

IDENTIFICATION OF ANTITRYPANOSOMAL AND ANTILEISHMANIAL LEADS FROM PLANT EXTRACTS

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Visceral leishmaniasis and African trypanosomiasis are diseases caused by protozoan parasites which contribute to high mortality and morbidity rates in developing countries. Currently there are no vaccines available for either of these disease and the drugs that are used for their treatment are toxic, difficult to administer and in some cases compromised by the development of resistance. The Werbovetz and Kinghorn labs are now working together to investigate natural products as a source of antiparasitic compounds that could be developed into drugs that are more effective, less toxic, less costly and administered orally.

Recent work in our laboratories has focused on the random screening of plants available in the College of Pharmacy for selective antiparasitic activity (phase 1) and the isolation of pure, biologically active compounds from selective plant extracts (phase 2). In phase 1, plant materials were first extracted with methanol, solvent was evaporated to a lesser volume and partitioned between distilled water and hexanes (hexanes were used to remove waxes and oils). The methanol/distilled water layer was then further partitioned with chloroform. The chloroform fraction was detannified with 1% aqueous sodium chloride then evaporated to provide a crude extract for activity assays to determine if the extracts possessed antileishmanial and/or antitrypanosomal activity. Active extracts were further examined against mammalian Vero cells to determine their toxicity. Among the plants shown to be non toxic, *Grindelia squarrosa* was selected for further investigation against African trypanosomes (IC_{50} vs *T. brucei* = 8 $\mu\text{g/mL}$) to begin phase 2. A large scale (2.2 kg) crude extract of *Grindelia squarrosa* was prepared in a slightly different but similar partition scheme as phase 1. All of the extracts (distilled water/methanol, hexanes, ethyl acetate, and water) were tested to determine their activity. The hexanes fraction displayed the greatest activity, with an IC_{50} of 8.5 $\mu\text{g/mL}$, and was selected for fractionation. As an initial attempt to isolate bioactive compounds, the extract was chromatographed using a silica gel column. Fractions 19 and 58 have shown the highest activity against *T. brucei*, with IC_{50} values of 0.7 $\mu\text{g/mL}$ and 1.3 $\mu\text{g/mL}$, respectively. Future work on this project will include further bioactivity-guided fractionation using the in vitro antitrypanosomal assay to select subfractions for further purification. Column chromatography, HPLC, and/or preparative TLC will be used to isolate pure compounds, and NMR spectroscopy and mass spectrometry will be used for structure elucidation of active compounds.

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