# Preliminary phytochemical and antileishmanial studies of the ethanolic extracts of *Pterodon pudescens*

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**ABSTRACT:** Pentavalent antimonials are the first choice for the treatment of human leishmaniasis. However in rural areas the traditional plants may be preferred for the treatment of lesions. In recent years a number of papers are published related to the natural products especially plant derivative with infectious diseases. The present work was undertaken to evaluate the antileishmanial activity of *Pterodon pubescens* which is a native tree widely distributed over the central region of Brazil and used in folk medicine as wine infusions to treat inflammatory disease. The phytochemical screening and the biological essay of ethanolic extract of *Pterodon pudescens* (PPE) leaves at the concentrations of 150, 300, 450, 600 µg/ml were tested *in vitro* in *Leishmania amazonensis*-infected macrophages to support its traditional medicinal use as a leishmaniasis remedy. Phytochemical screening of PPE has shown the presence of catechemical tannins, steroids, triterpenoids and flavonoids. The biological test suggests that PPE were found to control parasite burden of cell cultures in dose-dependent manner. These findings highlight the fact that the apparent potency of *Pterodon pudescens* compounds, together with their widely distribution over Latin America and Brazil, may represent a promising antileishmanial agent.

**Keywords:** *Pterodon pubscens*, *Leishmania amazonensis,* Plant extract

**RESUMO: Estudos preliminares sobre a fitoquimica e a atividade anti-leishmania de extratos etanólicos de *Pterodon pudescens*.** Antimoniais pentavalentes são a primeira escolha para o tratamento das leishmanioses humanas. No entanto, no interior brasileiro plantas tradicionais são usadas para o tratamento dessas lesões. De fato, recentes trabalhos tem relatado o potencial terapêutico de produtos naturais, especialmente derivados de plantas. Neste estudo avaliamos a atividade leishmanicida de *Pterodon pubescens*, uma árvore nativa, distribuída pela região central brasileira e usada em infusões para tratamento de inflamações. Foi realizada a análise fitoquímica e o ensaio *in vitro* em macrófagos infectados com *Leishmania amazonensis* em concentrações de 150, 300, 450, 600 µg/ml do extrato etanólico de folhas de *Pterodon pudescens* (PPE) para comprovar o uso tradicional desta planta como terapia para as leishmanioses. Os testes fitoquímicos indicaram a presença de taninos catequímicos, flavonas, esteroides, triterpenoides, flavonoides e xantonas. Os ensaios biológicos revelaram que o PPE foi capaz de controlar a carga parasitária em macrófagos de maneira dose dependente. Estes resultados corroboram com o potencial terapêutico de compostos de *Pterodon pudescens* e, junto com sua ampla distribuição no Brasil, podem representar promissor agente leishmanicida.

**Palavras-chaves:** *Pterodon pubscens*, *Leishmania amazonensis,* Extrato de planta

## INTRODUCTION

Leishmaniasis is a zoonotic complex of disease that displays widely different clinical manifestations in humans. The severity of the disease produced by several *Leishmania* species varies enormously, ranging from disfiguring scars and deformities caused by tegumentar leishmaniasis to death caused by viscerotropic species (Grimaldi & Tesh, 1993). In addition, over the last years, leishmaniasis have impaired economic development and placed enormous burdens on Leishmania-afflicted developing countries (2 million new cases are reported each year), and consequently

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continue to be an enormous global health challenge (Handman *et al*., 2008).

The first line of therapy for all forms of the disease is pentavalent antimonial, sodium stibogluconate, and meglumine antimoniate glucantime (Berman, 2006). The polyene antibiotic amphotericin B and diamidine pentamidine are second-line drugs, and miltefosine, a lysophospholipid analog, is the most recent drug approved for leishmaniasis treatment (Berman, 2006; Mishra *et al*., 2007). However several works indicate that current therapies frequently fail to eradicate the parasite from infected tissues, while also presenting serious side effects (Berman, 2006).

Natural product scaffolds have been the basis of the majority of current anti parasitic medicines. Molecules such as quinine, lapachol and artemisinin were originally isolated from herbal medicinal products. The success of these drugs has broadened the search for natural plant products as a source of novel drugs for leishmaniasis (Ginsburg & Deharo, 2011).

*Pterodon pubescens* (Leguminosae-Papilionoideae) is a native tree widely distributed over the central region of Brazil, which is used in folk medicine as wine infusions to treat inflammatory disease (Lorenzi, 2009). Several works suggest immuno modulatory and anti-protozoa effect of plant component of *Pterodon* genera without side effects observed (Cardoso *et al*., 2008; Menna-Barreto *et al*., 2008; Dutra *et al*., 2009; Coelho *et al*., 2004). Recently, anti-tumoral effects have been shown for *Pterodon pubescens* extracts using in vitro model of leukemia (Pereira *et al*., 2011). Nevertheless, the effects of this extract on leishmaniasis remain unknown. In this study, experiments were designed to address whether *in vitro* treatment of primary murine macrophages with PPE can modulate the cellular response to *Leishmania amazonensis* infection.

## MATERIAL AND METHODS

### *Plant material and Pterodon pubescens Extract (PPE) preparation*

*Pterodon pubescens* leaves were collected around Pontal do Araguaia city, Mato Grosso State, Brazil in September 2008. The taxonomic identity was confirmed by Dr Maryland Sanchez Lacerda from the Federal University of Mato Grosso (UFMT). A voucher specimen was deposited in the central herbarium of the UFMT (No: 1950). The air-dried and powdered leaves of *Pterodon pubescens* were extracted with ethanol at room temperature during fifty days, in a dark condition. After complete ethanol evaporation, at 45 ºC in dry chamber, all of the extracts were prepared at 100% concentration (g/ml).

### Phytochemical screening of extract

The major secondary metabolites classes such as Tannins, Phlobatannins, Saponins, Terpenoids, Steroids, Flavonoids, Alkaloids and Glycosides were screened according to the common phytochemical methods described previously (Harborne, 1998).

### In vitro antileishmanial activity studies

*L. amazonensis* (MHOM/BR/73/M2269) amastigotes were isolated from active skin lesions from BALB/c mice as previously described (Arrais-Silva *et al*., 2005). Primary mouse macrophages were obtained from normal BALB/c mice by peritoneal lavage, cultured in RPMI medium supplemented with antibiotics and 10% heat-inactivated fetal calf serum, on 24-well plates containing 13-mm diameter glass coverslips. Macrophages were exposed to *L. amazonensis* at an amastigote–macrophage ratio of 3:1 for 1 hr. After the exposure period, the cultures were washed to remove extracellular parasites and then incubated in the presence of different concentrations of the PPE at 37◦ C for 72 h. For the evaluation of the PPE anti leishmanial activity, the percentage of infected macrophages and number of amastigotes per macrophage were determined in culture cells stained with Giemsa and examined microscopically at ×1,000 magnification. Cell viability was determined by hemocytometer counts after staining with erythrosine B (Colhone *et al*., 2004). All experiments were repeated at least 3 times in triplicate wells, and the results are expressed as the mean ± SD. Statistical analyses were performed using the 2-tailed Student's *t*-test and Origin 6.0 (OriginLab Corp., Northampton, Massachusetts). A $p < 0.05$ value was considered to be significant (Arrais-Silva *et al*., 2005).

## RESULTS AND DISCUSSION

Phytochemical screening of PPE has shown the presence of catechemical tannins, flavons, steroids, triterpenoids, flavonoid and xanthins (Table 1). Although the mechanism of action of these secondary metabolites has not been evaluated in the present study, however, some of these metabolites have been found to exert their anti-parasite effects by elevating cell oxidation or by inhibiting protein synthesis (Philipson & Wright, 1990). The phytochemical investigation and isolation of various compounds from *P. pubescens* has led to the identification of xanthin and flavonoids with antibacterial, anticancer, antitumour, antitrypanosomal and antimalarial-like activities (Tran *et al*., 2003; Kamboj *et al*., 2010).

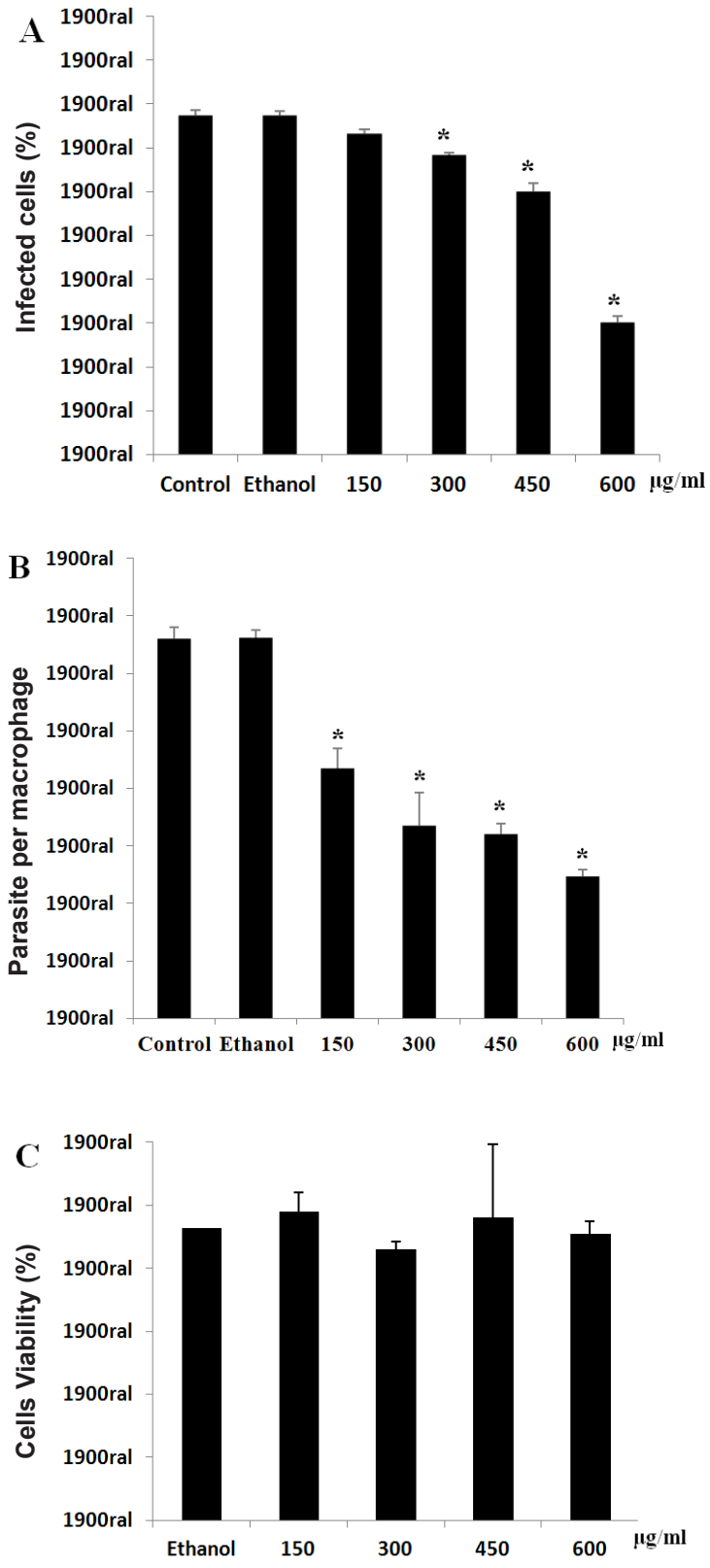


**Figure 1. Effect of PPE on *Leishmania amazonensis* infection of murine peritoneal macrophages.** Peritoneal macrophages of BALB/c were infected with *L. amazonensis* amastigotes for 1 hr. Cell cultures were washed and incubated under treatment conditions for 72 hr. The percentage of infected macrophages (**A**); the number of amastigotes per macrophage (**B**); and the percentage of viability cells by erythrosine-B staining (**C**). The results represent the mean ± SD of 3 experiments. The significance of the difference between cell cultures in control and treated is indicated in the figure. *p ≤ 0.01.

**Table 1. Preliminary phytochemical analysis of *P. pudescens leaves extract***

| Phytochemical test | Name of the test | Leaves extract |
|---|---|---|
| Phenols | $FeCl_3$ test | - |
| Pyrogallic Tannins | $FeCl_3$ test | - |
| Catequic Tannins | $FeCl_3$ test | ++ |
| Antocianins e antocianidins | HCl and NaOH test | - |
| Flavonoids | Flavonoids Shinoda test | + |
| Leucoanthocyanidins | HCl and NaOH test | - |
| Steroids | Lieberman-Burchard test | ++ |
| Pentacyclic Triterpenoids | Lieberman-Burchard test | + |
| Saponnins | Saponins Frothing test | + |

+: present, -: absent

Here experiments were undertaken to study the possible effects of PPE on murine macrophages infected with amastigotes of *L. amazonensis*. PPE caused a concentration-dependent decreasing infected cell when compared with non-treated conditions. In fact, infected cell cultures untreated to PPE failed control parasite burden and exhibited 77.4 % of infection, while infected macrophages exposed to 150, 300, 450 and 600 µg/ml of PPE treatment had shown consistently decreasing of infected cells to 73.2, 68.2, 60 and 30.1% respectively (Figure 1A).

There was also a significant reduction in the number of amastigotes per cell in PPE exposed macrophage cultures (1.2 amastigotes/cell at 600 µg/ml extract concentration) when compared with control cell conditions (3.3 amastigotes/cell) (Figure 1B).

Exposure of infected macrophages cultures to PPE failed to affect cell viability or morphology as evaluated by erythrosine-B staining (Figure 1C) (Colhone *et al*., 2004). In fact, previous reports indicate save treatment with *P. pudescens* extracts components without side effects observed (Dutra *et al*., 2009).

To summarize, we have shown that *Pterodon pubescens* leaves extract induces macrophage resistance to *L. amazonensis* without side effects to host cells. However whether the effects of PPE on macrophages observed *in vitro* play a role in tissue damage occurring during the course of leishmaniasis is a question to be addressed.

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