochondrial level, as showed by transmission electron microscopy. Furthermore, cells cultivated in the presence of 100 µg/ml of essential oil showed a decrease of activity of the succinate cytochrome *c* reductase enzyme, a typical mitochondrion marker, as compared to untreated cells.

Key words: *Herpetomonas samuelpessoai*, medicinal plants, *Ocimum gratissimum*, ultrastructure.

INTRODUCTION

During the last century the practice of herbalism has become mainstream throughout the world. In spite of the great advances observed in modern medicine, plants still make an important contribution to health care. This is due in part to the recognition of the value of traditional medical systems, particularly of Asian origin, and the identification of medicinal plants from indigenous pharmacopoeias, which have significant healing power. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto 2000, Lewis 2001). In Brazil, around 80,000 species of higher plants were described, which offer enormous prospects for discovering new compounds with therapeutic properties.

Among all families of the plant kingdom, members of the Lamiaceae have been used for centuries in folk medicine. *Ocimum gratissimum* L. (Lamiaceae), commonly known as "alfavaca", is naturally used in the treatment of different diseases, e.g., upper respiratory tract infections, diarrhea, headache, fever, ophthalmic

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and skin diseases, and pneumonia (Corrêa 1932, Onajobi 1986, Ilori *et al*. 1996). The *Ocimum* oil is also active against several species of bacteria (*Escherichia coli, Shigella, Salmonella* and *Proteus*) and fungi (*Trichophyton rubrum* and *T. mentagrophytes*) (El-Said *et al*. 1969, Begum *et al*. 1993, Nwosu and Okafor 1995, Nakamura *et al*. 1999, Orafidiya *et al*. 2000). Various sister species of *O. gratissimum*, e.g., *O. viride* Linn, *O. suave* Linn, *O. basilicum* Linn and *O. canum* Sims, have been reported for their numerous medicinal uses (Mshana *et al*. 2000).

Diseases caused by protozoa are responsible for mortality in tropical and subtropical countries. At the moment, the number of drugs available for the treatment of human and animal trypanosomiasis, amoebiasis, leishmaniasis, and malaria are limited. Considering the side effects and the resistance that pathogenic protozoan builds against these drugs, more attention should be given to the extracts and biologically active compounds which are isolated from plant species commonly used in herbal medicine (Essawi and Srour 2000).

Protozoa of the Trypanosomatidae family comprise a large number of species, some of which are agents of important illnesses, such as leishmaniasis, Chagas' disease and African trypanosomiasis. In addition, other non-pathogenic trypanosomatids have emerged as important models for the study of basic biological processes, including RNA editing and *trans*-splicing, organization of extranuclear DNA, antigenic variation, etc (De Souza and Motta 1999). *Herpetomonas samuelpessoai* is a non-pathogenic trypanosomatid that shares important antigens with *Trypanosoma cruzi*, the agent of Chagas' disease (Souza *et al*. 1974). *H. samuelpessoai* can be easily cultivated in a defined medium at 28°C and 37°C, and can induce a humoral and cell-mediated immune response (Roitman *et al.* 1972). Several Brazilian researcher groups have used this parasite as a model to study the biology of trypanosomatids. So, it may be suitable as a model for screening new trypanocidal drugs.

A previous screening of crude extracts of plants used in traditional medicine showed that the essential oil of *O. gratissimum* inhibited the growth of *H. samuelpessoai* (Holetz *et al*. 2002), however a detailed study of this natural product has not been carried out. In the present study we reported the effect of the essential oil from *O. gratissimum* on growth, viability, ultrastructural and biochemical alterations of *H. samuelpessoai* cultivated in defined or complex medium at 28°C and 37°C.

MATERIALS AND METHODS

Plant material. *Ocimum gratissimum* was collected in Maringá, Paraná, Brazil, identified, and a voucher no. HUM 9.613 is deposited at the Maringá State University Herbarium. Fresh leaves from the plant were cut into pieces and subjected to steam distillation. The distillate was then extracted with petroleum ether, which was removed carefully, and the essential oil was obtained. The oil was then stored at -20°C until needed.

Preparation of stock solutions. The stock solution (10 mg/ml) of essential oil from *O. gratissimum* was made in a chemically defined medium (Roitman *et al*. 1972). For this purpose, initially the essential oil was diluted in 2% Tween 80. From the stock solution, dilutions were made to obtain 250, 150, 125, 100, 62.5, 50, and 20 µg/ml. The stock solution of Eugenol (Biodinâmica Química e Farmacêutica Ltda) was prepared in a similar way, just considering its density (1.0664 g/ml) (Budavari *et al*. 1989). Benznidazole (N-benzyl-2-nitro-1-imidazolacetamide - Roche Pharmaceuticals, Rio de Janeiro, Brazil) was used as reference drug. Both stock solutions of Eugenol and Benznidazole were prepared in the defined medium.

Microorganism. *Herpetomonas samuelpessoai* (ATCC 30252) was maintained by weekly transfers in a chemically defined medium (Roitman *et al*. 1972) and distributed in 5 ml volumes in screw-capped tubes. Cells were grown at 28°C for 48 h and cultures were kept at 4°C.

Antiprotozoan activity. For experiment *H. samuelpessoai* was incubated in defined or complex medium (Roitman *et al*. 1972) containing different concentrations of essential oil, which were added only once to the cultures. Cells were grown in 13 x 100 mm tubes containing 1 ml of defined medium and the start inoculum consisted of the protozoan in logarithmic growth phase (1 x 10^6 cells/ml). After 24, 48, 72 and 96 h at 28°C or 37°C, the cell growth was estimated by counting in a haemocytometer (Improved Double Neubauer). As negative controls, defined medium alone and defined medium plus 2% Tween 80, were used. All experiments were performed in triplicate and the results expressed as log number cells/ml and as percentage of the growth inhibition at 72 h.

Viability assay. In order to evaluate the viability of the protozoa treated with essential oil, each solution was added to eppendorfs containing 1 x 10^7 cells in logarithmic growth phase. Immediately after the addition of the essential oil (time zero) and after 1, 2, 3, 6 and 24 h at 28°C, equal volumes (25 µl) of protozoan suspension and 0.4% erythrosine B were mixed and the cell viability was quantified by light microscopy. The preparations were made in duplicate and the percentage of viability was determined by counting at least 300 cells (Hodgkinson *et al*. 1980).

Light microscopy. To evaluate the morphologic changes induced by the essential oil of *O. gratis*simum in *H. samuelpessoai*, cells treated with 100 µg/ml of the oil in defined medium during 72 h, were collected by centrifugation, smear-dried onto slides, fixed in methanol and stained for light microscopy using the "Panótico Rápido LB" stain (Laborclin Prod. Lab. Ltda., Pinhais, Paraná State, Brazil). Images were obtained using an optic microscope Olympus CBA coupled to an Image Pro-Plus program, version 4.0 (Media Cybertennics).

Ultrastructural analysis. *Herpetomonas samuelpessoai* treated with 100, 125 or 150 µg/ml of the essential oil, 2% Tween 80 or

medium alone, at 28°C were collected by centrifugation in intervals of 24 h and for a period up to 96 h, washed in PBS and fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, containing 1.0 mM $CaCl_2$ at 4°C. Cells were then washed three times with 0.1 M sodium cacodylate buffer and postfixed for 1 h at room temperature in 1% osmium tetroxide plus 0.8% potassium ferricyanide and 5 mM $CaCl_2$. After rinsing, cells were dehydrated in acetone, incubated in an acetone-epon mixture (2:1, 1:1, 1:2) and embedded in Epon resin. Ultrathin sections obtained in a Reichert Ultracut E ultramicrotome were stained with uranyl acetate and lead citrate and observed in a Zeiss CEM - 900 electron microscope.

Biochemical analysis. Cells treated with 100 µg/ml essential oil and untreated cells were incubated at 28°C for 72 h, and harvested by centrifugation at 3000 *g* for 10 min in a refrigerated centrifuge Sorvall Super 21 (SL50T Rotor). Then, they were washed three times in PBS (pH 7.2), resuspended in a hypotonic solution (Tris-HCl 10 mM) containing a cocktail of protease inhibitors (antipain, aprotinin, leupeptin and pepstatin - 10 µg/ml each) and disrupted by sonication with 20 cycles of 2 s with 1 s rest between cycles on ice using an Ultrasonic Processor (CV 33 Model). The cell disruption was monitored with light microscope. Protein concentration was determined by Bio-Rad protein assay, using bovine serum albumin as a standard and following the manufacturer's instructions.

Succinate cytochrome *c* reductase activity was measured according to Masters *et al*. (1967). The reaction mixture contained 0.2 M phosphate buffer pH 7.4; 0.003 M EDTA pH 7.4; 0.6 M succinic acid adjusted to pH 7.4 with NaOH; 0.001 M cytochrome *c*. The specific activity was measured at 30°C, by following the reduction of cytochrome *c* at 550 nm.

RESULTS

A dose-dependent antiprotozoan effect of the essential oil of *O. gratissimum* is shown in Fig. 1. It was possible to observe an inhibitory effect of the essential oil on the cell growth when cells were treated with a high concentration of the drug in defined and complex media. The IC_{50} (50% inhibitory concentration) in both media at 28°C were 100 and 91 µg/ml, respectively. Eugenol inhibited *H. samuelpessoai* growth at the same concentration of essential oil and Benznidazole did not induce a representative inhibition in defined medium at 28°C. Tween 80, the dilution agent, and Petrolatum oil used as indifferent oil, showed no effect on the protozoa growth (data not shown). The effects of the essential oil on growth of *H. samuelpessoai* cultivated in defined or complex medium for 72 h, at 28°C or 37°C are shown in Table 1. It is possible to observe in this Table that the composition of the culture medium did not interfere with the growth inhibition of the flagellate when incubated at 28°C. On the other hand, *H. samuelpessoai* cultivated at 37°C in defined medium containing 50, 62.5 and 100 µg/ml of essential oil was inhibited more efficiently

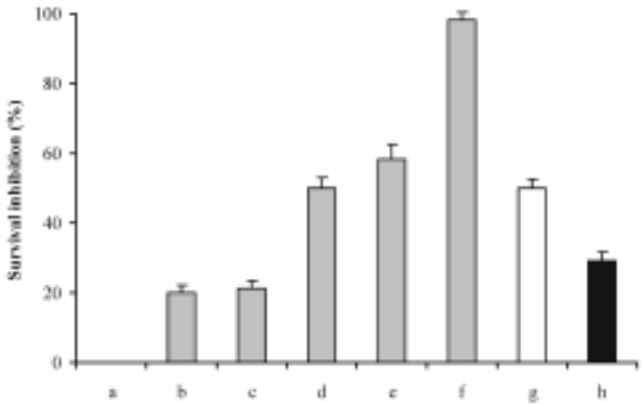


Fig. 1. Effect of essential oil from *O. gratissimum* (a - f); eugenol (g) and benznidazole (h) on growth inhibition of *Herpetomonas samuelpessoai* in defined medium at 28°C by 72 h. **a** - 20 µg/ml; **b** - 50 µg/ml; **c** - 62.5 µg/ml, **d** - 100 µg/ml; **e** - 125 µg/ml; **f** - 150 µg/ml; **g** - 100 µg/ml; h - 100 µg/ml. Results from three experiments in duplicate are shown as percentages of growth inhibition ± standard deviations.

Table 1. Effect of essential oil from *O. gratissimum* on growth of *Herpetomonas samuelpessoai* cultivated in defined and complex media at 28°C and 37°C, during 72 h.

	Protozoan growth inhibition (%)			
Concentration of essential oil (µg/ml)	Chemically defined medium		Complex medium	
	28°C	37°C	28°C	37°C
250	99.3	97.3	99.2	98.0
150	98.1	96.7	98.7	98.1
125	58.2	96.9	57.5	98.1
100	50.0	96.3	56.0	74.3
62.5	21.5	72.1	30.8	49.0
50	20.0	74.0	25.0	30.0
20	0.0	21.0	0.0	20.0

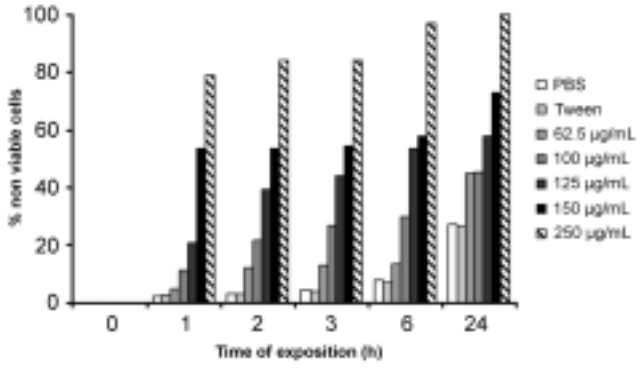
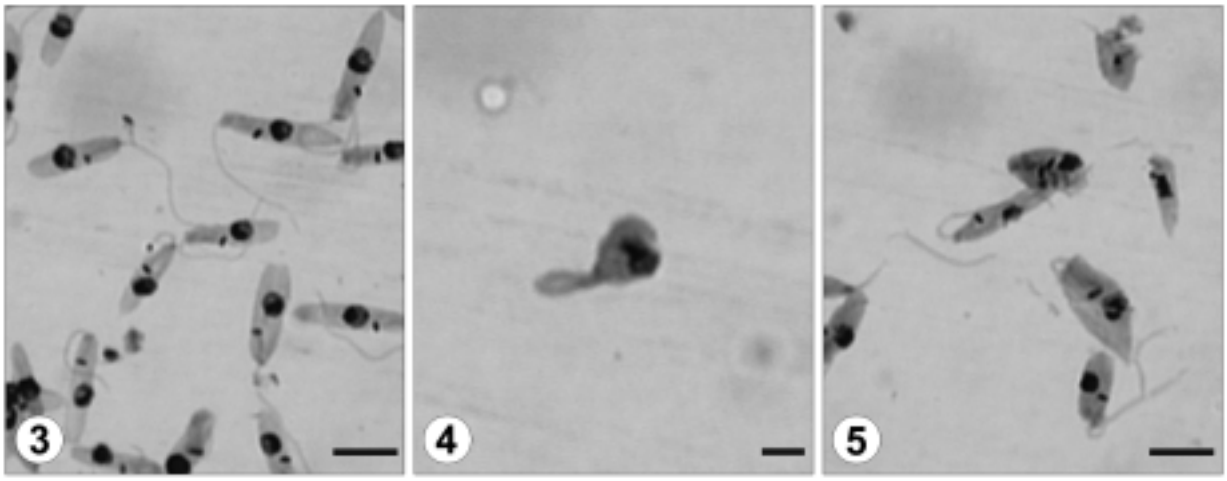


Fig. 2. Effect of essential oil on viability of *Herpetomonas samuelpessoai.*



Figs 3-5. Light microscopy of *Herpetomonas samuelpessoai* cultivated in defined medium in the presence of essential oil during 72 h at 28°C; **3** - control; **4, 5** - 100 µg/ml. Scale bar 5 µm.

than when cultivated in complex medium. The inhibitory activity of the essential oil was increased at 37°C in both culture media used.

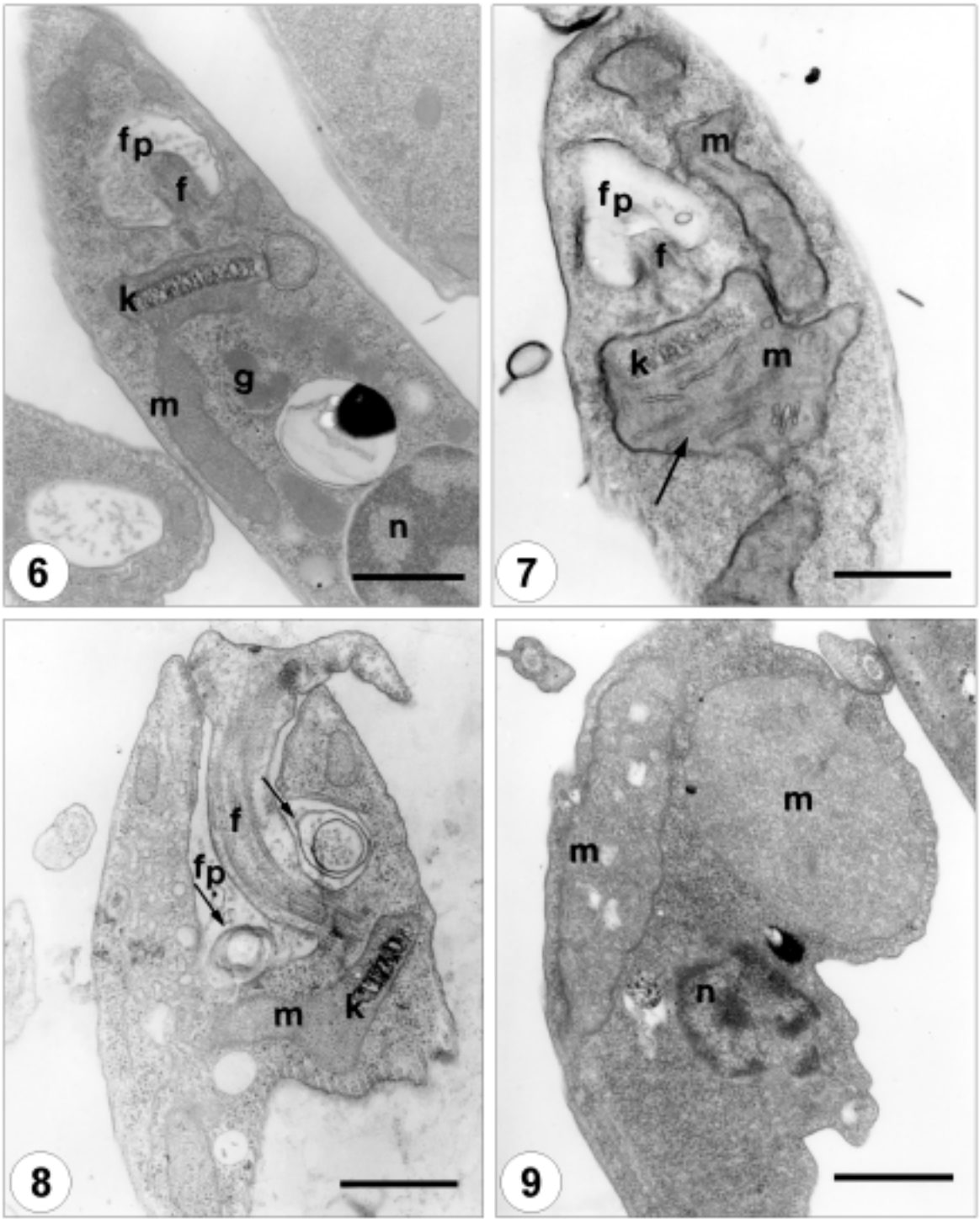
Viability of treated and control cells were assessed by the dye exclusion test. The percentage of non-viable cells, obtained by exposition of the protozoan to different concentrations of essential oil, is shown in Fig. 2. Treatment with 150 and 250 µg/ml of essential oil, in the first hour, reduced the parasite viability in 53.5% and 78.5% respectively. Low concentration of the drug did not interfere with the viability of protozoa. After 6 h of treatment, at concentration of 125, 150, and 250 µg/ml, lethality were 53.5, 58, and 97%, respectively. Morphologic alterations, such as cellular volume increase and irregular shape of the cells grown in chemically defined medium for 72 h containing 100 µg/ml of essential oil, were also observed (Figs 3-5).

In order to determine ultrastructural changes in the *H. samuelpessoai* treated with 100 µg/ml essential oil, electron microscopy analysis was carried out. It was possible to observe a considerable swelling of the mitochondrion, showing the inner mitochondrial membrane altered with a significant increase of the number of cristae and in some cells the mitochondrial matrix became less electron dense (Fig. 7). Concentric membranes within the flagellar pocket were also seen (Fig. 8). Similar alterations were observed when cells were cultured using eugenol (Fig. 9), which represent 67% of the composition of the essential oil, as demonstrated by Nakamura *et al.* (1999). These ultrastructural changes were not observed in cells cultured in the presence of Tween 80 (not shown) and in untreated cells, which showed a mitochondrion with well-distinguished membranes and a dense matrix (Fig. 6). In order to verify the activity of succinate cytochrome *c* reductase, an enzyme related to metabolism of this organelle, enzymatic assays were performed. The enzymatic activity of treated cells with the essential oil (2.0 ± 0.36 nmol/min/mg of protein) decreased about 5- fold in relation to untreated cells (9.7 ± 1.82 nmol/min/mg of protein).

DISCUSSION

Several species and varieties of plants of the genus *Ocimum* have been reported to yield oil of diverse nature, commonly known as basilic oils. Craveiro *et al.* (1981) reported some chemical components usually found in the essences of these plants, such as: eugenol, linalool, methyl cinnamate, camphor and thymol. It has been demonstrated that the eugenol isolated from *O. gratissimum* presents antibacterial (Nakamura *et al.* 1999) and antihelmintic activity (Pessoa *et al.* 2002). In the present study, when the IC_{50} of essential oil and eugenol in *H. samuelpessoai* were compared, a similar antiprotozoan activity was observed. Since eugenol is the principal constituent of the essential oil, this result suggests that it could be responsible for this effect.

Herpetomonas samuelpessoai can be easily cultivated in defined medium (free of macromolecules) showing its ability to synthesize macromolecules, for instance lipids, from compounds with low molecular weight. Silva and Oliveira (1985) reported that



Figs 6-9. Transmission electron microscopy of *Herpetomonas samuelpessoai* cultivated in defined medium with essential oil at 28°C for 72 h; **6** - control; **7, 8** - 100 µg/ml; **9** - 100 µg/ml of eugenol. Arrows showed swelling mitochondria (Fig. 7) and concentric membranes within flagellar pocket (Fig. 8). f - flagellum; fp - flagellar pocket; g - glycosome; k - kinetoplast; m - mitochondria; n - nucleus. Scale bar 1 µm.

H. samuelpessoai presents lower levels of lipids when cultivated in defined medium at 28°C and this difference is more evident in total sterol content. Rodrigues *et al.* (2002) suggested that ergosterol and its analogs are essential to the maintenance and organization of the mitochondrial membrane in trypanosomatids. Furthermore, it is well known that the cell membrane fluidity is highly dependent on the lipid composition. On the other hand, temperature increases causes disorganization of the hydrophobic portion of the fat acids chains present in the membrane increasing the fluidity of this structure (Granner 1990). It was reported that the lipid composition of the cell membrane changes drastically depending on the temperature and culture conditions in *H. samuelpessoai*. Fagundes *et al.* (1980) verified a substantial increase of the total lipids when these protozoa were incubated at 37°C suggesting a balanced reposition of membrane components necessary for the maintenance of the functionality of this structure. In addition, when this flagellate was cultivated in defined medium a decrease in stearic acid content was observed (Pinto *et al.* 1982). Thus, in the present study the medium constitution and incubation temperature may be the adjuvant factors, responsible for the action of the essential oil as antiprotozan agent.

Benznidazole is a drug used for the treatment of the Chagas' disease, which is caused by *Trypanosoma cruzi.* Considering that *H. samuelpessoai* belong to the same family, we used this drug to compare the effect of the essential oil. Yong *et al.* (2000) reported values of IC_{50} of benznidazole to the *T. cruzi* strains Dm_{28C} resistant and sensitive, of 6 and 5 µM, respectively. In the present study, culture of *H. samuelpessoai* in chemically defined medium at 28°C, were inhibited in 31% with concentration of 384 µM of benznidazole. As IC_{50} could not be determined even at 3840 µM, it is possible that *H. samuelpessoai* is naturally resistant to this compound.

Viability assay showed that cells treated with high concentrations of the essential oil caused more than 50% of cell death in the first hour of incubation. Similar results were obtained in lower concentrations of the oil after 24 h of treatment. These results were confirmed through the growth curves of the protozoa where high concentrations of the essential oil inhibited more than 95% of the growth.

Morphological alterations of *H. samuelpessoai* were observed after treatment with the essential oil, as seen by light microscopy. At ultrastructural level it was possible to observe significant alteration at the mitochondrion, such as a remarkable swelling and some modifications in the inner membrane like disorganization and increase of the number of cristae. We do not have an explanation to the exact mechanism involved in these alterations. However similar results have been reported in other trypanosomatids; for instance, Delorenzi *et al.* (2001), reported mitochondrial alterations when promastigote and amastigote forms of *Leishmania amazonensis* were treated with a purified indole alkaloid, obtained from the crude extract of stem of *Peschiera australis* (Apocinaceae). Analog alterations were observed in this organelle in promastigote forms of *L. amazonensis* when submitted to the treatment with a purified chalcone of *Piper anduncum* (Torre-Santos *et al.* 1999). Rodrigues *et al.* (2002) reported that the treatment of *L. amazonensis* with 22,26 - azasterol, an inhibitor of ergosterol synthesis, induced a marked alteration in the inner mitochondrial membrane, with the formation of elaborated and complex structures. Other studies reported similar alterations in epimastigote and amastigote forms of *T. cruzi* treated with different compounds (Lazardi *et al.* 1991).

Due to these ultrastructural alterations at the mitochondrion, the activity of a well-known marker enzyme involved in the respiratory chain, the enzyme succinate cytochrome *c* reductase, was evaluated. Cells treated with essential oil of *O. gratissimum* showed a decreasing in enzymatic activity as compared with control cells. Some studies have shown that the lincochalcone A, an oxygenated chalcone, showed a potent antimalarial and antileishmanial activity. This compound also altered the mitochondrial morphology and inhibited the activity of the mitochondrial dehydrogenase (Chen *et al.* 1994, Zhai *et al.* 1995). Recently these authors investigated the action mechanism of this chalcone focusing the respiratory chain of *L. major* and *L. donovani.* The authors showed that several important enzymes of the electron-transport systems were inhibited, including the enzyme succinate cytochrome *c* reductase (Chen *et al.* 2001).

In conclusion, the study of the medicinal plant effect on the trypanosomatid *H. samuelpessoai* here reported demonstrates the utility of this flagellate as a biologic model in the evaluation of cellular alterations induced by drugs. This model is capable of mimic events that happen in pathogenic microorganisms as *T. cruzi*, *Leishmania* sp., *T. brucei* and even in phytopathogenic

trypanosomatid. The results obtained with the essential oil of *O. gratissimum* open perspectives to find more effective drugs of vegetal origin, less toxic and available for low socioeconomic population in the treatment of these diseases.

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REFERENCES

Begum J., Yusuf M., Chowdhury U., Wahab M. A. (1993) Studies on essential oils for their antibacterial and antifungal properties. Part 1. Preliminary screening of 35 essential oils. *J. Sci. Ind. Res*. **28:** 25-34

Budavari S., O'Neil M., Smith A., Heckelman P. E. (Eds.). (1989) The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals. Merck & CO., Rahway

Calixto J. B. (2000) Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Braz. J. Med. Biol. Res*. **33:** 179-189

Chen M., Theander T. G., Christensen S. B., Hviid L., Zhai L., Kharazmi A. (1994) Lincochalcone A, a new antimalarial agent, inhibits in vitro growth of the human malaria parasite *Plasmodium falciparum* and protects mice from *P. yoelii* infection. *Antimicrob. Agents Chemother.* **38:** 1470-1475

Chen M., Zhai L., Christensen S. B., Theander T. G., Kharazmi A. (2001) Inhibition of fumarate reductase in *Leishmania major* and *L. donovani* by chalcones. *Antimicrob. Agents Chemother.* **45:** 2023-2029

Corrêa M. P. (1932) Dicionário das plantas úteis do Brasil. IBDF, Rio de Janeiro

Craveiro A. A., Fernandes A. G., Andrade C. H. S., Matos F. J. A., Alencar W. J. (1981) Óleos essenciais de plantas do Nordeste, Imprensa Universitária, Universidade Federal do Ceará, Fortaleza

Delorenzi J. C., Attias M., Gattas C. R., Andrade C. R., Pinto A. C., Henriques A. T., Bou-Habib D. C., Saraiva E. M. B. (2001) Antileishmanial activity of an indole alkaloid from *Peschiera australis. Antimicrob. Agents Chemother.* **45:** 1349-1354

De-Souza W., Motta M. C. M. (1999) Endosymbiosis in protozoa of the Trypanosomatidae family. *Fems Microbiol. Lett*. **173:** 1-8

El-Said F., Sofowora E. A., Malcolm S. A., Hofer A. (1969) An investigation into the efficacy of *Ocimum gratissimum* as used in Nigerian native medicine. *Planta Medica*. **17:** 195-200

Essawi T., Srour M. (2000) Screening of some Palestinian medicinal plants for antibacterial activity. *J. Ethnopharmacol*. **70:** 343-349

Fagundes L. J. M., Angluster J., Gilbert B., Roitman I. (1980) Synthesis of sterols in *Herpetomonas samuelpessoai*: Influence of growth conditions. *J. Protozool*. **27:** 238-241

Granner D. K. (1990) Membranas: Estrutura, Arranjo e Função. In: Harper: Bioquímica (Eds. R. K. Murray, P. A. Mayes and V. W. Rodwell). Atheneu, São Paulo, **42:** 454-472

Hodgkinson V. H., Herman R., Semprevivo L. (1980) *Leishmania donovani*: Correlation among assays of amastigote viability. *Exp. Parasitol*. **50:** 397-408

Holetz F. B., Ueda-Nakamura T., Dias-Filho B. P., Cortez D. A. C, Mello J. C. P., Nakamura C. V. (2002) Effect of plant extracts used in folk medicine on cell growth and differentiation of *Herpetomonas samuelpessoai* (Kinetoplastida, Trypanosomatidae) cultivated in defined medium. *Acta Scientiarum*. **24:** 657-662

Ilori M., Sheteolu A. O., Omonibgehin E. A., Adeneye A. A. (1996) Antidiarrhoeal activities of *Ocimum gratissimum* (Lamiaceae). *J Diarrhoeal Dis Res*. **14:** 283-285

Lazardi K.; Urbina J. A.; De Souza W. (1991) Ultrastructural alterations induced by ICI 195,739, a bis-triazole derivate with strong antiproliferative action against *Trypanosoma* (*Schizotrypanun*) *cruzi*. *Antimicrob. Agents Chemother.* **35:** 736-740

Lewis E. M. (2001) Should we be concerned about herbal remedies. *J. Ethnopharmacol.* **75:** 141-164

Masters B. S., Willians C. H. Jr, Kamin H. (1967) The preparation and properties of microsomal TPNH-cytochrome *c* reductase from pig liver. In: Methods in Enzymology (Eds. R. W. Estrabrook and M. E. Pullman). Academic Press, New York **10:** 565-573

Mshana N. R., Abbiw D. K., Addae-mensah I., Adjanohoun E., Ahyi M. R. A., Enow-Orock E. G., Gbile Z. O., Noamesi B. K., Odei M. A., Adunlami H., Oteng-Yeboah A. A., Sarpong K., Sofowora A., Tackie A. N. (2000) Traditional Medicine and Pharmacopoeia. Contribution to the Revision of Ethnobotanical and Floristic Studies in Ghana. Scientific, Technical and Research Commission of the Organization of African Unity

Nakamura C. V., Nakamura T. U., Bando E., Melo A. F. N., Cortez D. A. G., Dias Filho B. P. (1999) Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Mem. Inst. Oswaldo Cruz*. **94:** 675-678

Nwosu M. O., Okafor J. I. (1995) Preliminary studies of the antifungal activities of some medicinal plants against *Basidiobolus* and some other pathogenic fungi. *Mycoses*. **38:** 191-195

Onajobi F. D. (1986) Smooth muscle contracting lipid soluble principles in chromatographic fractions of *Ocimum gratissimum. J. Ethnopharmacol*. **18:** 3-11

Orafidiya O. O., Elujoba A. A., Iwalewa F. O., Okeke I. N. (2000) Evaluation of antidiarrhoeal properties of *Ocimum gratissimum* volatile oil and its activity against enteroagregative *Escherichia coli. Pharm. Pharmacol. Lett.* **10:** 9-12

Pessoa L. M., Morais S. M., Bevilaqua C. M. L., Luciano J. H. S. (2002) Anthelmintic activity of essential oil of *Ocimum gratissimum* Linn. and eugenol against *Haemonchus contortus. Vet. Parasitol*. **109:** 59-63

Pinto A. S., Pinto A. C., De Souza W., Angluster J. (1982) Fatty acid composition in *Herpetomonas samuelpessoai*: influence of growth conditions. *Comp. Biochem. Physiol*. **73b:** 351-356

Rodrigues J. C. F., Attias M., Rodriguez C., Urbina J. A., De Souza W. (2002) Ultrastructural and biochemical alterations induced by 22,26-azasterol, a $\Delta^{24(25)}$-sterol methyltransferase inhibitor, on promastigote and amastigote forms of *Leishmania amazonensis. Antimicrob. Agents Chemother.* **46:** 487-499

Roitman C., Roitman I., Azevedo H. P. (1972) Growth of an insect Trypanosomatid at 37°C in a defined medium. *J. Protozool*. **19:** 346-349

Silva R., Oliveira M. M. (1985) Investigations on phospholipids of *Herpetomonas samuelpessoai*: influence of growth conditions. *Comp. Biochem. Physiol.* **82b:** 173-177

Souza M. C., Reis A. P., Silva W. D., Brener Z. (1974) Mechanism of acquired immunity induced by "*Leptomonas pessoai"* against *Trypanosoma cruzi* in mice. *J. Protozool.* **21:** 579-584

Torres-Santos E. C., Moreira D. L., Kaplan M. A. C., Meirelles M. N., Rossi-Bergmann B. (1999) Selective effect of 2', 6'-dihydroxy-4'-methoxylchalcone isolated from *Piper aduncum* on *Leishmania amazonensis*. *Antimicrob. Agents Chemother.* **43:** 1234-1241

Yong V., Schmitz V., Vannier-Santos M. A., Lima A. P. C. A., Lalmanach G., Juliano L., Gauthier F., Scharfstein J. (2000) Altered expression of cruzipain and a cathepsin B-like target in a *Trypanosoma cruzi* cell line displaying resistance to synthetic inhibitors of cysteine-proteinases. *Mol. Biochem. Parasitol.* **109:** 47-59

Zhai L., Blom J., Chen M., Christensen S. B., Kharazmi A. (1995) The antileishmanial agent lincochalcone a interferes with the function of parasite mitochondria. *Antimicrob. Agents Chemother.* **39:** 2742-2748

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