SCREENING OF *Leishmania donovani* NUCLEOSIDE HYDROLASE ENZYME INHIBITORS (*Ld*NH) FROM EXTRACTS OF *Moringa oleifera* LAMARCK

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Introduction

The discovery of potential inhibitors is extremely important for the development of new drugs for the treatment of visceral leishmaniasis, a neglected disease responsible for high fatality rates.[1] The enzyme nucleoside hydrolase (NH) belongs to the purine salvage pathway and as the protozoan *Leishmania* also depends on this pathway for nucleotide synthesis, this pathway becomes an important source of biological targets.[2] In this sense, *Moringa oleifera* Lamarck is a medicinal plant native to northeastern India with numerous pharmacological properties, including antileishmanial activity. [3] Thus, the study aimed to screen *Ld*NH enzyme inhibitors from extracts of leaves and flowers of *M. oleifera*.

Material and Methods

Air dried leaves and flowers of *M. oleifera* were supplied by the Herbarium of the Federal University of Goiás (UFG). The extracts were prepared using two techniques: infusion and ultrasound-assisted solvent extraction. For each technique, experiments in duplicate were performed, using 3 g of leaves and 1 g of flowers dissolved, in that order, in 100 mL and 33 mL of either: ultra-pure water or water:ethanol (7:3). In the infusion extraction, the extract was subjected to a hot bath at 90°C for 1 hour, while using ultrasound, the extract was left for 20 minutes at room temperature. A further extraction was performed using ethyl acetate as diluent, which was subjected to 24 min under ultrasound. The extracts were dried using a vacuum freeze-dryer, when necessary, and the organic solvent was removed using a rotary evaporator.

The screening assay was prepared in triplicate using microtubes with control samples and microtubes with *M. oleifera* extracts at 200 µg/mL (50 µL from the stock solution at 4 mg/mL), 780 µL of phosphate buffer solution with NaCl (20 mM, pH 7.4, 300 mM NaCl), 150 µL inosine (substrate) at 5 mM, and 20 µL of magnetic particles (10 µg/mL) coated with *Ld*NH covalently immobilized, were added in both microtubes. After agitation, each microtube was submitted to a magnetic extraction for 30 seconds and the supernatant was collected for HPLC/DAD analysis.

IC_{50} values were determined for extracts with inhibition higher than 80%, in duplicate experiments. For the construction of the dose-response curves, extracts at 500 µg/mL, 200 µg/mL and 4 mg/mL were used for dilutions in the range of 0,5 - 300 µg/mL. Microtubes containing the same solutions used in the screening assay were submitted to magnetic extraction at the different extract concentrations, followed by removal of the supernatant, which was analyzed in the HPLC/DAD.

Chromatographic separation of inosine (substrate) and hypoxanthine (product) was achieved by a Supelco Ascentis C18 column (15 cm x 0,46 cm, 5 µm) using as mobile phase a solution containing triethylamine buffer (1% in water, v/v, acidified with AcOH, pH 6,0): methanol (95:5, v/v), at a flow rate of 0,8 mL/min and an injection volume of 20 µL. Detection was monitored at 249 nm.

Results and Discussion

*Ld*NH enzymes catalyze the hydrolysis of N-glycosidic bonds of ribonucleosides, such as inosine, leading to the formation of free ribose and the corresponding nitrogenous base, such as hypoxanthine (Figure 1).[4] The presence of an enzyme inhibitor decreases or completely prevents this reaction. The

percentage of inhibition can be determined by monitoring the decrease in the concentration of hypoxanthine in the presence of the inhibitor and comparing its concentration to the one found in a control sample. In the present work, this monitoring was performed by a chromatographic method previously developed and validated.[5]

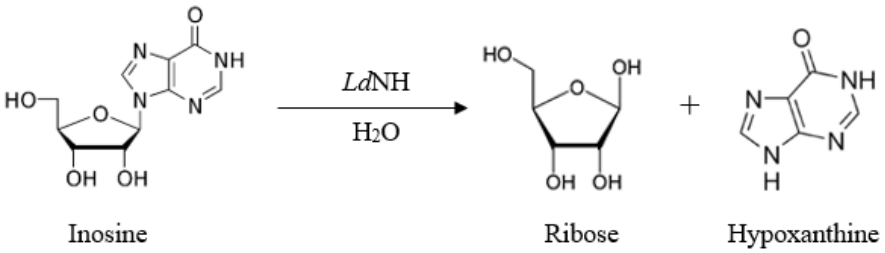

Figure 1: Hydrolysis of inosine by the enzyme *Ld*NH.

In the screening assays, extracts of leaves of *M. oleifera* prepared in the 3 solvents tested didn't show inhibition. On the other hand, the flower extracts AIEH and AUEH, obtained respectively by infusion and ultrasound using ethanol:water (7:3) as solvent showed inhibition 95.5% and 95.4% at 200 µg/mL, in that order. An inhibition of 26.8% was also observed in the flower extract obtained by ultrasound technique using ethyl acetate as solvent.

For the construction of dose-response curves, the inhibition percentage of the extracts AIEH and AUEH were evaluated in the concentration ranges 2.5 - 300 µg/mL and 0.5 - 200 µg/mL, respectively. The extracts AIEH and AUEH showed values for IC_{50} of 26.2 ± 4.63 µg/mL and 4.96 ± 0.52 µg/mL, respectively. Therefore, the most active extract in inhibiting *Ld*NH and promising for future application experiments was AUEH.

Conclusion

Through the screening of inhibitors in extracts of leaves and flowers of *M. oleifera*, extracts with inhibitory activity of *Ld*NH enzyme were found from *M. oleifera* flowers. The IC_{50} values evidenced that the most active extract was the one obtained by the ultrasound technique and extracted from ethanol:water (7:3) (AUEH).

Acknowledgments

The authors would like to acknowledge the Herbarium of the Federal University of Goiás (UFG) for the plant samples provided and the financial support from the Brazilian agencies CAPES (Finance Code 001) and FAPERJ (E-26/010.000978/2019, E-26/202.090/2019 e Edital Faperj 22/2016 - Emergency Support for Stricto Sensu Graduate Programs and Courses in the State of Rio de Janeiro Project E - 26/200.930/2017).

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