Effect of plant extracts used in folk medicine on cell growth and differentiation of *Herpetomonas samuelpessoai* (Kinetoplastida, Trypanosomatidae) cultivated in defined medium

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ABSTRACT. This work reports the effect of 15 medicinal plants on cell growth and differentiation of *Herpetomonas samuelpessoai,* a non-pathogenic trypanosomatid, used as biological model for its similar antigens to *Trypanosoma cruzi*. Crude extracts (1,000 μg/ml) or essential oil (250 μg/ml) were added in a defined medium. Cell growth was estimated by counting in Neubauer's chamber and cell differentiation was examined by light microscope. *Ocimum gratissimum, Lippia alba, Piper regnellii, Stryphnodendron adstringens,* and *Tanacetum vulgare* showed antiprotozoan activity, *Psidium guajava* and *Punica granatum* a lower activity and *Achillea millefolium, Eugenia uniflora, Mikania glomerata, Plantago major,* and *Spilanthes acmella* had no activity. In contrast, *Arctium lappa, Erythrina speciosa,* and *Sambucus canadensis* stimulated *H. samuelpessoai* growth. Only *L. alba* and *S. acmella* stimulated cell differentiation in this flagellate. These results indicate that medicinal plants possess active compounds against *H. samuelpessoai.* Thus, this protozoan seems to be a suitable model for screening plants containing trypanocidal drugs.

Key words: *Herpetomonas samuelpessoai,* medicinal plants, trypanosomatids.

RESUMO. Efeito de extratos de plantas utilizadas na medicina popular no crescimento e diferenciação celular de *Herpetomonas samuelpessoai* (Kinetoplastida, Trypanosomatidae) cultivada em meio definido. Neste trabalho, verificou-se o efeito de 15 plantas medicinais no crescimento e diferenciação celular de *Herpetomonas samuelpessoai*, um tripanosomatídeo não patogênico utilizado como modelo biológico, que apresenta antígenos semelhantes aos do *Trypanosoma cruzi*. Extratos brutos (1.000 μg/ml) ou óleo essencial (250 μg/ml) foram adicionados ao meio definido. O crescimento celular foi determinado pela contagem em câmara de Newbauer e a diferenciação celular examinada por microscopia ótica. *Ocimum gratissimum, Lippia alba, Piper regnellii, Stryphnodendron adstringens,* e *Tanacetum vulgare* mostraram atividade antiprotozoário, *Psidium guajava* e *Punica granatum* menor atividade e *Achillea millefolium, Eugenia uniflora, Mikania glomerata, Plantago major,* e *Spilanthes acmella* não apresentaram atividade. Por outro lado, *Arctium lappa, Erythrina speciosa,* e *Sambucus canadensis* estimularam o crescimento de *H. samuelpessoai* e *L. alba* e *S. acmella* a diferenciação celular deste flagelado. Estes resultados indicam que plantas medicinais possuem princípios ativos contra *H. samuelpessoai*, o qual parece ser útil como modelo para seleção de plantas que contém drogas tripanomicidas.

Palavras-chave: *Herpetomonas samuelpessoai,* plantas medicinais, tripanosomatídeos.

Introduction

Herbal medicine is used to treat various infectious diseases, in most of the world's cultures. In Brazil, around 80,000 higher plants species were described, offering enormous prospects for discovering new drugs in popular medicine. Focusing attention on the plants medicinally used by indigenous people is the most efficient way to identify plants that may contain bioactive substances (Schultes, 1994). Considering the enormous variety of higher plant species, their potential as new drug sources has not been completely explored. Only 17% of this plant group has been sistematically studied in the discovery of biologically active compounds (Soerjato, 1996). Plants have been

traditionally used for the treatment of diseases of different etiology. Plant extracts are used, for instance, as a source of medicinal agents to cure urinary tract infections, cervicitis, vaginitis, gastrointestinal disorders and skin infections, such as herpes simplex virus type 1 (Caceres *et al*., 1990; Meyer *et al*., 1996).

Diseases caused by protozoa are responsible for considerable mortality in the tropical and subtropical countries. New drugs are now required for amoebiasis, leishmaniosis, malaria and trypanosomiasis treatment. The crisis of reemerging infectious diseases and the resistance of many pathogens for current drugs has been widely recognized as serious and of immediate concern. In addition, the compounds used in parasitic illness treatment, such as benznidazole, nifurtimox, pentavalent antimonials, melarsoprol and pentamidine, are highly toxic, expensive and require long-term treatments (Wright and Phillipson, 1990; Croft *et al*., 1997; Delorenzi *et al*., 2001). The number of drugs available for human and animal trypanosomiases treatment is limited nowadays. Effective drugs are urgently needed as therapeutical alternatives for antiprotozoa chemotherapy, and the higher plants are a potential source of new antiprotozoa drugs.

Herpetomonas samuelpessoai is a non-pathogenic trypanosomatid, isolated from the predatory insect *Zelus leucogramus* (Hemiptera:Reduviidae) (Galvão *et al*., 1970), that shares important antigens with *Trypanosoma cruzi* (Souza *et al*., 1974). This protozoan is used by several research groups in Brazil, as model to study the biology of trypanosomatids, because it can be easily cultivated in a defined medium, either at 28ºC and at 37ºC. It is sensitive to antitrypanosomatid agents (Roitman and Roitman, 1972), and it can induce humoral and cell-mediated immune response. The undifferentiated promastigote form, in culture, can differentiate into opisthomastigotes via the paramastigote intermediate form (Nakamura and Pinto, 1989). These three forms are characterized by the kinetoplast position in relation to the nucleus. So it may be a suitable model to screen new trypanocidal drugs.

In this work, the 15 following plants, currently used in folk medicine, were studied: *Achillea millefolium, Arctium lappa, Erythrina speciosa, Eugenia uniflora, Lippia alba, Mikania glomerata, Ocimum gratissimum, Piper regnellii, Plantago major, Psidium guajava, Punica granatum, Sambucus canadensis, Spilanthes acmella, Stryphnodendron adstringens* and *Tanacetum vulgare*. All of them are used as infectious diseases treatment; that's why they were chosen to have their *in vitro* antiprotozoan activity investigated.

Material and methods

Plant material

The plants were collected in March 2001, in Maringá, State of Paraná, southern Brazil. The plants, after identified by the same researchers that accomplished the collection, were deposited and authenticated at Herbarium of Universidade Estadual de Maringá, Maringá, Paraná, Brazil. *S. adstringens* bark was collected during November, 1999 in São Jerônimo da Serra, Paraná, Brazil and the voucher herbarium specimen was deposited in the same herbarium.

Crude plants extracts and essential oil preparation

The plant parts selected were ground, macerated with ethanol-water (90-10%) for 48 h at 25°C and protected from sunlight. The hydroalcoholic extracts obtained were evaporated under vacuum, lyophilized, and the residues directly assayed against *Herpetomonas samuelpessoai*.

Stryphnodendron adstringens barks were dried in the dark, at room temperature, powdered and extracted by turbo-extraction in 70% acetone. Afterwards, the crude acetonic extract was evaporated under reduced pressure (Mello *et al*., 1996).

O. gratissimum fresh leaves were cut into pieces and subjected to steam distillation. The distillate was extracted with petroleum ether, later carefully removed to obtain the essential oil (Nakamura *et al*., 1999).

Stock solutions preparation

10 mg of hydroalcoholic and acetonic crude extracts were dissolved in 1 ml of phosphate buffer saline 0.01 M pH 7.2 (PBS). One hundred microliters from each stock solution were added to 0.9 ml of defined medium at 1,000 μg/ml final concentration.

For essential oil stock solution, 52.1 μl of *O. gratissimum* oil were solubilized in 52.1 μl of 2% tween 80 and 4,895.8 ml defined medium (10 mg/ml) and a 1:40 dilution was made, obtaining a 250 μg/ml concentration.

Microbial culture growth conditions

Herpetomonas samuelpessoai (ATCC 30252) was cultivated in defined medium (Roitman *et al*., 1972) at 28ºC, for 48 hours and kept at 4ºC. Cells were

grown in 5-ml volumes, in 16 x 150 mm screw-capped tubes.

Antiprotozoan activity

Herpetomonas samuelpessoai in logarithmic growth phase, at a 10^6/ml concentration, was incubated in defined medium, in the presence of 1,000 μg/ml of crude extracts or 250 μg/ml of essential oil. Experiments were carried out in 13 x 100 mm tubes containing 1 ml of the defined medium. After 72 hours at 28ºC, cell growth was estimated by counting in a hemocytometer (Improved Double Neubauer). All cultures were duplicated, and the results were expressed as log cells number/ml and as growth inhibition percent at 72 hours, in comparison to control.

Cell differentiation

Culture aliquotes were taken after 72 hours of incubation at 28ºC, to determine the percentages of the three *H. samuelpessoai* developmental forms: promastigotes, paramastigotes and opisthomastigotes. Cells were collected through centrifugation, stained with "Panótico Rápido LB" stain (Laborclin Prod. Lab. Ltda., Pinhais, Paraná, Brazil) and observed in a light microscope. A minimum of 200 organisms was examined on each preparation.

Results and discussion

Fifteen plant species, traditionally used to treat different diseases, were evaluated in this study and listed in Table 1. The ethnobotanical screening tests on *Herpetomonas samuelpessoai* growth inhibition of 14 plants crude extracts and *O. gratissimum* essential oil are shown in Figure 1. The antiprotozoan effect, expressed as growth inhibition percentage, was found at a 1,000 μg/ml concentration of plants hydroalcoholic extracts. *L. alba, T. vulgare, P. regnellii* inhibited 90.7%, 97.4%, and 99.5%, respectively. *O. gratissimum* essential oil at 250 μg/ml concentration showed 99.3% of growth inhibition. Additionally, *S. adstringens* also showed inhibitory activity. Its acetonic extract displayed an antiprotozoan effect with 75.3% of growth inhibition.

Table 1. Traditional use of plants species selected for antiprotozoan investigation

Species (family) (Herbarium number according to collector)	Local name	Part tested	Popular use[a]
Achillea millefolium L. (Compositae) (8424)	Mil-folhas, Erva-de- cortadura	Leaf	Flowers and leaves are used to treat wounds, ulcers, diarrhea, skin injuries, and gastrointestinal disorders (3, 4)
Arctium lappa Willd (Compositae) (8426)	Bardana, Bardana-maior, Orelha-de-gigante	Leaf	Leaves and stem are used to treat abscess, skin injuries, insect bites, mycosis, and genital affections (1)
Erythrina speciosa Andrews (Leguminosae) (8416)	Mulungu	Stem	The traditional usage indicates that *Erythrina* species could have analgesic, anti-inflammatory and antibacterial activity (3)
Eugenia uniflora L (Myrtaceae) (8419)	Pitanga	Leaf	Leaves are used for treatment of throat complaints (4)
Lippia alba (Mill.) N.E.Br. (Verbenaceae) (8421)	Erva-cidreira, Erva-cidreira-brasileira, Alecrim-do-campo, Chá-da-febre	Leaf	Its leaves are employed as an fusion or decoction in the treatment of gastrointestinal disorders, dysentery, colds and cough, as well as febrifuge (4)
Mikania glomerata Spreng (*Com*positae) (8420)	Guaco	Leaf	Leaves infusion used as antiseptic, anti-inflammatory, and antibacterial (4, 5, 6)
Ocimum gratissimum L. (Lamiaceae) (9613)	Alfavaca	Leaf	Leaves infusion are used for treatment of upper respiratory tract infections, diarrhea, headache, skin diseases, pneumonia, cough and fever (2)
Piper regnellii L. (Piperaceae) (8392)	Pariparoba, Capeba	Leaf	Leaf and root are used in the form of crude extracts, infusions or plasters to treat common infections (1)
Plantago major L. (Plantaginaceae) (8427)	Tanchagem, Erva-de-orelha	Leaf	Leaves and seeds are used as antiseptic, anti-inflammatory, and antibacterial (4, 6, 8)
Psidium guajava L (Myrtaceae) (8423)	Goiabeira	Leaf	Leaf, root, and bark extracts are used for treatment of diarrhea, leukorrhea, cholera, external ulcers, and skin diseases (4)
Punica granatum L (Punicaceae) (8417)	Romã	Fruit	Fruit is used against aphtha, diarrhea, intestinal parasites (4, 6)
Sambucus canadensis L. (Caprifoliaceae) (8422)	Sabugueiro	Leaf	Leaf, flower, and fruit extracts of parts of these plants have been used for respiratory and pulmonary disorders (cold, coughs, etc.) (4, 6)
Spilanthes acmella Mart. (Compositae) (8418)	Agrião-do-Brasil, Jambu	Leaf	A decoction or infusion of the leaves and flowers is recommended for stammering, toothache, stomatitis and throat complaints (4)
Stryphnodendron adstringens Mart. (Leguminosae) (3800)	Barbatimão	Bark	Bark is used for treatment of leukorrhoea, diarrhoea and as anti-inflamatory agent (7)
Tanacetum vulgare L. (Compositae) (8425)	Tanaceto, Erva dos vermes	Leaf	Leaves, flowers, and seeds are recommended as anti-inflammatory and helminthes infections (2)

A: Reference for uses: 1:Alzugaray (1983); 2:Corrêa (1984); 3:Cruz (1979); 4:Silva and Santana (1995); 5:Oliveira and Kisue (1989); 6:Biazzi (1996); 7:Neto (1987); 8:Zatta (1998)

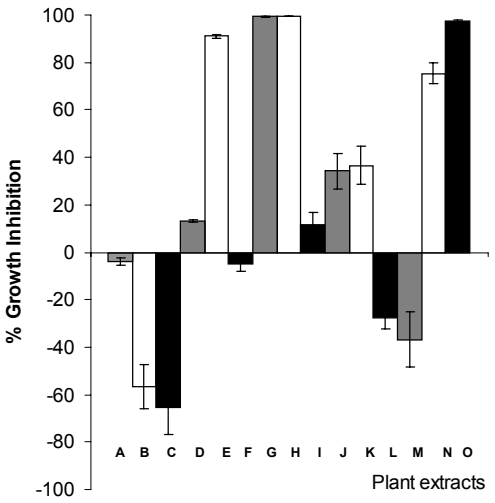

Figure 1. Effects of crude extracts and essential oil on *Herpetomonas samuelpessoai* growth, in defined medium, at 28ºC, for 72 hours. Positive values indicate growth inhibition percentage and negative values correspond to growth stimulation percentage. A. *Achillea millefolium;* B. *Arctium lappa;* C. *Erythrina speciosa;* D. *Eugenia uniflora;* E. *Lippia Alba;* F. *Mikania glomerata;* G. *Ocimum gratissimum;* H. *Piper regnellii;* I. *Plantago major;* J. *Psidium guajava;* K .*Punica granatum;* L. *Sambucus canadensis;* L.M. *Spilanthes acmella;* N. *Stryphnodendron adstringens;* O. *Tanacetum vulgare;* Data represent the mean of three replicates, with vertical bars indicating standard deviation

Numerous extracts of Brazilian medicinal plants have been screened for their antibacterial, antifungal, molluscicidal, antiprotozoan or antiviral activities (Alves *et al*., 2000; Holetz *et al*., 2002). The antibacterial activity of *O. gratissimum* essential oil and eugenol was reported by Nakamura *et al*. (1999). This plant is traditionally used in folk medicine to treat different diseases, e.g. upper respiratory tract infections, diarrhea, skin diseases, pneumonia, cough, fever and conjunctivitis (Corrêa, 1984; Onajobi, 1986). Natural products, such as alkaloids (harmine, vinblastine, ellipticine, and olivacine), terpenes (taxol, phorbol ester, tingenone), quinones (naphthoquinone β-lapachone and allyl-β-lapachone), and polyphenols (gossypol) have shown potent *T. cruzi* or *Leishmania brasiliensis* growth inhibition (Wright and Phillipson, 1990). Some Nigerian medicinal plants have been screened for trypanocidal properties (Adewunmi *et al*., 2001). Their extracts showed good activity on *Trypanosoma brucei* and *T. congolense*, suggesting that they might be a potential source of new and selective agents for the treatment of diseases caused by these protozoa. Recently, Weniger *et al*. (2001) reported the antiprotozoan activities of Colombian plants against several strains of *Plasmodium falciparum*, *Leishmania* sp. and *T. cruzi*.

Crude extracts of *A. millefolium, E. uniflora, M. glomerata, P. major, P. guajava,* and *P. granatum,* showed weak inhibitory activity. Addition of 2% tween 80 in defined medium did not interfere with protozoan growth (data not shown). In contrast, *A. lappa, E. speciosa, S. canadensis*, and *S. acmella* demonstrated stimulating effect on *H. samuelpessoai* growth. The percentages of growth stimulation were 56.5%, 65.6%, 27.5%, and 36.7% respectively (Figure 1). Previous studies demonstrated that *H. samuelpessoai* growth can be stimulated by low lithium chloride concentration (Nakamura and Pinto, 1989). This cation also stimulates growth in other, unrelated cell systems, such as mouse BALB-c 3T3 fibroblasts and mouse mammary epithelial cells (Ryback and Stockdale, 1981; Tomooka *et al*., 1983). Although the biological effect of these plants has not been demonstrated in other cell systems, a growth factor existence in these plants' extracts is an actual possibility.

Figure 2 summarizes the *H. samuelpessoai* cell differentiation in the presence of crude extracts after 72 h of incubation (end of log phase), at 28ºC. The percentages of promastigote, paramastigote, and opisthomastigote forms observed in untreated cells were 87%, 11% and 2%, respectively. Among the tested extracts, only two stimulated the protozoan cell differentiation. Proportions of paramastigote forms were 22% and 19%, when treated with *L. alba* and *S. acmella,* respectively. Other extracts did not interfere in *H. samuelpessoai* cell differentiation. Results obtained with 1,000 μg/ml of *P. regnellii* and *S. adstringens* extracts and 250 μg/ml of *O. gratissimum* essential oil showed a marked bluish color that did not allow different developmental stages identification (data not shown).

The *H. samuelpessoai* cell differentiation occurs in the stationary phase and when cell growth is inhibited; also, by high incubation temperature or after exposition to metabolic inhibitors like sodium butyrate, 2-deoxy-D-glucose, concanavalin A, lidocaine, dimethylsulphoxide and cholinergic drugs (Angluster *et al.,* 1977; Souza *et al*., 1980; Thomas *et al*., 1981; Castellanos *et al*., 1981; Nakamura and Pinto, 1989). The *H. samuelpessoai* differentiation mechanism is triggered by changes in the culture medium composition, incubation conditions and by adding substances to the culture medium, which interacts with cell components. Treatments that interfere with plasma membrane components are effective in triggering the differentiation process.

Considering the results obtained in this study, it is conceivable that the plants used in the popular medicine possess active compounds against *H. samuelpessoai*. Thus, it may be concluded that this non-pathogenic protozoan may be a suitable model for screening some medicinal plants which contain antitrypanosomatids drugs.

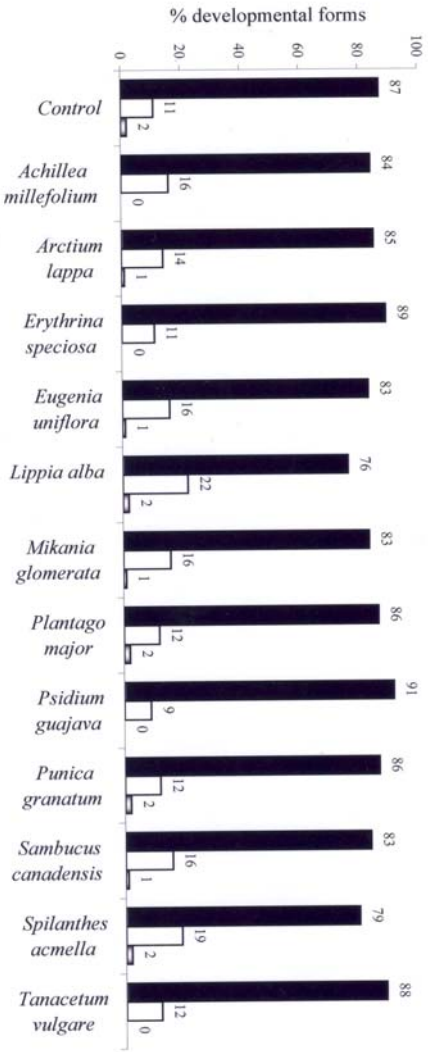

Figure 2. Percentages of the three developmental forms in *Herpetomonas samuelpessoai* culture, at 28ºC, after 72-h incubation, in the presence of plants' extracts. ■ Promastigotes; □ Paramastigotes; ◘ Opisthomastigotes

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References

ADEWUNMI, C. O. *et al*. Ethno-veterinary medicine: screening of Nigerian medicinal palnts for trypanocidal properties. *J. Ethnopharmacol.*, Limerick, v. 77, p. 19-24, 2001.

ALVES, T.M.A. *et al*. Biological screening of Brazilian medicinal plants. *Mem Inst Oswaldo Cruz*, Rio de Janeiro, v. 95, p. 367-373, 2000.

ALZUGARAY, D. *Plantas que curam*. São Paulo: Três Livros e Fascículo, v.I, e v.III, 1983.

ANGLUSTER, J. *et al*. Effect of 2-deoxy-d-glucose on differentiation of *Herpetomonas samuelpessoai*. *J. Parasitol*, Lancaster, v.63, p. 922-924, 1977.

BIAZZI, E. S. *Saúde pelas Plantas*. Tatuí: Casa Publicadora Brasileira, 1996.

CACERES, A. *et al*. Plants used in Guatemala for treatment of gastrointestinal disorders 1: screening of 84 plants against Enterobacteria. *J. Ethnopharmacol*., Limerick, v. 30, p. 55-73, 1990.

CASTELLANOS, G. B. *et al*. Induction of differentiation in *Herpetomonas samuelpessoai* by dimethyl sulfoxide. *Acta Trop*, Basel, v. 38, p. 29-37, 1981.

CORRÊA, M. P. *Dicionário das plantas úteis do Brasil e das exóticas cultivadas*. Rio de Janeiro: Instituto Brasileiro de Desenvolvimento Florestal, 1984.

CROFT, S. L. The current status of antiparasitc chemotherapy. *Parasitology*, Cambridge, v.114, p.3-15, 1997.

CRUZ, G. L. *Dicionário de plantas úteis do Brazil*. Rio de Janeiro: Civilização Brasileira, 1979.

DELORENZI, J. C. *et al*. Antileishmanial Activity of an Indole Alkaloid from *Peschiera australis*. *Antimicrob. Agents Chemother.*, Bethesda, v. 45, no.5, p. 1349-1354, 2001.

GALVÃO, A. B. *et al*. *Leptomonas pessoai* sp. n. (Trypanosomatidae, Kinetoplastida, Protozoa). *Revista Goiana de Medicina*, Goiânia, v. 16, p. 229-236, 1970.

HOLETZ, F.B. *et al*. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. *Mem. Inst. Oswaldo Cruz,* Rio de Janeiro, v. 97, n. 7, p. 1027-1031, 2002.

MELLO, J. C. P. *et al*. Flavan-3-ols and Prodelphinidins from *Stryphnodendron adstringens*. *Phytochemistry*, New York, v. 41, p. 807, 1996.

MEYER, J. J. M. *et al*. Inhibition of herpes simplex virus type 1 by aqueous extracts from shoots of *Helichrysum aureonitens* (Asteraceae). *J. Ethnopharmacol.*, Limerick, v.52, p. 41-43, 1996.

NAKAMURA, C. V.; PINTO, A. S. Biological effects of lithium chloride on the insect trypanosomatid *Herpetomonas samuelpessoai*. *Parasitology*, Cambridge, v. 99, p. 193-197, 1989.

NAKAMURA, C. V. *et al*. Antibacteryal activity of *Ocimum gratissimum* L. Essential Oil. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 94, no. 5, p. 675-678, 1999.

NETO, G.G. *Plantas utilizadas na medicina popular do Estado do Mato Grosso*. Brasília: CNPq Assessoria Editoral, 1987.

OLIVEIRA F.; KISUE G. *Fudamentos de farmaco-botânica*. Rio de Janeiro: Atheneu, 1989.

ONAJOBI, F. D. Smooth muscle contracting lipidic-soluble principles in chromatographic fractions of *Ocimum gratissimum. J. Ethnopharmacol.,*Limerick, v. 18, p. 3-11, 1986.

RYBAK, S. M.; STOCKDALE, F. E. Growth effects of lithium chloride in BALB/c 3T3 fibroblasts and Madin-Darby canine kidney epithelial cells. *Exp. Cell Res.,* New York, v. 136, p. 263-270, 1981.

ROITMAN, C. *et al*. Growth of an insect Trypanosomatid at 37ºC in a defined medium. *J. Protozool.*, Lawrence, v.19, p. 346-349, 1972.

ROITMAN, I.; ROITMAN, C. Increase growth inhibition of trypanosomatidal drugs at 37ºC. *J. Protozool.*, Lawrence, v. 19 (suppl), p. 59, 1972.

SCHULTES, R. E. Amazonian ethnobotany and the search for new drugs. *Symposium Ciba Foudation*, Amsterdam, v. 185, p. 106-112, 1994.

SILVA I.; SANTANA, D. M. G. *Noções sobre o organismo humano e utilização de plantas medicinais*. Cascavel: Assoeste-Editora Educativa, 1995.

SOERJATO, D. D.; Biodiversity prospecting and benefits haring: perspectives from the field. *J. Ethnopharmacol.*, Limerick, v. 51, p. 1-15, 1996.

SOUZA, E. T. *et al*. Concanavalian A-induced cell differentiation in the protozoan *Herpetomonas samuelpessoai. J. Parasitol.,* Lancaster, v. 66, p. 985-988, 1980.

SOUZA, M. C. *et al*. Mechanism of Acquired Immunity Induced by "*Leptomonas pessoai"* against *Trypanosoma cruzi* in Mice. *J. Protozool.,* Lawrence, v. 21, no. 4, p. 579-584, 1974.

THOMAS, E. M. *et al*. Changes in cell shape and induction of cell differentiation in the protozoan *Herpetomonas samuelpessoai* by cholinergic drugs. *Res. Commun. Chem. Pathol. Pharmacol.*, Westbury, v. 34, p. 81-88, 1981b.

TOMOOKA, Y. *et al*. Growth effect of lithium on mouse mammary epithelial cells in serum-free collagen gel culture. *J. Cell. Physiol.,* Philadelphia, v.117, p. 290-296, 1983.

WENIGER, B. *et al*. Antiprotozoal activities of Colombian plants. *J. Ethnopharmacol.*, Limerick, v. 78, p. 193-200, 2001.

WRIGTH, C. W.; PHILLIPSON, J. D. Natural products and the development of selective antiprotozoal drugs. *Phytother. Res*, London, v.4, p. 127-139, 1990.

ZATTA, I. M. *A farmácia da natureza*. São Paulo: Paulinas, 1998.

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