IN-VITRO BIOACTIVITY OF FRACTIONS FROM A LOCAL MEDICINAL PLANT ON HIV-1 REPLICATION, AND SELECTED FUNGAL AND BACTERIAL PATHOGENS

By

Awelani M. Mutshembele

Submitted In fulfilment of the requirements for the degree of
Master of Science in the subject Microbiology at the
University of Venda

To the
Department of Microbiology
School of Mathematics and Natural Sciences
University of Venda, Thohoyandou 0950
South Africa

March 2009

Supervisor: Dr Pascal O. Bessong
Co-supervisors: Prof Jacobus N. Eloff
Prof Larry Obi

SUMMARY

In an earlier pilot study, a crude methanol extract from the stem-bark of *Peltophorum africanum* was evaluated for anti-HIV activity. The extract showed a strong inhibition against HIV-1 RT DNA polymerase and RNase H activities. An oxidized gallotannin from the extract also inhibited HIV-1 reverse transcriptase and integrase in an enzyme cell- free system (Bessong *et al.*, 2005). To broaden, the scope of the investigation on *P. africanum* it was important to evaluate different fractions from the plant obtained with different solvent system, in an attempt of identify other possible inhibitory compounds. In addition, it was important to evaluate extracts of the plant for anti-HIV replicative properties in infectious cultures.

Methanol crude extract, fractions of the extract, and isolated compounds were evaluated for bioactivity against HIV-1 *reverse transcriptase* (RT). Fractions and compounds with high inhibition were also tested against viral replication in infectious cultures. Fractions were obtained by silica gel column chromatography and further purified by Sephadex-LH 20 column chromatography. Fractionation led to the isolation of a white powdered compound which was identified as bergenin by spectroscopic methods. Inhibition of HIV-1 RT was performed using a RT colorimetric assay. A total of 28 fractions were obtained. Fraction C2 inhibited RT activity by 92% at 250 µg/ml. Other fractions such as B4, B6, D3 and D6 gave 87%, 84%, 78% and 50% inhibition respectively. C2 had significant inhibition at 100 and 250 µg/ml ($P< 0.05$). In infectious cultures, evaluating intracellular mode of action, bergenin did not inhibit HIV-1 replication, while the strongest inhibition was observed for fraction C2 with a mean IC_{50} of 9.5 µg/ml based on p24 antigen production. C2 shows the best activity in the cell-free anti HIV assay and in infectious cultures. In addition C2 did not exhibit any cytotoxicity at concentrations less than 250 µg/ml in a CellTiter Cell Proliferation Assay. C2 is worthy of further chemical and biological analysis.

Bioactivity of fractions of the methanol crude extract of the stem bark of *Peltophorum africanum* against the fungi *Candida albicans*, and *Cryptococcus neoformans*; and the bacteria *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Enterococcus faecalis* was also investigated. To evaluate the inhibition of the plant extract and fractions on these fungi

and bacteria, the minimum inhibitory concentration (MIC), and bioautographic profiles were assessed. Twenty eight fractions were initially screened by bioautography and MIC. Pooling was done for similar fractions based on bioautographic activity. After two rounds of pooling five fractions gave the best active profiles against one or several of the organisms. These fractions were B4, D1, D7, H1, and H3. Based on the bioautography and MIC, B4 was the most active against *P. aeruginosa, S. aureus, E. coli*, and *E. faecalis*. The question to answer is whether B4 has a broad spectrum or non-specific mode of inhibition. Further studies will elucidate the chemical constituent or constituents of B4 and its mode of action.