TRYPANOCIDAL ACTIVITY OF TRITERPENE ACIDS ISOLATED FROM *MICONIA* SPECIES.

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Introduction

Chagas' disease is a chronic illness caused by the protozoan *Trypanosoma cruzi* afflicting over 18 million people in the three Americas, from Southern Argentine to the Southern United States, with a further 100 million at risk. Unfortunately, only two drugs are currently available to treat chagasic patients: nifurtimox and benznidazole. Besides presenting severe side effects and requiring long treatment, they are only effective in the acute phase of the disease. Drugs to substitute gentian violet, a dye used to sterilize banked blood to prevent transmission by transfusion are also required.
Continuing the search for potential trypanocidal compounds, the methylene chloride active extracts of *M. sellowiana* Naud. and *M. ligustroides* (DC.) Naudin. were fractionated and other triterpenes acids were identified and their activities against the trypomastigote blood forms of *T. cruzi* were evaluated. *In vivo* assays were also undertaken for the most active compounds.

Materials and Methods

M. sellowiana and *M. ligustroides* were collected along Franca-Claraval highway, São Paulo, Brazil and identified by Dr. Angela Borges Martins, Instituto de Biologia, UNICAMP.The aerial parts of the plants were dehydrated at 40°C, powdered and sequentially extracted by maceration with methylene chloride and ethanol at room temperature.
The fractionation of methylene chloride extract of *M. sellowiana* (12.3 g, VLC, silica gel) afforded 3 ursolic acid **,** a mixture of 2α-hydroxyursolic acid and maslic acid. The methylene chloride extract of *Miconia ligustroides* (6.5 g, VLC, silica gel) afforded urjnolic acid of mixture of ursolic acid and oleanoic acid. An aliquot of this mixture was purified by HPLC furnishing oleanoic acid [1]. All isolated compounds were tested *in vitro* against trypomastigote forms of *T. cruzi*. The *in vitro* trypanocidal assay was undertaken by using trypomastigote forms of *T. cruzi*, which were obtained by culture of $LLMCK_2$ cell lineage [2]. The *in vivo* assays were undertaken only for the most active compounds [3]

Results and Discussion

Results for *in vitro* tripanocidal activity indicates that ursolic acid and oleanoic acid were the most active amongst the isolated compounds, showing IC_{50} of 17.1 μM and 12.8 μM, respectively. The IC_{50} value obtained for mixture of ursolic acid and oleanoic acid was lower than those pure compounds. This can be indicative for a possible sinergistic effect. In contrast, a mixture of 2α-hydroxyursolic acid and maslic acid were much less potent than a mixture of ursolic acid and oleanoic acid. Finally, urjnolic acid displayed weak tripanocidal activity (IC_{50} 76.3 μM) when compared with the other triterpenes.
For the in vivo assay, ursolic acid showed the most significant reduction of parasites in the parasitemic peak (75.7%). In addition, the survival time was increased for all the treated animals.
The mechanism of the trypanocidal action of triterpene acids is still unknown. However, our results seem to indicate that the structural variables most consistently influencing the activity are the number of hydroxyl groups at C-2, C-23 and C-28.
Taking into account that a mixture of 2α-hydroxyursolic acid and maslic acid was much less active than mixture of ursolic acid and oleanoic acid, it is suggested that hydroxylation at C-2 results in a decrease in the tripanocidal activity. In the same manner, hydroxylation at C-23 (urjnolic acid) also is expected to lead to a decrease in the *in vitro* activity against *T. cruzi*.

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