

Evaluation of the Cytotoxic Activity of Aqueous Extract of *SchinusterebinthifoliusRaddi*

Arnóbio, A., O.N. Terra Jr., P.R.G. Lemos,
A.R. Silva, G.C. Maldonado and L.L.M. Cunha

Central State University of West Zone-Central Sector of Biological and Health Sciences,
Rio de Janeiro, 23.070-200, Brazil

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ABSTRACT

Phytotherapy consists in all pharmaceutical preparation using certain part of plants as a feedstock with known pharmacological effects for medicinal purposes. In order to a better understanding of the biological effects associated with herbal medicines, many scientific studies have been conducted and developed in recent years. *SchinusterebinthifoliusRaddi* (Aroeira) is native from Peru and is also found in Europe, Asia and some Latin American countries. This herbal remedy was chosen for study because of its wide use and interesting pharmacological actions. Among the effects documented in the literature, they are a potent antimicrobial agent. Its use as hydroalcoholic extract showed positive effect on cystotomy healing in rats and the studies indicate that *SchinusterebinthifoliusRaddi* vaginal gel is effective and safe for the treatment of bacterial vaginosis. The experimental model discussed in this study was the Minimum Inhibitory Concentration (MIC) in different strains of protozoa. Evaluation of the effects of the extract with *Trypanosomacruzi* and *Leishmaniaamazonensis* strains was conducted at different concentrations of a dilution series to allow a more sensitive analysis, in a wide concentration range. A static or toxic action can be distinguished by the color reaction which is involved rezazorina. The results indicate that in none of the tested strains were observed cytotoxic effects of aqueous extract. There was also no inhibitory action on the development of tested strains.

Keywords: Phytotherapy, *SchinusterebinthifoliusRaddi*, MIC, *Trypanosomacruzi*, *Leishmaniaamazonensis*

1. INTRODUCTION

Plants (herbs) with medicinal activities have gained great attention of the population, especially in developing countries (Silveira *et al.*, 2008). For instance, in Nigeria, such as in some developing countries, 80% of the population uses traditional medicine based on phytotherapy for the treatment of many diseases (Odetola *et al.*, 2006). The herbal therapy is a branch of medicine that uses natural products for therapeutic purposes (Osungunna and Adedeji, 2011). Its wide popular use generates attention of the health authorities and managers, creating incentives in the form of public

investment. According to the World Health Organization, about 75% of the population living in developing countries, essentially depends on phytotherapy for their primary health care, mainly due to economic difficulties (WHO, 2008), it is notable the increasing acceptance of phytotherapy by health professionals and the raising of primary care by the population (Homar, 2005). Natural products from higher plants can provide a new source of antimicrobial agents (Oskay *et al.*, 2005), such as antioxidants (Sepulveda-Jimenez *et al.*, 2009; Malathi *et al.*, 2012), as antiseptics, anti-inflammatories (Bonjar, 2004) or another possible innovative mechanisms of action. Worth noting

Corresponding Author: Arnóbio, A., Central State University of West Zone-Central Sector of Biological and Health Sciences,
Rio de Janeiro, 23.070-200, Brazil

that there is more than one hundred chemical substances which were derived from plants for drugs and medicines (Ashafa *et al.*, 2008).

Actually, there is a great perspective and interest about using these plants, mainly arousing the students and researchers in related fields (Brandao *et al.*, 2001; Burta *et al.*, 2008; Kawsar *et al.*, 2008; Ranjbar *et al.*, 2011). However, most of these herbal medicines have no well-known toxicology description (Capasso *et al.*, 2000). Using herbs carelessly can induce health damage, even if their cytotoxicity shall be low (Silveira *et al.*, 2008).

SchinusterebinthifoliusRaddi is a large tree, thin and scaly bark. It has compound leaves with lanceolate leaflets, pointed and flowers arranged in multiple pedicles, small and greenish, white or yellow. It is a native plant from Peru and is also found in Europe, Asia and America (Coutinho *et al.*, 2006).

The bark, leaves and inner bark are among the main components of *S. terebinthifoliusRaddi* reported in scientific studies (El-Massry *et al.*, 2009). One of the effects documented in the literature is that they are a potent antimicrobial agent. Its use as hydroalcoholic extract showed positive effect on cystotomy healing in rats and the studies indicate that *SchinusterebinthifoliusRaddi* vaginal gel is effective and safe for the treatment of bacterial vaginosis (Amorim and Santos, 2003). The treatment with *S. terebinthifoliusRaddi* was also effective in treating denture stomatitis (Soares *et al.*, 2010).

The strains applied here comprise a group of etiologic agents responsible for a number of diseases classified as neglected. Although there is no funding for researches related to these diseases, the knowledge produced has not translated into significant therapeutic advances, such as new drugs, vaccines and diagnostic methods (Brazil, 2010). *Trypanosomacruzi* (*T. cruzi*) is a well-known flagellated protozoan, etiologic agent of Chagas disease, also known as trypanosomiasis. Chagas disease is an illness of high mortality and morbidity due to the prevalence of diseases associated with vectors in the Americas and thirdly relevant after malaria and leishmaniasis (Silva *et al.*, 2009).

Leishmaniaamazonensis (*L. amazonensis*), is one of the main agents of diffuse cutaneous leishmaniasis, which doesn't respond to treatments currently known (Mendonca-Filho *et al.*, 2004). Species of *Leishmania* are obligate intracellular parasites of macrophages and dendritic cells (Alexander *et al.*, 1999).

The experimental model discussed in this study was the Minimum Inhibitory Concentration (MIC) in

different strains of protozoa. Evaluation of the effects of the extract with strains was conducted at different concentrations of a dilution series to allow a more sensitive analysis, in a wide concentration range, the toxicity or static can be distinguished by staining reaction in which rezazorina is involved.

The aims of this study were to evaluate the effect of aqueous extract in different strains of protozoa and evaluate the cytotoxicity of the strains from the different tested concentrations.

2. MATERIALS AND METHODS

2.1. Plant Gathering

The *S. terebinthifoliusRaddi* fresh leaves were collected in the properties of the Municipal Environment, Agriculture and Fisheries of the Municipality of Itaguaí (SMMAAP-Itaguaí/RJ), amounting approximately 2.83 kg. They were cleaned, dried and stored in the freezer of Analysis Laboratory Chemical Biological (LAQB) of Centro Universitário Estadual da Zona Oeste (UEZO) for approximately 16 h at 28°C. The exsiccata species were deposited in the Herbarium of the National Museum of UFRJ for certification of a botanist.

2.2. Extraction and Preparation of the Aqueous Extract

The aqueous extract was obtained from fresh leaves prepared by its infusion, according to Brazilian Herbal Pharmacopoeia form (Brazil, 2011), with some modifications.

Each 2 L of water were used 800 g of fresh leaves.

The water was boiled up to 100°C in a beaker and then was transferred to another vessel containing fresh leaves that were ground into electrical process.

After homogenization, the solution achieved a temperature of 70°C and held standing for 30 min in an enclosed environment.

After that the solution was filtered through a porcelain funnel with the aid of filter paper and vacuum pump (Nevoni Model: 14014POC).

The extract was stored in amber bottles, identified and maintained under refrigeration (Freezer Blood Plasma-Indrel Model: GPS 10d), until its lyophilization.

2.3. Culture of Parasites

L. amazonensis promastigotes were maintained by weekly transferences in Brain Heart Infusion medium (BHI) supplemented with 10% Fetal Bovine Serum (FBS) at 26°C.

The parasites were held for periodic inoculations using hamsters (Rosa *et al.*, 2003).

2.4. Evaluation by Minimal Inhibitory Concentration (MIC)

Promastigotes of *L. amazonensis* were incubated at 26°C for 120 h in fresh medium (BMHI) supplemented with 10% FBS in the presence of different concentrations (500, 250, 125, 62.5, 31.25 $\mu\text{g mL}^{-1}$) and the concentration of 500 $\mu\text{g mL}^{-1}$ was the initial to a serial dilution of the aqueous extract (cell growth was determined by visible turbidity) in order to evaluate the MIC, as previously described (Newton *et al.*, 2002).

The strains of *T. cruzi* in promastigote form, Ty and MD, were maintained under the same conditions as in *L. amazonensis* at PBHIL medium, both supplemented with 10% FBS.

The used plates were incubated at 28°C for 72 h, then 25 μL of resazurin added to each well and again incubated for 2 h at 28°C, then the toxic action on protozoa from turning staining provided by resazurin could be observed.

Three controls have been used: only the culture medium, culture medium associated with the extract and a medium associated with protozoa.

The MIC was considered the lowest concentration of each substance used to prevent the growth of *L. amazonensis in vitro* (Rosa *et al.*, 2003).

3. RESULTS

3.1. Inhibition of Parasite Growth

The results indicate that none of the tested strains in this study was observed cytotoxicity of aqueous extract. Also there was no inhibitory effect on cell growth from different concentrations of the extract.

Pink coloration was observed in all wells, meaning cell viability. The initial concentration of 500 $\mu\text{L mL}^{-1}$ of extract was tested in combination with more concentrations of 250, 125, 62.5, 31.25 $\mu\text{g mL}^{-1}$ and control solutions.

This result shows that there was cell viability after 72 h of incubation; it is noteworthy that the experiment was conducted in duplicate.

4. DISCUSSION

For the purpose of better understanding the biological effects associated with herbal medicine, many scientific studies have been conducted.

The species *S. terebinthifoliusRaddi* was chosen for this study due to its wide use and interesting pharmacological actions.

Besides being used against uterine disorders in general, the leaves and bark are also used for rheumatism treatment, powerfully acting as a curing agent for ulcers and wounds, showing febrifuge and purgative effects (Guerra *et al.*, 2000; Martinez *et al.*, 1996; 1997).

As in other experiments, cytotoxicity was evaluated by survival of the bacteria in liquid and solid medium. There was no toxicity in any used concentrations (10, 20, 30 and 40 mg mL^{-1}), including those which were acting as protector of Stannous Chloride (SnCl_2) harmful action (Lemos *et al.*, 2011).

Other studies suggest that bacterial extract wasn't able to induce a toxic activity or bactericides, under the gel condition for treatment of bacterial vaginosis and has a slower rate of healing compared to its control (Leite *et al.*, 2011). Those results along with these obtained in this study suggest that the aqueous extract of *S. terebinthifoliusRaddi* has no considerable toxic action under the proposed models.

Some studies have identified compounds with antifungal properties, especially schinol and identified new biphenyl 4'-ethyl-4-methyl-2, 2', 6,6'-tetra [1,1'-biphenyl] -4, 4' -dicarboxylate, these compounds have an effective action of *Paracoccidioides brasiliensis*, but it takes more attention to the mechanisms of action and toxicity of these compounds using combining therapeutic actions, leading to an effector action against different strains (Johann *et al.*, 2010).

The MIC is a technique that has proven efficacious in the evaluation of toxicity in different cell models. It is often used in research to evaluate the effects of extracts or components on these cells, from which one can get a new therapeutic approach.

In this study, the MIC model discussed resulted in survival of three different strains, showing that the extract had no therapeutic effect, but it wasn't able to kill or inhibit their growth either, indicating a low or negligible toxicity in cellular models.

5. CONCLUSION

According to the results, it can be speculated that none of the concentrations used in aqueous extract of *SchinusterebinthifoliusRaddi* has shown a static and cytotoxic effects in any of the different strains used for MIC.

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