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* showed 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.

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 systematically 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, leishmaniasis, 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 trypanosomiasis treatment is limited nowadays. Effective drugs are urgently needed as therapeutic 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_, _Passion 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 25ºC 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
Effect of plant extracts on *Herpetomonas samuelpessoai*

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

**Antiprotozoan activity**

*Herpetomonas samuelpessoai* in logarithmic growth phase, at a 10^7/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 28°C, 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 28°C, 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” 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 plant species selected for antiprotozoan investigation

<table>
<thead>
<tr>
<th>Species (family) (Herbarium number)</th>
<th>Local name</th>
<th>Part tested</th>
<th>Popular use’</th>
</tr>
</thead>
<tbody>
<tr>
<td>Achillea millefolium L. (Compositae) (8424)</td>
<td>Mil-folhas, Erva-de-cortadura</td>
<td>Leaf</td>
<td>Flowers and leaves are used to treat wounds, ulcers, diarrhea, skin injuries, and gastrointestinal disorders (3, 4)</td>
</tr>
<tr>
<td>Armoracia lapidifera Wild (Compositae) (8426)</td>
<td>Bardana, Bardana-maior, Orilha-de-pijage</td>
<td>Leaf</td>
<td>Leaves and stem are used to treat abscess, skin injuries, insect bites, mycosis, and genital affections (1)</td>
</tr>
<tr>
<td>Erythrina speciosa Andrews (Leguminosae/Mutungu) (8416)</td>
<td>Stem</td>
<td>The traditional usage indicates that <em>Erythrina</em> species could have analgesic, anti-inflammatory and antibacterial activity (3)</td>
<td></td>
</tr>
<tr>
<td>Eugenia uniflora L. (Myrtaceae) (8419)</td>
<td>Pitanga</td>
<td>Leaf</td>
<td>Leaves are used for treatment of throat complaints (4)</td>
</tr>
<tr>
<td>Lippia alba (Mill.) N.E.Br. (Verbenaceae) (8421)</td>
<td>Erva-cidreira, Erva-cidreira-brasileira, Alcemin-do-campo, Chá-da-febre</td>
<td>Leaf</td>
<td>Its leaves are employed as an infusion or decoction in the treatment of gastrointestinal disorders, dysentery, colds and cough, as well as fever (4)</td>
</tr>
<tr>
<td>Mikania glomerata Spreng (Compositae) (8420)</td>
<td>Guaco</td>
<td>Leaf</td>
<td>Leaves infusion used as antiseptic, anti-inflammatory, and antibacterial (4, 5, 6)</td>
</tr>
<tr>
<td>Oxynum gratissimum L. (Lamiaceae) (9613)</td>
<td>Alfíaca</td>
<td>Leaf</td>
<td>Leaves infusion are used for treatment of upper respiratory tract infections, diarrhea, headache, skin diseases, pneumonia, cough and fever (2)</td>
</tr>
<tr>
<td>Piper regnellii L. (Piperaceae) (8392)</td>
<td>Pariparoba, Capeba</td>
<td>Leaf</td>
<td>Leaf and root are used in the form of crude extracts, infusions or plasters to treat common infections (3)</td>
</tr>
<tr>
<td>Plantago major L. (Plantaginaceae) (8427)</td>
<td>Tanchagem, Erva-de-orelha</td>
<td>Leaf</td>
<td>Leaves and seeds are used as antiinflammatory, and antibacterial (4, 6, 8)</td>
</tr>
<tr>
<td>Psidium guajava L. (Myrtaceae) (8423)</td>
<td>Goiabeira</td>
<td>Leaf</td>
<td>Leaf, root, and bark extracts are used for treatment of diarrhea, leukorrhea, cholera, external ulcers, and skin diseases (4)</td>
</tr>
<tr>
<td>Punica granatum L. (Punicaceae) (8417)</td>
<td>Romá</td>
<td>Fruit</td>
<td>Fruit is used against aphtha, diarrhea, intestinal parasites (4, 6)</td>
</tr>
<tr>
<td>Sambucus canadensis L. (Caprifoliaceae) (8422)</td>
<td>Sabugueiro</td>
<td>Leaf</td>
<td>Leaf, flower, and fruit extracts of parts of these plants have been used for respiratory and pulmonary disorders (cold, coughs, etc.) (4, 6)</td>
</tr>
<tr>
<td>Spilanthes acmella Mart. (Compositae) (8418)</td>
<td>Agrião-do-Brasil, Jambu</td>
<td>Leaf</td>
<td>A decoction or infusion of the leaves and flowers is recommended for stammering, toothache, stomatitis and throat complaints (4)</td>
</tr>
<tr>
<td>Styphnoloden adeninum Mart. (Leguminosae) (3900)</td>
<td>Barbatinho</td>
<td>Bark</td>
<td>Bark is used for treatment of leukorrhea, diarrhoea and as anti-inflammatory agent (7)</td>
</tr>
<tr>
<td>Tanacetum vulgare L. (Compositae) (8425)</td>
<td>Tanaceto, Erva dos vermes</td>
<td>Leaf</td>
<td>Leaves, flowers, and seeds are recommended as anti-inflammatory and helmintes infections (2)</td>
</tr>
</tbody>
</table>

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, diarrhoea, skin diseases, pneumonia, cough, fever and conjunctivitis (Corrêa, 1984; Onajobi, 1986). Natural products, such as alkaloids (harmane, 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 braziliensis* 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 1 summarizes the *H. samuelpessoai* cell differentiation in the presence of crude extracts after 72 h of incubation (end of log phase), at 28°C. 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.
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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 28°C, after 72-h incubation, in the presence of plants’ extracts.](image)

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**References**


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