Isolation of leishmanicidal natural products produced by one species of Asteraceae

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Introduction: The Leishmaniasis, anthropozoonosis caused by protozoa of the genus *Leishmania*, have been expanding territorially. The therapeutic alternatives available present limitations due to the occurrence of serious adverse effects. The fact that the flora is an important source of drug leaders justify the search for leishmanicidal compounds of natural origin. Therefore, the objective of this project is to isolate leishmanicidal compounds of one species of Asteraceae family, using modern methods of chromatography.

Experimental part: Ethanolic leaves extract was fractionated by HSCCC using the biphasic system hexane/ethyl acetate/methanol/water (2:3:2.5:2.5). The fractions obtained were evaluated by TLC (silica gel 60F²⁵⁴) with ethyl acetate/methanol/water/acetic acid (100:13.5:10:5) and NP/PEG as spray reagent to group similar fractions. The obtained fractions were tested in murine macrophages infected with *L. amazonensis* (PINHEIRO, Diagn Micr Infec Dis, v71, 273, 2011). The fractions 1, 10 and 12 were tested at a concentration of 20µg/mL and the others in 50µg/mL. The percentage of inhibition was determined by counting under a optical microscope.

Results/Discussion: They were obtained 241 fractions in the HSCCC fractionation, which were grouped in 21 final fractions. After 48h of incubation with the infected macrophages 5 fractions were active in the test without damages to macrophages cells. They showed percentage inhibition of the parasite between 54 and 100%. The active fractions were analyzed by TLC and HPLC and these analyses showed that different classes of natural products (coumarins, flavonoids and terpenoids) may be responsible for leishmanicidal activity. The preparative HPLC method to isolate the bioactive compounds has been determined.

Conclusion: The use of HSCCC technique was adequate and allowed the semi-purification of the crude extract. The good results showed by the five fractions support the need for the isolation of the active natural products that have been already in progress.

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